Committee for Risk Assessment

RAC

Annex 1

Background document

to the Opinion proposing harmonised classification and labelling at EU level of

Granulated copper

EC Number: 231-159-6
CAS Number: 7440-50-8

CLH-O-0000001412-86-216/F

The background document is a compilation of information considered relevant by the dossier submitter or by RAC for the proposed classification. It includes the proposal of the dossier submitter and the conclusion of RAC. It is based on the official CLH report submitted to public consultation. RAC has not changed the text of this CLH report but inserted text which is specifically marked as 'RAC evaluation'. Only the RAC text reflects the view of RAC.

Adopted

8 June 2018

CLH report

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation),
Annex VI, Part 2

Substance Name: Copper, granulated

EC Number: 231-159-6

CAS Number: 7440-50-8

Index Number: -

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Part A.

1 PROPOSAL FOR HARMONISED CLASSIFICATION AND LABELLING

1.1 Substance

Table 1: Substance identity

Substance name:	Copper, granulated
EC number:	231-159-6
CAS number:	7440-50-8
Annex VI Index number:	-
Degree of purity:	min 99.0% (w/w)
Impurities:	See annex I (confidential)

1.2 Harmonised classification and labelling proposal

Table 2: The current Annex VI entry and the proposed harmonised classification

	CLP Regulation
Current entry in Annex VI, CLP Regulation	-
Current proposal for consideration by RAC	Eye Irrit. 2 – H319 Aquatic Chronic 2 – H411
Resulting harmonised classification (future entry in Annex VI, CLP Regulation)	Eye Irrit. 2 – H319 Aquatic Chronic 2 – H411

1.3 Proposed harmonised classification and labelling based on CLP Regulation

Table 3: Proposed classification according to the CLP Regulation

CLP Annex I ref	Hazard class	Proposed classification	Proposed SCLs and/or M-factors	Current classification [1)]	Reason for no classification [2)]
2.1.	Explosives	None	-	None	Conclusive but not sufficient for classification
2.2.	Flammable gases	None	-	None	Not relevant
2.3.	Flammable aerosols	None	-	None	Not relevant
2.4.	Oxidising gases	None	-	None	Not relevant
2.5.	Gases under pressure	None	-	None	Not relevant
2.6.	Flammable liquids	None	-	None	Not relevant
2.7.	Flammable solids	None	-	None	Conclusive but not sufficient for classification
2.8.	Self-reactive substances and mixtures	None	-	None	Conclusive but not sufficient for classification
2.9.	Pyrophoric liquids	None	-	None	Not relevant
2.10.	Pyrophoric solids	None	-	None	Conclusive but not sufficient for classification
2.11.	Self-heating substances and mixtures	None	-	None	Conclusive but not sufficient for classification
2.12.	Substances and mixtures which in contact with water emit flammable gases	None	-	None	Conclusive but not sufficient for classification
2.13.	Oxidising liquids	None	-	None	Not relevant
2.14.	Oxidising solids	None	-	None	Conclusive but not sufficient for classification
2.15.	Organic peroxides	None			Not relevant
2.16.	Substance and mixtures corrosive to metals	None			Conclusive but not sufficient for classification
3.1.	Acute toxicity - oral	None	-	None	Conclusive but not sufficient for classification
	Acute toxicity - dermal	None	-	None	Conclusive but not sufficient for classification
	Acute toxicity - inhalation	None	-	None	No data
3.2.	Skin corrosion / irritation	None	-	None	Conclusive but not sufficient for classification
3.3.	Serious eye damage / eye irritation	Eye Irrit 2 – H319	-	None	
3.4.	Respiratory sensitisation	None	-	None	No data

3.4.	Skin sensitisation	None	-	None	Conclusive but not sufficient for classification
3.5.	Germ cell mutagenicity	None	-	None	Conclusive but not sufficient for classification
3.6.	Carcinogenicity	None	-	None	Conclusive but not sufficient for classification
3.7.	Reproductive toxicity	None	-	None	Conclusive but not sufficient for classification
3.8.	Specific target organ toxicity –single exposure	None	-	None	No data
3.9.	Specific target organ toxicity – repeated exposure	None	-	None	Conclusive but not sufficient for classification
3.10.	Aspiration hazard	None	-	None	No data
4.1.	Hazardous to the aquatic environment	Aquatic Chronic 2-H411	-	None	
5.1.	Hazardous to the ozone layer	None		None	No data

[1)] Including specific concentration limits (SCLs) and M-factors
[2)] Data lacking, inconclusive, or conclusive but not sufficient for classification

Labelling:

Signal word: Warning

Hazard pictogram: GHS07, GHS09

Hazard statements: H319, H411

Proposed notes assigned to an entry: None

2 BACKGROUND TO THE CLH PROPOSAL

2.1 History of the previous classification and labelling

There is no harmonised classification for copper metal or copper metal in the form of granules (See section 1.2 of Part A of the report).

2.2 Short summary of the scientific justification for the CLH proposal

Copper, granulated has been approved in Regulation (EU) 528/2012 (Biocidal Products Regulation) on 1st January 2017. The data sources used for this CLH report include the data available in the biocidal dossier and the dataset of the updated REACH registration dossier as available on January 2017.

A classification for eye irritation is proposed for copper, granulated. Data on copper flakes (RAC opinion adopted in December 2014, CLH-O-0000001412-86-30/F) and data of bioelution are used to

support the non-classification for acute toxicity, skin irritation and sensitisation. No toxicological classification is proposed for the other endpoints: repeated toxicity, carcinogenicity, mutagenicity and reprotoxicity; based on a read-across from available studies on copper sulphate such as already presented for the other copper salts assessed by the RAC (RAC opinions on copper flakes and nine copper compounds adopted by RAC in December 2014).

With regard to acute aquatic classification and by taking into account the recommendations of Annex IV of the Guidance on the Application of the CLP criteria:

- the dissolved metal ion concentrations after a period of 7 days at a loading rate of 1 mg/L are below the acute ERVs of the dissolved form of copper, whatever the pH range. Therefore, **granulated copper does not have acute aquatic classification.**

With regard to long-term aquatic classification and by taking into account the recommendations of Annex IV of the Guidance on the Application of the CLP criteria:

- the estimated dissolved metal ion after a period of 28 days at a loading rate of 0.1 mg/L are below the chronic ERVs at all pH-classes (6, 7 and 8). Therefore, granulated copper does not have aquatic chronic 1 classification
- the estimated dissolved metal ion after a period of 28 days at a loading rate of 1 mg/L are above the chronic ERVs at at pH 6 and 7. Therefore, granulated copper is classified Aquatic chronic 2.

Therefore, **granulated copper is proposed to be classified as Aquatic Chronic 2.**

2.3 Current harmonised classification and labelling

There is no harmonised classification for copper metal or copper metal in the form of granules (See section 1.2 of Part A of the report).

2.4 Current self-classification and labelling

The classification applied by the applicant of the active substance dossier in the scope of Regulation (EU) 528/2012 (Biocidal Products Regulation) is the following:

- Aquatic Acute 1, H400 – Very toxic to aquatic life
- Aquatic chronic 3, H412 - Harmful to aquatic life with long lasting effects

RAC general comment
Granulated copper is a form of copper metal defined by its particle size and specific surface area. In the CLP guidance, the default diameter for massive metal is 1 mm. If the diameter of a sphere for massive copper is > 1 mm, the corresponding surface area is < 0.67 mm^2/mg (<6.74 cm^2/g). However, granulated copper particles are cylindrical with a length greater than 1 mm (range: 0.9 – 6.0 mm; mean: 2.1 mm) and width below 1 mm (range: 0.494 – 0.949 mm; mean: 0.706 mm), and a surface area of 25.6 cm^2/g (significantly above the limit for massive). As such, it is considered to be between massive (defined as a sphere with a diameter >1 mm and a surface area of <6.74 cm^2/g) and the powder form (diameter of <0.2 mm and a surface area of 240 cm^2/g) of copper.

RAC previously evaluated CLH proposals for ten other copper compounds from the same dossier submitter (DS) (France). For these copper compounds, as well as now for granulated copper, the dossier submitter stated that where systemic toxicity is concerned, the toxicologically relevant moiety is the Cu^{2+} ion, which is released to a different degree from all the copper compounds. A comparison of the bioavailability (and hence toxicity) of various copper compounds showed that bioavailability is highest for the most soluble compound copper sulphate. Consequently, the use of copper sulphate data would represent a worst-case scenario for the determination of the systemic toxicity of relatively insoluble copper compounds (such as granulated copper). For the assessment of the systemic endpoints, with no data available on granulated copper itself, the dossier submitter therefore proposed to read-across from data on the different copper compounds previously evaluated. The present CLH report is thus similar to the other ten copper compounds for STOT RE, germ cell mutagenicity, carcinogenicity and reproductive toxicity. The test substance in studies reported in these common sections is most often copper sulphate pentahydrate, but sometimes also other copper compounds have been tested.

RAC previously considered the dossier submitter's proposal to group the information on copper containing substances together for consideration of STOT RE and the CMR endpoints. RAC noted then that differences in solubility and other physico-chemical properties may potentially impact the toxicity of the various copper compounds, in particular locally after inhalation exposure. RAC noted further that the anions, in particular thiocyanate, might also be a contributing factor to the toxicity. However, these aspects were not addressed in the CLH reports on the ten copper compounds, whereas RAC concluded that these would need a more detailed analysis. But as none of the studies with copper sulphate pentahydrate or the other tested copper substances yielded positive evidence for the classification for these endpoints, RAC at that time did not pursue the aspect of grouping the copper containing substances any further. For the present evaluation of granulated copper, RAC maintains this position, whilst noting that for granulated copper (with no anion present) only toxicity of the Cu^{2+} ion is relevant.

For the assessment of the local and acute human health endpoints (acute toxicity, STOT SE, skin irritation/corrosion, eye damage/irritation and skin sensitisation), the dossier submitter proposed to read-across from the data on the previously evaluated coated copper flakes, in combination with a comparative analysis of particle and solubility characteristics. With granulated copper being a particle with the aforementioned defined size, it was argued that copper massive, copper powder and coated copper flakes are the most representative forms for extrapolation. Since toxicity data are only available for coated copper flakes, read-across from this substance was proposed. RAC's considerations on this read-across in combination with the comparative analysis are presented in the respective local and acute endpoint sections.

3 JUSTIFICATION THAT ACTION IS NEEDED AT COMMUNITY LEVEL

There is no harmonised classification for copper metal or copper metal in the form of granules (See section 1.2 of Part A of the report).

Harmonised classification and labelling (CLH) for coated copper flakes and nine copper compounds has been recently submitted by the ANSES and recommendations for the corresponding forms have been adopted by the RAC in their opinions of 4 December 2014. The adopted CLH have been published in the 9th ATP of the CLP.

Granulated copper is an active substance in the meaning of Regulation (EU) 528/2012 (Biocidal Products Regulation) that has been recently approved. It is not covered by any of the classification entry previously discussed.

In accordance with Article 36(2) of the CLP Regulation, granulated copper shall therefore be subjected to a full harmonised classification and labelling. Therefore, this proposal considers all physical, health and environmental hazard classes.

Part B.

SCIENTIFIC EVALUATION OF THE DATA

1 IDENTITY OF THE SUBSTANCE

1.1 Name and other identifiers of the substance

Table 5: Substance identity

Substance name	Copper, granulated
EC number:	231-159-6
EC name:	Copper
CAS number:	7440-50-8
CAS name:	Copper
IUPAC name:	Copper
CLP Annex VI Index number:	-
Molecular formula:	Cu
Molecular weight range:	63.55 g/mol

Structural formula: Cu

1.2 Composition of the substance

Table 6: Constituents (non-confidential information)

Constituent	Typical concentration	Concentration range	Remarks
Copper, granulated (CAS 7440-50-8)		≥ 99.0% (w/w)	

Table 7: Impurities (non-confidential information)

Impurity (Name and numerical identifier)	Concentration range (% w/w minimum and maximum)	Current CLH in Annex VI Table 3.1 (CLP)	The impurity contributes to the classification and labelling
Nickel (CAS 7440-02-0)	Max 0.001%	Skin Sens. 1; H317 Carc. 2; H351 STOT RE 1; H372 Aquatic Chronic 3; H412	no
Arsenic (CAS 7440-38-2)	Max 0.0005%	Acute Tox. 3 *; H301 Acute Tox. 3 *; H331 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	no
Cadmium (CAS 7440-43-9)	Max 0.0001%	Acute Tox. 2 *; H330 Muta. 2; H341 Carc. 1B; H350 Repr. 2; H361fd STOT RE 1; H372 Aquatic Acute 1; H400 Aquatic Chronic 1; H410	no
Lead (CAS 7439-92-1)	Max 0.0008%	Lact.; H362 Repr. 1A; H360FD	no
Sodium (CAS 7440-23-5)	Max 0.01%	Water-react. 1; H260 Skin Corr. 1B; H314	no

Further information is presented in the confidential annex I.

Table 8: Additives (non-confidential information)

Additive	Function	Typical concentration	Concentration range	Remarks
Not applicable				

The substance as manufactured is copper with a cylindrical shape. The length ranges between 0.9 and 6.0 mm with a mean at 2.1mm and the width ranges between 0.494 and 0.949mm with a mean at 0.706mm.

For the purposes of this CLH report, the substance has been defined as granulated copper and is considered between massive and powder form:

- In the CLP guidance, copper massive is defined as a sphere with a diameter > 1mm and with a corresponding surface area of < 0.67 mm^2/mg (<6.74 cm^2/g).

The particles of the substance granulated copper are cylindrical with size higher than 1mm for one dimension (the length ranges between 0.9 and 6.0 mm with a mean at 2.1mm) and with the other size below 1 mm (the width ranges between 0.494 and 0.949mm with a mean at 0.706mm) whereas the surface area of the substance granulated copper has been found to be 25.6cm^2/g which is significantly above the limit for massive. Therefore the substance granulated copper cannot be defined as massive form.

- The substance granulated copper cannot also be defined as a powder because the active substance is not classified by inhalation based on the particle size and copper powder can have lower particle size which will lead to a classification by inhalation.

1.2.1 Composition of test material

The purity and the tested material is specified when available and/or relevant in the different parts of the CLH report.

Some information in the literature shows that nanomaterials containing copper compounds may exist. However, the information available in the biocidal dossier does not seem to indicate that the substance exists under this form for these applications.

In this context, it was decided not to take into consideration the potential nanoform of copper compounds in this report and thus the present CLH dossier is proposed for granulated copper. A specific dossier and hazard evaluation may be necessary for nanoforms of this substance.

1.2.2 Physico-chemical properties

The tested material for physico-chemical properties is copper powder which is not consistent with the specification of the active substance as manufactured (granulated copper targeted by the present CLH report). See further details in the confidential annex. Differences between the tested material and the substance as manufactured are not considered to impact the measured physico-chemical properties:

melting point, relative density and solubility in water. Therefore the results of the measured physico-chemical properties of the test material are acceptable for granulated copper.

Table 9: Summary of physico - chemical properties

Property	**Value**	**Reference**	**Comment (e.g. measured or estimated)**
State of the substance at 20°C and 101,3 kPa	Course, granular solid Red brown Slight, metallic odour	Hughes 2013; Particulate Copper Metal Analysis; Technical Summary Report TSR 13 01	
Melting/freezing point	1059-1069°C	Liipo, J. et al (2010); Characterisation of copper powder, Outotec Research Oy report number 10113-ORC-T	Measured, method EC A.1
Boiling point	Not necessary as boiling point will occur at temperatures greater than 360°C based on the melting point of granulated copper	-	-
Relative density	8.78	Liipo, J. et al (2010); Characterisation of copper powder, Outotec Research Oy report number 10113-ORC-T	Measured, method EC A.3
Vapour pressure	It is not required to test vapour pressure as the melting point is above 300ºC	-	-
Henry's law constant	Not possible to calculate without vapour pressure value	-	-
Surface tension	Not required for substances with a water solubility of < 1 mg l^{-1}	-	-
Water solubility	pH 6,34-7.56 at 20°C: 1 mg/L The solubilisation results of the oxido-reduction reaction of the copper metal into ionic copper. Cu(0) → Cu(I) → Cu (II) At low pH, these reactions are promoted.	Liipo, J. et al (2010); Characterisation of copper powder, Outotec Research Oy report number 10113-ORC-T	Measured, method OECD 105
Partition coefficient n-octanol/water	Not relevant for the ecotoxicological risk assessment, due to the specific absorption mechanism of copper.	-	-

Flash point	Not required because the active substance is a solid	-	-
Flammability	Not highly flammable based on chemical composition and experience in use. Granulated copper is thermally stable up to 1000°C.	- Liipo, J. et al (2010); Characterisation of copper powder, Outotec Research Oy report number 10113-ORC-T	-
Explosive properties	Based on the chemical composition and experience in use, it is considered that the test according to EC method A.14 would give a negative result for granulated copper.	-	-
Self-ignition temperature	No data	-	-
Thermal stability	Stability in inert atmosphere (nitrogen) and in air atmosphere up to 1000°C was confirmed. In air, the only possible evolution is oxidation to copper oxides (CuO and others).	Liipo, J. et al (2010); Characterisation of copper powder, Outotec Research Oy report number 10113-ORC-T	
Oxidising properties	No oxidising properties based on chemical composition and experience in use.	-	-

Granulometry	The length ranges between 0.9mm and 6.0mm, with a mean length (on the 150 measured granules) at 2.1mm. 90% of the particles are below 3mm and 50% of the particles are below 2mm. The width ranges between 0.494mm and 0.949mm. There are two maxima at 0.65mm and at 0.87mm with an average particle width (on the 150 measured granules) at 0.706mm. 90% of the particles are below 0.9mm and 50% of the particles are below 0.65mm.	Gordon Fern (2015) Particle size distribution by optical and Scanning Electron Microscopy : Copper granules from Arch Timber Report SNP-079	Measured (optical and Scanning Electron Microscopy) The length of the granules has been determined by measuring the length of 50 granules, this has been repeated three times with different samples of 50 granules. The cross section of the diameter which is equivalent to the width of the granule (viewed top-down) has been determined by a SEM analysis. The three previous samples have been used. Granule width was measured at 3 points along the length.
Specific surface area	25.6 cm^2/g	Hughes 2013; Particulate Copper Metal Analysis; Technical Summary Report TSR 13 01	Measured (BET)
Solubility in organic solvents	Granulated copper is soluble in aqueous monoethanolamine but is practically insoluble in organic solvents.	Hughes 2013; (a) Dissolution of Copper Granules; Technical Summary Report TSR 13 02 (b) Analysis of Solids from Copper Amine Solution; Technical Summary Report TSR 13 03	Measured, in-house method
Stability in organic solvents and identity of relevant degradation products	Not required. The active substance as manufactured does not include any organic solvents.	-	-
Dissociation constant	Not relevant, metallic copper cannot dissociate in water, due to its structure. Granulated copper is slightly soluble in water and the solubilisation results of oxido-reduction reaction of the copper metal into ionic copper. Any addition of acid would result in reaction with the copper	-	-

Viscosity	Not required because the active substance is a solid	-	-
Reactivity towards container material	No reactivity towards PP bags	-	Experience in use

2 MANUFACTURE AND USES

2.1 Manufacture

See confidential annex I.

2.2 Identified uses

Granulated copper is intended to be used as a biocidal active substance in wood preservative products (PT8 according to Regulation (EU) 528/2012), for the preventive preservation of wood in Use class 1, 2, 3 and 4 as defined in the EN 335[1]. The active substance is restricted to industrial use only, in timber treatment plants operated by trained personnel.

[1] Since 2007 and the revision of the EN335-1, use classes had replaced hazard classes.

3 CLASSIFICATION FOR PHYSICO-CHEMICAL PROPERTIES

Table 10: Summary table for relevant physico-chemical studies

Method	Results	Remarks	Reference
Flammability - Ignition	Not highly flammable Based on the chemical composition and experience in use, a determination of flammability was not carried out as this test could be predicted to give a negative result for granulated copper. Moreover granulated copper is thermally stable up to 1000°C	Theoretical assessment	- Liipo, J. et al (2010); Characterisation of copper powder, Outotec Research Oy report number 10113-ORC-T
Flash point	Not required as the active substance is a solid	-	-
Explosive properties	Based on the chemical composition and experience in use, it is considered that the test according to EC method A.14 would give a negative result for granulated copper.	Theoretical assessment	-
Oxidizing properties	No oxidising properties based on chemical composition and experience in use.	Theoretical assessment	-

3.1 Explosivity

Based on the chemical composition and experience in use, it is considered that the test would give a negative result for granulated copper.

As a metallic powder, granulated copper dust clouds may explode but explosivity due to powder forms is not covered by the CLP criteria.

3.2 Flammability

Not highly flammable. Granulated copper is thermally stable up to 1000°C.

As a metallic powder, granulated copper dust clouds may ignite but flammability due to powder forms is not covered by the CLP criteria.

3.3 Oxidizing potential

Based on the chemical composition and experience in use, it is considered that granulated copper has no oxidizing properties.

RAC evaluation of physical hazards

Summary of the Dossier Submitter's proposal

Granulated copper is a stable inorganic compound. Based on the chemical composition and experience in use, the dossier submitter does not expect granulated copper to have flammable, explosive or oxidising properties. Moreover, granulated copper is indicated to be thermally stable up to 1000 °C. The dossier submitter therefore proposed no classification for physical hazards.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC supports **no-classification for physical hazards**, as proposed by the dossier submitter.

4 HUMAN HEALTH HAZARD ASSESSMENT

Repeated Toxicity/CMR endpoints

Considering that in mammalian the toxic form of any copper is the Cu^{2+} ion, a read across with the data on the different salts previously evaluated for classification (copper sulphate, dicopper oxide, copper hydroxide, copper oxide, copper carbonate, copper thiocyanate, coated copper flakes, copper oxychloride and Bordeaux mixture) will be used for assessment of repeated toxicity, mutagenicity, carcinogenicity and reprotoxicity of granulated copper. Therefore, the report of these endpoints is similar to the previous CLH reports for copper compounds.

In mammalian toxicity, it is considered that the toxic form of any copper compound is the Cu^{2+} ion. This is shown through the comparison of bioavailability and hence toxicity of the most soluble (copper sulphate) and relatively insoluble copper salts. In fact, the use of copper sulphate data would represent a worst-case scenario for the determination of the systemic effect of relatively insoluble copper compounds in mammalian toxicity. This has also been confirmed in a series of bioavailability studies conducted by several authors who have compared the bioavailability of copper sulphate to other copper salts including copper oxide, coated copper flakes, copper thiocyanate and copper carbonate. Moreover, in another study copper was administered orally to bile-canulated rats, as copper sulphate, copper hydroxide, copper oxychloride, Bordeaux mixture, tribasic copper sulphate and copper (I) oxide. There were no differences in absorption, copper levels in plasma, liver or bile, or in excretion rates between the five forms and copper sulphate. This study demonstrates bioequivalence between the five forms and copper sulphate, such that repeated dose toxicity studies on copper

sulphate, or on only one of the five forms, may be considered representative of the other forms for systemic effects.

In 2010, Rodriguez *et al.* assessed the relative/dissolution of copper ions from copper materials and copper compounds in gastric mimetic fluid (pH 1.5). The results show a highest solubility of copper sulphate. Therefore in order to reduce animal testing, as copper sulphate release more ion Cu^{2+} than the other copper compounds and it is considered that the toxic form is the Cu^{2+} ion, read-across from copper sulphate data can be applied to all long term studies by oral routes , as a worst case.

In conclusion, for systemic effects of granulated copper, read-across is performed on copper sulphate data such as done for the different salts previously evaluated for classification and labelling (copper sulphate, dicopper oxide, copper hydroxide, copper oxide, copper carbonate, copper thiocyanate, coated copper flakes, copper oxychloride and Bordeaux mixture). The endpoints concerned by this approach are repeated dose toxicity, carcinogenicity, mutagenicity and reprotoxicity**. Indeed, as there is no counter anion here, the toxicity of ion Cu^{2+} only seems to be relevant.**

Acute toxicity

No data on granulated copper are available for acute toxicity, irritation and sensitization. The acute toxicity will be based on coated copper flake as data are available for coated copper flake only (no data are available for massive and powder forms).

For acute toxicity, biosolubility is the main parameter leading the toxicity. Data on biosolubility on the different forms (massive, powder and coated copper flakes) have thus been taken into account.

The study of Rodriguez *et al., 2010* (already mentioned above) submitted under REACH dossier by the applicants demonstrates that granulated copper would not induce toxicity compared to other forms of copper when particle size and surface area for example, are taken into account. Three forms of copper are identified in the REACH dossier on the basis of particle size and surface area, i.e. copper wire massive, copper powder and coated copper flake. Coated copper flake is a special designation in which the copper flake has been coated with stearic acid/zinc stearate. The coating stabilises the flake in small particle sizes (preventing aggregation), resulting in a higher surface area and the potential of higher bioavailability. All three forms of copper are relatively insoluble in water at high and neutral pH, but differences in solubility become evident at lower pH (pH 1.5 in simulated gastric fluid solution). At lower pH the coated copper flake appears to be significantly more soluble than the other forms of copper and is therefore more bioavailable.

Table 11: Comparaison of particle size and surface area between granulated copper, copper wire massive and copper powder used in the study (Rodriguez et al., 2010)

	Particle size (diameter) min -max	**Surface area**
Copper wire massive a) sphere equivalent*	130 µm	-
Copper wire massive b) sphere equivalent*	400 µm	-
Copper wire massive c) sphere equivalent*	1000 µm	-
Granulated copper (assessed in this dossier)	1086 µm	0.00256 m^2/g
Copper powder	100-200 µm	0.024 m^2/g
Coated copper flake	8-10 µm	2.9 m^2/g

**Three wires of different diameter were tested. The particle size was expressed as a diameter. As the material tested in the Rodriquez study (2010) was cut pieces the diameter is assumed based on the spherical equivalent. Hence the designation as copper sphere equivalent.*

As shown in Table 11, the physico-chemical parameters of granulated copper (diameter: 1086 µm; specific area of 0.00256 m^2/g) assessed in this dossier could be considered in the range of massive wire and powder copper. Therefore, as only data are available for the coated copper flake, which represents a worst case for the granulated copper, the read-across approach will be based on this form.

Rodriguez *et al*, 2010 assessed the relative *in vitro* release/dissolution of copper ions from copper materials and copper compounds in gastric fluids. The *in vitro* test follows the ASTM D 5517-07 protocol, using HCl 0.07N (pH 1.5). The results from this test give a conservative measure of biosolubility (bio-elution test) because only solubility in the gastric fluid is assessed and the homeostatic mechanisms at the level of the intestine and liver are ignored. The resulting value is termed biosolubility is defined as the fraction of a substance that is soluble under physiological conditions and therefore "potentially available" into systemic circulation.

The copper compounds tested include: copper sulphate ($5H_2O$), copper wires (representing massive copper materials), copper powder (0.1-0.2 mm diameter) and coated copper flakes. Loading rates between 200 mg/L and 2 g/L were assessed.

The results are expressed as % mass recovered at the end of the bio-elution test and compared with the results obtained from soluble copper sulphate. The influence of surface area on biosolubility, of relevance to copper in powder and massive forms, was also evaluated.

Table 12 below presents the results obtained in the study of Rodriguez et al. (2010). All copper present in copper sulphate at 2g/L was solubilised (99.95%). Coated copper flake samples showed solubility between 41.6 and 71.5%, at 2 g/L loading, and 200 mg/L loading, respectively. The results for copper in powder form showed a coefficient of variation between the replicas of 66% at sample loading of 2 g/L, with 7% copper release. On the other hand, at 200 mg/L loading, 1.1% copper solubility was found with a lower coefficient of variation between vessels. The high variability at the higher loading rate is possibly related to abrasion of the particles. The "massive" copper material, tested as wires at different mass loadings (59 to 478 mg/L) and surface loadings (67 – 516 mm^2/L) consistently showed a solubility of 0.1%. The results are summarized in table below:

Table 12: Relative biosolubility of copper and copper compounds, assessed from the recovery of copper after bioelution tests in gastric fluids (pH 1.5) (Rodriguez *et al.*, 2010).

Material Tested	Composition	Bioelution recovery (as % of Cu content)
Copper wire massive	>99.9% Cu	0.096 – 0.105
Copper powder	99.7% Cu, 0.3% Cu_2O	1.1 – (7.03*)
Coated copper flakes	93.7% Cu, 2.6% Cu_2O, 3.89% LOI**	42 – 71
Copper sulphate	25.45% Cu	100

*The results at the higher loading rate show unacceptably high variability (CV of 66%), possibly related to abrasion of the particles during the test. The results of this test are therefore not considered as reliable. ** Loss on ignition, as a measure of the organic content.

For the copper massive and copper powder materials, the results are also expressed as surface loading (table 13 below). For the massive material, the linear regression observed between the dissolved copper concentration (µg Cu/L) and the surface loading (mm^2/L) allows the calculation of an average surface-specific release rate of 0.9 $µg/mm^2$ (figure 1 below). For the powder, a reliable surface-specific release rate of 0.5 $µg/mm^2$ was measured. For consistency, the slightly more conservative value, recorded for the massive material (0.9 $µg/mm^2$) was retained for all non-coated forms (massive and powder).

Table 13: Biosolubility of copper as a function of the particle surface area as obtained from bioelution tests in gastric fluids (pH 1.5) (Rodriguez et al., 2010)

Material tested	Surface loading (mm^2/L)	Bioelution recovery (µg Cu/l)	Surface-specific release ($µg/mm^2$)
Copper massive (wire)	67-516	62-460	0.89-0.92
Copper powder	4800	2208	0.5*

*The results from the lower loading rates are more reliable and these were therefore retained.

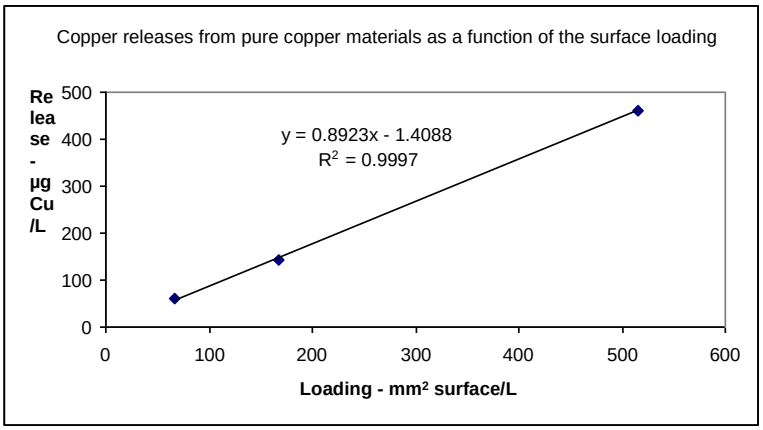


Figure 1: Release of copper from wires with different amounts of surface exposed to the gastric mimetic fluid.

Copper release from the blank and copper wires of 67.3, 167.8 and 516.1 mm2/L surface loading, equivalent to the surface of spheres of 0.13, 0.4 and 1 mm in diameter, respectively, tested at concentrations of 2 g/L.

Finally, the study from Rodriguez *et al*., 2010 demonstrates a large variability in the gastric biosolubility of copper-bearing materials. The recorded copper biosolubility, relative to copper sulphate, were as follows: copper coated flakes, 42 – 71%; copper powder, 1.1%; copper massive forms, 0.1%. The coated copper flake is thus the most bioavailable form of copper. The granulated copper would be comprised between massive forms and powder.

This conclusion can also be seen in 28-day transformation/dissolution tests in solutions at pH6 (Rodriguez *et al.*, 2011; 2012 and Klawonnet al., 2013) presented in the following Table 14.

Table 14: Biosolubility of copper as a function of the particle surface area as obtained from bioelution tests in gastric fluids (pH 1.5) (Rodriguez *et al.*, 2010)

Form of copper	Surface area (mm^2/mg)	Loading tested	Transformation/Dissolution at pH6 µg dissolved Cu-ions/L	
			7-days	28-days
Massive (1mm)	0.67	1 mg	1	3.4
Powder (10 µm-1mm)	67 – 107	1 mg	28 – 44	112 – 176
Flakes (10 µm)	2080 - 2900	1 mg	720	744 - 813

In conclusion, for acute toxicity, there is no available data for granulated copper. Among the different forms of copper, studies are available for only coated copper flake which represents the appropriate form for the read-across approach to granulated copper.

Irritation and sensitisation
No data on granulated copper are available for irritation and sensitization. As granulated copper is a powder, copper massive, copper powder and coated copper flake are the most representative forms for extrapolation. The irritation and sensitization endpoints will be based on coated copper flake as data are available for coated copper flake only (no data are available for massive and powder forms).

For irritation and sensitisation, the solubility is not the only physico-chemical parameter to take into account for this kind of toxicity. Therefore, as there is no available data for the granulated copper, a read-across from the coated copper flake studies is proposed as worst case to fulfil the datagaps on irritation and sensitization endpoints.

4.1 Toxicokinetics (absorption, metabolism, distribution and elimination)

4.1.1 Non-human information

The following summary of toxicokinetics of the copper ion Cu^{2+} is derived from the pesticide and biocide assessment reports made for the review of copper compounds under directive 91/414/EEC and 98/8/EEC.

Absorption

Absorption in both rats and humans varies according to diet. For humans: on a copper-adequate diet, absorption is 36 %, on a low copper diet 56 %, and on a high copper diet 12%. Similar figures have been obtained for rats.

Distribution

After oral absorption, when entering interstitial fluid and blood plasma, absorbed copper initially becomes bound to two proteins; albumin and transcuprein. Although the affinity of transcuprein for copper is higher than that of albumin, copper ions are freely exchangeable between them. Most of the copper bound to albumin and transcuprein is rapidly transported via portal blood to the liver (main organ of regulation), although some also goes directly to other tissues, especially to the kidney. The liver controls the distribution of copper to the rest of the body via the bloodstream, bound to ceruloplasmin.

By other routes of exposure (mainly inhalation), absorbed copper does not pass first by the liver, therefore, a wider distribution through the body is possible.

Metabolism

Metabolism does not occur. Copper is a monatomic ion and cannot be metabolised. It is however used in every cell in the body, and every cell can regulate its copper content. Many enzymes and other proteins containing copper have been described.

Interspecies differences

Albumin, one of the major copper transport proteins of the blood, contains histidine in position 3 which is essential for tight binding of copper. In dogs and pigs, this histidine is replaced by a tyrosine, and consequently the albumin does not have the same affinity for copper. Dog and pig albumins have several low-affinity sites for copper, but albumin is still an effective transport protein in those species. Dogs show unusually high levels of copper in the liver, ten times the levels in other species. While dog liver rapidly took up copper injected intravenously, dogs do not appear to be able to excrete copper via the bile as readily as other species. It is possible that dogs express the WND protein less than other species resulting in accumulation of copper in the liver. Based on these differences in albumin structure and the liver of the dog, it was concluded that the dog is not a good animal model for human risk assessment of copper.

Accumulation

Accumulation does not occur except in cases of genetic disease or chronic administration of exceptionally high doses (60 mg/person/day), where copper accumulates in the liver.

Excretion

Excretion in most species is *via* the bile, in a trypsin-independent protein fragment such that entero-hepatic circulation does not occur. A significant amount of copper is excreted bound to metallothioneins contained in intestinal brush border cells sloughed off and lost in faeces. Minor amounts are also excreted in urine and from skin and hair.

Excretion is rapid. An oral dose of 20 mg Cu/kg to rats was completely eliminated from the liver by 48 h. Blood plasma levels did not increase during this period.

Bioequivalence

As previously mentioned, in mammalian toxicity, it is considered that the toxic form of any copper compound is the Cu^{2+} ion.

The use of copper sulphate data would represent a worst-case scenario for the determination of the systemic effect of relatively insoluble copper compounds in mammalian toxicity. This has also been confirmed in a series of bioavailability studies conducted by several authors who have compared the bioavailability of copper sulphate to other copper salts including copper oxide, coated copper flakes, copper thiocyanate and copper carbonate. Moreover, in another study copper was administered orally to bile-canulated rats, as copper sulphate, copper hydroxide, copper oxychloride, Bordeaux mixture, tribasic copper sulphate and copper (I) oxide. There were no differences in absorption, copper levels in plasma, liver or bile, or in excretion rates between the five forms and copper sulphate. This study demonstrates bioequivalence between the five forms and copper sulphate, such that repeated dose toxicity studies on copper sulphate, or on only one of the five forms, may be considered representative of the other forms for systemic effects.

4.1.2 Human information

Literature review on ADME

Copper is a micronutrient. It is essential for life and is employed in all living cells. It is used in many enzyme systems, particularly in energy transfer where the property of electron transfer is exploited in photosynthesis and catabolism. It has been the subject of intense research.

Copper is present in almost all foods, with some foods (nuts, shellfish, chocolate) naturally containing more than 20 ppm copper.
Most human diets naturally include between 1 and 2 mg/person/day of copper, with some containing up to 4 mg/person/day. Copper levels in blood and tissues are generally stable. The body is able to maintain a balance of dietary copper intake and excretion that allows normal physiological processes to take place.

As with all micronutrients (minerals), copper is absorbed, used, stored and excreted. This applies at the level of the individual cell, at the organ and at the level of the whole organism. The cell membrane transport mechanisms for copper have been studied extensively, and the genetic codes for the individual transporter proteins are very similar in many different organisms: bacteria, fungi and fish, indicating that the process is ancient.

The copper transport mechanisms at the level of the organism form part of the system of homeostasis, the process by which the levels of copper in the body (and ultimately the cell) are regulated. Copper can be considered to show a flattened "U"-shaped dose-response curve.

*The left side of the "U" curve represents defici*ency, where intake is less than the requirement. This can be lethal, especially in children, where copper is needed for growth. Copper deficiency is associated with growth retardation, anaemia, skin lesions, impaired immunity, intestinal atrophy, impaired cardiac function,

reproductive disturbance, neurological defects and skeletal lesions. Copper is essential for normal physiological function such as cellular respiration, free radical defence, synthesis of melanin, connective tissue, iron metabolism, regulation of gene expression, and normal function of the heart, brain and immune system.

The central near-horizontal part of the "U" curve represents homeostasis, where intake and excretion are balanced, and copper levels are said to be normal.

The right-hand part of the "U" represents toxicity or excess copper disease.

The natural homeostatic regulation of copper means that an individual on a low copper diet will retain more of an artificial dose of copper than an individual on a high copper diet.

4.1.3 Summary and discussion on toxicokinetics

Copper is widely distributed in biological tissues, where it occurs largely in the form of organic complexes, many of which are metalloproteins and function as enzymes. Copper enzymes are involved in a variety of metabolic reactions, such as the utilisation of oxygen during cell respiration and energy utilisation. They are also involved in the synthesis of essential compounds, such as the complex protein of connective tissues of the skeleton and blood vessels, and in a range of neuroactive compounds concerned in nervous tissue function. Copper is present in almost all foods, most human diets naturally include between 1 to 2 mg/person/day of copper, with some containing up to 4 mg/person/day. Copper levels in blood and tissues are generally stable; the body is able to maintain a balance of dietary copper intake and excretion that allows normal physiological processes to take place. Up to 93 % of the copper in the blood is bound to the enzyme caeruloplasmin, with the majority of the rest bound to albumin and amino acids; there is strong evidence that absorbed copper is never released free in the blood or in the cells.

A bioequivalence study was performed to compare copper hydroxide, copper oxychloride, Bordeaux mixture, tribasic copper sulphate and copper (I) oxide with copper sulphate pentahydrate on bile cannulated rats. Absorption, distribution and excretion rates were similar between the six variants of copper following oral ingestion of 20 mg Cu/kg bw; liver was the principal organ of regulation of copper and main excretion was via the bile. Liver copper levels increased significantly following dosing with T_{max} at 12 hours; depuration was rapid, with levels returning to control by 48 hours after dosing. Plasma concentrations in both control and dose rats remained unchanged.

Oral absorption of copper varies according to the diet, for humans a copper-adequate diet results in 36 % absorption, while a low copper diet results in 56 % absorption and a high copper diet in 12 % absorption. Similar figures were found in rat, 50 % oral absorption was considered for this species. Distribution was directly from the intestine to the liver, which controls the distribution of copper to the rest of the body via the bloodstream, bound to ceruloplasmin. Metabolism does not occur. Copper does not accumulate except in cases of genetic disease or chronic administration of high doses, where copper accumulates in the liver. Excretion is rapid, via the bile, in a trypsin-independent protein fragment such that entero-hepatic circulation does not occur. Significant amounts of copper are excreted bound to metallothioneins contained in intestinal brush border cells sloughed off and lost in faeces; minor amounts are also excreted in urine and from skin and hair.

4.2 Acute toxicity

No acute toxicity test was realised with granulated copper. However, the read-across of acute toxicity of biocidal granulated copper from coated copper flake is proposed (see below for the justification).

Table 15: Summary table of relevant acute toxicity studies for coated copper flakes

Route	Method Guideline	Species Strain Sex no/group	Dose levels Duration of exposure	Value LD50/LC50 (mg/kg bw)	Remarks	Reference
Oral	OECD 423 GLP Acute Toxic Class Method	Rat; Sprague-Dawley; one group of 3 females and one group of 3 males and 3 females	2000 (F) and 200 (M and F) mg/kg bw; single dose; 14 day post exposure period	**LD_{50} = 300-500**	3/3 and 0/6 deaths at 2000 and 200 mg/kg bw. Clinical signs at 2000 mg/kg bw (hunched posture, lethargy, pilo-erection, decreased respiratory rate, laboured respiration, ataxia, pallor of the extremities, emaciation and tiptoe gait) and 200 mg/kg bw (hunched posture in one male). Surviving animals showed normal bodyweight gain. Gross necropsy findings (at 2000 mg/kg bw only): abnormally red lungs, dark liver, dark kidneys, haemorrhagic gastric mucosa, sloughing of the non-glandular epithelium of the stomach and haemorrhagic small and large intestines. **Acute Tox 4 – H302 (CLP)** **Xn, R22 (Annex VI of 2001/59/EC).**	Sanders A. (2001d)
Dermal	OECD 402 GLP Limit Test	Rat; Sprague-Dawley; one group of 5 males and 5 females	2000 mg/kg bw; single 24 hour exposure; 14 day post exposure period	**LD_{50} > 2000**	There were no deaths or clinical signs of systemic toxicity in this study. Signs of dermal irritation had resolved within two to seven days after dosing. All animals showed expected gains in bodyweight during the study. No abnormalities were noted at necropsy.	Sanders A. (2001b)

4.2.1 Non-human information

4.2.1.1 Acute toxicity: oral

Based on the available acute oral toxicity data, LD_{50} between 300 and 500 mg/kg bw, coated copper flakes is classified as harmful if swallowed (H302).

No acute toxicity data are available for granulated copper (assessed in this dossier). It should be noted that copper granulate and coated copper flakes are produced via very different processes. Coating is needed to obtain very fine flakes with a high surface area.

The surface area of coated copper flakes (8 – 10 µm) was measured as 2.9 m^2/g (Liipo *et al*., 2010) whereas a typical powder was measured as 0.024 m^2/g. The surface area of granulated copper is lower, 0.00256 m^2/g. A much higher reactivity and solubility of the coated copper flakes, compared to granulated copper, can therefore be anticipated.

The discussion on classification of granulated copper therefore includes comparative bioavailability and read across from toxicity data obtained for copper flakes and copper compounds.

With oral administration of soluble and insoluble copper substances, copper is present in the GIT, at least in part, in the ionic form and is therefore available for absorption. Relative bioaccessibility in gastric fluids of 0.1 and 1% were noted for respectively copper massive and copper powder, compared to 42 - 71% for the coated copper flakes, 77 – 94% for CuCl and 100% for $CuSO_4$ (see Table 12).

Considering the much lower biosolubility in gastric fluids, of copper powder and massives compared to copper flakes, no classification for copper powder and copper massives is proposed. Therefore no classification is proposed for granulated copper. Indeed, the granulated copper fulfils criteria corresponding more to a powder than to a massive form.

Further evidence for the absence of concerns related to "acute oral toxicity" of granulated copper can be obtained from the toxicity data, expressed as "biosoluble" copper for other forms of copper.

The biosoluble effects levels are calculated in the table below as the LD_{50} (mg substance/kg bw) x % Cu x % biosoluble (see Table 16 below).

Table 16: Acute toxicity of copper compounds, expressed as external doses of substance and calculated as internal dose, using the biosolubility data from previous table.

Source Material Tested	Administered LD_{50} (mg substance/kg bw)	Cu Biosolubility (%)	LD_{50} as biosoluble Cu (mg Cu/kg bw)
Cu flakes; 98% Cu	300 – 500 (5 µm)	42 – 71 (8.5 µm)	231 (121 – 341) (5 µm)
$CuSO_4$; 25.4% Cu	481	100	123
CuCl; 63.78% Cu	336	77 – 94	144 - 201

A linear relationship was observed between the surface area and the copper biosolubility in gastric fluids. Linear extrapolation demonstrates that a relative Cu biosolubility of 6 to <17% is needed to be outside of the criteria for an oral classification entry. Indeed, considering the limit maximum of 2000 mg/kg/d to not classify an active substance, a release between 6 and 17% are necessary to obtain the range of LD_{50} as biosoluble Cu of 121-341 mg Cu/kg bw, which lead to a classification, considering the purity of granulated copper of 98.7%: 121/(98.7%x2000) and 341/(98.7%x2000). The relative biosolubilities of copper powder and of copper massive materials are respectively 1% and 0.1% (values < 6%). In consequence, powder and massive forms will not be classified for acute oral toxicity.

Finally, in comparing the submitted data on the particle size and surface area of granulated copper with the copper massive and powder data detailed in the table above, this confirms that the granulated copper would not be classified by the oral route.

To conclude, based on all these data, there is no need for an acute oral classification for granulated copper.

4.2.1.2 Acute toxicity: inhalation

There is no acute inhalation toxicity study available. Indeed, granulated copper is not available as inhalable particles of copper powder as the average particle size of granulated copper significantly > 100 µm (diameter: 1086 µm) and it is non-bioavailable. Therefore no classification by the inhalation route is proposed contrary to coated copper flake (Acute Tox 3). For coated copper flake, the particle size is lower (10 µm) with a high surface area, which can enter to respiratory tract contrary to granulated copper and induces local but also systemic toxicity.

In conclusion, the classification of the coated copper flake cannot be extrapolated to granulated copper; no classification is warranted for granulated copper.

4.2.1.3 Acute toxicity: dermal

Coated copper flake is not classified by dermal route. In this context, considering all the data mentioned above and that none of the assessed copper compounds is classified for skin, no toxicity for granulated copper is suspected.

4.2.1.4 Acute toxicity: other routes

No data

4.2.2 Human information

Inhalation

Little information is available on acute effects in humans and inhalation of copper-containing materials.

Published studies on acute effects in humans appear to have focussed on metal fume fever (MFF)[2] and possible association with copper exposure. This subject has been reviewed extensively by Borak *et al* (2000) with the aim of establishing whether there is an association between exposure to copper and MFF. The review was based on seven reports, identified in a literature search as the only reports that contained original descriptions of copper-exposed workers who developed symptoms consistent with MFF. These seven reports are summarised below.

The earliest publication by Hansen (1911) provided a brief report of MFF-like symptoms in 10 males working in a research foundry where scrap copper was melted. The symptoms occurred as an isolated incident. No qualitative or quantitative data concerning exposure were provided. The isolated nature of this incident was considered by Borak *et al* to indicate an association with exposure to contaminants other than copper.

Koelsch *et al* (1923) reported the occurrence of symptoms that included chest discomfort, shivering, nausea and fever in 10 men performing hot rolling of copper bars in a rolling mill. The symptoms, which had not previously been associated with the process, resolved in 24 hours. No qualitative or quantitative exposure data were presented. As with the previous study, the isolated nature of this incident suggested to Borak *et al* that contaminants other than copper were involved.

Friberg and Thrysin (1947) reported MFF-like syndrome in approximately 50 workers involved in cleaning reactor ovens where pulverised copper was used as a catalyst. During the cleaning task, heads and faces of the workers were reported to be covered in dust consisting mainly of cuprous and cupric oxides. Initial symptoms included throat discomfort, burning eyes, nausea and headache, followed by flu-like symptoms, nausea,

2 Metal fume fever (MFF) is a transient illness which appears to develop 4-12 hours after occupational exposure to metal fume. MFF presents as an influenza-like illness with cough and dyspnoea followed by fever, sweating and shivering. Other accompanying clinical signs and symptoms are nausea, headache, weakness, a sweet metallic taste, and muscle and joint pain.

vomiting, diarrhoea and chest discomfort. In many workers, symptoms persisted for more than 72 hours. Quantitative exposure data was not provided. Dust particles were reported to range from 1-15 µm diameter, with more than 70% >5 µm. Given that MFF is typically associated with fine particles (< 1 µm diameter), Borak *et al* considered that the study did not support association between copper and MFF. Further, the heavy exposure indicated in this study is not generally associated with occurrence of MFF.

Schiotz (1949) reported the occurrence of initial symptoms such as metallic taste, throat dryness and slight chest oppression, followed by shivering, sweating and fever among seven workers involved in pulverising cuprous oxide during the production of marine paint. Symptoms subsided after 20-30 hours. Quantitative exposure data were not provided, although the described working conditions indicated very high levels of exposure.

Gleason (1968) reported symptoms in workers exposed to dust generated during polishing of copper plates with aluminium oxide abrasives. Symptoms were reportedly similar to "the onset of a common cold with chills or warmth, stuffiness of the head, etc". Lower respiratory symptoms were not reported, nor were other symptoms characteristic of MFF. Quantitative exposure data were limited to a single breathing zone sample, indicating 0.12 mg/m^3, although the study's author suggested exposure levels may have been "two or three times" higher. In this report, symptoms persisted for several weeks until ventilation was introduced, a feature which is not usually associated with MFF. In view of the absence of many symptoms characteristic of MFF and the persistence of the reported symptoms, Borak *et al* considered that the condition was unlikely to be MFF. Further, co-exposure to aluminium oxide was also likely, a metal also implicated in MFF aetiology.

Hopper (1978) described the single case of a foundry worker who developed an isolated episode of symptoms which included headache, cough, chest pain, chills and shortness of breath. Symptoms occurred shortly after exposure to a molten alloy of copper, beryllium and aluminium, which was poured into vessel containing alcohol and adhesive glue. Exposure data were not presented. Borak *et al* noted the co-exposure to other metals which have been implicated in MFF aetiology and the likely exposure to other potentially harmful substances. Consequently this case-report was not considered as providing evidence of an association between copper and MFF.

Armstrong *et al* (1983) reported symptoms of MFF in a group of 26 workers after cutting brass pipes (containing 90% copper, 10% nickel, and smaller amounts of zinc) with torches in a confined space. Symptoms included fever, chills, headache, dyspnoea and nausea. Exposure data for the different metals were not provided, although a description of the process indicated that high exposure levels were likely. As with the previous two studies, Borak *et al* considered that co-exposure to other metals implicated in MFF prevented identification of copper as the causative agent.

None of the seven studies covered by the review provided adequate exposure data, qualitative or quantitative, to enable identification of the causative agent(s) associated with the reported symptoms. Further, as noted by Borak *et al*, there was a lack of any occupational pattern associated with the MFF symptoms, as indicated by the range of industrial processes covered (foundry work, rolling mill, paint production, metal polishing and pipe cutting). The conclusion of Borak *et al* was that, based on the seven studies identified in the literature search, there is insufficient evidence to conclude that exposure to copper dust or fume causes MFF. Based on data which are currently available, this conclusion would appear to be justified.

Dermal

There are no published data on acute dermal effects of copper or copper compounds.

Oral

Self-poisoning

Self-poisoning with copper sulphate is rare in western countries but has been a common method of suicide among low income groups in some areas of India. The most extensive study concerns 48 cases, including 7 fatalities (15%), admitted to one hospital in Delhi and 5 fatalities reported to other Dehli hospitals (Chuttani *et al*, 1965). The most frequent symptoms observed in subjects were nausea, epigastrial burning and vomiting. In addition, diarrhoea was reported in 14 patients (29%). Biopsy examination of fatalities indicated deep

erosions in gastric mucosa, haemorrhage in the stomach and small intestine and oedema in the sub mucosa. Jaundice of variable severity occurred in 11/48 cases (23%). In the more severe cases, palpable liver enlargement, significantly elevated serum glutamic oxaloacetic transaminase (SGOT, 252.4 ±142 IU) and elevated bilirubin (112 ±8.9 mg/litre) were observed. Biopsy examination of liver tissue from fatalities showed centrilobular necrosis and biliary stasis. Post-mortem examination also indicated swollen and congested kidneys with glomerular swelling and necrosis of tubular cells. Anuria was reported in 13/48 patients (27%) and oliguria in 5/48 (10%). Red discolouration of urine was observed, with haemoglobinuria confirmed in some patients. These findings suggest haemolysis and are consistent with other reports. Haematocrit and serum/plasma appearances were not reported. Serum or blood levels of copper in the cases were elevated 2- or 3-fold compared to normal values. Estimated quantities of copper ingested were based on patients' accounts and therefore are unreliable. Consequently, this study provides no reliable data which can be used for human hazard assessment.

Subsequent case reports describe massive overdoses of copper sulphate (175 g) by a 22 year-old Indian male (Mittal, 1972) and 250 g by a 42 year old US male (Jantsch *et al*, 1985). Both patients survived following rapid chelation therapy with single or multiple injections of dimercaprol. The amounts ingested were considerably greater than the highest estimated dose reported by Chuttani and co-workers (1965). It therefore seems probable that survival of these patients was attributable to immediate chelation therapy.

Accidental ingestion

The ingestion of a relatively small amount of copper sulphate (3 g), together with an equal amount of zinc sulphate, by an 86 year-old female patient has also been reported (Hantson *et al*, 1996). The patient was admitted to hospital vomiting blue/green material and she had diarrhoea. Gastric lavage, dehydration and chelation therapy with dimercaprol were performed. The patient then suffered hypotension, bronchial inflammation and ulceration and a decline in respiratory function. These symptoms were interpreted as corrosive pneumonitis. The patient was placed on a mechanical ventilator for three days and subsequently made a complete recovery. In this case, the symptoms may have been exacerbated by the patient's age and health status, but may also have been mitigated to some extent by the co-ingestion of zinc sulphate which may have served to limit copper uptake and the severity of the systemic effects.

Therapeutic treatment

Systemic effects, including renal damage and thrombocytopaenic purpura, were reported in a 17-year old boy who was given 1% copper sulphate (2 mg/day) orally for treating vitiligo (Pande and Gupta, 1969).

4.2.3 Summary and discussion of acute toxicity

No acute toxicity was conducted with granulated copper. The acute toxicity of biocidal granulated copper is based on coated copper flake studies.

A study proposed under REACH dossier by applicants shows that when the particle size and surface area are taken into account, the toxicity can differ by different release rate of cupric ion.

The surface of coated copper flake was measured as 2.9 m^2/g whereas a typical powder was measured as 0.024 m^2/g. A much higher reactivity and solubility of coated copper flake than granulated copper can therefore be anticipated (more close to powder criteria). Relative bioaccessibility in gastric fluids of 0.1 and 1% were noted for copper massive and copper powder, respectively, compared to 42-71% for coated copper flake and 100% for copper sulphate.

Evidence for the absence of concerns related to "acute oral toxicity" of copper in granulate and massive forms can be obtained from the toxicity data, expressed as "biosoluble" copper for respectively copper flakes, and $CuSO_4$. The biosoluble effects levels are calculated as the LD_{50} (mg substance/kg bw) x % Cu x % biosoluble (see Table 16).

Finally, in comparing the submitted data on the particle size and surface area of granulated copper with the copper massive and powder data, this confirms that the granulated copper would not be classified by the oral route.

4.2.4 Comparison with criteria

Oral:

A linear relationship was observed between the surface area and the copper biosolubility in gastric fluids. Linear extrapolation demonstrates that a relative Cu biosolubility of 6 to <17% is needed to be outside of the criteria for an oral classification entry. Indeed, considering the limit maximum of 2000 mg/kg/d to not classify an active substance, a release between 6 and 17% are necessary to obtain the range of LD_{50} as biosoluble Cu of 123-341 mg Cu/kg bw, which lead to a classification. The relative biosolubilities of copper powder is 1%, further confirming that for copper granulates, there is no need for an acute oral classification entry.

Based on the results of the acute oral toxicity study, coated copper flake is classified as Acute Tox 4 – H302 according to the classification criteria as given in CLP regulation (LD_{50} = 300-500 mg/kg bw for males and females). Comparing the submitted data on the particle size and surface area of granulated copper with the copper massive and powder data above, this indicates that the granulated copper would also not be classified by the oral route.

Inhalation:

There is no acute inhalation toxicity study available. Indeed, granulated copper is not available as inhalable particles compared to coated copper flake which is classified Acute Tox 3. For coated copper flake, the particle size is small with a high surface area meaning that the substance can enter into respiratory tract and induces local but also systemic toxicity.

In conclusion, the classification of the coated copper flake cannot be extrapolated to granulated copper; no classification is guaranteed for granulated copper.

Dermal:

Coated copper flake is not classified by dermal route. In this context, considering all the data mentioned above and that none of the assessed copper compounds is classified for skin, no toxicity for granulated copper is suspected.

4.2.5 Conclusions on classification and labelling

No classification is proposed by oral, inhalation and dermal routes.

RAC evaluation of acute toxicity
Summary of the Dossier Submitter's proposal In the absence of acute toxicity data for granulated copper, the dossier submitter used data available on the acute oral and dermal toxicity of coated copper flakes (Sanders 2001a/b) in combination with comparative physico-chemical data (particle size and surface area, solubility) to arrive at a proposal for no classification for all three routes of administration.

In an *in vitro* bio-elution assay (Rodriguez *et al.*, 2010), copper compounds (copper sulphate, copper wire, copper oxide, cuprous chloride, coated biocidal and non-biocidal copper flakes, and copper powder) were tested for their potential to release bioaccessible metal to artificial gastric juice. A two hour test was performed at pH 1.5, using 2 g/L and 200 mg/L sample loading. The test was carried out in HCl 0.07N with an agitation rate of 171 rpm, at 37 °C in darkness. The samples were agitated for 1 hour and left to stand for another hour. The results (expressed as % mass recovered at the end of the bio-elution test as compared to the results obtained for soluble copper sulphate) and the physico-chemical parameters of the tested copper compounds are presented in the table below.

Table. *Bio-elution of copper compounds (Rodriguez et al., 2010)*

Material Tested	**Composition**	**Particle size**	**Surface area**	**Bio-elution recovery (as % of Cu content)**	
				200 mg/L loading	**2 g/L loading**
Copper wire massive	>99.9% Cu	0.13(a), 0.4(b), 1(c) mm	<6.74 cm^2/g		0.096-0.105
Copper powder	99.7% Cu, 0.3% Cu_2O	0.1-0.2 mm	240 cm^2/g	1.1	7*
Coated copper flakes	93.7% Cu, 2.6% Cu_2O, 3.89% LOI**	0.008-0.01 mm	29000 cm^2/g	60-71	42-44
Copper chloride		n/a	n/a	77	94
Copper oxide		n/a	n/a	84	68
Copper sulphate	25.45% Cu	n/a	n/a		100
Granulated Copper	>99% Cu	1.086 mm	25.6 cm^2/g		

*The results at the higher loading rate show unacceptably high variability (CV of 66%), possibly related to abrasion of the particles during the test. The results of this test are therefore not considered as reliable. ** Loss on ignition, as a measure of the organic content.

According to the dossier submitter, granulated copper (diameter: 1.086 mm; specific area of 25.6 cm^2/g) assessed in this dossier could be considered in the range of massive wire and powder copper (with relative biosolubilities of 0.1 and 1.1%, respectively), less so in the range of coated copper flakes. However, as data are available on coated copper flakes but not on massive or powder copper, read-across from coated copper flakes is proposed, acknowledging that this would represent a worst-case for granulated copper, given the relatively high biosolubility of coated copper flakes.

The CLH report included two acute toxicity studies with coated copper flakes in rats. In an oral study, conducted according to OECD TG 423, the LD_{50} value for males and females combined was estimated to be between 300 and 500 mg/kg bw (Sanders, 2001d), resulting in classification as Acute Tox. 4; H302 for coated copper flakes. In a dermal study, conducted according to OECD TG 402, no mortalities were seen, resulting in an LD_{50} value

>2000 mg/kg bw (Sanders, 2001b) and thus no classification for coated copper flakes (dermal route).

In view of the much lower biosolubility in gastric fluids of copper powder, massive and (presumably) granulates compared to coated copper flakes, no classification is proposed for granulated copper in contrast with the coated copper flakes. In support of the 'no classification', the dossier submitter presented acute toxicity data for other forms of copper, expressed as "biosoluble" copper (see the table below).

***Table**. Acute toxicity of copper compounds, expressed as external doses of substance and calculated as internal dose, using the biosolubility data from Table 1*

Source material tested	**LD_{50} (mg substance/ kg bw)**	**Cu Biosolubility (%)**	**LD_{50} as biosoluble Cu (mg Cu/kg bw)**
Cu flakes; 98% Cu	300 – 500 (0.005 mm)	42 – 71 (0.0085 mm)	231 (121 – 341) (0.005 mm)
CuSO4; 25.4% Cu	481	100	123
CuCl; 63.78% Cu	336	77 – 94	144 - 201

A linear relationship was observed in the Rodriguez *et al.* (2010) study when plotting the bio-elution against the surface area for the three sizes of copper wire tested. Extrapolation to 1.086 mm (the surface area of granulated copper) indicates a bio-elution of 6-17% is at least required (granulated copper consists for over 99% of copper) to reach sufficient Cu^{2+} concentrations that would cause effects warranting classification for Acute Tox. 4. Since the estimated surface area of granulated copper is somewhere between copper powder and massive copper forms, the bio-elution is expected to be small, between 0.1 and 1.1%. Therefore no effects are expected that warrant classification for acute oral toxicity.

For the dermal route, the dossier submitter concluded that no classification for granulated copper is warranted, given that coated copper flakes are not classified for acute dermal toxicity, nor in fact are any of the other previously evaluated copper compounds.

For the inhalation route, read-across to coated copper flakes was not applied, given that in contrast to coated copper flakes granulated copper is not available as inhalable particles (with average particle size significantly >0.100 mm) and is non-bioavailable. Therefore no classification by the inhalation route is proposed contrary to coated copper flakes (classified as Acute Tox. 3; H332).

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that no acute toxicity data are available specifically for granulated copper. The CLH report contains acute toxicity data on coated copper flakes, from which the dossier submitter proposed to read-across to granulated copper for the oral and dermal route. Additionally, supporting information was provided for the read-across argumentation that

is focused around particle size, surface area and solubility characteristics of various copper compounds.

The information provided in the bio-elution test with artificial gastric juice suggests that the bio-elution of granulated copper will be very small upon oral administration, leading to a bioavailability that is likely too small to illicit effects that warrant classification for acute oral toxicity. The bio-elution characteristics of granulated copper supposedly lie somewhere between copper powder and copper wire (massive) in terms of particle size and surface area. This seems to be supported by an apparent linear relation between surface area and the bio-elution of the different sizes of copper massive tested. Whereas copper powder, copper wire and copper flakes are forms of copper without counter ions that could affect the biosolubility, RAC considers it plausible that the bio-elution of granulated copper is more in the range of copper powder and copper wire than in the range of copper flakes (much smaller particles and unknown effect of coating on bio-elution). Yet, RAC notes the ongoing debate on the applicability of the bio-elution concept, without internationally agreed guidelines for the conduct of bio-elution techniques/studies available at the moment and without data to show a systematic relationship between bio-elution and systemic availability. RAC notes some further uncertainties as to the current study. For instance, the bio-elution measured for the various copper compounds is based on an *in vitro* test simulating bioavailability in the stomach only. Therefore there is an underlying assumption that the bio-elution ratio (difference) between copper compounds tested in *in vitro* gastric fluid is similar in comparison to bio-elution *in vivo* passing through several organs/tissues. Additionally, the *in vitro* bio-elution was performed during one hour agitation plus one hour static while *in vivo* the agitation and timeframe might be different. RAC finally notes that the comparison between biosolubility and acute toxicity/LD_{50} data was only done for highly (bio)soluble copper compounds, not for the much less (bio)soluble copper powder/copper massive. No data were thus presented showing that copper compounds of more similar (bio)solubility to granulated copper have an LD_{50} value above the cut-off of 2000 mg/kg bw for classification. RAC concludes that in the absence of sufficient data, no proposal for classification for acute oral toxicity can be made for granulated copper.

RAC notes that for acute dermal toxicity, the proposed read-across from coated copper flakes is hampered by the comparative biosolubility data being based on a bio-elution test using a fluid mimicking gastric juice rather than artificial sweat. RAC however also notes that none of the ten previously evaluated copper compounds was classified for acute dermal toxicity, not even the most soluble one (copper sulphate pentahydrate). In view of this, RAC does not expect granulated copper to present this hazard and therefore supports the proposed no classification for acute dermal toxicity.

No read-across was proposed for acute inhalation toxicity because the particle size of granulated copper was considered too large for inhalation. Although the available particle size information provides no specific data regarding the fraction of inhalable particles present in granulated copper, based on the available granulometry indicating length ranges between 0.9 and 6.0 mm and width ranges between 0.494 and 0.949 mm, the presence of a substantial percentage of inhalable particles is considered unlikely. RAC therefore concurs with the DS that no classification for acute inhalation toxicity is warranted for granulated copper.

In summary, **no classification** is the RAC conclusion for acute toxicity of granulated copper **via any route of administration**.

4.3 Specific target organ toxicity – single exposure (STOT SE)

4.3.1 Summary and discussion of Specific target organ toxicity – single exposure

Data of coated copper flake are reported in this part as representing a worst case for granulated copper. The human being has well recorded homeostatic mechanisms to control excess copper levels in the body by a combination of decreased absorption and increased excretion. Human epidemiological data is available however information is limited regarding doses consumed and exposure. Acute toxicity in humans is infrequent and generally results from ingestion of contaminated foodstuffs/beverages, for suicide purposes.

A paper by Chuttani (Chuttani *et al*, 1965) reviewed 53 cases of copper sulphate poisoning with ingestion varying between 1 and 100g. Jaundice was recorded as a symptom with post mortem examinations showing that the liver had signs of severe histological changes. A kidney biopsy showed swelling and necrosis in two patients, and following an autopsy of patients who had died, a congested kidney was observed. Emesis and irritation of the gastric mucosa was observed in all patients.
A case was reported where a male ingested an estimated 175g of copper sulphate, renal damage was observed (Mittal, 1972).

In acute animal studies with copper the following clinical signs and necropsy findings were observed.

Considering the study of Sanders A. (2001d), a group of three fasted females Sprague-Dawley rats 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. 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.

Considering the inhalation exposure, groups of 5 or 10 Sprague-Dawley rats (five males and/or 5 females) were exposed during 4 hours to a dust atmosphere of copper at mean achieved atmospheric concentrations of 2.13, 1.68, 1.12, 0.84 or 0.59 mg/l in the study of Wessen C.M. (2001).
4/5, 2/5, 5/10, 4/5 and 1/5 animals died at 2.13, 1.68, 1.12, 0.84 and 0.59 mg/l, respectively. All deaths occurred within 24 h of exposure to the test substance.
Common clinical observations noted during the study included decreased respiratory rate, laboured respiration, noisy respiration, hunched posture and piloerection. There were instances of increased respiratory rate, cyanosis, ataxia, pallor of the extremities and ptosis whilst tiptoe gait, lethargy, sneezing and hypothermia were noted sporadically.
Reduced bodyweight gain or weight loss was noted in most surviving animals during the first week of the recovery period. All surviving animals showed an overall body weight gain at theend of the treatment period.
Gross necropsy of premature decedents and surviving animals showed abnormal findings in the lungs (enlarged, dark patches, pale, pale patches, dark foci, fluid filled, discolouration, haemorrhagic, abnormally dark) and less frequently in the liver (patchy pallor), small intestine (gaseous distension, dark contents) and large intestine (gaseous distension).

Considering the dermal exposure, copper, moistened with arachis oil BP, was applied to the shaven, intact dorsal skin (approximately 10% of body surface) of 5 male and 5 female Sprague Dawley rats at 2000 mg/kg bw under a semi-occlusive bandage during 24h in the study of Sanders A. (2001b).

There were no deaths or clinical signs of systemic toxicity in this study. All animals showed expected gains in bodyweight during the 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. No abnormalities were noted at necropsy.

4.3.2 Comparison with criteria

There was no clear evidence of any specific toxic effects on a target organ or tissue in experimental studies. Clinical signs of toxicity were observed after single exposures to copper but were transient in nature and are considered to be unspecific signs of general acute toxicity.

4.3.3 Conclusions on classification and labelling

No classification as STOT-SE under regulation (EC) 1272/2008 is proposed. No classification or SCLs are considered necessary.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)

Summary of the Dossier Submitter's proposal

The proposal for this endpoint is based on read-across from coated copper flakes as no data for granulated copper are available. The data for coated copper flakes is considered by the dossier submitter as a worst-case scenario for granulated copper because the solubility as an indicator for bioavailability is much higher, and toxicity is considered to be caused by the copper ion.

No clear evidence of specific target organ toxicity was reported in the acute toxicity studies with coated copper flakes. Clinical signs of toxicity were transient in nature and they were considered to be unspecific signs of general acute toxicity. Acute toxicity in humans is infrequent and generally results from ingestion of contaminated foodstuffs/beverages, for suicide purposes. As this led to no classification for STOT SE for coated copper flakes, the dossier submitter concluded that also no classification is warranted for granulated copper for STOT SE.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that no data are available for granulated copper. RAC however also notes that neither coated copper flakes nor any of the other nine previously evaluated copper compounds was classified for STOT SE. This was due to the fact that in the acute toxicity studies available for these copper compounds the effects observed were mostly general and transient in nature and not indicative of specific target organ toxicity, narcotic effects or respiratory tract irritation. Furthermore, in human self-poisoning cases with copper

sulfate the most frequently observed symptoms (nausea, epigastric burning, vomiting, diarrhoea) were also indicative of non-specific, general acute toxicity. In view of this, RAC supports the proposed **no classification for STOT SE for granulated copper**.

4.4 Irritation

4.4.1 Skin irritation

Table 17: Summary table of relevant skin irritation studies of coated copper flake

Species	Method	Average score 24, 48, 72 h		Reversibility yes/no	Result	Reference
Rabbit, New Zealand White	OECD 404 GLP (3 animals)	Erythema	Oedema	No irritation was seen in this study.	**Non-irritant**	Sanders A. (2001a)
		0,0,0	0,0,0			

4.4.1.1 Non-human information

Based on data detailed in the introduction of the Human Health Hazard assessment, it can be concluded that the coated copper flake represents the most soluble form of copper and that irritation studies on the coated copper flake should therefore be considered worst case in terms of bioavailability.

Coated copper flake is not hazardous or classified as skin irritant according to the EU criteria for classification.

4.4.1.2 Human information

No data

4.4.1.3 Summary and discussion of skin irritation

Coated copper flake is not hazardous or classified as a skin irritant.

Considering the relative high potential bio-solubility of coated copper flakes compared to copper powder and copper massive materials it can be concluded that granulated copper do not need to be classified for eye/skin irritation.

4.4.1.4 Comparison with criteria

Read-across from coated copper flake data.

4.4.1.5 Conclusions on classification and labelling

Coated copper flake does not support classification for skin irritation under CLP regulation criteria, thus no classification is proposed for copper granulate.

RAC evaluation of skin corrosion/irritation

Summary of the Dossier Submitter's proposal

The proposal for this endpoint is based on read-across from coated copper flakes as no data for granulated copper are available. The CLH report included one skin irritation study with rabbits, conducted with coated copper flakes according to OECD TG 404 (Sanders, 2001a). Since no erythema or oedema was observed in any animal at any time point, this study resulted in no classification for skin irritation for coated copper flakes. Considering the relatively high biosolubility of coated copper flakes compared to granulated copper, the dossier submitter considered coated copper flakes to represent a worst-case scenario for granulated copper. Because coated copper flakes were not classified for skin irritation, it was concluded that granulated copper does not need to be classified for skin irritation either.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

No data are available for granulated copper. RAC notes that for skin irritation the proposed read-across from coated copper flakes is hampered by the comparative biosolubility data being based on a bio-elution test using a fluid mimicking gastric juice rather than artificial sweat. However, when looking at the skin irritation studies available for all ten previously evaluated copper compounds, RAC notes that none warranted classification, irrespective of the degree of solubility (a potential indicator of copper ion irritancy) of the copper compound tested. Given that not even the most soluble form (copper sulphate pentahydrate) was classified for skin irritation, RAC does not expect granulated copper to require classification either and therefore supports the proposed no classification of granulated copper for skin corrosion/irritation.

4.4.2 Eye irritation

Table 18: Summary table of relevant eye irritation studies of coated copper flake

Species	Method	Average Score (24, 48, 72 hr)				Reversibility yes/no	Result	Reference
Rabbit, New Zealand White	OECD 405 GLP (3 animals)	Cornea Opacity	Iris Lesion	Conjunctiva Redness	Conjunctiva Chemosis	Yes	**Non-irritant ***	Sanders A. (2001c)
		0,1,2	0,0.7,1	1.7,1.7,2	0.7,1,1.7			

*Results were considered irritant by RAC (RAC opinion, December 2014)

4.4.2.1 Non-human information

Based on data detailed previously, it can be concluded that irritation studies on the coated copper flake should be considered worst case in terms of local effects for granulated copper.

Coated copper flake is classified as eye irritant in a RAC opinion adopted in December 2014. In contrast to the dossier submitter, RAC concluded that coated copper flakes fulfilled the criteria for classification and that it should therefore be classified as Eye Irrit. 2 – H319.

Considering the local effects, coated copper flake is a worst case for granulated copper, thus the same classification is proposed for granulated copper.

4.4.2.2 Human information

No data.

4.4.2.3 Summary and discussion of eye irritation

Coated copper flake is classified as eye irritant. Consequently, the same classification is proposed for granulated copper.

4.4.2.4 Comparison with criteria

Coated copper flakes caused signs of irritation in the available eye irritation study. All effects were shown to be reversible within 21 days. Whereas the mean scores over 24-72 h were below the threshold values for classification for iritis, conjunctival redness and chemosis (≥1, ≥2 and ≥2, respectively), the mean score for corneal opacity over 24-72 h was at or above the threshold value for classification (≥1) in 2 of the 3 tested animals. The mean score over all three animals (1) was also at this threshold value

4.4.2.5 . Conclusions on classification and labelling

Copper flakes fulfil the criteria for classification as Eye Irrit. 2 – H319. By applying read-across, granulated copper should be classified as Eye Irrit. 2 – H319 but does not meet the classification criteria for skin irritation.

RAC evaluation of serious eye damage/irritation

Summary of the Dossier Submitter's proposal

The proposal for this endpoint is based on read-across from coated copper flakes as no data for granulated copper are available. The CLH report included one eye irritation study with rabbits, conducted with coated copper flakes according to OECD TG 405 (Sanders, 2001c). Coated copper flakes caused signs of irritation in the available eye irritation study. All effects were shown to be reversible within 21 days. Whereas the mean scores over 24-72 h were below the threshold values for classification for iritis, conjunctival redness and chemosis (≥1, ≥2 and ≥2, respectively), the mean score for corneal opacity over 24-72 h was at or above the threshold value for classification (≥1) in 2 of the 3 tested animals. The mean score over all three animals (1) was also at this threshold value. Based on these results, coated copper flakes were classified with Eye Irrit. 2; H319. The same classification was proposed for granulated copper.

Comments received during public consultation

Industry, supported by a MSCA, indicated that due to the specific form of granulated copper, it is not suitable for testing in eye irritation studies. On the one hand, the substance is a very coarse material which on contact with the eye could be considered as a possible cause of physical trauma. On the other hand, it would quickly be physically removed by anyone exposed to the substance because of its relatively large size. From that perspective, industry considered classification not warranted. With solubility being a potential indicator of copper ion irritancy, industry supported that for the purpose of classification, read-across based on solubility is possible with other copper compounds tested for eye irritation. Based however on a comparison of available transformation-dissolution data and results of eye irritation studies for (in descending order of copper release) copper sulphate pentahydrate (classified), coated copper flake (classified), dicopper oxide (classified) and copper oxide (not classified), they argued that for copper granulate, with an even lower release of copper than copper oxide, classification as an eye irritant is not warranted.

Assessment and comparison with the classification criteria

Given the specific form and particle size of granulated copper, RAC does not consider the proposed read-across from coated copper flakes appropriate and further notes that it is unclear whether the observed eye effects in the study with coated copper flakes were due to the particles causing mechanistic irritation or by dissolution of copper resulting in irritating effects. Also the contribution of the coating is unclear. RAC concludes that in the absence of relevant data, **no proposal for classification for eye irritation can be made** for granulated copper. RAC acknowledges though that the testing of solids for eye irritation may have issues related to physical stress.

4.4.3 Respiratory tract irritation

No data available.

4.5 Corrosivity

See skin irritation section 4.4.1.

4.6 Sensitisation

4.6.1 Skin sensitisation

Table 19: Summary table of relevant skin sensitisation studies coated copper flake

Species	Method	Number of animals sensitized/total number of animals	Result	Reference

Guinea-Pig, Dunkin Hartley	OECD 406 (M&K) GLP (5 control and 10 test animals)	0/10	**Non-sensitising**	Sanders A. (2001e)

4.6.1.1 Non-human information

Under the conditions of this test, coated copper flake produced a 0 % (0/10) sensitisation rate, thus is not considered skin sensitizing.

Considering the relative high potential bio-solubility of coated copper flakes compared to copper granulates, it can be concluded that granulated copper does not need to be classified for skin sensitization (based on data detailed in Human Health Hazard assessment).

4.6.1.2 Human information

The few cases of skin sensitisation from exposure to copper or its compounds reported in the literature are restricted to clinical case reports involving small numbers of patients, and in evaluation of a case-series of patients from dermatology clinics.

Allergic dermatitis with positive patch tests

Barranco (1972) reviewed the literature and noted that only six cases of allergic contact dermatitis to copper have been reported by then – 3 cases occurred as a result of contact with brass (copper and zinc alloy). The other cases were due in each case to $CuSO_4$, copper metal, and copper in jewellery respectively. To evaluate the prevalence of skin sensitisation to a range of metals encountered in the ceramics industry, Motolese and co-workers (1993) assessed 190 enamellers and decorators by patch tests. While the patch tests showed several cases positive to other metals, there was only a single case of a positive patch test to red copper oxide in the group.

Sterry and Schmoll (1985) described contact urticaria with a positive patch test in a patient exposed to copper (II)-acetyl acetonate used in self-adhesive disinfection pads applied to the skin.

Cross-reactivity

Metal objects such as spectacle frames have caused dermatitis (Gaul, 1958), but the role of copper in these cases is uncertain, as there is often concomitant exposure to other known sensitisers such as nickel compounds. Cross-reactivity between copper and other metal sensitisers have been documented. Hackel and co-workers (1991) described a patient with palladium sensitisation who also reacted positively to patch tests using nickel sulphate and $CuSO_4$ (1% petrolatum preparation). Nordlind (1992) showed cross reactivity between $CuSO_4$ and mercuric chloride in patients with oral lesions associated with mercury amalgam restorations.

Skin reactions following use of copper IUD

Barkoff (1976) reported a case of a woman who developed urticaria a month after insertion of a copper-based intra-uterine contraceptive device (IUD). Skin patch tests using 1% $CuSO_4$ solution were negative, but scratch tests using the same test material resulted in an erythematous flare reaction.

Romaguera and Grimalt (1981) described four women who developed papulo-erythematous skin lesions between 1 and 4 months after insertion of a copper-containing IUD. Patch tests were positive for 2% $CuSO_4$ in all four cases, although one of the patients also tested positive to nickel sulphate. All four patients improved after removal of the IUDs and provision of topical treatment.

The first report of IUD-induced copper sensitisation was by Barranco (1972) who obtained a positive patch test with 5% $CuSO_4$ solution. Other subsequent similar reports include those by Frenz and Teilum (1980) and

Rongioletti *et al* (1985) who demonstrated a positive patch test reaction to 1% $CuSO_4$ in water in a housewife with a 2-month history of dermatitis, and a copper-containing IUD inserted a few weeks before the onset of symptoms. Removal of the IUD resulted in abatement of the symptoms. Pujol *et al* (1998) reported a case of a woman with a 2-year history of recurrent non-pruritic skin eruption and abdominal pain. It was reported that the woman had had a copper-containing IUD "placed 12 years earlier". Whilst not clearly stated, this suggests that the same IUD remained in place for the whole period and therefore represents misuse. Patch tests were positive for $CuSO_4$ (2%) and for nickel and cobalt salts. Symptoms resolved after removal of the IUD. The authors suggest the copper-containing IUD as a cause for the dermatitis. However, it is possible that the other substances to which the patient reacted with a positive patch test may be the causative factor.

In an assessment of 37 female patients with side-effects following usage of a copper impregnated IUD, Joupilla *et al* (1979) showed that skin tests to copper were negative despite a history of skin rashes experienced by ten of the patients after insertion of the IUD. Allergy to copper was therefore not thought responsible for the skin and other side effects.

Prevalence of allergic dermatitis from copper salts

To establish the prevalence of irritant and allergic contact dermatitis from pesticides, Lisi *et al*. (1987) patch tested 652 outpatients with pre-existing skin disorders. 564 subjects were tested with 1% $CuSO_4$, of which 4 cases (<1%) demonstrated an allergic reaction, with none of the cases deemed to have an irritant reaction to $CuSO_4$. The inclusion of 2% $CuSO_4$ in a routine patch test series assessing 1190 eczema patients over a three-year period showed a positive reaction to $CuSO_4$ in only 13 patients. Copper salts are not common as skin sensitiers (Karlberg, 1983).

These findings indicate the relative rarity of copper compounds in comparison to other metals as a cause of allergic contact dermatitis.

4.6.1.3 Summary and discussion of skin sensitisation

Coated copper flake is not a skin sensitiser. This result is extrapolated to granulated copper.

4.6.1.4 Comparison with criteria

Read-across from coated copper flake data.

4.6.1.5 Conclusions on classification and labelling

No classification is proposed for skin sensitisation for granulated copper.

RAC evaluation of skin sensitisation
Summary of the Dossier Submitter's proposal In the absence of data for granulated copper, the dossier submitter proposed to read-across from coated copper flakes. In the CLH report a guinea pig maximisation test (GPMT), conducted with coated copper flakes is included, as well as some human data. In the GPMT test, conducted according to OECD TG 406, intradermal and topical induction doses of coated copper flakes were 0.1% (w/w) and 50% (w/w) at days 1 and 7, respectively (Sanders, 2001e). Animals were challenged with 25% (w/w) and 50% (w/w) at day 21. No reactions were seen in any of the tested (n=10) or control (n=5) animals.

A few clinical cases of allergic dermatitis upon copper exposure and skin reactions following use of copper-based intrauterine contraceptive devices have been reported, but overall the findings indicate that in comparison with other metals, copper was relatively rarely a cause of allergic contact dermatitis.

On the basis of the data above, coated copper flakes were concluded not to be skin sensitising, and were therefore not classified as such. Considering the relatively high potential biosolubility of coated copper flakes compared to granulated copper, the dossier submitter considered coated copper flakes to represent a worst-case for granulated copper, and concluded that granulated copper does not need to be classified for skin sensitisation either.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that for skin sensitisation the proposed read-across from coated copper flakes is hampered by the comparative biosolubility data being based on a bio-elution test using a fluid mimicking gastric juice rather than artificial sweat. RAC however also notes that none of the ten previously evaluated copper compounds was classified for skin sensitisation, based on the results of skin sensitisation studies with these copper compounds, in combination with evidence for a very low skin skin sensitising potential of copper compounds in humans. In view of this, RAC supports the proposed **no classification of granulated copper for skin sensitisation**.

4.6.2 Respiratory sensitisation

No data available

4.7 Repeated dose toxicity

As mentioned in introduction of the human health hazard assessment, a metabolism/bioequivalence study has been performed to demonstrate that the ion, as present in the form of copper sulphate, is similarly or more bioavailable to the other forms of copper following oral administration. Data from studies with the sulphate, and other forms that liberate the copper ion, may be used in the assessment process. Table 20 below presents a summary of relevant repeated dose toxicity studies with copper and copper compounds.

Note: the terms copper sulphate, cupric sulphate, copper sulphate pentahydrate and cupric sulphate pentahydrate have been used by various authors in studies quoted. These terms all refer to the same substance, $CuSO_4.5H_2O$, properly known as cupric sulphate pentahydrate, but more typically called copper sulphate.

Table 20: Summary table of relevant repeated dose toxicity studies

Method	Results	Remarks	Reference
Oral			
Rat Fisher 344/N 5/sex/dose/species Copper sulphate pentahydrate Drinking water 15 days 0, 300, 1000, 3000, 10000 ppm Correspond to 0, 10, 29, 45, 36 mg Cu/kg bw/d in males rats and 0, 10, 26, 31, 31 mg Cu/kg bw/d in females rats	10000 ppm: all rats died or were killed moribund. Clinical signs included ruffled fur, emaciation, abnormal posturing, hypoactivity, dyspnoea, tremors and prostration. 3000 ppm: Significant ↓ mean bw gains. ↓water consumption (poor palatability of the solution). 300 and 1000 ppm: ↑ size and number of protein droplets in epithelial cells of the proximal convoluted tubules of the kidney of males. LOAEL of 300 ppm (equivalent to 10 mg Cu/kg bw/d)	No guideline GLP Deviation: 15d instead of 28 days Purity: 99-100%	Hébert, C.D., Elwell, M.R., Travlos, G.S., Fitz, C.J. and Bucher, J.R. (1993)
Mice B6C3F1 5/sex/dose/species Copper sulphate pentahydrate Drinking water 15 days 0, 300, 1000, 3000, 10000 ppm Correspond to 10, 24, 58 and 133 mg copper/kg bw/d in males mice and 15, 36, 6 and174 mg copper/kg bw/d in females mice	> 3000 ppm: mortality, significant ↓ mean bw gains. ↓water consumption Microspcopic cellular depletion in several tissues. NOAEL: 1000 ppm (equivalent to 24 or 36 mg Cu/kg bw/d for males and females)	No guideline GLP Deviation: 15d instead of 28 days Purity: 99-100%	Hébert, C.D., Elwell, M.R., Travlos, G.S., Fitz, C.J. and Bucher, J.R. (1993)
Rat Fisher 344/N 5/sex/dose/species Copper sulphate pentahydrate Feeding studies 15 days 0, 1000, 2000, 4000, 8000, 16000 ppm	≥ 2000ppm: Chronic inflammation of the liver.Hyperplasia and hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach. Depletion of haematopoetic cells in bone marrow occurred. A minimal to mild decrease in erythroid haematopoesis was seen in the spleens.There was an increase in the	No guideline GLP Deviations: 15 days instead of 28 days. Purity: 99-100%	Hébert, C.D., Elwell, M.R., Travlos, G.S., Fitz, C.J. and Bucher, J.R. (1993)

Correspond to 23, 44, 162, 196, 285 mg Cu/kg bw/d in males mice and 23,46, 92, 198, 324 mg Cu/kg bw/d in females rats	number and size of protein droplets in the cytoplasm and lumen of the renal cortical tubules in the male and female rats similar to that seen in the drinking water studies. NOAEL of 1000 ppm (equivalent to 23 mg Cu/kg bw/d)		
Mice B6C3F1 5/sex/dose/species Copper sulphate pentahydrate Feeding studies 15 days 0, 1000, 2000, 4000, 8000, 16000 ppm in diet Correspond to 0, 43, 92, 197, 294, 717 mg Cu/kg bw/d in males mice and 0, 53, 104, 216, 398, 781 mg Cu/kg bw/d in females mice	16000ppm: ↓ significantly bw gains in female. ↓ mean food consumption. ≥ 2000ppm: minimal hyperplasia and hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomachs. NOAEL of 1000 ppm (43 and 53 mg Cu/kg bw/d in male and female, respectively)	No guideline GLP Deviations: 15 days instead of 28 days. Purity: 99-100%	Hébert, C.D., Elwell, M.R., Travlos, G.S., Fitz, C.J. and Bucher, J.R. (1993)
Rat Fisher 344/N 10/ animals sex/dose Copper sulphate pentahydrate Feeding studies 90 days 0, 500, 1000, 2000, 4000, 8000 ppm in diet Corresponds to 8, 16, 32, 66, 140 mg Cu/kg bw/d in male and 9, 17, 34, 68, 134 mg Cu/kg bw/d in female rats	≥4000ppm: ↓bw gain; Haematological changes. Hyperplasia and hyperkeratosis in the forestomac mucosa, probably as a result of irritant effects of the compound. ≥ 2000ppm: histological changes in the liver and kidney were recorded. NOAEL of 1000 ppm in rat (16 or 17 mg Cu/kg bw/d for males and females)	No guideline GLP Purity: 99-100%	Hébert, C.D., Elwell, M.R., Travlos, G.S., Fitz, C.J. and Bucher, J.R. (1993)
Mice B6C3F1 10/ animals sex/dose Copper sulphate pentahydrate Feeding studies 90 days 0, 1000, 2000, 4000, 8000 and 16000 ppm in diet Corresponds to 44, 97.2, 187.3, 397.8, 814.7 mg Cu/kg bw/d in male and 52.2, 125.7, 266.7, 536 and 1058 mg Cu/kg bw/d in female mice	≥4000ppm: ↓bw gain; Hyperplasia and hyperkeratosis in the forestomac mucosa, probably as a result of irritant effects of the compound. NOAEL of 2000 ppm (97.2 mg Cu/kg bw/din male and 125.7 mg cu/kg bw/d in female) in mouse	No guideline GLP Purity: 99-100%	Hébert, C.D., Elwell, M.R., Travlos, G.S., Fitz, C.J. and Bucher, J.R. (1993)
Rats 4 males per group (9 groups) Copper sulphate Feeding study 1, 2, 3, 6, 9 or 15 weeks 2000 mg/kg diet	The treatment was associated with reduced bodyweight gains and toxicity to the liver and kidneys. Toxicity (including hyperplasia and cellular damage) was marked at 6 weeks of dietary administration, but by 15 weeks, animals had shown almost	No guideline No GLP	Haywood, S (1980a)

Correspond to 165 mg Cu/kg bw/day	total adaptation and recovery at the cellular level. NOAEL < 165 mg Cu/kg/d		
Rats Wistar 4 males/group Copper sulphate Feeding study Exposure during 2, 3, 4, 5, 6 or 15 weeks 0, 3000, 4000, 5000 or 6000 ppm Correspond to 150, 200, 250 or 300 mg Cu/kg bw/day)	Toxicity after 6 weeks followed by regeneration of the liver up to 5000 ppm. 6000 ppm resulted in unsustainable liver damage and death by six weeks.	No guideline No GLP	Haywood, S. (1985)
Rats 4 males/group Copper sulphate Feeding study Killed at intervals of 1, 2, 3, 6, 9 and 15 weeks 0 or 2000 ppm Cu Correspond to 200 mg/kg bw/day in the young rat, or 100 mg/kg bw/day in the older rat	Dietary copper, administered at high levels to weanling rats was associated with increased blood and plasma copper concentrations after six weeks (with an initial transient rise in plasma concentration in the first week) to reach a maximum at nine weeks. Similarly, ceruloplasmin activity increased significantly at six weeks. Alanine aminotransferase activity rose gradually from the first week to reach a maximum at nine weeks. Alkaline phosphatase activity and bilirubin concentration showed no change. The changes in enzyme activity and ceruloplasmin levels coincide with liver toxicity seen at higher levels in subsequent studies, and may reflect increased competence to manage high levels of copper following the initial insult.	No guideline stated No GLP	Haywood, S. and Comerford, B. (1980b)
Inhalation			
Guinea pigs 6 male/group exposed daily for 5 minutes aerosols Inhalation 0.4% aqueous solutions of either copper oxychloride (containing 50% copper) or copper oxychloride (containing 37.5% copper) plus zineb (16%). Animals killed after 60, 120, 200, 270 and 420 periods of exposure	After 70 days of exposure animals showed copper inclusions within swollen Kupffer cells and histiocytes in the portal tracts and subcapsular areas. In three animals killed after 270 days of exposure, a close association was noted between the lesion reported and perisinusoidal and portal fibrosis. The exposure of limited numbers of animals to copper formulations indicates that animals show similar lesions to humans.	No guideline No GLP	Pimentel (1969)

Rat Sprague Dawley 10/ animals sex/dose for low, med-low and med-high dose and 20/ animals sex/dose for control and high dose Cuprous oxide Dust aerosol Whole-body inhalation exposure as a 6-hour/day exposure 0, 0.2, 0.4, 0.8 and 2 mg/m^3 for 1, 2, 3, or 4 weeks 13-week recovery period	Following a 13-week recovery period at 2 mg/m^3, there were no test substance related effects on hematology parameters, BALF parameters, or lung, lymph node or nasal histopathology. The effects on lung weights were greatly reduced, but still slightly detectable following the recovery period. But there were no microscopic findings or changes in BALF parameters that correlated with the higher lung weights at the recovery necropsy.	OECD 412 GLP	Kirkpatrick, 2010
Dermal			
Rabbit 5/sex/groups 3 weeks exposure Copper hydroxide 1000 or 2000 mg/kg/day of the formulation Correspond to 500 or 1000 mg Cu/kg bw/day)	2000 mg/kg: 3 deaths not related to treatment. Body weight loss. Increased incidence of dermal necropsy findings. The skin of treated animals was discoloured blue by test material. NOAEL = 1000 mg/kg bw/d (=500mg Cu/kg bw/d)	OECD 410 No GLP Purity not stated	Painter O.E. (1965)

4.7.1 Non-human information

4.7.1.1 Repeated dose toxicity: oral

Reference: Hébert, C.D, (1993)
Guideline: No
GLP: Yes
Several studies were realised:

- Studies on rats and mice by drinking exposure during 15 days,
- Studies on rats and mice by diet exposure during 15 days,
- Studies on rats and mice by diet exposure during 92 days.

Duration of treatment: 15 days
Deviations:

- Study duration is less than recommended,
- no haematology or clinical chemistry investigations,
- adrenals and spleen are not weighted at necropsy.

These deficiencies do not, however, necessarily compromise the validity of the data generated.

Five males and five females Fischer 344/N rats and 5 males and 5 females B6C3F1 mice were exposed to copper sulphate pentahydrate at concentrations of 0, 300, 1000, 3000, 10000 ppm in the drinking water.
Five others males and females Fischer 344/N rats and 5 males and females B6C3F1 mice were exposed to copper sulphate pentahydrate at concentrations of 0, 1000, 2000, 4000, 8000 and 16000 ppm in the diet.

During the studies, clinical observation and mortality were reported. At termination all animals were given a full macroscopic examination and body and organ weights (liver, thymus, right kidney, right testis, heart, lungs, brain) were determined. Histopathological examination was performed on control animals (plant diet or untreated drinking water), any unscheduled kill animals, all animals in the highest dose group with 60%

survival rate and all animals in higher dose groups. Target organs (liver, kidney, forestomach) were examined to a no-effect level in lower exposure groups.

Drinking water studies results:

Table 21: Body weight, water and compound consumption in rats

	Rats Dose level (ppm)				
	0	**300**	**1000**	**3000**	**10000**
Male					
Final body weight (g)	169	171	174	88**	-
Water consumed (g/day)	17.9	17.3	14.7	4.8	1.0
Calculated compound consumption (mg/kg/day)	0	41	113	175	140
Female					
Final body weight (g)	139	141	131	75**	-
Water consumed (g/day)	16.3	15.3	11.3	3.2	0.9
Calculated compound consumption (mg/kg/day)	0	39	102	121	120
	Mice Dose level (ppm)				
	0	**300**	**1000**	**3000**	**10000**
Male					
Final body weight (g)	27.2	27.7	26.5	21.1**	-
Water consumed (g/day)	4.8	3.6	2.4	1.6	1.1
Calculated compound consumption (mg/kg/day)	0	41	95	226	524
Female					
Final body weight (g)	22.2	21.5	21.1	14.6**	-
Water consumed (g/day)	5.6	4.1	2.8	1.3	1.1
Calculated compound consumption (mg/kg/day)	0	58	140	245	683

** $P < 0.01$

Clinical signs of both rats and mice in the highest two groups included ruffled fur, emaciation, abnormal posturing, hypoactivity, dyspnoea, tremors and prostration. Animals from the two highest groups also showed a decreased water consumption, which was attributed to poor palatability of the cupric sulphate solution.Final mean body weight gains for surviving animals of both species from the 3,000 ppm groups were significantly reduced.

All rats and all mice in the 10,000 ppm groups and one female rat, one male mouse and three female mice in the 3,000 ppm groups died or were killed moribund during the study.

Any changes in absolute organ and relative organ weights were attributed to the lower body weights of animals receiving 3,000 ppm, rather than a direct toxic effect of treatment. Microscopic lesions in rats were limited to an increase in the size and number of protein droplets in epithelial cells of the proximal convoluted tubules of the kidney of males in the 300 and 1,000 ppm groups. No kidney lesions were observed in female rats or in mice of either sex. The only microscopic lesion in mice was cellular

depletion, present in numerous tissues in mice from the two highest dose groups and which was attributed to the marked decrease in water consumption and body weight gain in these groups.

Concentrations of cupric sulphate above 3,000 ppm were lethal to rats and mice within two weeks. Slight kidney changes were observed in male rats at 300 and 1,000 ppm but female rats and mice of both sexes were not affected.

Feeding studies results:

Table 22: Body weight, food and compound consumption in rats

	Dose level (ppm)					
	0	**1000**	**2000**	**4000**	**8000**	**16000**
Male						
Final body weight (g)	184	186	183	178	151**	122**
Food consumed (g/day)	14.6	15.2	14.7	14.4	13.3	9.2
Calculated compound consumption (mg/kg/day)	0	92	180	363	777	1275
Female						
Final body weight (g)	138	139	138	136	128*	106*
Food consumed (g/day)	11.4	11.6	11.2	11.7	11.7	7.1
Calculated compound consumption (mg/kg/day)	0	89	174	637	769	1121

* $P < 0.05$.
** $P < 0.01$

Table 23: Body weight, food and compound consumption in mice

	Dose level (ppm)					
	0	**1000**	**2000**	**4000**	**8000**	**16000**
Male						
Final body weight (g)	25.1	25.1	25.4	24.6	23.6	23.6
Food consumed (g/day)	4.4	4.1	4.5	4.7	3.3	4.0
Calculated compound consumption (mg/kg/day)	0	168	362	773	1154	2817
Female						
Final body weight (g)	21.2	21.4	20.2	20.8	20.2	20.0*
Food consumed (g/day)	4.1	4.3	4.0	4.3	3.8	3.7
Calculated compound consumption (mg/kg/day)	0	210	408	849	1563	3068

* $P < 0.05$.

No animals died or were killed during the study. Final mean body weights gains of male and female rats of 8,000 and 16,000 ppm groups and of female mice receiving 16,000 ppm were significantly lower than the controls. These decreases were attributed to decreased feed consumption in animals, considered to be due to the poor palatability of the feed mixture rather than to specific cupric sulphate toxicity.

Changes in organ weights and organ to body weight ratios were sporadic and were considered to be related to decreased body weights rather than to toxicity of the cupric sulphate.

Microscopic findings in rats at 2,000 ppm and above included hyperplasia and hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach. A similar finding was observed in mice but the severity was minimal. This was considered due to the irritant effects of cupric sulphate and the authors noted that there were no adverse effects on the health of the animals. Additionally in rats, chronic active inflammation of the liver characterised as minimal to mild mononuclear inflammatory cell infiltrate was observed in males at 8,000 ppm (4/5) and 16,000 ppm (5/5) and in females at 16,000 ppm (3/5). Depletion of haematopoetic cells in bone marrow occurred in male and female rats in the 8,000 and 16,000 ppm groups, consisting of a decreased cellularity of bone marrow erythroid/myeloid elements and an increase in the prominence of fat cells normally present in the bone shaft. In several high dose animals bone mass (cortex and trabecular density) was reduced when compared to controls. This was considered a consequence of reduced body weight gain rather directly related to treatment. A minimal to mild decrease in erythroid haematopoesis was seen in the spleens of rats in the 16,000 ppm group. There was an increase in the number and size of protein droplets in the cytoplasm and lumen of the renal cortical tubules in the male and female rats of the three highest dose groups, similar to that seen in the drinking water studies.

Microscopic findings were more severe in rats than in mice and at levels of 2,000 ppm and above included hyperplasia and hyperkeratosis of the squamous mucosa of the limiting ridge of the stomach. This finding was minimal in mice, and may have been associated with the sulphate ion, rather than copper. Administration at 4,000 ppm and above was associated with inflammation of the liver, changes in the kidney similar to the drinking water study and changes in bone marrow cells.

Duration of treatment: 92 days:
Deviations:

- No ophthalmoscopy was performed,
- adrenals were not weighted at necropsy.

Copper sulphate pentahydrate was administered in the diet to groups of 10 male and 10 female Fischer 344/N rats at dietary levels of 0, 500, 1,000, 2,000, 4,000 and 8,000 ppm for 92 days. Also groups of 10 male and 10 female B6C3F1 mice received treated diet at levels of 0, 1,000, 2,000, 4,000, 8,000 and 16,000 ppm for 92 days. Chemical analyses of the formulations showed that they were within ±10% of theoretical concentrations.

Clinical signs and mortality were reported but schedule for observations were not indicated.

Bodyweights and organ weights (liver, thymus, right kidney, right testis, heart, lungs and brain) were determined at the termination of the study for all rats and mice.

Haematology and clinical chemistry evaluations (haematocrit, haemoglobin concentration, mean cell volume, platelets, erythrocyte count, total and differential leukocyte count, reticuloocyte count, blood urea nitrogen, creatinine, total protein, albumin, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase, 5'-nucleotidase, bile salts) were performed on Days 5 and 21 on supplemental rats (10 animals/sex/per group) and on the main study rats on Day 92 (termination).

Urinalysis (clarity, colour, volume, specific gravity, creatinine, glucose, total protein, aspartate aminotransferase (AST), N-acetyl-3-glucosaminidase (NAG)) was performed on Day 19 on supplemental rats (10 animals/sex/per group) and on the main study rats on Day 92.

Gross necropsy was performed on all animals.

Histopathology was performed on decedents, all control animals, on animals from the highest dose group with 60% survival rate and on any higher dose group animals. Target organs (liver, kidney and forestomach) were examined to a no-effect level in the lower exposure groups.

No mortality was reported. There were no clinical signs observed that could be directly attributed to treatment among rats and mice.

Food consumption was generally similar to the controls in all groups in both rats and mice except for the highest dose group in the rat (8,000 ppm).

Final mean body weight gains were significantly reduced in male rats in the two highest dose groups (4,000 and 8,000 ppm) and in female rats in the highest dose group (8,000 ppm).

Treated mice showed a dose-related reduction in body weight gain that occurred earlier than in the rat and was more severe at the higher dose levels.

Haematology showed significant changes in rats of both sexes at all time points but generally limited to the 2,000, 4,000 and 8,000 ppm dose groups. Initially (day 5), significant increases in haematocrit (HCT), haemoglobin (HGB), platelet count and erythrocytes (RBC) were seen in the 8,000 ppm group which were consistent with polycythemia related to dehydration. Also on day 5, significant decreases in reticulocyte count, mean cell volume (MCV), and mean cell haemoglobin (MCH) were noted in high-dose animals. By Day 21, HCT and HGB levels were significantly decreased for male rats in the 2 highest dose groups and female rats in the 3 highest dose groups together with MCV and MCH and these persisted until the end of the study. Significant increases in RBC and reticulocytes were noted in high dose males at the end of the study.

Clinical chemistry showed significant elevations of alanine aminotransferase (ALT) and sorbitol dehydrogenase (SDH) activities throughout the study, indicating hepatic injury. Decrease in alkaline phosphatise (AP) activity were noted on days 5 and 21 in both sexes in the two highest dose groups, but AP activity had returned to control levels by day 92. Total protein and albumin concentrations were significantly decreased and urea nitrogen increased in the two highest dose groups at all time points. Variations occurred in other parameters with reversal of trends at differing time points.

Significant changes in urinalysis parameters included an increase in aspartate aminotransferase (AST) and N-acetyl-β-D-glucosaminidase (NAG) and 5'-nucleotidase (5'NT, males only) activities in the two highest dose groups.

Clinical pathology and urinalysis are summarised on the table below:

Table 24: Selected clinical pathology and urinalysis parameters at termination in rats

Parameter	Dose level (ppm)				
	0	1000	2000	4000	8000
Male					
HCT (%)	47.9	47.5	48.1	44.8**	40.2**
HGB (g/dL)	14.3	14.0	14.3	13.4**	12.2**
RBC (106/µL)	8.88	8.84	9.06	9.23	9.55**
Reticulocytes (106/µL)	0.15	0.17	0.15	0.20	0.27**
MCV (fL)	54.0	53.8	53.1*	48.8**	42.1**
MCH (pg)	16.0	15.9	15.7**	[2]	12.8**
ALT (IU/L)	51	78	108**	494**	563**
5'-NT (IU/L0)	33.6	33.3	32.5	39.3**	36.5**
SDH (IU/L)	22	32	42**	197**	282**
Total protein (g/dL)	6.6	6.6	6.5	6.5	6.1**
Albumin (g/dL)	4.6	4.6	4.5	4.5	4.3**
Urea nitrogen (mg/dL)	21.6	20.6	20.7	22.1	23.5*
Urine AST (IU/mg creatinine)	0.08	NR	0.09	0.10	0.43**
Urine NAG (IU/mg creatinine)	0.09	NR	0.10	0.11**	0.22**
Female					
HCT (%)	48.6	47.9	47.9	47.7	43.9**
HGB (g/dL)	14.5	14.4	14.2	14.2	13.2**
RBC (106/µL)	8.48	8.41	8.48	8.44	8.51
Reticulocytes (106/µL)	0.13	0.12	0.14	0.13	0.15
MCV (fL)	57.2	57.0	56.3	56.5*	51.5**
MCH (pg)	17.0	17.2	16.8*	16.9	15.5**
ALT (IU/L)	44	38	37	84*	214**
5'-NT (IU/L0)	34.5	40.0	36.7	36.8	31.8
SDH (IU/L)	16	19	16	34**	96**
Total protein (g/dL)	6.6	6.8	6.9	6.3**	5.7**
Albumin (g/dL)	4.8	4.9	5.1	4.6**	4.0**
Urea nitrogen (mg/dL)	17.1	19.7**	18.0*	20.6**	22.9**
Urine AST (IU/mg creatinine)	0.04	NR	0.07**	0.15**	0.96**
Urine NAG (IU/mg creatinine)	0.10	NR	0.11	0.16**	0.38**

* $P < 0.05$

** $P < 0.01$

Data taken from NTL report in preference to published paper (NR = not reported in either)

Generally, absolute organ weights of both species were reduced in the two highest dose groups when compared with the controls and the relative organ weights were similar or increased with decreasing body weight. It was considered that the changes could be attributed to the lower final mean body weight in the higher dose groups.

Gross and histopathology observations showed (tables 25 and 26):

- For forestomach:

Gross lesion in rats was characterized by an enlargement of the limiting ridge in all animals in the 4000 and 8000 ppm groups and in 7 females and 9 males in the 2000 ppm group.

In mice, the limiting ridge had focal white discoloration of the squamous mucosa where it forms a junction with the glandular gastric mucosa.

Histopathological findings included dose-related minimal to moderate hyperplasia with hyperkeratosis of the squamous mucosa of the forestomach from 2000 ppm, at the site of the limiting ridge. Severe incidences of this lesion were often accompanied by an increase in the number of inflammatory cells and/or oedema in the lamina propria of the limiting ridge. Rats were more severely affected than mice at similar dose levels. The difference between the species may be associated with the lower stomach pH (less acidic) in the rat. It may be anticipated that the hydrochloric acid in the stomach may react with copper sulphate to produce copper chloride and sulphuric acid. This acid may have caused the irritation, and the rat stomach, being adapted to a less-acidic environment, showed more effects than the mouse.

- For liver:

There was a dose-related increase in the incidence and severity of chronic inflammation in the livers of rats, characterised by multiple foci of a mixture of mononuclear inflammatory cells. Staining of the livers for the presence of copper showed a presence in the 4,000 and 8,000 ppm groups. At 8,000 ppm staining had a clear periportal to midzonal distribution and consisted of a few to numerous red granules of 1 to 2 mm in the cytoplasm of hepatocytes. At 4,000 ppm staining was periportal and there was a marked reduction in the number of cells stained and in the number of granules per cell. Positive minimal staining of livers for copper was evident in the high dose male and female mice and consisted of only a few positive-staining hepatocytes in the entire liver section.

- For kidneys:

Changes in the kidneys included an increase in the size and number of cytoplasmic protein droplets present in the epithelium of proximal convoluted tubules of rats at doses of 2,000 ppm and higher and were less severe in females than in males. Many of the protein droplets in the male rats had large irregular crystalline shapes, which were not present in the females. Minimal nuclear enlargement (karyomegaly) in renal tubule cells was present in the high dose group. Degeneration of renal tubule epithelium was present in three females from the 8,000 ppm group. Positive staining for copper was seen in the kidneys at 4,000 ppm, and to a greater extent, in the 8,000 ppm groups and consisted of red granules in the cytoplasm of the renal tubule epithelium and a diffuse red staining of the protein droplets in the cytoplasm and the tubule lumen. There was no staining for copper in the kidneys of any mice.

In conclusion, administration of copper sulphate pentahydrate to rats and mice for 92 days via the diet produced hyperplasia and hyperkeratosis in the forestomach mucosa, although this may be associated with the sulphate ion, rather than copper.

The NOAEL for this lesion was 1,000 ppm for rats and 2,000 ppm for mice.

In rats damage to the liver was produced with a NOAEL of 1000 ppm for males and 2000 ppm for females.

In rats damage to the kidney was produced with a NOAEL of 1000 ppm for both sexes.

A NOAEL for mice could not be derived for liver and kidney toxicity as lesions were not seen in these organs even at the highest concentration.

Sperm morphology and vaginal cytology were also realised. There were no changes in testis, epididymis or cauda epididymis weight, or spermatid counts or sperm motility in males of either species at any dose level. Similarly, there were no changes in oestrous cycle length or in the timings in each phase of the cycle in females of either species.

Table 25: Rats - histopathological findings - incidence and severity

	Incidence and mean severity ( ) at dose level (ppm)					
	0	**500**	**1000**	**2000**	**4000**	**8000**
Male						
Forestomach, hyperplasia and hyperkeratosis	0	-	-	10 (1.6)	10 (2.8)	10 (2.8)
Liver, inflammation	0	-	0	1 (1.0)	10 (1.0)	10 (1.9)
Kidney, droplets	0	-	0	3 (1.0)	10 (2.0)	10 (2.5)
Kidney, karyomegaly	0	-	0	0	0	10 (1.0)
Female						
Forestomach, hyperplasia and hyperkeratosis	0	-	-	7 (1.3)	10 (2.5)	10 (2.5)
Liver, inflammation	0	-	0	0	6 (1.2)	10 (1.9)
Kidney, droplets	0	-	1 (1.0)	9 (1.0)	10 (1.0)	10 (1.0)
Kidney, karyomegaly	0	-	0	0	0	10 (1.1)
Kidney, degeneration	0	-	0	0	0	3 (1.3)

Mean severity (in brackets) based on number of animals with lesions 1, minimal; 2, mild; 3, moderate; 4, marked

Table 26: Mice -histopathological findings - incidence and severity

	Incidence and mean severity ( ) at dose level (ppm)					
	0	**1000**	**2000**	**4000**	**8000**	**16000**
Male						
Forestomach, hyperplasia and hyperkeratosis	0	-	0	2 (1.0)	6 (1.0)	10 (1.6)
Female						
Forestomach, hyperplasia and hyperkeratosis	0	-	0	5 (1.0)	8 (1.0)	10 (1.7)

[a] mean severity (in brackets) based on number of animals with lesions 1, minimal; 2, mild; 3, moderate; 4, marked

Reference: Haywood, S. (1980a)
Guideline: No
GLP: No

Male weanling rats of uniform age and weight were allocated to nine groups of four animals. Groups 1 to 6 were fed powdered laboratory diet (Spillers expanded) to which 2000 mg/kg diet as $CuSO_4$ (equivalent to 165 mg Cu/kg/d) had been added, for up to 15 weeks. Groups 7 to 9 received unsupplemented diet and served as controls.
Rats on the copper supplemented diets from groups 1 to 6 were killed in weeks 1, 2, 3, 6, 9 and 15 respectively. Two control animals were killed at the same time.
Animals were exsanguinated under ether anaesthesia and liver and kidneys were dissected free and weighed. Slices of the liver and kidney were preserved for histological examination; other parts were frozen (-70°C) and triplicate samples analysed for copper content following acid digestion using atomic absorption spectrophotometry.

There were no deaths.

Animals receiving copper showed reduced body weight gain, and reduced liver weight. The liver to body organ weight ratio was similar in all groups.

Macroscopic liver changes were recorded from week 6, when clearly defined peripheral areas of necrosis were recorded in the right and median lobes. By week 9, pale areas were still visible but not so clearly defined, and by week 15 the livers were apparently normal, except for fine scarring on the lobular surface.

Histological changes were noted from week 2, with hypertrophy of the periportal parenchymal cells. Copper was present in the outer zones of lobules in sections stained with rubeanic acid.
In week 3, inflammatory foci were present, restricted to the periportal zone. Lesions consisted of aggregates of hypertrophied hyperchromatic parenchymal cells, some of which showed signs of necrosis. There was marked deposition of copper in outer zones of the lobules, pericanalular in distribution.
By week 6 there were marked changes in the livers of all animals, although there was considerable individual variation. The changes were always more severe in the right and median lobes. Necrosis was widespread, with marked cellular inflammatory reaction consisting of polymorpho-nuclear neutrophil leukocytes and mononuclear cells. There was extensive copper in the cells, considered to be lysosome-bound copper. There was also bile duct hyperplasia and some attempted regeneration of still-viable cells.
By week 9 there was extensive regeneration of parenchymal tissue, and individual cells were normal in size, with plentiful glycogen. Necrosis was limited to a cuff of cells in the periportal zone and the cellular response had subsided but was still present. Copper had largely disappeared from the rubeanic acid-stained sections.
By week 15, all livers showed advanced healing, although there was still architectural distortion of the right and median lobes. Bile duct hyperplasia was still present, and necrotic remnants consisting of eosinophilic (hyaline) bodies occasionally with nuclear material, were present in portal areas.

In the kidney, macroscopic changes were limited to greenish discolouration in some animals at week 6.

Histological changes were noted from week 3, when small eosinophilic droplets were present in the cytoplasm of the proximal convoluted tubules. Extrusion of the droplet-containing cells into the lumen of the tubule was common. Copper was not detected in the rubeanic acid stained sections. By week 6 there were marked changes in the proximal convoluted tubule, although there was considerable individual variation in degree. The cytoplasmic droplets were larger, more numerous and assumed the appearance of green globules. Rubeanic acid staining revealed copper in particulate form, and in the droplets visible in the H & E stained sections. In some kidneys there was extensive desquamation of the epithelial cells of the proximal convoluted tubule, with the lumen frequently obliterated by debris. Regeneration was also evident among surviving cells, with mitosis common. The remainder of the nephron was unaffected.
By week 9 the regeneration was mostly completed, with copper still present in rubeanic acid stained sections.
At week 15, regeneration was complete, with little particulate copper in the rubeanic acid stained sections.

Analysis of copper content in both liver and kidney matched the rubeanic acid staining; both rose to maximum values in week 6, after which levels fell.

Dietary administration of copper as sulphate was associated with histological changes in the liver and kidney, reaching a maximum after six weeks of treatment, followed by recovery to week 15. Initially copper accumulated with little effect, but from 2-3 weeks, histological changes were evident in both tissues. Accumulation eventually caused a crisis, associated with severe necrosis, followed by regeneration and recovery.

Reference: Haywood, S. (1985)
Guideline: No
GLP: No

Male weanling Wistar rats were caged in fours and allocated to groups receiving 0, 3000, 4000, 5000 or 6000 ppm copper as copper sulphate for up to 15 weeks. All rats were fed a standard laboratory diet (Labsure

Animal Diet, RHM Agriculture South Ltd., with a copper content of 10 ppm). Animals were regularly inspected and weighed. In each dietary group, a cage of four rats was killed after 2, 3, 4, 5, 6 and 15 weeks of dietary administration. At necropsy, the liver and kidneys were removed for histological examination. Kidney and parts of the right median liver lobe were preserved for histological examination (H & E or Gomoris reticulin stain for general histopathology, and rubeanic acid and rhodanine for copper, and orcin for 'copper-associated protein); other samples were frozen (-70ºC) and triplicate samples analysed for copper content following acid digestion using atomic absorption spectrophotometry

Rats at 3000 ppm showed reduced weight gain, with 'staring' coats between weeks 4 and 5, but by week 15, coats were described as sleek and the animals active, although they weighed less than controls (202 g compared to 438 g for controls).
Rats at 4000 and 5000 ppm showed clinical deterioration between 3 and 4 weeks and subsequent recovery.
Rats at 6000 ppm showed no weight gain. Two animals died in week 2, and by week 6 the remaining animals showed weight loss and deteriorating condition and were sacrificed.

Control mean liver copper concentration was 17.8 μg/g dry weights.
At 3000 ppm, liver copper concentration rose rapidly to 4780 μg/g dry weight between 4 and 5 weeks, but fell significantly to 2412 μg/g dry weight at week 6. By week 15, copper content had fallen further to the same level as at week 2 (approximately 1500 μg/g dry weight).
At 6000 ppm, maximum liver concentrations occurred at week 2 (approximately 3800 μg/g dry weight), and fell only to 2000 μg/g dry weight by week 6, when the animals were terminated.

Renal copper concentration in controls was 34 μg/g dry weights.
Renal copper concentration at 3000 ppm rose more slowly than in the liver, with a maximum of 1188 μg/g dry weight between 4 and 5 weeks which was maintained to 15 weeks.
In the kidney, copper concentration at 6000 ppm continued to rise to week 4, when it equalled the liver value (approximately 2500 μg/g dry weight).

Similar patterns occurred in the liver and kidney at 3000 and 4000 ppm, although the maximum occurred earlier at week 3 (values not stated in paper).

Histological findings in the liver at up to 5000 ppm showed an earlier onset, but were essentially similar to those seen in the earlier study, with hepatic hypertrophy and necrosis, followed by regeneration and recovery. At 6000 ppm necrotic changes were evident in the first week, increased in severity to weeks 2-3, and resulted in chronic hepatitis at 6 weeks.

Renal histopathology at 3000 ppm was similar to that seen at 2000 ppm in the earlier study. However, at 4000 and 5000 ppm, the findings showed earlier onset and correlated with the earlier liver findings. Findings were more marked, with numerous copper-staining granules and droplets in the cells of the proximal convoluted tubule. Extrusion of droplets and exfoliation of whole cells was common in the distal or collecting tubules, with extensive degeneration in many proximal tubules, with the occlusion of the lumen by copper-containing debris and its passage into the distal tubule. By week 15, regeneration was complete. The author concluded that the kidney has the capacity to excrete copper as well as the liver in cases of copper overload, and excrete high doses of copper via the urine.

Dietary doses of 6000 ppm (approximately equivalent to 300 mg/kg bw/day) of copper produced unsustainable liver damage by 6 weeks of administration. Doses of between 3000 and 5000 ppm (approximately equivalent to 150 and 250 mg/kg bw/day) result in liver and kidney damage after between 2 and 5 weeks, with subsequent full recovery by week 15. Regeneration of both organs takes place at 5000 ppm and below, and the kidney appears to develop the capacity to excrete copper when the liver is overloaded.

Reference: Haywood, S. and Comerford, B. (1980b)
Guideline: No
GLP: No

Copper sulphate was administered in the diet to six groups of four male weanling rats at a level of 2,000 mg copper/kg diet. Three similar groups of rats received unsupplemented diet and served as controls. Animals from group 1 to 6 were killed at intervals of 1, 2, 3, 6, 9 and 15 weeks and 2 control animals were killed at each of these time.
During the necropsy of each animal, a blood sample was withdrawn from the vena cava.
The samples (5-10 mL) were taken into lithium heparin anti-coagulant; 1 mL was retained for copper analysis and plasma obtained from the remainder. Copper content was determined in the whole blood and plasma. Alanine aminotransferase (GPT), ceruloplasmin (plasma copper oxidase), alkaline phosphatase and bilirubin were determined on plasma.

Copper content in blood and plasma: the copper concentration in plasma rose significantly after Week 1 but fell to normal at Week 2. Both the plasma copper and the blood copper concentrations increased significantly at Week 6 and thereafter although a slight fall occurred in Week15.

Plasma enzyme activities and bilirubin concentrations: GPT activity was significantly greater than the control value at Week 1 and thereafter rose to a maximum activity around 6 to 9 weeks which was maintained until Week 15. This early rise in activity coincided with the time of pathological changes in the liver seen at higher dose levels but there was not the subsequent decline to parallel the regeneration of the liver.

Alkaline phosphatase activity did not differ greatly from the control value throughout the trial.
Ceruloplasmin activity was similar to the control value for the first three weeks but was high at Week 6 and thereafter.
Bilirubin concentration was similar to the control throughout the trial
Dietary copper, administered at high levels to weanling rats (2,000 ppm, equivalent to 200 mg/kg bw/day in the young rat, or 100 mg/kg bw/day in the older rat) was associated with increased blood and plasma copper concentrations after six weeks (with an initial transient rise in plasma concentration in the first week) to reach a maximum at nine weeks. Similarly, ceruloplasmin activity increased significantly at six weeks. Alanine aminotransferase activity rose gradually from the first week to reach a maximum at nine weeks. Alkaline phosphatase activity and bilirubin concentration showed no change.

The changes in enzyme activity and ceruloplasmin levels coincide with liver toxicity seen at higher levels in subsequent studies, and may reflect increased competence to manage high levels of copper following the initial insult.

4.7.1.2 Repeated dose toxicity: inhalation

Reference: Pimentel, J.C. (1969)
Guidelines: Not standard
GLP: No

Four groups of six guinea pigs housed in poorly ventilated glass cages were treated, by inhalation, as follows: one group was untreated and served as controls; one group was treated with a finely pulverised Bordeaux mixture (solution of copper sulphate neutralised with hydrated lime). This was done three times a day, such that the atmosphere of the cage was completely saturated with the spray. The second group was similarly treated with a solution of wine tartar using a Flit spray gun and the fourth group treated three times a day with sulphur dioxide fumes produced by burning 'sulphur wicks' such as are used for the disinfection of wine vats. The animals were treated daily for at least 6 months. The guinea pigs were radiographed at the start of the study, at the second month and at the end of the 6 months treatment. Radiographic changes were noted in the animals treated with Bordeaux Mixture. Four of these animals were sacrificed at the end of treatment and two were retained untreated for three months when further radiographs were taken before sacrifice. Histopathological examination of the pulmonary lesions was performed, including staining for copper.
The lungs of the animals exposed to sulphur dioxide showed scanty intra-alveolar cells containing yellow/dark brown granules; staining indicated the presence of sulphur-containing amino acids. The wine tartar spray

treated animals showed occasional inter-alveolar cells with a brown/yellow granular pigment that stained for copper. The Bordeaux Mixture-treated animals killed at the end of treatment showed micronodular lesions, characterised by foci involving a variable number of alveoli filled with plugs of desquamated macrophages with inclusions of a substance rich in copper. Additionally, in one guinea pig small histocytic granulomas were seen in the septa with the appearance of fibro-hyaline scars similar to those found in human cases. In the two animals killed three months after exposure an apparently total regression of the lesions was noted on the radiograph. Microscopic examination revealed fibrous bands, small groups of alveoli filled with macrophages, hyaline deposits and small areas of condensation of the reticulin fibres of the septa in regions not involved when using the routine stains.

Reference Kirkpatrick (2010)
Guideline: OECD 412
GLP: Yes
Deviations: None

Cuprous oxide was administered via whole-body inhalation exposure as a 6-hour/day exposure duration to male and female Sprague Dawley Crl:CD(SD) rats for 1, 2, 3, or 4 weeks (5 days/week), test substance-related effects observed at exposure levels of 0.2, 0.4, 0.8 and 2.0 mg/m^3 (particle size of 1.725 µm MMAD +- 1.73 µm GSD).
For the core study, 20 males and 20 females per concentration (control and high) and 10 males and 10 females per concentration (low, med-low and med-high) were used. For the satellite study which evaluate whether a plateau was observed when a time course was conducted for effects following 5, 10 and 15 exposures, 10 males and 10 females per exposure (control and high) and time point (1,2 or 3 weeks) were used.

After 4 weeks of exposure, there was an exposure concentration-related increase in microscopic findings in the lung, and increased lung, bronchial lymph node, and mediastinal lymph node weights. Lung histopathology included alveolar histiocytosis, acute inflammation, and perivascular mononuclear cell infiltrates. At 0.2 mg/m^3, alveolar histiocytosis was minimal, progressing to moderate severity at 0.8 and 2.0 mg/m^3.

Higher blood neutrophil counts were observed following 4 weeks of exposure to cuprous oxide. Inhalation exposure resulted in higher LDH, total protein, and total cell counts, and a higher proportion of neutrophils in the bronchoalveolar lavage fluid of rats following 1, 2, and 3 weeks of exposure (2.0 mg/m^3 group on study days 5, 12, and 19) and following 4 weeks of exposure at the end of exposure evaluation (0.2 mg/m^3 or higher, except 0.4 mg/m^3 or higher for total cell count, at study week 3 after a minimum of 20 exposures as the first week of exposure is study week 0). In the nasal cavity after 4 weeks of exposure, findings considered test substance-related were minimal olfactory epithelium degeneration in a small number of males from the 0.8 and 2.0 mg/m^3 groups and mild subacute inflammation in a small number of males from the 2.0 mg/m^3 group. Most test substance-related effects at 2.0 mg/m^3 appeared to show a peak in the effect prior to completion of 4 weeks of exposure and therefore, the results were consistent with a possible plateau. Only lung weights and the incidence of lymphoid hyperplasia of the bronchial lymph node in males appeared to continue to increase relative to control through 4 weeks of exposure.

At the lowest exposure level of 0.2 mg/m^3, the inflammatory effects in the alveoli were minimal and present in only 2 of 10 animals. There was no microscopic evidence for alveolar epithelial or endothelial cell injury or the presence of edema at any exposure level.

Following a 13-week recovery period at 2 mg/m^3, there were no test substance related effects on hematology parameters, BALF parameters, or lung, lymph node or nasal histopathology. The effects on lung weights were greatly reduced, but still slightly detectable following the recovery period. But there were no microscopic findings or changes in BALF parameters that correlated with the higher lung weights at the recovery necropsy.

4.7.1.3 Repeated dose toxicity: dermal

Reference: Paynter, O.E. (1965)
Guideline: OECD 410
GLP: No
Deviations: Yes

- Animals were treated five days per week for three weeks, instead of continuously for 28 days, as recommended,
- haematology and histological investigations were performed, but the number of parameters investigated was smaller than the modern guideline,
- the test was performed on a formulation, not on the technical material.

Copper hydroxide (wettable powder formulation KOCIDE101) was applied as a 53% w/v aqueous suspension to the shaved backs of adult albino rabbits. Suspensions of test material were applied at 1000 and 2000 mg/kg bw/day of the formulation which represente 500 and 1000 mg/kg bw/day copper as hydroxide. The control group consisted of 5 males and 5 females and the test groups of 10 males and 10 females. Animals were treated for five days per week for three weeks. Half of the animals by sex in each group were subject to mild abrasion of the skin prior to dosing at the beginning of each week. The dose site was covered with a light gauze bandage. Animals were treated for 6 – 8 hours per day. At the end of each exposure, bandage and collar were removed and the treated area washed lightly with water and wiped dry. All animals were sacrificed three or four days after the last application and necropsied. Sections of liver, kidney and skin were preserved.

There were two deaths in the low dose group and three deaths in the high dose group. None of the deaths in dose groups could be conclusively related to the test material; all deaths were considered to be due to apparent gastroenteritis. There were no indications of irritation.

There was an overall mean bodyweight loss in the high dose group. There were no adverse effects on food consumption.

There were no adverse effects of treatment on haematological or urinalysis parameters.

Necropsy and histopathology revealed degenerative changes in the skin. Five control animals showed minimal findings such as focal leukocyte infiltration of the dermis or focal peri-follicular thickening. There were no histological findings in the skin of low dose animals. Ten high dose animals (five abraded and five intact skin) showed skin abnormalities at histopathology. Findings included epidermal thickening, focal leukocyte infiltration of the dermis, keratin thickened or distorted, atrophied hair follicles. There were single instances of dermal fibrosis, dermal oedema, eschar formation and slight ulceration.

4.7.1.4 Repeated dose toxicity: other routes

No data available.

4.7.1.5 Human information

O'Donohue, J.W. (1993) reports a case of chronic self-administration. A 26-year-old Irishman took 30 mg Cu/day for two years (apparently without ill effect), then increased the dose to 60 mg Cu/day in the third year and suffered liver failure.

Araya, M, (2001) reports that relatively low concentrations of free copper in water induce nausea in humans. In an international trial, 179 individuals were given water containing copper sulphate at 0, 2, 4, 6 or 8 mg Cu/L in a 200 mL bolus of water (equivalent to a dose of 0, 0.4, 0.8, 1.2 and 1.6 mg Cu). Subjects were monitored for nausea and other symptoms. The no-adverse-effect-level for nausea was 4 mg Cu/L. However, this represents a taste effect of a soluble copper salt in water. Copper sulphate is a gastric irritant, and the nausea is probably associated with irritation of the stomach. Natural levels of copper in food include 6 mg/kg (= ppm)

for shrimp and liver, 10 mg/kg for mushrooms, and 27 mg/kg for dark (bitter) chocolate. Consumption of 200 g of shrimp or liver in a meal, or 160 g of mushrooms, or 50 g of dark chocolate (which would each provide the same amount of bound copper as was administered in drinking water in the drinking water nausea study) would not be expected to induce nausea.

Araya et *al.* (2003) report another study, in which copper sulphate was administered by the same protocol than the previous investigation but in bottled spring water rather than deionised water and using an entirely female study population (n=269). Consistent with the previous study, nausea was the earliest and most commonly reported gastrointestinal symptom, occurring mostly within 15 min of copper ingestion with a no-adverse-effect-level of 4 mg Cu/L.

Olivares et *al.*(1998) report a study in which the effect of copper supplementation in the drinking water at the level of 2 mg/L was investigated in formula-fed and breast-fed infants from 3 to 12 months old in Chile. This study failed to demonstrate any adverse effects in infants who had consumed water with a copper content during the first 12 months of life. The only observed effect in children with copper relative to control was an increase in ceruloplasmine at 9 months only.

Other human epidemiological data are available and summarised in section 4.10.

4.7.1.6 Other relevant information

No data available.

4.7.1.7 Summary and discussion of repeated dose toxicity

Oral route:
Several studies were available for the assessment of the toxicity after repeated administration:

- 15-days drinking water studies in rat and mice
- 15-days feedings studies in rat and mice
- 15-weeks feedings studies in rat
- 90-days feedings studies in rat and mice
- Human data

2 week drinking study:
All rats and all mice in the 10000 ppm groups and one female rat died, one male mouse and three female mice in the 3000 ppm groups died or were killed during the study. Clinical signs of both rats and mice in the highest two groups included emaciation, abnormal posturing, hypoactivity, dyspnoea, tremors and prostration. Final mean body weight gains for surviving animals of both species from the 3000 ppm groups were significantly reduced. Animals from the two highest groups also showed a decreased water consumption, which was attributed to poor palatability of the cupric sulphate solution. Microscopic lesions in rats were limited to an increase in the size and number of protein droplets in epithelial cells of the proximal convoluted tubules of the kidney of males in the 300 and 1000 ppm groups. No kidney lesions were observed in female rats or in mice of either sex.

2 week feeding study: No animals died or were killed during the study. Final body weights of male and female rats of 8,000 and 16,000 ppm groups and of female mice receiving 16,000 ppm were significantly lower than the controls. Mean food consumption for rats in the 16,000 ppm group and for mice in the 8,000 and 16,000 ppm groups was lower than controls. These decreases were considered to be due to the poor palatability of the feed mixture rather than to specific cupric sulphate toxicity. Microscopic findings in rats at 2,000 ppm and above included hyperplasia and hyperkeratosis of the squamous mucosa on the limiting ridge separating the forestomach from the glandular stomach. A similar finding was observed in mice but the severity was minimal. This was considered due to the irritant effects of cupric sulphate and the authors noted that there were no adverse effects on the health of the animals. Additionally in rats, chronic active inflammation of the liver

characterised as minimal to mild mononuclear inflammatory cell infiltrate was observed in males at 8,000 ppm (4/5) and 16,000 ppm (5/5) and in females at 16,000 ppm (3/5). Depletion of haematopoetic cells in bone marrow occurred in male and female rats in the 8,000 and 16,000 ppm groups, consisting of a decreased cellularity of bone marrow erythroid/myeloid elements and an increase in the prominence of fat cells normally present in the bone shaft. In several high dose animals bone mass (cortex and trabecular density) was reduced when compared to controls. This was considered a consequence of reduced body weight gain rather directly related to treatment. A minimal to mild decrease in erythroid haematopoesis was seen in the spleens of rats in the 16,000 ppm group. There was an increase in the number and size of protein droplets in the cytoplasm and lumen of the renal cortical tubules in the male and female rats of the three highest dose groups, similar to that seen in the drinking water studies.

90-day feeding studies (Hebert, 1993):

Fischer rats (10 males and 10 females per group) were treated with copper sulphate (hydrated salt) administered in the diet at doses of 0, 500, 1,000, 2,000, 4,000 and 8,000 ppm for 92 days. B6C3F1 mice (10 males and 10 females) were treated to concentration of 0, 1,000, 2,000, 4,000, 8,000 and 16,000 ppm for 92 days. All rats and mice, except one female rat in the 1,000 ppm group (accidental death), survived to the end of the study. Final mean body weight gains were significantly reduced in male rats in the two highest dose groups (4,000 and 8,000 ppm) and in female rats in the highest dose group (8,000 ppm). Treated mice showed a dose-related reduction in body weight gain that occurred earlier than in the rat and was more severe at the higher dose levels. Food consumption was generally similar to the controls in all groups in both rats and mice except for the highest dose group in the rat (8,000 ppm).

Significant changes in haematology were noted in rats of both sexes at all time points but generally limited to the 2,000, 4,000 and 8,000 ppm dose groups. The effects were more marked in males. Significant increases in haematocrit (HCT), haemoglobin (HGB), platelet count and erythrocytes (RBC) were seen in the 8,000 ppm group which were consistent with polycythemia related to dehydration. By Day 21, HCT and HGB levels were significantly decreased together with MCV and MCH and these persisted until the end of the study. Significant increases in RBC and reticulocytes were noted in high dose males at the end of the study. There was a dose-related increase in the incidence and severity of chronic inflammation in the livers of rats, characterised by multiple foci of a mixture of mononuclear inflammatory cells.

Staining of the livers for the presence of copper showed a presence in the 4,000 and 8,000 ppm groups. At 8,000 ppm staining had a clear periportal to midzonal distribution and consisted of a few to numerous red granules of 1 to 2 mm in the cytoplasm of hepatocytes. At 4,000 ppm staining was periportal and there was a marked reduction in the number of cells stained and in the number of granules per cell. Positive minimal staining of livers for copper was evident in the high dose male and female mice and consisted of only a few positive-staining hepatocytes in the entire liver section.

Changes in the kidneys included an increase in the size and number of cytoplasmic protein droplets present in the epithelium of proximal convoluted tubules of rats at doses of 2,000 ppm and higher, and was less severe in females than in males. Many of the protein droplets in the male rats had large irregular crystalline shapes, which were not present in the females. Minimal nuclear enlargement (karyomegaly) in renal tubule cells was present in the high dose group. Degeneration of renal tubule epithelium was present in three females from the 8,000 ppm group. Positive staining for copper was seen in the kidneys at 4,000 ppm, and to a greater extent, in the 8,000 ppm groups and consisted of red granules in the cytoplasm of the renal tubule epithelium and a diffuse red staining of the protein droplets in the cytoplasm and the tubule lumen. There was no staining for copper in the kidneys of any mice. There was a reduction in iron-positive granules in the cytoplasm of splenal macrophages in the 8,000 ppm group, which was also evident but less prominent in the 2,000 and 4,000 ppm dose groups.

Human epidemiological data

Data are available however information are limited regarding doses consumed and exposure. The information is based on estimated quantities of copper ingested, which are reliant on patient accounts and are therefore biased, or effects observed are of differing severity which are not consistent with reported copper exposure concentrations. A case study is available detailing an individual who consumed 30mg/day of copper as a dietary supplement (well above the Tolerable upper intake level of 5 mg/day suggested by the Scientific Committee on Food) for 2 years (10 times the RDA) with no apparent ill effects. He then increased the copper intake to 60mg/day and was finally admitted to hospital showing signs of malaise and jaundice. His symptoms

included cirrhosis of the liver and six weeks after admission to hospital he was given a liver transplant and made a good postoperative recovery (O'Donohue *et al*, 1993).

Inhalation exposure:
Two studies are available and were performed in guinea-pigs and rats. Other data are available in human in the chronic/cancerogenicity section (See 4.10.2).

The study of Pimentel (1969) was not performed according to standard guideline. In this study, Guinea-pigs showed interstitial pulmonary lesions, possibly leading to respiratory insufficiency (without a NOAEL).

In human, similar pulmonary lesion were seen after inhalation of Bordeaux mixture. However, in theses epidemiological data analysis different confusing situation were identified (smoking, wood dust, arsenic, etc…) and therefore no link could be established.

Furthermore, in the 4-weeks inhalation toxicity study, performed with current guideline in rat, there was an exposure concentration-related increase in microscopic findings in the lung, and increased lung, bronchial lymph node, and mediastinal lymph node weights. Lung histopathology included alveolar histiocytosis, acute inflammation, and perivascular mononuclear cell infiltrates. However, following a 13-week recovery period, the effects on lung weights were greatly reduced and the microscopic findings were no more observed. As the effects were reverible, they are not considered as severe or significant;

Dermal exposure:
Three-week dermal exposure in rabbit show slight dermal effects above 500 mg Cu/kg bw/d**.**

4.7.1.8 Summary and discussion of repeated dose toxicity findings relevant for classification as STOT RE according to CLP Regulation

Oral route:
Target organs of copper upon oral administration were the liver (inflammation), kidneys (histopathological changes) and hyperplasia and hyperkeratosis of the forstomach in rats, haematological changes were also observed in this specie, while mice were less sensitive, showing adverse effects only in the stomach. Thus, minimal to moderate effects were observed at dose equivalent to 32 mg Cu/kg bw/d (liver-kidney). More severe effects were observed above 66 mg Cu/kg bw/day (forestomach-haematological change);

Inhalation route:
The 4-weeks study in rat, performed with standard guideline is considered the most relevant.
In this study, no irreversible adverse effects were observed up to 2 mg/m^3 Cu.

Dermal route:
No adverse effects were observed at or below 500 mg/kg bw Cu in the available study (3 weeks exposure).

4.7.1.9 Comparison with criteria of repeated dose toxicity findings relevant for classification as STOT RE

Evaluation of non-human data

Oral route:
Two 90-d studies are available and were performed in rat and mice.
In the 90-day study in rat, at the dose of 32 mg copper /kg bw/day, liver and kidney changes were observed but were not considered "significant/severe" effects. At the dose of 66 mg copper/kg bw/day, hyperplasia and hyperkeratosis in the forestomach mucosa and haematological changes (haematocrit and haemoglobin levels were significantly decreased, together with mean cell volume and mean cell haemoglobin) were observed.

These effects were in the harmful cut-off values for classification cat 2 (10-100 mg/kg) in CLP regulation but were considered as not enough relevant to classify this substance. In effect, the haematological change observed were significant but were not notable as the decrease was inferior to 10% of the control values. The effects on forestomach were probably the results of irritant effect of sulphate ($CuSO_4$) and therefore were not relevant for copper.
In the 90-day study in mice, no adverse effects were observed below 100 mg/kg bw/d.

Overall, in the available studies in mice and rat, it was considered that no serious adverse effects were observed below the harmful cut-off values for classification Cat 2 (10-100 mg/kg bw) in the CLP regulation.

Inhalation route:
In the 4-weeks study in rat performed with standard guideline No serious adverse effects were observed at the maximum tested concentration (2 mg/m^3). Therefore, no classification is warranted according to the CLP criteria.

Dermal route:
In the 3 weeks study performed in rabbit, no adverse effects were observed at or below 500 mg/kg Cu. No classification is therefore necessary according to the CLP criteria

Evaluation of human data:
Human epidemiological data is available however information is limited regarding doses consumed and exposure. The information is based on estimated quantities of copper ingested, which are reliant on patient accounts and are therefore biased, or effects observed are of differing severity which are not consistent with reported copper exposure concentrations.
A case study is available detailing an individual who consumed 30mg/day of copper as a dietary supplement (well above the Tolerable upper intake level of 5 mg/day suggested by the Scientific Committee on Food) for 2 years (10 times the RDA) with no apparent ill effects. He then increased the copper intake to 60mg/day and was finally admitted to hospital showing signs of malaise and jaundice. His symptoms included cirrhosis of the liver and six weeks after admission to hospital he was given a liver transplant and made a good postoperative recovery (O'Donohue et al, 1993).

Other human epidemiological data are available and summarised in section 4.10.

Weight of evidence of all data, including human incidents, epidemiology, and studies conducted in experimental animals, do not to support classification for specific target organ toxicity following repeated exposure.

4.7.1.10 Conclusions on classification and labelling of repeated dose toxicity findings relevant for classification as STOT RE

No classification is considered necessary for repeated exposure.

RAC evaluation of specific target organ toxicity– repeated exposure (STOT RE)
Summary of the Dossier Submitter's proposal No data on granulated copper are available in the CLH report. However, in light of the proposal to read-across between the different copper compounds for systemic endpoints (see section "RAC general comment" above), the dossier submitter included in the CLH

report several animal studies with repeated exposure to other copper compounds (predominantly copper sulphate pentahydrate) for various durations and routes, as well as some human data.

Hébert *et al.* (1993) reported on oral 15-day drinking water and feeding studies and 90-day feeding studies in both rats and mice, all conducted with copper sulphate pentahydrate but none were guideline compliant. In addition, three studies where copper sulphate was administered in the diet at one or several doses for up to 15 weeks and animals sacrificed at several intervals, were also reported (Haywood, 1980, 1985; Haywood & Comerford, 1980). One OECD TG 412 compliant 28-day rat inhalation study which was conducted with dicopper oxide (Kirkpatrick, 2010) was included together with an older non-guideline compliant study, where guinea pigs were exposed via inhalation to Bordeaux mixture for about 6 months (Pimentel & Marques, 1969). Finally, an OECD TG 410 compliant dermal rabbit study is included (Paynter, 1965), with exposure to copper dihydroxide for 3 weeks (5 days per week).

With regard to available human data, a human case study of chronic oral self-administration of copper causing liver failure (O'Donohue *et al.*, 1993) was reported together with human volunteer studies demonstrating nausea associated with copper sulphate in drinking water (Araya *et al.*, 2001, 2003). Human case studies of chronic inhalation exposure to Bordeaux Mixture causing pulmonary lesions were also reported (e.g. Pimentel & Marques, 1969; Pimentel & Menezes, 1975, 1977).

Inhalation exposure to dicopper oxide resulted in no irreversible adverse effects up to the highest dose tested in rats (2 mg/m^3). Following dermal exposure to rabbits, degenerative skin abnormalities were only observed at 1000 but not at 500 mg copper/kg bw/day. Human data were poorly reported and doses are difficult to estimate. Following oral exposure in rats, target organs of copper were the liver (inflammation), kidneys (histopathological changes) and forestomach (hyperplasia and hyperkeratosis), with some evidence of haematological changes. Mice were less sensitive, with adverse effects limited to the forestomach. According to the dossier submitter, no serious adverse effects were observed in the available oral studies below the cut-off value for classification (100 mg/kg bw/day for a 90-day study). After considering all available human and animal data, the dossier submitter concluded that they do not support classification for specific target organ toxicity following repeated exposure.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that no data on granulated copper are available. The CLH report contains data on other copper compounds (predominantly copper sulphate pentahydrate), from which the dossier submitter proposed to read-across to granulated copper. In view of the considerations presented in the section "RAC general comment", RAC has not pursued the aspect of grouping any further. RAC concludes that in the absence of relevant data, **no proposal for classification for specific target organ toxicity following repeated exposure can be made for granulated copper**.

4.8 Germ cell mutagenicity (Mutagenicity)

Copper has been extensively investigated in a series of mutagenicity studies in various salts. The majority of studies were from the literature, but there are three core guideline compliant GLP studies.

Table 27: Summary table of relevant *in vitro* and *in vivo* mutagenicity studies

Method	Results	Remarks	Reference
In vitro			
Ames *S. typhimurium* TA 98, TA100, TA1535, TA1537, TA102. Copper sulphate pentahydrate Five concentration: 50, 100, 200, 400 and 800 µg/plates +/- metabolic activation system Positive and negative controls Pre-incubation: 1 hour with metabolic activation	Cytotoxicity at 800 µg/plates and at 200 and 400 µg/plates with S9 +S9: negative -S9: negative	OECD 471 GLP Purity: 99-100.5%	Ballantyne (1994)
Ames *S. typhimurium* TA 102 Copper sulphate 10, 30, 100, 300, 100 and nM/plate Positive and negative controls Triplicate	+S9: Not investigated -S9: negative	No guideline No GLP Lack of data	Marzin, D.R., Phi, H.V. (1985)
Ames *S. typhimurium* TA 98, 100, 1535, 1537, 1538, and *E. coli.*WP2 Copper sulphate Up to 5000 µg/plate. Oxine copper Up to 50 µg/plate.	**Copper sulphate** Cytotoxicity not stated. +S9: negative -S9: negative **Oxine copper** Cytotoxicity above 5µg/plate +S9: negative -S9: negative	Guidelines followed with lacks. No GLP	Moriya, M., Ohta, T., Watanabe, K., Miyazawa, T., Kato, K. Shirasu, Y. (1983)
In vitro UDS Primary rat hepatocytes Copper sulphate Concentrations : 7.9, 15.7, 41.4, 78.5µM (incubation 20h) +/- hydroxyurea Positive and negative controls Triplicate	Lowest concentration non-cytotoxic Highest concentration moderately cytotoxic +S9: Not investigated -S9: Positive Significant stimulation of 3H-thymidine incorporation into the DNA, both in presence and absence of hydroxyurea and at all concentrations (dose-dependent).	Guideline not stated No GLP Lacks of data	Denizeau, F., Marion, M. (1989)

Ames *S. typhimurium* TA 98, 100, 1535, 1537, 1538. Technical Bordeaux mixture First replicate: 33, 100, 333, 1000, 3333, 10000 µg/plate Second replicate: 312.5, 625, 1250, 2500, 5000, 10.000 µg/plate. Third: 1000,2000, 3000, 4000, 5000, 6000µg/plate (TA 98 and 100)	+S9: negative -S9: negative	OECD 471 GLP Deviation: lack of strain TA 102 or *E. coli.*WP2 Purity: not stated	Dillon, D. M., Riach, G. C. (1994a)
Ames *S. typhimurium* TA 98, 100, 1535, 1537, 1538 Copper oxychloride. First: 33, 100, 333, 1000, 3333, 10000µg/plate Second: 312.5, 625, 1250, 2500, 5000, 10000 µg/plate Third: 1000, 2000, 3000, 4000, 5000, 6000 µg/plate (TA 98 and 100)	Toxic effects observed above 3333µg/plate +S9: negative -S9: negative	OECD 471 No GLP Deviation: only 2 tested strain Purity: 98.3%	Dillon, D. M., Riach, G. C. (1994b)
Ames *S. typhimurium* TA 98, TA100 Copper Nordox Technical 0.1, 1.0, 10, 20 µg/plate.	+S9: negative -S9: negative		Bossotto, A., Allegri, R., Chujman, A., Terceño, A., Mannocci, S. (2000)
Ames *S. typhimurium* TA 98,TA102, TA1535, TA1537 Copper chloride 160ppm and 200ppm (no more precision).	+S9: negative -S9: negative	Guideline not stated No GLP Deviations: lack of data Purity: not stated	Wong P.K. (1988)
Rec-assay Cold incubation assay in recombination-repair deficient strains of *Bacillus Subtilis* CuCl and $CuCl_2$	+S9: Not investigated -S9: negative	Non-guideline study No GLP Deviations: Lack of information on concentrations. Purity: not stated	Kanematsu, N., Hara, M., Kada, T. (1980)
UDS and SCE assays CHO V79 cells Copper (II) nitrate	+S9: Not investigated -S9: Assay showed binding to DNA and weak positive SCE	Non-guideline study Deviations: Lack of information on concentrations. No positive control, experimented not duplicated. Purity: not stated	Sideris, E.G., Charalambous, A.T. Katsaros, N. (1988)

In vivo			
Mouse micronucleus CD-1 mice 5/sex/groups Copper sulphate Oral gavage First: LD50 745 mg/kg Main study: 2 days at 447 mg/kg (i.e. 113.76 mg Cu/kg). Twice on consecutive days Sacrifice 24 and 48h, after second treatment. Positive control	Negative Decreased ratio of PCE to NCE after 24h compared to vehicle control indicated that copper sulphate had been absorbed into the bone marrow.	OECD 474 GLP Purity: 99-100.5%	Riley, S.E. (1994)
UDS Rat (hepatocytes) Wistar rats. 6 males/group Copper sulphate Oral gavage 632.5, 2000 mg/kg (equivalent to 161 and 509 mg copper/kg/day), once sampling times: 12-14h or 2-4h post dosing positive and negative controls	Negative No production of a group mean net grain counter greater than 1.0 in primary cultures of hepatocytes treated with 3H thymidine. No more than 1.0 % of cells found in repair at either dose.	No guideline GLP Purity: 99-100.5%	Ward, P.J. (1994)
Swiss mice Groups of 3 mice Copper sulphate Bone marrow chromosome aberration study, micronucleus assay and sperm abnormality assay in the mouse ip injection **<u>Bone marrow chromosome aberration study</u>** IP inj, doses 5, 10, 20 mg/kg,. Mice killed after 6h (20 mg/kg), 24h (5, 10, 20mg/kg) and 48h (20mg/kg). Other group with IP inj.of 4 mg/kg/day during 5days. Mice killed 24h after the last injection	**<u>Bone marrow chromosome aberration</u>** Aberrations such as gaps more frequent than, breaks, fragments, exchange of rings. Greatest effect with IP inj. **<u>Micronucleus:</u>** Significant dose-dependent increase in the incidence of micronuclei. **<u>Sperm abnormality assay:</u>** Significant dose-related increase in the mean number of abnormal sperm (head shapes, tail attachments and double tailed sperm).	Guideline not stated No GLP Lack of information	Bhunya, S.P. Pati, P.C. (1987)

Oral or SC: dose: 20mg/kg; sacrifice 24 h later. **<u>Micronucleus</u>** 5, 10, 20mg/kg/day; 2 inj. at 24h interval. Mice killed 6h after the 2nd. inj. **<u>Sperm abnormality assay</u>** IP inj. Doses: 1, 2, 4 mg/kg/day 5 consecutive days. Sacrifice 35 days after the first inj. 500 sperm examined for each animal.			
Bone marrow chromosome aberration Swiss albino Mice 6 mice/groups Copper sulphate IP injection Doses: 1.1, 1.65, 2.0, 3.3, 6.6 mg/kg. Sacrifice of 6 mice at 6, 12, 24h, after treatment for each dose Positive control: Mitomycine C.	Positive The aberrations induced were mainly of the chromatid type and only in the higher dose groups were chromosomal breaks significantly enhanced. There were positive trends with increasing dose for the number of chromosomal aberrations per cell and the % damaged cells at all hours of exposure.	Guideline not stated (meet OECD 475) No GLP	Agarwal, K., Sharma, A. Talukder, G. (1990)
Mouse micronucleus Male CBA mice 5 animals/group Copper sulphate i.p. injection Doses: 6.6, 13.2, 19.86 mg/kg. Sacrifice of 6 mice at 24h (all doses) or 48h (6.6 mg/kg), after treatment. Positive controls: Cyclophosphamide and Vincristine sulphate.	Negative Reduced PE/NE ratio indicated that copper sulphate had been absorbed into the bone marrow. Deviation: Statistical analyses not performed	Guideline not stated GLP: not stated Deviation: no statistical analysis Purity: not satated	Tinwell, H. Ashby, J. (1990)

4.8.1 Non-human information

4.8.1.1 In vitro data

The *in vitro* systems, particularly those involving isolated mammalian cells, may not be valid in the risk assessment of copper. Copper absorbed by the body is always bound , and transfer from blood/plasma to cells is regulated such that copper passed through the cell membrane is also bound to metallothioneins within the cell, before being incorporated in various enzymes. The *in vitro* tests bypass these strict control mechanisms and effectively present the cell with a totally artificial situation of excess free copper ion. The free copper ion is highly reactive, and the presence of high quantities of free ion in cell cultures will cause disruption of the cellular processes.

As *in vitro* data are not appropriate to assess genotoxicity of copper (Arce, 1998) and that several data *in vivo* were available, *in vitro* data are only summarized in the table above.

4.8.1.2 In vivo data

Reference: Ward, P. J. (1994)
Guideline: No
GLP: No
Deviations: None

Doses of 623.5 and 2000 mg/kg bw of copper II sulphate pentahydrate were administered to male Wistar rats by gavage at a dose volume of 10 mL/kg bw to groups of 6 rats. Doses were administered on two occasions separated by 2 hours. Negative control animals received water only. Positive control animals received an oral dose of 2-Acetamidofluorene, suspended in corn oil at 75 mg/kg (experiment 1) or dimethylnitrosamine, suspended in water at 10 mg/kg (experiment 2). After 12-14 hours (experiment 1) or 2-4 hours (experiment 2), the rats were killed and the livers perfused with collagenase to provide a primary culture of hepatocytes. Cultures were made from 5 animals per group and were treated with [^{3}H] thymidine. Slides were treated with photographic emulsion to prepare autoradiograms, and examined microscopically. The net grain count, the number of grains present in the nucleus minus the number of grains in 3 equivalent areas of cytoplasm, was determined.

Negative (vehicle) controls and positive controls confirmed the validity of the assay. Treatment with 632.5 or 2000 mg/kg bw copper II sulphate (equivalent to 161 or 509 mg copper/kg bw) did not produce a group mean net grain count greater than -1.0, nor were there any more than 1.0% cells found in repair at either dose (table 28).

Copper II sulphate pentahydrate has no genotoxic activity in the in vivo rat liver UDS assay.

Table 28: Group mean net grain counts for experiment 1 and 2

12-14 hour sacrifice time

Dose (mg/kg)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG≥5)	
	Mean	SD	Mean	SD	Mean	SD
0 water	-1.3	0.6	0	-	-	-
632.5	-1.3	0.3	10.2	6.4	0.6	0.9

2000	-1.0	0.3	5.5	0.9	1	1
75 2-AAF	12.7	0.9	13.7	0.8	90.0	4.0

2-4 hour sacrifice time

Dose (mg/kg)	Net nuclear grain count (NG)		Net grain count of cells in repair		Percent of cells in repair (NG≥5)	
	Mean	SD	Mean	SD	Mean	SD
0 water	-2.2	0.3	0	-	-	-
632.5	-2.2	0.2	0	-	-	-
2000	-3.2	0.5	0	-	-	-
10 DMN	17.2	2.8	17.3	2.7	99.6	0.9

Reference: Riley, S. E. (1994)
Guideline: OECD 474
GLP: Yes
Deviations: Yes

- Only one dose tested in the main study.

Copper II sulphate pentahydrate was administered orally by gavage to groups of male and female CD-1 mice. In the main study, mice were treated on two consecutive days at 447 mg/kg bw/day to groups of 5 male and female mice, that were killed either 24 or 48 hours after the second dose. Groups of mice were also dosed on two consecutive days with vehicle (distilled water) only and killed either 24 or 48 hours after the second dose, and other groups of 5 male and 5 female mice were dosed with the positive control cyclophosphamide dissolved in purified water at 80 mg/kg bw and killed after 24 hours.
Erythrocytes of bone marrow were observed in all animals, in order to determine polychromatic/normochromatic erythrocytes ratio and frequency of micronucleated PCE/1000 cells determined.

Several animals in the main study died prior to scheduled sacrifice (5 out of 10 males and 3 out of 10 females), indicating that it would not have been possible to administer the test material at a significantly higher dose. Mice treated with copper II sulphate pentahydrate showed decreased ratios of polychromatic erythrocytes (PCE) to normochromatic erythrocytes (NCE) when sampled after 24 hours, compared to concurrent vehicle controls, indicating that copper II sulphate pentahydrate had been absorbed into the bone marrow. The PCE/NCE ratios seen in animals sampled at 48 hours were similar to those of control animals. Mice treated with copper II sulphate pentahydrate exhibited frequencies of micronucleated PCE which were similar to vehicle controls at all sampling times. There were no instances of statistically significant increases in micronucleus frequency for any group receiving the test chemical at either sampling time. The positive control animals exhibited increased numbers of micronucleated polychromatic erythrocytes, such that the frequency of micronuclei was significantly greater than in concurrent controls (table 29).

Table 29: Summary of group mean findings

Treatment group (mg/kg) twice	Kill time (hours)	Sex	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000)	
				Per sex	Per treatment group
Vehicle control	24	♂	1.07	0.4	0.35

		♀	1.20	0.3	
	48	♂	1.44	0.38	0.33
		♀	0.83	0.3	
447	24	♂	0.70	0.6	0.5
		♀	0.84	0.4	
	48	♂	1.12	0.5	0.45
		♀	1.32	0.4	
Positive control CPA, 80+	24	♂	0.52	26.87	28.07
		♀	0.48	29.27	

Conclusion: Copper II sulphate pentahydrate did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice at 447 mg/kg bw/day (equivalent to 113.76 mg copper/kg bw/day), a dose at which limited mortality was observed.

Reference: Bhunya, S.P. and Pati, P.C. (1987)
Guideline: No
GLP: No
Deviations: Yes

- Only three animals per group were used,
- no positive control group,
- in the micronucleus test animals were killed 6 h after the last injection.

Three parameters were analysed:

- Bone marrow chromosome aberration assay,
- micronucleus assay,
- sperm abnormality assay.

Bone marrow chromosome aberration assay:
Swiss mice, with an average body weight of 25g, were administered hydrated copper sulphate, by a single intraperitoneal injection, at dose levels of 5, 10 and 20 mg/kg and groups of three were killed after 6 h (20 mg/kg), 24 h (5, 10 and 20 mg/kg) and 48 h (20 mg/kg). Another group of animals was administered the test article at a dose level of 20 mg/kg divided into 5 equal parts, each part administered daily by intraperitoneal injection (4 mg/kg/d during 5 days) and the animals were killed 24 h after the last injection. A similar group of animals was administered double distilled water and served as controls. Further groups of animals were given a single administration of the test article orally or by subcutaneous injection at a dose level of 20 mg/kg and were killed after 24 h. Groups of animals were given double distilled water by similar methods to serve as controls. Colchicine was used, shortly before sacrifice, as a spindle inhibitor. Bone marrow smears were prepared and 100 metaphases per animal were scored for aberrations.

Micronucleus assay:
The test article was administered to groups of three Swiss mice by two intraperitoneal injections, separated by 24 h, at dose levels of 5, 10 and 20 mg/kg. A similar group received double distilled water and served as controls. The animals were killed 6 h after the second injection. Bone marrow smears were prepared and 1,000 erythrocytes per animal scored for micronuclei.

Sperm abnormality assay:
The test article was administered to groups of three Swiss mice by intraperitoneal injection at dose levels of 5, 10 and 20 mg/kg, each dose being split into five equal parts and each part being injected daily at 24 h intervals. A similar group received double distilled water and served as controls. The animals were killed 35 days after the first injection. Sperm were collected from the cauda epididymides and slides prepared. Five hundred sperm from each animal were examined and sperm abnormalities categorised. Statistical analyses were performed on each series of tests.

Bone marrow chromosome aberration assay:
Treatment induced aberrations such as gaps, breaks, fragments, double minutes, exchanges and rings, with gaps being more frequent than breaks. Repeated exposure of fractionated doses induced less aberration than that of the equivalent dose as a single dose. The greatest effect was produced when copper sulphate was administered by intraperitoneal injection (table 30).

Table 30: Chromosomal aberrations (%)

Kill (h)	**Dose level (mg/kg)**			
	0	**5**	**10**	**20**
Single intraperitoneal injection				
6	-	-	-	4.00*
24	-	4.00*	4.66*	5.00*
48	0.70	-	-	4.33*
Multiple intraperitoneal injection				
120	-	-	-	4.00*
Oral				
24	0.66	-	-	4.00*
Subcutaneous injection				
24	0.66	-	-	4.66*

* Statistically significant using an equality of proportion test

In all cases, chromosomal aberrations were predominantly chromatid gaps and when gaps are excludes, results were similar to negative controls.

Micronucleus assay:
There was a significant dose-dependent increase in the incidence of micronuclei (table 31).
However, a statistically significant increase in the frequency of nuclei in lysis compared to controls was also reported for all doses investigated, indicating that all the doses of copper sulfate used in this study were cytotoxic.

Table 31: Bone marrow counts (mean %)

	Dose level (mg/kg)			
	0	**5**	**10**	**20**
Number cells examined	3000	3000	3000	3000
Poly and normochromatic erythrocytes with micronuclei	0.15	0.98	1.41	1.76
Poly/normochromatic ratio	0.88	1.10	1.10	1.10
Immature white cells with micronuclei	0.06	0.40	0.88	1.23
Nuclei in lysis	-	0.20	0.30	0.46
Total	0.21	1.58*	2.59*	3.45*

* Statistically significant using an equality of proportion test

Sperm abnormality assay:

There was a significant dose-related increase in the mean number of abnormal sperm. Varieties of abnormal sperm were induced, including various head shapes, tail attachments, double headed and double tailed sperm (table 32).

Table 32: Incidence of sperm abnormality

	Dose level (mg/kg)

	0	**5**	**10**	**20**
Number sperm examined	1500	1500	1500	1500
Number abnormal sperm	62	87	166	231
Mean %	2.06	5.80*	11.60*	15.40*

* Statistically significant using an equality of proportion test

Conclusion: Results indicated that copper sulphate solution administered by intra-peritoneal injection (where free copper is injected directly to the abdominal cavity) caused mutagenic activity in bone marrow cells and in sperm. However, this route of administration is inappropriate, as it avoids the normal processes of copper absorption and distribution.
Chromosomal aberration study in vivo where copper is administered orally (the natural route, whereby uptake is controlled by homeostatic mechanisms) is positive (at 20 mg/kg). However, chromosomal aberrations were predominantly chromatid gaps and when gaps are excludes, results were similar to negative controls. Moreover, only three animals are used whereas in the guideline 10 animals are recommended.

Dose, route and time influenced significantly the frequency of chromosomal aberration, incidence of micronucleus and sperm abnormality. The study deviated from the guideline and the findings are consequently considered to be unreliable.

Reference: Agarwal, K., Sharma, A. and Talukder, G. (1990)
Guideline: No. Generally meets requirements of OECD 475
GLP: No.
Deviations: Yes

- Groups of six male mice were used for each dose level at each time point,
- no cytotoxicity was observed and reported in this study,
- only 50 metaphase plates from each 6 animals per dose were scored, whereas OECD guideline 475 require that “At least 100 cells should be analysed for each animal.

The test article, copper sulphate pentahydrate in isotonic saline, was administered by intraperitoneal injection to groups of Swiss albino male mice at dose levels of 1.1, 1.65, 2.0, 3.3 and 6.6 mg/kg. Prior to sacrifice (1.5 h) the mice were injected with 4 mg/kg colchicine, a spindle inhibitor. Groups of six mice were killed at 6, 12 and 24 h after treatment for each dose. A similar group of mice was treated with 1.5 mg/kg mitomycin C (a positive control article) and then animals killed after 6 h. Bone marrow smears were prepared by standard methods and 50 metaphases from each of the six animals from each group were scored for aberrations, excluding gaps.

The aberrations induced were mainly of the chromatid type (isochromatid breaks and chromatid gaps) and only in the higher dose groups were chromosomal breaks significantly enhanced. When gaps were excluded, there were positive trends with increasing dose for the number of chromosomal aberrations per cell and the % of cells with at least one chromosomal aberration at all time points and doses investigated. Further analysis of the data demonstrated that both chromosome aberrations/cell and % of cells with at least one chromosomal aberration (excluding gap) were significantly higher at 6h compared to 12 and 24h at all doses investigated, indicating a relative early onset of clastogenesis. The highest concentration of copper sulphate produced higher values in the chromosomal aberrations per cell and % damaged cells at 6 and 12 h exposure than the positive control, mitomycin C (Table 33).

Table 33: Chromosomal aberrations

Exposure (h)	Mitomycin C 1.5 mg/kg	Dose level (mg/kg)					
		0	1.1	1.65	2	3.3	6.6
Chromosome aberrations (excluding gaps)							
6	0.077	0.017	0.053	0.060	0.073	0.067	0.100
12		0.017	0.023	0.040	0.037	0.050	0.087

24		0.010	0.037	0.047	0.047	0.040	0.050
% damaged cells with at least 1 aberration							
6	7.667	1.670	5.330	6.000	7.330	6.670	10.00
12		1.670	2.330	4.000	3.670	5.000	8.670
24		1.000	3.670	4.670	4.670	4.000	5.000

Conclusion: Results show that copper sulphate is a moderate clastogenic agent in mice causing a significant increase in aberrations at higher dose levels. Intraperitoneal injection bypasses the natural mechanisms for binding copper, and is not an appropriate route to assess oral exposure.

Reference: Tinwell, H. and Ashby, J. (1990)
Guideline: No but very close
GLP: No
Deviations: Yes

- Statistical analyses were not performed.

The study was performed as a direct response to the previous study and published in the same journal. Hydrated copper sulphate dissolved in sterile deionised water was administered by a single intraperitoneal injection to groups of six male CBA mice at dose levels of 6.6, 13.2 and 19.86 mg/kg. Other groups of six mice of the same age, sex and strain were given distilled water and served as controls. Positive control articles, cyclophosphamide and vincristine sulphate dissolved in sterile deionised water, were administered to groups of mice at dose levels of 65 mg/kg (two mice) and 0.1 mg/kg (one mouse), respectively. A dose volume of 10 mL/kg was used. The animals were killed 24 h (all doses) or 48 h (6.6 mg/kg dose only) after treatment. Bone marrow smears were prepared and 2,000 polychromatic erythrocytes (PE) were assessed for micronucleated PE. The ratio of PE to normocytes (NE) was determined from 1,000 erythrocytes. Statistical analyses were not performed as the results were considered to be obvious.

No toxicity was reported at the lowest dose level (6.6 mg/kg) during the course of the experiment. At the other two dose levels, the animals were reported as appearing subdued. In addition, a marked depression in erythropoiesis (reduced PE/NE ratio) was observed at both 13.2 and 19.86 mg/kg, indicating cytotoxicity. The dose of 19.86 mg/kg (60% of LD50) was estimated to be the maximum tolerated dose. Copper sulphate failed to induce micronuclei in the bone marrow at any of doses or time points investigated. These results conflict with those of the preceding study.

Table 34: Mean bone marrow counts

		Dose level (mg/kg)					Cyclophos-phamide (65 mg/kg)	Vincristine sulphate (0.1 mg/kg)
		0 Test 1	0 Test 2	6.6	13.2	19.8		
MPE/1000 PE	at 24 h	2.6	1.5	3.3	2.1	2.0	65.25	10.5
	at 48 h			2.5				
PE/NE ratio	at 24 h	0.9	0.9	1.0	0.5	0.45	0.7	0.7
	at 48h			0.9				

Conclusion: Copper sulphate did not induce micronuclei in the bone marrow of mice. As this conflict with a preceding study and the age and sex of the animals were the same, there is the possibility of a strain specific bone marrow response, although no precedent exists.

4.8.2 Human information

No data available.

4.8.3 Other relevant information

Literature review on copper genotoxicity:

Reference: Arce, G. T. (1998), Griffin, Unpublished report.

This report is a summary of evidence from the literature. The report emphasises the essential nature of copper including its presence in the cell nucleus associated with stabilising genetic materials and with DNA polymerases. Copper appears to be essential for the replication of DNA, and transcription of RNA.
The report notes that *in vitro* systems, particularly those involving isolated mammalian cells, may not be valid in the risk assessment of copper. Copper absorbed by the body is always bound, and transfer from blood/plasma to cells is regulated such that copper passed through the cell membrane is also bound to metallothioneins within the cell, before being incorporated in various enzymes. The *in vitro* tests bypass these strict control mechanisms and effectively present the cell with a totally artificial situation of excess free copper ion. The free copper ion is highly reactive, and the presence of high quantities of free ion in cell cultures will cause disruption of the cellular processes. These effects may be manifest as gene mutations, but their occurrence is not evidence for mutagenic activity of copper, but shows that the proper concentration of copper is vital for the correct functioning of all cells.
Copper has rarely been found to be mutagenic alone. In combination with certain chemicals or UV light, it can cause mutation by allowing the production of hydroxyl radicals, where excess copper is the catalyst producing oxidation through the Cu (II)/Cu(I) redox cycle. The report also notes that copper, like iron, has been shown to be responsible for inducing mutations through the formation of metal-generated free radicals, often in the presence of another chemical.
One such report cited the role of copper in DNA strand breaks when the chemical menadione is added to Chinese hamster fibroblast cultures. No additional copper was added. There is enough natural copper present in the cells: menadione induced the release of sufficient stored copper from the cell to produce hydrogen peroxide through the redox reaction, which produced sufficient oxygen free radicals to cause DNA damage. Similar studies have been performed with UV light, hydroquinone and ascorbic acid.

4.8.4 Summary and discussion of mutagenicity

The potential mutagenicity of copper compounds has been investigated in a number of in vitro assays in bacterial and mammalian cells, and in several in vivo assays.

Ames tests were negative. Two *in vitro* studies were positive. The *in vitro* UDS positive results is considering not relevant as the *in vivo* UDS study was negative. The SCE weak positive result is considered equivocal due to the lack of information in this study.

Two *in vivo* tests performed by the oral route (a micronucleus assay and a UDS test of Riley, S.E., 1994 and Ward, P.J., 1994, respectively) presented no concern about their validity and were negative. Only the chromosomal aberrations study of Bhunya (1987) presented positive results at 20 mg/kg. However, chromosomal aberrations were predominantly chromatid gaps and when gaps are excludes, results were similar to negative controls. Moreover, only three animals are used whereas in the guideline 10 animals are recommended. Consequently, the findings were not considered.
Results of these studies provide no evidence that copper compounds are mutagenic *in vivo* upon oral administration.

Following non-oral exposure, two tests via ip (intra-peritoneal) (Bhunya, S.P., 1987 and Argawal, K., 1990) showed positive results, although they had some short comings: no positive control had been used for one, a low number of animals had been used and a low number of cells examined, and both studies were no GLP. Moreover, this route of administration is inappropriate, as it avoids the normal processes of copper absorption and distribution. Furthermore, these results are in conflict with an additional well-conducted negative *in vivo* micronucleus study *via* intraperitoneal injection (Tinwell, H., 1990).

To conclude, a number of studies have been performed, but several suffer of deficiencies. Consideration of the weight of evidence from *in vitro* and *in vivo* tests, leads to the conclusion that copper compounds are likely not mutagenic.

Overall, data indicates that copper compounds do not meet the criteria for classification as a genotoxic.

4.8.5 Comparison with criteria

1) Criteria in the CLP classification:

A substance shall be classified in category 2 for germ cell mutagenicity endpoint if the substance causes concern for humans owing to the possibility that they may induce heritable mutation in the germ cells of humans. This classification is based on:

- Positive evidence obtained from experiments in mammals and/or in some cases from in vitro experiments, obtained from:
    - Somatic cell mutagenicity tests in vivo, in mammals (mammalian bone marrow chromosome aberration test, mouse spot test or mammalian erythrocyte micronucleus test); or
    - Other in vivo somatic cell genotoxicity test (UDS or SCE assay) which are supported by positive results from in vitro mutagenicity assays (in vitro mammalian chromosome aberration test, in vitro mammalian cell gene mutation test or bacterial reverse mutation test).

2) Comparison with criteria:

For copper compounds, positive results were observed for bone marrow micronucleus assay (Bhunya, 1987) and bone marrow chromosome aberration assays (Bhunya, 1987 and Agarwal, 1990) when the substance was administered by **intra-peritoneal route**. However, this route is considered as inappropriate as it avoids the normal process of copper absorption and distribution. And another bone marrow micronucleus assay (Tinwell, 1990), with less deficiency than the Bhunya's study, was available and gave negative result. Moreover, two in vivo reliable test (bone marrow micronucleus assay (Riley, 1994), UDS in hepatocyte cells (Ward, 1994) performed by the oral route (natural route, whereby uptake is controlled by homeostatic mechanisms) were negative.

4.8.6 Conclusions on classification and labelling

In this context, the available data do not support a classification for mutagenicity endpoints.

However, there was insufficient evidence to exclude a local genotoxic potential of copper as some studies by I.P route were positive (but with a low reliability) and that UDS and SCE *in vitro* tests without metabolic activation were also positive.

RAC evaluation of germ cell mutagenicity
Summary of the Dossier Submitter's proposal No data on granulated copper are available in the CLH report. However, in light of the proposal to read-across between the different copper compounds for systemic endpoints, (see section "RAC general comment" above), the dossier submitter included in the CLH report, mutagenicity studies with other copper compounds (predominantly copper sulphate pentahydrate).

Ten *in vitro* studies were very briefly summarised in tabular form. Three Ames tests conducted with copper sulphate (pentahydrate) and another four conducted with Bordeaux Mixture, dicopper chloride trihydroxide, copper Nordox Technical and copper chloride as well as a rec-assay with copper chloride were all reported as negative. An unscheduled DNA synthesis (UDS) test conducted with copper sulphate in primary hepatocytes and an UDS and sister chromatid exchange (SCE) assay with copper nitrate in Chinese hamster V79 cells showed positive results in the absence of metabolic activation. The dossier submitter did not discuss these studies further in the report, as *in vitro* data are not considered appropriate to assess the genotoxic potential of copper. This is because absorbed copper is normally always bound to proteins in the body, whereas the *in vitro* tests present the cells with free copper, which is highly reactive.

Five *in vivo* studies are included in the CLH report, all conducted with copper sulphate pentahydrate. A negative mouse bone marrow micronucleus assay (Riley, 1994) and a negative rat liver USD assay (Ward, 1994) administering copper sulphate pentahydrate by gavage are presented. In addition, three studies administering copper sulphate pentahydrate by intra-peritoneal (IP) injection to mice are included. Two bone marrow chromosome aberration assays were concluded as positive as well as a sperm abnormality assay and one out of two micronucleus assays (Bhunya & Pati, 1987; Agarwal et al., 1990; Tinwell & Ashby, 1990). Mice also scored positive for bone marrow chromosome aberrations following oral and subcutaneous administration of copper sulphate pentahydrate (Bhunya & Pati, 1987). Considering that the IP route bypasses the normal processing of copper in the body, that there were conflicting results for two IP micronucleus assays, and that two reliable studies via the oral route (where uptake is controlled by homeostatic mechanisms) were negative, the dossier submitter concluded that the available data do not support classification for germ cell mutagenicity for copper compounds, including granulated copper.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that no data on granulated copper are available. The CLH report contains data on other copper compounds (predominantly copper sulphate pentahydrate), from which the dossier submitter proposed to read-across to granulated copper. In view of the considerations presented in the section "RAC general comment", RAC has not pursued the aspect of grouping any further. RAC concludes that **in the absence of relevant data no proposal for classification for germ cell mutagenicity can be made for granulated copper**.

4.9 Carcinogenicity

Table 35: Summary table of relevant carcinogenicity studies

Method	Results	Remarks	Reference
Rat Sprague-Dawley Daily in diet Sodium copper chlorophyllin 20/sex/group exposed for 104 weeks 2/sex/group exposed for 10 weeks and 3/sex/group exposed for 52 weeks 0.1, 1 or 3% (=2.7, 27 or 80 mg Cu/kg bw/day)	3% 22% Survival *vs* 30% in control. Plasma copper level slightly elevated (303 µg/100ml *vs* 180µg/100mL in control). There were no indication of increased tumour incidence at 104 weeks	Guideline not stated No GLP Deviations: Number of animals too small to concluded on a carcinogenic potential. Numbers of organs were not examined.	Harrisson, J.W.E., Levin, S.E., Trabin, B. (1954)
Rat Sprague-Dawley Daily in diet Copper sulphate 25/sex/group for 44 weeks 530, or 1600 ppm (=27 or 80 mg Cu/kg bw/day)	1600 ppm Marked reduction in bw in comparison to control. ↓food efficiency. Moderate ↑ in blood urea nitrogen. ↑ liver, kidney, stomach weight. Icteric pigmentation and abnormal cytoplasmic staining properties of liver. ≥ 530 ppm Marked accumulation of copper levels in liver and kidneys.	Guideline not stated No GLP Deviations: No report but a published paper. Number of animals too small to concluded on a carcinogenic potential. Numbers of organs were not examined. The study duration is short: 44 weeks.	Harrisson, J.W.E., Levin, S.E., Trabin, B. (1954)
Rat Sprague-Dawley Daily in diet Copper gluconate 25/sex/group for 44 weeks 1600 ppm (=80 mg Cu/kg bw/day)	1600 ppm 90% of the animals died between the fourth and eight month. Marked reduction in bw in comparison to control. ↑ in blood urea nitrogen. Marked accumulation of copper levels in liver and kidneys. ↑ liver, kidney, stomach weight. Icteric pigmentation and abnormal cytoplasmic staining properties of liver.	Guideline not stated No GLP Deviations: No report but a published paper. Number of animals too small to concluded on a carcinogenic potential. Numbers of organs were not examined. The study duration is short 44 weeks.	Harrisson, J.W.E., Levin, S.E., Trabin, B. (1954)

Rats 4 male weanling /groups For 1, 2, 3, 6, 9, and 15 weeks In diet 2000 ppm copper (equivalent to approximately 200 mg/kg bw/day)	No deaths. ↓body weight gain.↓ liver weight. Copper content in both liver and kidney rose to maximum values in week 6, after which levels fell. Dietary administration of copper as sulphate at 2000 ppm was associated with histological changes to the liver and kidney, reaching a maximum after six weeks of treatment, followed by recovery to week 15. Initially copper accumulated with little effect, but from 2-3 weeks, histological changes were evident in both tissues. Accumulation eventually caused a crisis, associated with severe necrosis, followed by regeneration and recovery.	No guideline study No GLP Deviations: too short to be used for carcinogenicity assessment.	Haywood (1980)
Rats Wistar Male weanling Copper sulphate 4/groups for 1, 2, 3, 4, 5, 6 and 15 weeks In diet 0, 3000, 4000, 5000 or 6000 ppm approximately equivalent to 150, 200, 250, 300 mg/kg bw/day	<u>3000 ppm</u> ↓body weight gain. Liver copper concentration rose rapidly between 4 and 5 weeks but fell significantly at week 6. By week 15, copper content had fallen to the same level as at week 2. Renal copper concentration rose more slowly than in the liver, with a maximum between 4 and 5 weeks. This concentration declined very slightly to week 15. Liver and kidney damage between 2 and 5 weeks, subsequent full recovery. Renal histopathology at 3000 ppm was similar to that seen at 2000 ppm in the earlier study. <u>4000 and 5000 ppm</u> Clinical deterioration between 3 and 4 weeks and subsequent recovery. Liver and kidney damage between 2 and 5 weeks, with subsequent full recovery. The findings showed earlier onset and were correlated with the earlier liver findings. Findings were more marked. <u>6000 ppm</u> No weight gain. Two animals died in week 2. At week 6 remaining animals showed weight loss and deteriorating condition and were sacrificed. Maximum liver concentrations at week 2 and fell only by week 6.	No guideline study No GLP Deviations: too short to be used for carcinogenicity assessment.	Haywood, S., (1985)

	In the kidney, copper concentration rise until week 4, when it equalled the liver value. Necrotic liver changes evident in the first week, increased in severity to weeks 2-3, and resulted in chronic hepatitis at 6 weeks.		
Rat Male weanling Sequential kills 15, 20, 29 and 52 we Diet Copper sulphate 3000ppm for 52 weeks (250 mg Cu/kg bw/d) 3000 ppm for 15 weeks followed by 6000 ppm for 3 weeks	Animals treated with copper at 3000 ppm for one year showed no long-term evidence of liver toxicity: an adaptive response was shown similar to the earlier shorter study, and at 52 weeks, copper concentrations were lower than at 15 weeks. Animals previously treated with copper at 3000 ppm for 15 weeks that were then given 6000 ppm (double the dose) for three weeks did not show altered liver copper concentrations, whereas previously untreated rats of the same age and strain given 6000 ppm copper showed moderate to severe hepatocellular necrosis.	No guideline study No GLP Deviations: Yes This study can not be considered as a key study, as it only focus on growth rate and liver copper content. The longest of the 3 experiments(52 weeks) does not allow the assessment of the carcinogenic potential of copper.	Haywood, S., Loughran, M. (1985)
Carcinogen co-administration Rats 5/sex/groups Exposed for 16 or 19 months In diet or in finely ground maize *Liver carcinogen: p*-dimethylaminobenzene (DMAB) at 0.9% w/w Copper acetate and/or ferric citrate were also added at 0.5% and 2.0% respectively to some groups	Copper, when added to rat diets containing the known carcinogen *p*-dimethylaminobenzene significantly reduced the incidence of liver tumours, and delayed the onset of histological changes leading to cirrhosis and hyperplasia.	No guideline study No GLP The design of the study did not permit assessment of tumour incidence of copper administered alone. However, if copper were to have any carcinogenic action either alone, or as a co-carcinogen, this type of study would certainly have shown an increased incidence of tumours, and an earlier onset.	Howell, J.S. (1958)
Investigation of the effects of oral CuSO4 on the incidence of 7,12-dimethylbenz(α)anthracene (DMBA) induced ovarian tumours, tumours of the breast and lymphomas in C57BL/6J mice Mouse C57BL/6J Female 10-12 animals/group Copper sulphate pentahydrate	The incidences of ovarian tumours after 46 weeks were 0/10, 0/12, 11/11 and 6/11 in the untreated controls, copper treated mice, DMBA-treated mice and DMBA-copper-treated mice respectively. This suggests that copper sulphate may possibly inhibit DMBA-induced tumour development. CuSO4 had no effect on the incidence of DMBA-induced adenomas of the lung, lymphomas and breast tumours.	No guideline study No GLP Purity not stated	Burki, H.R. and Okita, G.T. (1969)

46 weeks Oral drinking water 198g/L			
Rat Sprague Dawley 50-58 animals/male/group 9 months Oral diet Copper sulphate The excess Cu diet contained 800 ppm Cu as $CuSO_4$	Liver necrosis (3/32) and transitional nodules in the liver (1/32) was observed at 40 mgCu/kg/bw/day whereas one kidney tumour (1/42) was observed in the low Copper group (not thought significant). Decreased body weight gain and increased mortality were found in the high copper group. Exposure to known carcinogens increased the incidence of liver necrosis and transitional nodules and each induced a similar incidence of liver tumours in rats fed excess copper or copper-deficient diets. In the DMN group, 17/30 rats on the copper-deficient diet and kidney tumours compared to 0/29 given excess copper. The incidence of AAF-induced extrahepatic neoplasms was apparently reduced by the excess copper diet. (5/30 vs 11/27).	No guideline study No GLP Purity not stated	Carlton, W.W. and Price, P.S. (1973)

4.9.1 Non-human information

4.9.1.1 Carcinogenicity: oral

Reference: Harisson (1954)

Guideline: No

GLP: No (Prior to GLP).

Deviations:

- This is not a report but a published paper in *J. American Pharm ASS.*,
- number of animals too small, with several interim sacrifices, all being not due to bad conditions,
- due to the small number of rats per group it is impossible to make any conclusion on a carcinogenic potential,
- number of organs were not examined**,**
- adrenals were not weighed at necropsy, clinical chemistry parameters not performed..

Potassium sodium copper chlorophyllin **(104 weeks, but interim sacrifices, see below).**

Twenty males and 20 females Sprague-Dawley rats were dosed with 0, 0.1, 1, and 3 % of potassium sodium copper chlorophyllin. in the diet (equivalent of 53, 530 and 1600 ppm copper in the diet or equivalent of 2.7, 27 and 80 mg Cu/kg b.w./day). The animals were observed at least three times a week for mortality and clinical observations. Body weights, food and water consumption were measured weekly.
During the course of the study 5 males and 5 females from each group were paired for mating for a period of one week.
The females were allowed to litter and rear pups to maturity. Numbers of pups born and the number raised to maturity were counted.
Haematology and urinalysis were performed at regular intervals throughout the study.
Necropsies were performed after 10 weeks (2 animals per sex per group), 52 weeks (3 animals per sex per group)and 104 weeks (up to 10 animals per sex per group) and organ weights (heart, lungs, liver, spleen, kidneys, stomach, brain, uterus, ovaries, seminal vesicles testes) were determined. Samples of liver, kidneys and spleen were examined for copper and iron content from animals killed after 10, 52 and 104 weeks.
Histopathology was performed on all animals from the 52-week kill and at terminal sacrifice. Plasma and faecal samples were taken after 62 days and analysed for copper and 'chlorophyllin' content.

Mortality: Control group 30 %, group 0,1 % in diet 18 %, group 3 % in diet 22 %. There is no indication, in the published paper, for the 1 % in diet group.

Bodyweight: At 3% (80 mg/kg), there is a slight decrease in comparison to controls but there were no significant differences in body weights and body weight gains in the chlorophyllin treated animals compared with the controls over the 104 weeks of the study.

Food consumption and food efficiency were similar for all groups.

Mating: Not all females were pregnant, although the period allowed for mating was only 1 week. Mean numbers of pups born were 7.2 for controls and 6.5 to 9 for the treated groups. The number of pups raised to maturity was 5.2 for the controls and 4.5 to 6.2 for the treated groups. There were no differences that could be attributed to treatment. The report does not state the duration of pre-mating treatment.

Haematology and urine examinations: There were no differences in any of the parameters measured including the oxygen carrying capacity of haemoglobin.

Plasma chlorophyllin and plasma copper:

Table 36: Plasma chlorophyllin and copper

Diet	Chlorophyllin	Copper
	µg/mL	µg/100 mL
Control	None	189
2.7 mg/kg b.w	None	174
27 mg/kg b.w	58	196
80 mg/kg b.w	116	303

Plasma copper levels were slightly elevated in the high dose group.

Tissue stored copper: The high dose animals had a slightly higher liver copper concentration (not significant) after two years treatment compared with the controls. Kidney and spleen copper contents of the chlorophyllin treated animals were similar to the controls.

Necropsy, organ weights and histopathology: Organ weight analysis and necropsy findings at the interim and final kills were not adversely affected by treatment.

Findings at terminal kill included ventricular oedema, areas of pulmonary consolidation, occasional liver tumour and occasional cystic areas, retention cysts and minor congestion of the kidneys, pituitary tumours, hyperplasia of the lymphoid tissue of the small intestine and occasional atrophy of the reproductive organs. The study authors reported that these findings were distributed among control and test groups and were consistent with the age and strain of animals. No detail on the incidence of these tumours was available.

There were no significant differences in organ weight ratios of the chlorophyllin treated animals.

At 1600 ppm, the kidneys, liver, stomach, small intestine and spleen of animals sacrificed after 52 weeks, showed only tinctorial changes with no cell injury. All sections of control and test animals showed interstitial scarring, tubular atrophy, and dilatated tubules filled with hyaline material and minor inflammatory changes in kidney, at termination. Apart from minor adrenal cortical changes of a cystic and old hemorrhagic nature in the cortex of 2 high level animals and a small adenoma at the same dose there were no adverse effects at histopathological examination of the chlorophyllin treated animals.

There was no observation of increased tumour incidence in rats at 104 weeks.

Copper administered to rats as potassium sodium copper chlorophyllin showed moderate adverse effects following prolonged (104 weeks) dietary administration at 1600 ppm (*ca* 80 mg Cu/kg bw/day). NOAEL = 27 mg Cu/kg bw/day.

- **Copper sulphate (42 weeks)**

Twenty-five males and 25 females Sprague-Dawley rats were fed diets containing copper sulphate, equivalent in copper content to the copper in the 3 and 1% potassium sodium copper chlorophyllin diets, i.e. 1,600 ppm and 530 ppm (equivalent of 80 and 27 mg Cu/kg b.w./day), respectively for up to 44 weeks A third control group received the basal diet only. Similar data were collected throughout this study as in the study with potassium sodium copper chlorophyllin.

An interim sacrifice was carried out at 33 weeks in which 4 animals from the control group and 4 animals from the group fed 1600 ppm Cu were sacrificed. The balance of the animals was continued in the study, and all surviving animals of all groups were sacrificed at 40 – 44 weeks.

Mortality: A higher proportion of the high dose sulphate treated animals died compared to controls.

Bodyweight: The growth of animals on the high level of $CuSO_4$ was adversely affected by treatment. This was readily discernible at the 26th week, when male control animals and animals receiving 530 ppm Cu weighed at least 50% more than animals on the 1600 ppm Cu intake. Animals of both sexes receiving 530 ppm copper as sulphate showed body weights that were essentially similar to controls.

Food consumption and food efficiency:. Although the intake of food was less during the first twelve weeks, the gain in weight per gram of food consumed was similar for all groups

Blood and urine examinations: Blood nonprotein nitrogen levels were high in the high dose (83 mg% with expected range = 60-70 mg%).

Tissue-stored copper: The liver copper levels were several times higher than the controls or the chlorophyllin treated animals and were produced in relatively shorter time. In the high dose sulphate treated animals showed higher levels in kidney and spleen than the chlorophyllin treated animals.

Necropsy, organ weights and histopathology: Treated animals (killed in Weeks 33 and 42) findings in the high dose groups included bronzed kidneys exhibiting sharp demarcation between the cortex and the medulla; bronzed or yellowish livers; hypertrophied ridges between the cardiac and peptic portions of the stomach, occasional ulcers and some blood; bloody mucous in the intestinal tract.

Some slight differences in the organ weight ratios in the treated animals were probably related to the lower body weights of the treated animals rather than a direct result of treatment. Stomach weight ratios of the high dose female animals were increased compared with controls.
Other organs examined were heart, lungs, liver, spleen, kidneys, uterus, ovaries, seminal vesicles, testes and brain. There were increase of liver, kidneys and stomach weights at 1600 ppm.

Histopathology was performed on the organs of animals in the 1600 ppm group (sacrificed at 30 to 35 weeks), and also on the liver, kidney and testes of animals in the 530 ppm group (sacrificed at 40 to 44 weeks). The following organs were normal in all animals: Spleen; adrenals; small intestine; large intestine; stomach; sciatic nerve. The livers of animals in the 1600 ppm group showed well-defined abnormalities of a toxic nature in both males and females; icteric pigmentation was increased and cytoplasmic staining properties were abnormal. The kidneys of animals in the 1600 ppm group showed minor changes. Varying degrees of testicular degeneration were noted in both treatment groups; the ovaries of the females were not noticeably affected to any degree. The kidneys, liver and testes of all the control animals were found to be normal. No microscopic evidence of neoplasms was reported.

Copper administered to rats as sulphate showed adverse effects following prolonged (but limited to 44 weeks) dietary administration at 1600 ppm and a far less extent at 530 ppm (equivalent to approximately 27 mg Cu/kg bw/day). The NOAEL is ≤ 27 mg Cu/kg bw/day).

- ***Copper gluconate*** **(42 weeks)**

Guideline: No
GLP: No
Deviation: Yes

- Number of animals too small (25 males and 25 females per group). Only one or two group(s) of treated animals,
- the study duration is short 44 weeks. Due to the short duration it is impossible to make any conclusion on a carcinogenic potential,
- number of organs not convenient.

Twenty-five males and 25 females were fed diet containing copper gluconate with a copper equivalent to 1,600 ppm or 80 mg Cu/kg b.w./day up to 44 weeks.

Mortality: Ninety percent died between the fourth and eight month

Bodyweight: There was a very marked reduction of bodyweight gains, from week 8 in males and week 26 in females.

Food consumption and food efficiency: Slight variations were observed, although the intake of food was less during the first twelve weeks, the gain in weight per gram of food consumed was similar for all groups.

Blood and urine examinations: Blood nonprotein nitrogen levels were high in the high dose (109 mg% with expected range = 60-70 mg%).

Copper content of tissues: The liver copper levels were several times higher than the controls or the chlorophyllin treated animals and were produced in relatively shorter time. The very high levels seen in the gluconate treated animals correlated with the high death rate and the high blood non-protein nitrogen in these animals. In the high dose gluconate treated animals showed higher levels in kidney and spleen than the chlorophyllin treated animals.

Necropsy, organ weights and histopathology: Treated animals findings included bronzed kidneys and livers, hypertrophied limiting ridges in the stomach with occasional ulcers and bloody mucous in the intestinal tract. The stomachs of some animals were sometimes flabby and distended.

Some slight differences in the organ weight ratios in the gluconate treated animals were probably related to the lower body weights of the treated animals rather than a direct result of treatment. Stomach weight ratios of the high dose gluconate animals were increased compared with controls. The uterus and ovary weight ratios were reduced in the gluconate treated females, and mean testis weight was slightly reduced in the gluconate treated males at 42 weeks.
There were minor histopathological changes, but not consistent, in the kidney sections of the high dose animals. Icteric pigmentation was increased in the liver with abnormal cytoplasmic staining properties.
There were no observations of increased tumour incidence

Copper administered to rats as gluconate showed marked adverse effects following prolonged (but limited to 44 weeks) dietary administration at 1600 ppm (equivalent to approximately 27 mg Cu/kg bw/day).NOAEL is < 1600 ppm or < 80 mg Cu/kg bw/day.

Table 37: Copper content of tissues (mg Cu/100 g tissue)

Week	**Dose level (%) potassium sodium copper chlorophyllin**			
	0	**0.1**	**1**	**3**
Liver – Males				
10	0.41	0.47	0.58	0.56
52	0.78	1.46	0.81	1.06
104	1.82	1.47	1.85	2.18
Liver – Females				
10	0.48	0.57	0.74	0.56
52	1.09	1.14	2.43	2.14
104	1.10	1.85	2.02	3.71
Kidney – Males				
10	1.07	1.47	1.58	1.48
52	2.08	1.52	1.83	2.11
104	3.45	2.03	2.35	2.48
Kidney – Females				
10	1.72	1.52	1.57	1.65
52	4.46	2.44	3.79	2.97
104	2.25	2.55	3.19	3.22

Spleen – Males				
10	0.96	0.52	0.40	0.68
52	1.83	2.92	3.05	2.36
104	3.38	3.34	2.75	3.01
Spleen – Females				
10	1.59	0.46	0.72	0.52
52	4.00	3.26	3.46	3.61
104	6.96	1.92	2.34	2.96
	Dose level (ppm) copper sulphate and copper gluconate			
	0	**530 sulphate**	**1600 sulphate**	**1600 gluconate**
Liver – Males				
Term	1.16	12.47	38.28	75.1
Liver – Females				
Term	1.78	32.36	45.77	56.6
Kidney – Males				
Term	2.48	3.49	15.83	59.57
Kidney – Females				
Term	3.53	6.91	12.11	54.1
Spleen – Males				
Term	3.34	5.63	13.91	12.39
Spleen – Females				
Term	4.83	5.12	6.07	13.77

The two studies of Haywood 1980 and 1985 are summarized in the repeated toxicity part.

Reference: Haywood, S., and Loughran M. (1985)

Guideline: No

GLP: No

Male weanling Wistar rats were given 3000 ppm copper as copper sulphate via the diet for one year (equivalent for 250 mg Cu/kg b.w/day). At 15, 20, 29 and 52 weeks, groups of three or four rats were weighted, killed and the livers examined. In a second experiment, sixteen male weanling Wistar rats were fed diet containing 3000 ppm copper as copper sulphate for 15 weeks. At 15 weeks, four rats were killed and the livers examined as before. The remaining animals were given a diet containing 6000 ppm copper as copper sulphate (equivalent for 500 mg Cu/kg b.w/day) for a further three weeks at which time they were also killed and the livers examined. A further 16 rats were given control diet for 15 weeks, four were killed and the livers examined, and the remaining rats were also given the diet containing 6000 ppm copper as copper sulphate. At 18 weeks the animals were killed and the livers examined.

There were no deaths reported.

In the first experiment, at 52 weeks, the control group mean body weight was 513 g, and the group mean body weight of rats receiving 3000 ppm was 433 g. Mean liver copper concentration in the treated animals was 1303 μg/g at 15 weeks and fell to 440 μg/g at 52 weeks.

In the second experiment, the change of diet at 15 weeks to 6000 ppm did not affect the condition of the 'primed' rats previously fed copper at 3000 ppm, but the unprimed group were lethargic with ruffled coats. Liver copper content of the 'primed' group did not alter significantly (1395 μg/g compared to 1342 μg/g at week 18) at the change of diet, but liver copper content of the unprimed group rose from 18.0 μg/g at week 15 to 1835 μg/g at week 18 – higher than the primed animals.

Histologically, at 15 weeks, animals that had received 3000 ppm showed complete lobular recovery with only some fine residual scarring and some hyalinised cells in the portal areas, in line with recovery seen in earlier studies. There were no further changes in primed animals receiving 6000 ppm for the additional three weeks. In the animals with no previous copper supplementation, there was moderate to severe hepatocellular necrosis with an associated inflammatory response after 3 weeks administration of diet containing 6000 ppm.

Animals treated with copper at 3000 ppm for one year showed no long-term evidence of liver toxicity: an adaptive response was shown similar to the earlier shorter study, and at 52 weeks, copper concentrations were lower than at 15 weeks. Animals previously treated with copper at 3000 ppm for 15 weeks that were then given 6000 ppm (double the dose) for three weeks did not show altered liver copper concentrations, whereas previously untreated rats of the same age and strain given 6000 ppm copper showed moderate to severe hepatocellular necrosis.

No information on tumors development was available in this study.

The following studies did not permit assessment of tumour incidence of copper administered alone but showed a beneficial effect of copper when administered together with known carcinogens. In this context, theses studies must be only be considered illustrative. However, if copper were to have any carcinogenic action either alone, or as a co-carcinogen, this type of study would certainly have shown an increased incidence of tumours, and an earlier onset. It did neither.

Reference: Howell, J.S. (1958)

Guideline: No

GLP: No

During experiment A, groups of 5 male and 5 female rats received the known carcinogen *p*-dimethylaminoazobenzene in either standard laboratory diets or maize supplemented with ferric acid and copper acetate for their whole lifespan. Liver biopsies were performed regularly. Experiment B was performed to confirm the inhibitory effect of copper acetate. Groups of 5 male and 5 female rats received dimethylaminobenzebe (DMAB) in maize with or without ferric acid at 2% or copper acetate at 0.5%. In addition, groups with alternating feeding were included to reduce the likelihood of copper acetate interfering with DMAB absorption in the gut. The animals were sacrificed when palpable liver tumours were observed. Spleen weights were determined and histopathology of liver and spleen was conducted.

Copper, when added to rat diets containing the known carcinogen p-dimethylaminobenzene significantly reduced the incidence of liver tumours, and delayed the onset of histological changes leading to cirrhosis and hyperplasia.

It was concluded that copper has a beneficial effect in reducing the action of the carcinogen. The study indicates that copper has no carcinogenic potential when administered in the diet.

Reference: Carlton, W.W. and Price, P.S., (1973)

Guideline: No

GLP: No

Deviations: Yes

A study was carried out to determine whether a high level of Cu would have an inhibitory effect on the induction of neoplasia by acetylaminofluorene (AAF) or dimethylnitrosamine (DMN) and to determine

whether the incidence of neoplasia would be increased, or whether neoplasms would appear earlier in rats fed a diet low in Cu.

Six experimental groups of Sprague-Dawley rats were included in this study. Three groups were fed a basal diet containing 1 ppm Cu ("Cu-deficient diet") (equivalent for 0.05 mg Cu/kg bw/d) and a further 3 groups received the basal diet supplemented with $CuSO_4$ to give a Cu concentration of 800 ppm ("excess-Cu diet") (equivalent for 40 mg Cu/kg bw/d). Within each of these two dietary regimens, one group received DMN in the drinking water and the other received AAF in the diet. Groups without these carcinogens served as controls. The initial number of animals used in each group was as follows: Cu-deficient control, 50 rats; Cu-deficient-DMN, 74 rats; Cu-deficient-AAF, 55 rats; excess-Cu-control, 58 rats; excess-Cu-DMN, 102 rats; excess-Cu-AAF, 65 rats. The numbers in each group varied because preliminary studies showed that higher DMN concentrations were toxic.

DMN was added to the drinking water for 6 months at a concentration of 50 ppm for 4 days out of every 8. Similarly, AAF was added to the diets for 6 months at a concentration of 0.06% for 4 days out of every 8.

After 90 days, 5 rats from each diet group were killed. Each 30 days thereafter, an additional 5 animals from each group were killed. Spleen, kidneys, lungs, heart, thyroid gland, adrenals, duodenum and pancreas were taken from each animal and fixed in 10% formalin. The liver was divided into 2 portions; one of which was retained for analysis of Cu content; the other was fixed in formalin. Liver and enlarged neoplastic kidneys were weighed prior to fixation. Fixed tissues were processed, sectioned and stained with H&E for histological examination.

Liver and kidney Cu concentrations were determined by atomic absorption spectrophotometry. The analyses were run in triplicate and precautions were taken to prevent Cu-contamination of the tissues.

Rats fed the Cu-deficient control diet consistently had the highest mean bodyweights. Mean weights of other groups decreased in the following sequence: Cu-deficient-DMN; excess-Cu control and excess-Cu DMN had similar mean weights; Cu-deficient AAF; excess-Cu-AAF. AAF was considered to be markedly toxic.

After 3 months, mortality in the 6 groups was as follows:

Table 38: Mortality after 3 months and at termination

	Mortality after 3 months	Study termination
Cu-deficient control	2%	16% (minimum)
Cu-deficient-DMN	38%	57% (maximum)
Cu-deficient-AAF	15%	-
Excess-Cu control	33%	45%
Excess-Cu -DMN	69%	-
Excess-Cu-AAF	39%	54%

Macroscopic investigations showed that:

- Livers from control rats fed both Cu-deficient and excess-Cu diets were grossly normal.

The incidence of hepatic neoplasms in DMN-treated rats was similar for the Cu-deficient and excess-Cu groups. Livers of rats fed the Cu-deficient-DMN diet for 3 or 4 months varied in appearance from those that were grossly normal to those with severe macroscopic changes. Some were tan-coloured and slightly swollen. Features of livers from rats fed this diet for 5-8 months included: swelling, colour variation, presence of clear cysts, haematocysts and/or neoplasms. Livers from excess-Cu-DMN rats were either normal or slightly off-colour after 3 and 4 months. Few further changes were observed after 5 and 6 months, except for prominent capsular vessels. Cysts, swollen lobes and haematocysts occurred in livers of rats fed for 7 months. Livers from 4 rats killed after 8 months were more severely affected; haematocysts were observed in 2 livers and a neoplasm in one other.

Gross hepatic lesions were observed at monthly samplings in Cu-deficient-AAF rats. At 3 months, these included discoloration, enlargement and presence of focal pale areas. After 4 months, a few clear cysts were also present. Later, livers were pale, cystic and enlarged. Neoplasms of varying size were found in all lobes.

At 3 months, the surface of the liver of one rat fed the excess-Cu-AAF diet was converted into a mass of nodules. This was also seen in one or more livers at the other autopsy periods, and was more marked on the visceral surface. Clear cysts were also present peripherally after 5 months. Increased hepatic size, cysts and small white foci appeared after 6 months. Neoplasms were larger after 7 months, and all livers at 8 months had clear cysts, neoplasms and capsular nodularity.

The numbers of hepatic neoplasms in AAF-treated rats on the Cu-deficient and excess-Cu diets were similar and it appeared that the concentration of Cu had no effect upon the incidence of hepatic neoplasms. However, the latency period may have been slightly increased, as hepatocellular carcinomas and metastases occurred 1 month later in the excess-Cu group.

- Kidneys grossly enlarged with neoplasms were seen after 5 months in Cu-deficient-DMN rats.

The kidneys of 4/5 rats had neoplasms of various sizes. After 6 months, neoplasms were present in all 5 rats. Grossly apparent neoplasms were present in 3/5 rats examined after 7 months. Only one renal neoplasm was obvious at autopsy in 5 rats killed after 8 months. 3/13 rats on this treatment which died during the study had grossly apparent renal neoplasms.

- Abnormalities observed at autopsy in Cu-deficient-DMN rats included pale, expanding masses in the lungs of 2 rats.

Grossly detectable neoplasms were observed in the lungs of excess-Cu-DMN rats after 7 and 8 months.

Neoplasms at locations other than the liver were most numerous in Cu-deficient-AAF rats. After 5 months, 3 rats had grossly obvious neoplasms in one or more of the following locations: ventral throat area, middle of side, groin area and base of ear. After 6 months, neoplasms were noted in the lungs of 2 rats and in the spleen of another. At month 7, neoplasms were present in the ventral thorax, spleen, abdomen, perianal region, base of ear, right rear leg and small intestine.

Fewer extrahepatic neoplasms were found in excess-Cu-AAF rats (17% compared with 40% in the excess-Cu-AAF). Those that occurred werelocated at the base of the ear, along the lateral abdomen and in the lungs. It was considered that the Cu supplement acted to reduce the number of extrahepatic neoplasms.

No gross abnormalities were observed in the urinary bladder of animals in any group.

Histopathology showed that:

- Commonly occurring non-neoplastic lesions in the livers of carcinogen-treated rats included biliary-ductule cell hyperplasia, proliferation of biliary ducts and the presence of haematocysts.

Transitional nodules were localized groups of hepatocytes showing only minimal deviation of nuclear morphology and no compression of the surrounding parenchyma. Hepatomas were larger foci of hepatocytes showing changes in nuclear morphology and causing compression of the surrounding parenchyma. Hepatocellular carcinomas were large, highly cellular neoplasms showing marked alterations in nuclear and cytoplasmic morphology, containing areas of necrosis and blood cysts and invading blood and lymph vessels. In addition to hepatomas and hepatocellular carcinomas, a fibrosarcoma and cholangiocarcinoma were observed in Cu-deficient-DMN rats. Hepatomas, hepatocellular carcinomas, cholangiomas and one cholangiocarcinoma were observed in livers of Cu-deficient-AAF rats. The Cu level of the diet appeared to have no effect on the incidence rate of hepatic neoplasms.

- Fibrosarcomas, adenomas and adenocarcinomas were seen in kidneys of Cu-deficient-DMN rats.

One fibrosarcoma was found in a kidney from a rat fed the Cu-deficient control diet. No renal neoplasms were observed either grossly or microscopically in the rats from other groups killed for autopsy. One renal adenoma was observed in a rat that died after 7 months on the excess-Cu-DMN treatment.

- Neoplasms in locations other than liver and kidneys included those of the lung, spleen, skin and - intestine.

The neoplasms observed included adnexal gland adenocarcinomas, keratoacanthomas, splenic lymphoma, alveolar-cell adenomas and adenocarcinomas, adenocarcinoma arising from the epithelium of the intestinal mucosa, squamous cell carcinomas of the skin and lungs, fibrosarcoma of the dermis and a rhabdomyosarcoma. The incidences of these neoplasms were less in rats receiving excess Cu and a carcinogen.

Table 39: Incidence of hepatic lesions and neoplasms in rats fed copper-deficient and excess-copper diets with DMN or AAF treatment and killed at monthly intervals for autopsy.

		Incidence (%)* of						
Experimental group	**Total no. of rats killed**	**Liver necrosis**	**Transitional nodule**	**Hepatomas**	**Hepatocellular Carcinomas**	**Metastase**	**Kidney neoplasm**	**Other neoplasm**
Copper deficient:								
Control	42	0.0	0.0	0.0	0.0	0.0	2.4	0.0
+ DMN	30	30.8	76.7	23.3	10.0	0.0	56.7	30.0
+ AAF	27	22.2	100.0	92.6	40.7	3.7	0.0	40.0
Excess-copper diet:								
Control	32	9.4	3.1	0.0	0.0	0.0	0.0	0.0
+ DMN	29	55.2	82.8	27.6	13.8	0.0	0.0	24.1
+ AAF	30	30.0	100.0	90.0	30.0	10.0	0.0	16.7

DMN – 50 ppm dimethylnitrosamine in drinking-water for 4-day periods alternating with distilled water.
AAF – 0.06% acetylaminofluorene in the diet for 4-day periods alternating with control diet.
* Percentage of rats affected

To conclude

Liver: livers from excess-Cu control rats confirmed the occurrence of liver necrosis and transitional nodules in 3/32 and 1/32 animals, respectively. Neither of these lesions was found in the livers of animals fed a Cu-deficient diet. Exposure to DMN and AAF increased the incidence of liver necrosis and transitional nodules, and each induced a similar incidence of liver tumours in rats fed both the Cu-deficient and excess-Cu diets. It was concluded that the Cu level of the diet had no effect on the incidence of hepatic neoplasms.

Kidney: In the DMN group, 17/30 rats on the Cu-deficient diet had kidney tumours compared with 0/29 given excess Cu. There were no kidney tumours in the AAF-treated groups.

Other organs: The incidence of AAF-induced extra-hepatic tumours was apparently reduced by the excess-Cu diet (5/30, compared with 11/27 in the Cu-deficient group).

Reference: Burki, H.R. and Okita, G.T. (1969)

Guideline: No

GLP: No

A study was carried out to investigate the effects of oral CuSO4 on the incidence of 7,12-dimethylbenz(α)anthracene (DMBA)-induced ovarian tumours, tumours of the breast and lymphomas in C57BL/6J mice and of tumours of the lung in strain A mice. The study was divided into four separate experiments, designated A, B, C and D.
In all cases, CuSO4 was dissolved in drinking water at a concentration of 198 mg/l (equivalent to approximately 50 mg Cu^{2+}/l or 10 mg Cu/kg b.w/day). CuSO4-treated animals had access to the solution ad libitum over the entire experimental period.

Experiment A: CuSO4 was administered in the drinking water of 5 female mice (C57BL/6J) aged 4 – 6 months. Two weeks after commencement of copper treatment, the mice received an intravenous (i.v.) injection of 0.75 mg dimethylbenz(α)anthracene (DMBA), a known carcinogen. A second group of 5 mice received DMBA alone. Five untreated mice served as controls. The experiment was terminated 74 weeks after DMBA treatment.

Experiment B: CuSO4 was administered in the drinking water of 11 female mice (C57BL/6J) aged 12 – 15 weeks. After commencement of copper treatment, the mice received an i.v. injection of 0.75 mg DMBA.
A second group of 11 mice received DMBA alone. Ten untreated mice and 12 mice receiving CuSO4 served as controls. The experiment was terminated 44 weeks after DMBA treatment.

Experiment C: CuSO4 was administered in the drinking water of 9 female mice (strain A) aged 12 – 16 weeks. After commencement of the copper treatment, the mice received an i.v. injection of 0.75 mg DMBA and, 12 days later, an intraperitoneal (i.p.) injection of 0.5 mg DMBA. Ten other mice received 0.75 mg DMBA i.v., and 0.5 mg DMBA i.p only. Nineteen untreated mice and 12 mice receiving CuSO4 served as controls. The experiment was terminated 33 weeks after the first DMBA treatment.

Experiment D: CuSO4 was administered in the drinking water of eighteen pseudopregnant C57BL/6J female mice (i.e. virgins housed with vasectomised males), each of which also received 6 dermal applications of 0.5 ml of a 0.5% DMBA solution in olive oil at biweekly intervals. A separate group of 19 pseudopregnant females received dermal applications of DMBA, but did not receive CuSO4 in their drinking water. Eleven untreated mice and 17 pseudopregnant mice receiving CuSO4 served as controls. The experiment was terminated 50 weeks after the first DMBA treatment.

Animals in all experiments were observed daily. All mice found dead and those sacrificed were subject to post-mortem evaluation. Sections of the liver, lung, kidney, spleen, thymus, ovaries and all tumour-like structures were fixed in 10% formalin in phosphate buffer at pH 7.4. Specimens were embedded in wax, sectioned for light microscopy and stained by haematoxylin and eosin. Vaginal smears were also taken and stained with Wright's stain.

Experiments A and B: The incidences of ovarian tumours in Experiment A after 76 weeks were 0/5, 4/5, and 0/5 in the untreated controls, DMBA-treated mice and DMBA plus Cu-treated mice, respectively. The incidences of these tumours in Experiment B after 46 weeks were 0/10, 0/12, 11/11 and 6/11 in the untreated controls, copper-treated mice, DMBA-treated mice and DMBA/copper treated mice respectively. The results of these two experiments suggested that CuSO4 may inhibit DMBA-induced tumour development.
The incidences of lymphomas in Experiment A were 0/5, 1/5, and 5/5 in the untreated controls, DMBA-treated mice and DMBA plus Cu treated mice respectively. Although these results implied that incidence of lymphomas were greater in DMBA plus CuSO4-treated mice than in those receiving DMBA only, this finding could not be repeated in Experiment B (incidences of lymphoma 1/10, 2/12, 3/11 and 3/11 in the untreated controls, Cu-treated mice, DMBA-treated mice and DMBA plus Cu-treated mice, respectively). It was therefore concluded that CuSO4 had no effect on the induction of lymphomas by DMBA.

Experiment C: Tumour incidence in the 12 mice given CuSO4 alone (1 breast tumour, 2 lymphomas and no lung or ovarian tumours) was similar to that in the 19 untreated controls (2 lymphomas, no breast, lung or ovarian tumours). CuSO4 had no effect on the incidence of DMBA-induced lung adenomas (incidence 4/9 in DMBA plus Cu-treated mice and 4/10 in mice treated with DMBA only), although it appeared to prolong the survival of DMBA-treated mice (mean survival 28 weeks compared with 19 weeks in mice treated with DMBA only), and to slightly reduce the total number of tumours seen, as compared with mice given DMBA only.

Experiment D: No information was given on the tumour incidence in mice given CuSO4 alone. However, mice given DMBA plus CuSO4 had a greater number of mammary tumours (9 tumours amongst an original group of 18) than those given DMBA alone (5 tumours amongst an original group of 19). This increase was attributed to the greater longevity of Cu-treated mice.

No toxic effects were observed in otherwise untreated mice fed CuSO4 at the concentration used in these four experiments.

DMBA was injected or administered by skin paintings to C57BL/6J and to strain A female mice kept on a diet supplemented with CuSO4. It was found that CuSO4 had no effect on the incidence of DMBA-induced adenomas of the lung, lymphomas and breast tumours. CuSO4 did not prevent the induction of pre-cancerous lesions in the ovary, but may have delayed the development of granulosa cell tumours.

4.9.1.2 Carcinogenicity: inhalation

No data available.

4.9.1.3 Carcinogenicity: dermal

No data available.

4.9.2 Human information

In the VRA, a number of epidemiological studies have investigated the health hazards, including cancers (most frequently lung cancer) and non-malignant diseases, associated with occupational exposures in the copper mining, copper smelting and refining, and copper alloy industries. Most of these studies are confounded by numerous factors including co-occurring exposures to known carcinogenic compounds, such as arsenic; lack of consideration of individual exposures; failure to consider smoking status; and the use of biomarkers of copper status, such as serum copper levels, that are altered by the disease state. None of the available studies provide convincing evidence that copper plays an aetiological role in the development of cancer in humans.

Copper mining

Two cohort mortality studies of the same population of over 7000 copper miners in Tongling, China have addressed the risks associated with copper mining (Chen *et al,* 1993; 1995). In these studies, lung cancer mortality was found to be significantly increased, for underground miners and for drilling miners (both $p<0.01$). Cigarette smoking was a partial contributor to this excess mortality. Other cancers (oesophagus, stomach and liver) were also studied, but did not demonstrate a statistically significant increase. Although these were large, well-conducted epidemiological studies, the Chinese miners and smelters are subject to different genetic and environmental influences. Chinese workers are also likely to be subject to very different occupational exposures than European copper miners, such as those in Sweden. Although limited information regarding occupational exposures to dust and ionizing radiation was included in the two Chinese studies, exposure specifically to copper compounds was not measured. The results should therefore be extrapolated to European workers with caution. No well-designed epidemiological studies of European copper miners were available for review.

Copper smelting

The majority of the epidemiological studies have reported on large populations of copper smelter workers in the USA, at Anaconda in Washington State (Welch *et al*, 1982; Viren and Silvers, 1994), Tacoma in Montana (Enterline *el al,* 1995) and the Gila basin region of Arizona (Marsh *et al,* 1997; 1998). Additionally, the cancer risk of environmentally exposed residents in Arizona has been investigated by the latter authors. Other reports have described occupationally exposed populations in China (Chen *et al,* 1995), Japan, Sweden, (Welch *et al,* 1982; Viren and Silvers, 1994) and at a nickel copper smelter in Finland (Karjalainen *et al,* 1992).

Ten studies of copper smelters were identified which predominantly studied lung cancer mortality in populations of smelter workers, in most cases, focussing on the association with arsenic exposure. Potential involvement of copper in cancer mortality did not feature in any of these studies. Most of these studies demonstrated a statistically significant increase in lung cancer mortality. Of these, four out of five found a linear increase in the excess relative risk of respiratory cancer with increasing exposure to airborne arsenic

(Pinto *et al,* 1978; Welch *et al,* 1982; Viren and Silvers, 1994; Lubin *et al,* 2000; Enterline *et al,* 1995). Four of these five studies were from populations in the USA, the other study analysed two published cohort studies from the USA and Sweden. It is notable that none of the available studies present exposure data for copper or other pollutants (apart from nickel exposure at a nickel/copper smelter in the study by Karjainen *et al,* 1992).

Community-based studies have reported some positive evidence for the association between lung cancer risk and reported copper smelter related employment, however there was little evidence of a positive association between lung cancer mortality and residential exposure to smelter emissions (Marsh *et al,* 1997; 1998).

Several studies have also examined mortality from other cancers. Results reported show little concordance. Some studies demonstrated no statistically significant increased mortality for other cancers (Pinto *et al,* 1978), while others demonstrated statistically significant increases in mortality due to other causes (Chen *et al,* 1995; Welch *et al,* 1982; Lubin *et al,* 2000; Enterline *et al,* 1995). There is little consistency between studies with respect to sites of excess non-respiratory cancers; urinary tract cancer (Welch *et al,* 1982), cancer of the large intestine and bone cancer (Enterline *et al,* 1995).

Copper refining

A single study has been published on mortality among 4,802 workers in nine US copper and zinc refineries with the aim of determining whether any excess mortality was associated with specific refining operations (Logue *et al* 1982). As 74% of the study population were employed in copper refining only, causes of mortality were separately analysed for this group, involving 335 decedents. [In the study report, this group is misleadingly referred to as the "cohort exposed only to copper"]. In this cohort, statistically significant increases were demonstrated for all cancers (63 observed; 61.01 expected; SMR 128) and for cancer of the digestive tract (20 observed; 15.71 expected; SMR 157). The significant excess mortality due to all cancers, including respiratory cancers, among this cohort was largely attributable to one plant which unlike the other study plants had its refinery adjacent to a smelter. A number of workers at this refinery had transferred from the smelter. It is therefore possible that previous occupational exposure could have contributed to the excess cancer mortality. This study provides no qualitative or quantitative exposure data, or data on smoking history. Consequently, association between exposure and the excess cancer mortality reported cannot be explored.

Copper alloys

A single cohort study of mortality in 347 copper cadmium alloy workers has been reported, focussing on the relationship between cadmium oxide exposure and mortality from lung cancer and non-malignant diseasea of the respiratory system (Sorahan *et al* 1995). This study showed a statistically significant increased risk of mortality from chronic, non-malignant respiratory disease in workers exposed to cadmium oxide fume, but found no increase in risk for lung cancer.

Serum copper levels and cancer

Several studies have investigated the possible association between serum copper concentrations and cancer risk. However these investigations are complicated by the fact that alterations in serum copper concentration may be related to the disease-state. Therefore epidemiological studies investigating serum copper levels only following diagnosis of cancer provide little useful information regarding the possible causal role of copper in cancer (Cavallo *et al,* 1991, Dabek *et al,* 1992, Prasad *et al,* 1992).

In the few prospective studies where copper serum levels were measured prior to diagnosis, there is no convincing evidence that dietary intake or serum-copper levels play an aetiological role in carcinogenisis. For example, Coates *et al* (1989) investigated serum copper levels in a cohort diagnosed with a range of cancers up to 10 years prior to diagnosis with cancer. This study found that there was only a positive correlation between copper levels and cancer risk in cases diagnosed fewer than 2-4 years after blood draw. In cases diagnosed more than 2-4 years after collection of the blood sample there was no statistically significant relationship. Cancer is a complex multistage process generally regarded to take many years to develop clinical features. If elevated copper serum levels were truly a risk factor for cancer, an association between copper serum levels and subsequent disease would have been expected to be maintained in cases where blood samples where taken many years prior to diagnosis. A single cohort study of over 5000 healthy women from Guernsey,

studied between 1968 and 1975 investigated the influence of hormonal and other factors on breast cancer (Overvad *et al*, 1993). This study reported an association between raised serum copper levels and the risk of developing breast cancer. However, the authors concluded that elevated serum copper was probably disease mediated or an incidental association, rather than causal.

In summary, although serum copper appears to be elevated in some cancer patients and may be a potential marker of disease-state there is little or no convincing evidence that dietary copper plays an aetiological role in human cancer.

Genetic diseases in human:

Two rare genetic diseases of copper in the human provide evidence that copper is not carcinogenic following systemic absorption. These are Wilson's disease (WD) and Menkes' disease (MD). The following data were extracted from the Draft Assessment Report of copper compounds.

Wilson's disease is a defect in the ATPase for copper transport ATP7B (or WND), expressed mainly in the liver, resulting in faulty copper transport, impaired incorporation of copper into ceruloplasmin, impaired copper biliary excretion, and copper accumulation in the liver and brain. Hepatic copper levels range from 200 to 800 µg/g dry weight (normal range 20 to 50 µg/g), and patients present with hepatic cirrhosis and fatty infiltration of the liver. Urinary copper is much higher than normal (as in rats given sufficiently high oral doses to cause liver toxicity). Treatment is by chelation therapy using D-penicillamine, such that intestinal absorption is reduced, and chelated copper complexes are excreted in the urine, and liver and body levels are kept below levels at which liver disease occurs. Zinc therapy (orally as zinc sulphate) acts to induce excess metallothionein in the intestinal cells. Metallothionein has a stronger affinity for copper than zinc. The copper remains bound in the gut cells, which are then sloughed off, and the copper is lost. In the second or third decade of the disease, neurological symptoms can occur. Copper accumulation in the brain causes degeneration of the basal ganglia, resulting in defective movement, slurred speech, difficulty in swallowing, facial and other muscular spasms, dystonia and poor motor control. Depression and schizophrenia have been reported. Copper may also be deposited in the cornea (Kayser-Fleischer rings).

Menkes disease is an X-linked copper deficiency disease that is usually fatal in early childhood.
The symptoms result from a defect in the MNK protein, producing an inability to export copper from cells, particularly from the basal membrane of the small intestine, where copper is absorbed. This leads to very high concentrations of copper in sloughed intestinal cells, but the failure to export the "absorbed" copper to the bloodstream results in an effective copper deficiency for the rest of the body. The disease shows progressive mental retardation, hypothermia, seizures, poor muscle tone, feeding difficulties, jaundice, diarrhoea and a general failure to thrive. There are abnormalities of connective tissue with deformities of the skull, long bones and ribs. The hair is abnormal with a wiry texture and a spiral twist.

Both diseases result from genetic defects where the subject is unable to produce respectively the copper ATPases ATP7B and ATP7A. These are members of the human cation-transporting P-type ATPase family. The P-type ATPases are a large group of membrane proteins that utilise the energy of ATP hydrolysis to transport various ions across cell membranes. During the catalytic cycle the γ-phosphate of ATP is transferred to the invariant aspartic acid residue within the nucleotide-binding site of ATPase with the formation of acylphosphate intermediate: this property distinguishes the P-type ATPases from other cation-transporting pumps. Over 100 Ptype ATPases have been described. The loci of the encoding genes have been identified for both WD and MD. Both pump copper across cell membranes. The MD pump (ATP7A) is the pump that actually moves copper through the basal membrane of the intestinal epithelial cells so that copper enters the hepatic portal system where it binds to albumin, transcuprein and histidine to reach the liver. In the MD subject, ATP7A is inactive, and copper from the diet accumulates in the intestinal epithelial cells, bound to induce metallothionein. The presence of copper within the cell induces the production of more metallothionein, and the copper-metallothionein complex accumulates during the life of the cell. When the cells are sloughed off into the intestinal lumen, as is the normal course of events, the cells and the copper within them are excreted

in the faeces, and the copper is lost to the body. Subjects with Menkes' disease can still absorb small amounts of copper. Copper accumulates in fibroblasts and in the kidney of Menkes' disease subjects, but there is no evidence of increased incidence of cancer in these tissues either. Menkes' disease is effectively a disease of copper deficiency. In terms of risk assessment of copper in the normal human, the accumulation of copper in the intestinal epithelium on Menkes' subjects can be considered as the equivalent of an excessive oral dose of copper to the epithelial cells.

Carcinogens of the intestine may act by irritation or some other means to cause proliferation of the intestinal epithelium that eventually results in hyperplasia and tumour formation. MD subjects do not suffer from increased incidence of cancer of the intestine. This shows conclusively that excess copper in the intestinal cells does not cause cancer or long-term toxicity in that tissue. Wilson's disease (WD) involves the other ATPase previously referred to, ATP7B. In normal humans, this enzyme is primarily active in hepatocytes. It is involved in the trans-Golgi network (TGN). Copper absorbed by the hepatocyte via the inbound membrane pump hCTR1 (human copper transporter protein 1) and is bound to metallothionein within the cell. It may be bound by ATP7B to ceruloplasmin (a protein that binds up to 6 copper ions tightly and transports them to various tissues for use, including the brain. If there is excess copper in the hepatocyte, ATP7B is induced to traffic to vesicular compartments (lysosomes) and directly to the apical membrane, where copper is secreted from the cell bound to a trypsin-independent fragment of ceruloplasmin and excreted in the bile. In WD, ATP7B is inactive and the absorbed copper accumulates in the hepatocytes bound to metallothionein. The bile of WD subjects does not contain copper. In the hepatocyte, excess copper may accumulate in mitochondria, in the cytoplasm and in lysosomes, bound to metallothionein. Eventually the cell's copper storage capacity is exceeded.

Mitochondrial damage occurs and eventually the hepatocyte dies, whence the cell contents are released to the circulation, depositing copper in extrahepatic tissues. Wilson's disease thus leads to massive accumulation of copper in the liver. The disease usually manifests in late adolescence, and is ultimately fatal if not treated, but death is from liver failure, not from cancer. Treatment involves administration of penicillamine, which forms a copper complex capable of urinary excretion. There is no evidence of increased incidence of liver cancer in WD subjects. This shows that even massive accumulation of copper in the target organ, the liver, does not result in cancer in the human. Accumulation of copper leads to cell death, but this is only in the presence of excessive copper concentrations, brought about by a genetic condition resulting in the disruption of the natural homeostatic mechanisms for copper.

It should be noted that Wilson's disease is genetic, and the accumulation of copper and resulting liver failure occur under the natural levels of copper in the diet, not as a result of exposure to excessive levels of copper in the environment. However, the accumulation of copper in the liver may be taken as a model for accumulation of excess copper in a toxicity study, and the conclusion drawn that chronic high liver levels do not result in increased incidence of cancer.

Vineyard sprayer's lung: an occupational disease

Reference: Pimentel, J.C. and Marques, F. (1969)
Guideline: No
GCP: No.

Case reports of two male rural workers, whose main occupations were spraying vineyards using 'home-made' Bordeaux Mixture (solution of copper sulphate neutralised with hydrated lime) and/or cleaning the tartar from wine presses, admitted to the Thoracic Surgery Centre for investigation. In both cases tuberculosis had been diagnosed some months previously and had been treated. In one case there was improvement but not complete clearing and as his sputum was persistently negative for tubercle bacilli surgical lung biopsy was proposed. Similarly in the other case after improvement with treatment the symptoms reappeared on his return to work and lung biopsy was performed. The paper notes that the Bordeaux Mixture used to be applied to vines up to 14 times a season. The preparation of Bordeaux Mixture on the farm, from copper sulphate and lime is not relevant to the purchase of factory-prepared materials, as the home-made preparation is imprecisely neutralised, leading to excess of either copper sulphate or lime in the preparation. The home-made preparation was also applied by relatively primitive methods, e.g. by hand using a rush broom, or manual sprayers. Such

practices should not be taken into account when assessing the application of modern commercial formulations with modern machinery at the significantly lower application rates (approx. 8 kg/Ha compared to >24 kg/Ha historically). The paper also describes an inhalation study in guinea pigs.

In Case 1, lung lesions had a focal distribution and corresponded to three distinct patterns; a varying number of alveoli filled with desquamated macrophages, granulomas in the alveoli septa and fibro-hyaline nodules which seemed to be the scars of the granulomas. Copper was found in the granular material contained in the intra-alveolar macrophages. Similar findings were present in Case 2. In a separate experimental study using guinea pigs, similar findings were reproduced.

This investigation showed the need for protective measures for workers while spraying and that lung biopsy was required for the correct identification of this type of condition. The fact that the condition has not been reported in the recent literature indicates that the condition was primarily associated with uncontrolled use of 'home-made' product without any protective measures, and that modern application techniques for copper products are not associated with the condition. It does highlight the need for respiratory protection.

Reference: Pimentel, J.C. and Menezes, A.P. (1975)
Guideline: No
GCP: No.

Three cases of death were examined, one was an alcoholic and all were rural workers involved with spraying vineyards using Bordeaux Mixture, a copper sulphate solution neutralised with hydrated lime (referred to in this summary as "home-made" Bordeaux mixture). All had characteristic pulmonary lesions described previously for vineyard sprayers using 'home-made' Bordeaux Mixture. Livers were examined histopathologically either at necropsy or from percutaneous biopsy material. Various staining techniques for the sections were used, including histochemically for copper. The sections were also viewed using ordinary and polarised light.

In all cases hepatic changes were found consisting of proliferation and diffuse swelling of Kupffer's cells and the formation of well defined histiocytic or sarcoid-type granulomas all with inclusions of copper. These lesions were always found near the portal tracts. The identification of copper within the lesions characterises the nature of these granulomas. The lesions were different from those observed in conditions such as primary biliary cirrhosis in which copper deposits can be found in hepatocytes; granulomas containing copper are never found. In the present condition, copper deposits were never found in the hepatocytes.

The occupational exposure to 'home-made' Bordeaux Mixture, the characteristic pulmonary lesions of vineyard sprayer's lung and the presence of copper in the liver of these patients define this new variety of hepatic granulomatosis.

Reference: Pimentel, J.C. and Menezes, A.P. (1977)
Guideline: No
GCP: No

The livers of 30 rural workers who sprayed vineyards with Bordeaux Mixture (solution of copper sulphate with hydrated lime) for periods that varied from 3 to 45 years were studied. The paper states that spraying was carried out from 15 to 100 days per year, and 600 litres of mixture were sprayed each day by each worker. As has been observed previously, these practices from more than 25 years ago, using home-made Bordeaux mixture and primitive application techniques and significantly higher application rates should not be used in a risk assessment of factory-produced copper plant protection products, applied using modern engineering equipment and protective clothing, at modern (lower) application rates.

The spleens of four of cases were also examined. All cases with other possible causes of liver damage, such as hepatitis, alcoholism etc were excluded. Several stains were used for sections including those for histochemical localisation of copper. Various light forms including conventional, polarised, phase contrast and interference microscopy were used. Normal livers were used as controls.

The pathological findings were varied and included diffuse and focal swelling and proliferation of Kupffer cells, (diagnostic, and present in all cases), histiocytic and sarcoid-like granulomata (7 cases) fibrosis of variable degree in the perisinusoidal, portal and subcapsular areas (8 cases), accompanied by atypical proliferation of the sinusoidal lining cells, one case of liver angiosarcoma, micronodular cirrhosis (3 cases) and idiopathic portal hypertension (2 cases). Abundant deposits of copper were revealed, by histochemical techniques, within pulmonary and hepatic lesions. These cases were characterised by long-term exposure. The single case of angiosarcoma was in a man who had sprayed vineyards with 'copper sulphate' from the age of 18 to 53 (35 years). The average exposure in the cases of fibrosis was 29 years, and the two cases of cirrhosis followed exposure for 28 and 30 years.

The presence of abundant deposits of copper within the liver suggest a relationship between the occupational exposure and liver disease. This is explored further in following summaries.

Reference: Villar, T.G. (1974)
Guideline: No
GCP: No

Description of 15 consecutive patients admitted to Lisbon University Hospital, and review of earlier papers (cited above). Patients were 35 to 76 years of age, average 54 years. Patients had all been exposed to Bordeaux Mixture. The periods of exposure were not stated for all subjects, but some had been exposed for over 20 years. Most had used 'manual pulverizers carried on their backs', although one subject had used a rush broom. Seven of the patients smoked, one had been exposed to pigeon droppings and another to wood dust. Lung x-rays, biopsies, autopsies (where deceased) and histopathology were performed.

The initial diagnosis was Vineyards Sprayer's Lung (VSL) in three cases, pigeon fancier's lung in one case, tuberculosis in five cases, and pulmonary granulomatosis in two cases. In all cases, VSL was subsequently noted. The paper noted that in some cases, the condition remained clinically "silent" until a bronchiopulmonary bacterial or viral infection , or exposure to some other dust triggered further progression of the disease. It is interesting that the authors made an association between lung cancer and VSL, both in the Abstract (describing it as 'remarkable') and in several places in the paper, ignoring the relationship between lung cancer and cigarette smoking. The paper contained no information as to which of the patients had smoked, only that seven of the fifteen had smoked.

Fifteen patients suffering from VSL were in some cases initially misdiagnosed, but all followed chronic exposure to Bordeaux Mixture. The authors noted that three patients also showed lung cancer, and that seven patients had smoked cigarettes, although the paper gave no information as to the smoking habits of the patients with lung cancer, preferring to emphasise a "remarkable incidence" of lung cancer in patients with VSL.

Reference: Villar, T.G. and Nogueira, T. (1980)
Guideline: No
GCP: No

The study cites a review of 20,000 autopsies of (presumably Portuguese) rural workers. Vineyard Sprayer's Lung (VSL) was identified in 832 cases (retrospectively), corresponding to 4% of all autopsies and 20% of those with respiratory symptoms.

The paper also cites 33 patients admitted to Lisbon University Hospital. There is no information in the paper to determine if some of these patients had been described previously in an earlier paper (5.9.2/04). It is worthy of note that the description of the single female in this study matches closely the single female in the previous study, and it is reasonable to assume that the fifteen cases in the earlier paper have been included in this paper. Where possible, lung function tests were performed, as were biopsies, autopsies, and histopathology.

The age range of the patients was 35 to 76 years, average 53 years. Twenty-four percent were stated to be medium to heavy smokers (8 of the 33 cases), although number of non-smokers was not stated. The single

female in the study was stated to have sprayed vines from the ages of 10 to 14, and to have suffered pneumonia at the age of 50, during which she developed diffuse progressive fibrosis. She then presented with lung disease and was diagnosed with VSL. There were seven cases of lung cancer. The paper is seriously compromised in that there are no data to correlate smoking, which is known to be associated with lung cancer, and exposure to Bordeaux Mixture and VSL.

The author repeats an earlier conclusion that VSL is associated with high incidence of lung cancer, but ignores any possible association with cigarette smoking.

Reference: Plamenac, P. Santic, Z., Nikulin, A. and Serdarevic, H. (1985)
Guideline: No
GCP: No

Study of workers in the former Yugoslavia (Listica, Herzegovina) using "home-made" Bordeaux Mixture prepared by neutralising copper sulphate solution with lime. Unlike previous studies in Portugal, the study also recorded the smoking habits of the workers examined. The author performed some particularly stomach-churning sputum analyses in workers professionally exposed to regular inhalation of Bordeaux Mixture, who at the time of investigation showed no sign of pulmonary or any other disease. Sputum specimens were obtained from 52 exposed rural workers and 51 unexposed rural workers, from the same region who did not work in vineyards and did not come into contact with copper. These acted as controls. Sputum samples were obtained by morning cough on three consecutive days. Only expectorated material containing pulmonary macrophages was accepted as sputum. Sputa samples were fixed in 75% alcohol, embedded in paraffin and sections stained with H & E. These were then tested for iron (Turnbull stain)and for copper with rubeanic acid and benzidine.

Smokers produced sputa containing abnormal columnar cells in all cases. Macrophages containing copper granules in the cytoplasm were found in 64% of workers engaged in vineyard spraying, compared to none in the control group. Sputum specimens were evaluated for eosinophils, respiratory spirals, respiratory cell atypia and squamous metaplasia. Abnormal findings were more frequent in smokers than non-smokers. Atypical squamous metaplasia was observed in 29% of smokers who were vineyard workers, but only in 5% of cases in the non-smoking vineyard sprayers. There was enhanced expectoration of sputum in a high percentage of vineyard sprayers and in smoking controls, indicating that exposure to copper and cigarette smoke affects the respiratory epithelium.

Exposure to (home-made) Bordeaux Mixture in vineyard spraying affects the sputum. Smoking appears to exacerbate the effects.

Reference: Menzes, A.P., and Pimentel, J.C. (1996)
Guideline: No
GCP: No

Abstract only. Summarises changes seen in liver of patients with Vineyard Sprayer's Lung, and notes that similar liver lesions have been recorded in the livers of workers exposed to other pathogenic dusts (cement, cork, fur, mica and wood).
The foreign material could be identified within the lesions, using appropriate histological and histochemical techniques. It would appear that inhaled particulates can be transported to the liver, and can cause liver changes.

The authors conclude that the identification of foreign materials stored by the liver can be an important diagnostic tool in inhalatory disease.

4.9.3 Other relevant information

Reference: Stoner, G.D., Shimkin, M.B., Troxell, M.C., Thompson, T.L. and Terry, L.S. (1975)
Guideline: No
GLP: No

Cupric acetate (one of several metallic compounds investigated) in 0.85% sodium chloride solution was administered by intra-peritoneal injection to groups of 10 male and female Strain A/Strong mice at dose levels of 36, 90 and 180 mg/kg body weight. The injections were given three times a week for eight weeks (24 injections). Similar groups of mice were given 0.85% sodium chloride solution (24 injections), a single injection of urethan (positive control at 20 mg/animal) or remained untreated. The mice were weighed every 2 weeks during the injection period and at monthly intervals thereafter. They were killed 30 weeks after the first injection and their lungs removed and fixed in Tellyesniczky's fluid. After 1 to 2 days milky-white nodules on the lungs were counted; a few nodules were examined histopathologically to confirm the adenoma. Other selected organs (liver, intestines, thymus, kidney, spleen, salivary and endocrine glands) were examined histopathologically. Statistical analyses were performed.
Mean numbers of lung tumours in the vehicle and untreated control mice were similar indicating that occurrence was not significantly affected by the injections (table below). In the positive control the results demonstrated that the strain A was suitable for the induction of lung tumours. In mice treated with cupric acetate there was no statistically significant response to the numbers of tumours produced although the high dose produced a mean of 2.0. This result was based on only five surviving animals.

Table 40: Measurement of lung tumours

Treatment	**Dose level (mg/kg)**	**Number of survivors**	**Animals with lung tumours (%)**	**Mean number lung tumours/animal**
0.85% NaCl solution	NA	19/20	37	0.42
Urethan 20 mg	NA	18/20	100	21.6
Untreated	NA	19/20	31	0.28
Cupric acetate	180	5/20	60	2.00
	90	18/20	50	0.56
	36	15/20	27	0.40

The average numbers of tumours per lung increased in a dose-dependent manner but was not stastically significant. There was no evidence for any other tumors in the limited number of organs investigated. However, this study presented some deficiencies to assess carcinogenicity properties as the term of exposure, the inadequate numbers of animals, inappropriate exposure route and limited histopathological investigation.

4.9.4 Summary and discussion of carcinogenicity

Copper has been administered orally to rats in long term studies up to two years in duration. None of the studies presented below meets exactly the requirements of the International Guidelines, but they do show conclusively that copper has no carcinogenic activity.

Three types of studies have been performed:

- investigative toxicity studies demonstrating the long-term effects of very high dose levels (Haywood S., 1980 and 1985; Haywood S. and al., 1985),
- co-administration with known carcinogens to demonstrate that copper is effective at reducing the incidence and delaying the onset of tumours (Howell, J.S., 1958; Burki, H.R. and al. 1969; Carlton W.W. and al. 1973) and
- a two-year dietary administration study (Harrisson J.W.E., 1954).

The investigative toxicity studies, which were up to 52 weeks in duration, showed that dietary dose levels equivalent to 250 mg Cu/kg bw/day were associated with initial (week 6) liver damage including hypertrophied hyperchromatic parenchymal cells, necrosis and marked inflammatory reaction, and kidney damage to the proximal convoluted tubule. Both liver and kidney showed complete recovery between 9 and 15 weeks of continued copper administration, through to scheduled termination at 52 weeks. Subsequently, these animals

were able to tolerate even higher doses of copper, up to 300 mg/kg bw/day, even though this dose was lethal to naïve rats. There were no indications of pre-cancerous changes, and no tumours, up to 52 weeks administration (scheduled termination). The studies investigated high doses only; there was no attempt to derive no-effect levels.
The co-administration study was designed to show effects of copper when administered with a known liver carcinogen to two strains of rats for up to 19 months, and is one of several in the literature. The study showed that co-administration of copper significantly reduced the incidence and onset of liver tumours, which occurred at very high incidence in groups receiving the carcinogen without additional copper, and at control incidence in some groups receiving the carcinogen and additional copper. Thus copper has apparently a beneficial effect on liver cancer induction by a known carcinogen. It can also be concluded that copper has no activity as a cocarcinogen, or promoter (if copper had been a promoter, the liver tumours would have arisen earlier in the rats exposed to the carcinogen plus copper).
The two-year dietary study compared the administration of copper as sulphate or as gluconate with copper as potassium sodium copper chlorophyllin. The study showed that there was no increase in incidence of any tumour type after two years dietary administration of potassium sodium copper chlorophyllin at 3% dietary inclusion (approximately 80 mg Cu/kg bw/day).
But these studies suffered of real insufficiencies.

Copper is an essential nutrient, naturally present in almost all foodstuffs. Humans are exposed to copper in the diet from weaning as an essential micronutrient. Most western diets contain between 1 and 2 mg Cu/person/day. As such the population is exposed to copper in the diet every day. The various natural mechanisms for regulating copper in humans were described previously.

There are genetic abnormalities which lead to accumulation of copper in the liver, kidney and in the brain (Wilson's disease), and in the intestinal epithelium, kidney and fibroblasts (Menkes' disease). Both diseases can be fatal if not treated, but there is no evidence for increased incidence of cancer in victims of either Wilson's or Menkes' disease, despite the chronic high tissue copper levels.

The condition known as Vineyard Sprayer's Lung (VSL) has been reported in several papers, mostly from Portugal, but also from the former Yugoslavia. The condition is characterised by lung lesions with a focal distribution corresponding to three distinct patterns; a varying number of alveoli filled with desquamated macrophages, granulomas in the alveoli septa and fibro-hyaline nodules which appear to be the scars of the granulomas. Hepatic changes included proliferation and diffuse swelling of Kupffer's cells and the formation of well defined histiocytic or sarcoid-type granulomas all with inclusions of copper. These lesions were always found near the portal tracts. The identification of copper within the lesions characterised the nature of these granulomas. Copper deposits were never found in hepatocytes. The papers describe the preparation on-site of Bordeaux Mixture, as a copper sulphate solution neutralised with hydrated lime, and primitive application techniques at higher rates than those used in modern agriculture, where Bordeaux Mixture is formulated under controlled conditions in dedicated factories, and applied using modern machinery by workers wearing appropriate protective equipment. Most of the published findings date from the 1970s and 1980s. Some of the papers were compromised because the authors did not adequately describe the smoking habits of the subjects, only noting that certain subjects were heavy smokers. The Yugoslav paper surveyed smoking and non-smoking rural workers, including those which did and those which did not use home-made Bordeaux mixture, and found that there were indications of adverse effects in users of Bordeaux Mixture that were exacerbated by smoking.

Bordeaux Mixture is a highly complex mineral mixture. If the reaction of the lime and copper sulphate is not strictly controlled, the resulting mixture may not be sufficiently neutralised, and may contain significant amounts of plaster and gypsum, in a form that if inhaled, may result in lung disease. One paper also notes that similar liver lesions to those in VSL have been recorded in workers exposed to other pathogenic dusts (cement, cork, fur, mica and wood), where the inhaled dust has been transported, presumably by macrophages, to the liver.

In these epidemiological data analysis different confusing situation were identified (smoking, wood dust, arsenic, etc…). On the other hand, the IPCS publication (IPCS, 1998) on epidemiological studies excluded a link between Lung cancer and copper compound inhalation exposure.

Based on the limited information available in epidemiological studies, the link between Vineyard Sprayers Lung and lung cancer cannot be established.

The weight of evidence in humans and rats is that copper is not carcinogenic.

4.9.5 Comparison with criteria

Classification in category 1A for carcinogenicity is not considered as there are no human data of concern. Classification in category 1B requires human data that establish a causal relationship between human exposure and development of cancer and/or sufficient evidence to demonstrate clear animal carcinogenicity from well conducted animal studies. A substance shall be classified in category 2 for carcinogenic endpoint if the substance is suspected as human carcinogen. The placing of a substance in this category is done on the basis of evidence obtained from human and/or animal studies, but which is not sufficiently convincing to place the substance in category 1, based on strength of evidence together with additional consideration.

For copper compounds, no increase incidences of tumors were observed in the different animal studies by oral route. Moreover, there are two genetic conditions in human (Wilson's disease and Menkes' disease) that result in major alterations in copper absorption, distribution and excretion. Wilson's disease (where copper is absorbed in the intestine but cannot be pumped out of the liver to bile) leads to accumulation of copper in the principal target organ, the liver, and also in the kidney, brain and the cornea. People with Menkes' disease (where copper is absorbed by intestinal cells but cannot be pumped out of these cells to the hepatic portal system) can only absorb minimal amounts of copper, and show chronic accumulation of copper in the intestinal epithelium and high levels in kidney and in fibroblasts. Human subjects with these conditions may die of the condition itself (if untreated), but they do not show any increased incidence of cancer. If abnormally high levels of copper are present over long periods in an organ or tissue, yet there is no association between the high copper levels and cancer in these organs or tissues, in chronic disease, then it is reasonable to conclude that copper is not carcinogenic in these tissues.

4.9.6 Conclusions on classification and labelling

In this context, the available data do not support a classification for the carcinogenic endpoint.

RAC evaluation of carcinogenicity

Summary of the Dossier Submitter's proposal

No data on granulated copper were provided in the CLH report. However, in light of the proposal to read-across between the different copper compounds for systemic endpoints, (see section "RAC general comment" above), the dossier submitter referred in the CLH report to several long-term animal studies with other copper compounds and to human data on copper exposure.

Several animal studies administering copper compounds in either drinking water or diet of rats and mice for various periods of time (up to two years) are presented. However, none meet the guidelines for carcinogenicity testing and several have shortcomings when it comes to evaluating carcinogenicity, such as short duration. None of the studies showed an indication of carcinogenic potential of copper administered systemically. Co-administration of copper with known carcinogens appeared to lower the risk of tumour formation in some cases.

Several cohort or epidemiological studies in humans exposed to copper through copper mining, smelting and refining were briefly summarised in the CLH report. The dossier

submitter concluded that they provide little evidence for increased risk of cancer with exposure to copper compounds. Reference was also made to reports of the occupational disease Vineyard Sprayer's Lungs (VSL) associated with exposure to home-made Bordeaux Mixture. Due to poor reporting and possible confounders such as smoking, the dossier submitter concluded that a link between lung cancer and VSL cannot be established. There are two rare genetic diseases of copper in humans (Wilson's disease and Menkes' disease), but there is no evidence of increased incidences of cancer in patients with either disease, despite the chronic high tissue copper levels.

The dossier submitter concluded that the weight of evidence assessment in humans and animals concluded that copper is not carcinogenic and that therefore no classification for carcinogenicity is warranted for copper compounds, including granulated copper.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that no data on granulated copper are available. The CLH report contains data on other copper compounds (predominantly copper sulphate pentahydrate), from which the dossier submitter proposed to read-across to granulated copper. In view of the considerations presented in the section "RAC general comment", RAC has not pursued the aspect of grouping any further. RAC concludes that **in the absence of relevant data no proposal for classification for carcinogenicity can be made for granulated copper**.

4.10 Toxicity for reproduction

Table 41: Summary table of relevant reproductive toxicity studies

Method	Results	Remarks	Reference
Fertility			
2-generation study Sprague-Dawley rats 30/sex/group Oral, diet Copper sulphate pentahydrate 0, 100, 500, 100 or 1500 ppm equivalent to in actual doses (P1-F1): 0, 1.53-2.65, 7.7-13.3, 15.2-26.7, and 23.6-43.8 mg/kg body weight/day	*Parental toxicity* No treatment related effect on mortality, clinical signs, bw gain, food consumption, food efficiency in either sex in any generation. At 1500 ppm: ↓ spleen weight in female. ↓ liver iron concentration in P1 females at 1500 ppm. *Fertility effects* No adverse effects on fertility, general reproductive performance or offspring viability and growth. *Offspring effects* At 1500 ppm: ↓ spleen weight in F1and F2 male and female weanlings. ↑ Brain copper concentration in F1 females and F1 and F2 male weanlings. ↓ plasma iron concentration in F2 male and female weanlings. ↑ liver copper concentration in F1 female. 1000ppm: ↑ liver copper concentration of F1 males and F1 and F2 male and female weanlings. The majority of effects are reported in weanlings and in dams at the end of lactation - the food intake and compound consumption data show that both of these "populations" were consuming significantly higher amounts of diet than towards the end of the pre-mating maturation periods, and that the spleen effects are not seen in males at termination, when compound consumption is much lower. From this it may be concluded that the spleen effects may be transient even at high doses, and that when the dietary intake i.e. dose level is reduced, the spleen effect diminishes.	OECD 416 GLP	Mylchreest, E. (2005)
Fertility (cross mating) Wistar rats	No differences between treated and control groups in any of the parameters studied (pregnancy rate, implantation, resorption, live foetuses, gross fetal anomalies, duration of gestation, litter size, number of live young, gross anomalies, litter and mean pup weight throught the weaning.	No GLP	De la Iglesia F. W. *et al* (1973)

20 females/groups Gavage Copper gluconate 0, 3 or 30 mg/kg/day			
Fertility/ teratology Mouse Copper sulphate 0, 500, 1000, 1500, 2000, 3000 or 4000 ppm 4000ppm correspond approximately to 570 mg/kg bw/d	No adverse effects on mating performance and pregnancy rate.	No GLP Deviations: No detail given on the size of the groups. The study did not measure maternal bw gains or maternal liver histology or copper content.	Lecyk, M. (1980)
Developmental toxicity			
Teratology NZW Rabbit 22 females/group Gavage Copper hydroxide 0, 6, 9 or 18 mg/kg/day	<u>Maternal toxicity</u> 3 deaths and 2 abortions (subsequently sacrified) at 18 mg/kg/day. Animal found dead showed diarrhoea, red staining, weakness and irregular respiration. Marked initial weight loss at and above 9 mg/kg bw/d. Mean weight gain was 31% and 72% at 9 and 18 mg/kg bw/d, respectively. Marked inappetance during the initial part of the treatment period. <u>Developmental effects</u> ↓ mean foetal bw at 18 mg/kg bw/d (9% lower than control). 3 treated foetus and 1 control animal have malformations. These malformations were considered spontaneous and unrelated to treatment.↑ Incidence of foetal skeletal findings at 9 and 18 mg/kg/day.	OECD 414 GLP Purity: 61.14% w/w	Munley, S. (2003a to d)
Teratology Rat Gavage Copper gluconate 0, 0.1, 3 or 30 mg/kg/day	No maternal or developmental effects	No GLP Deviations: Partial summary. Treatment duration too short (day5-15 of pregnancy). Size of the groups not given.	De la Iglesia F. W. *et al* (1972a)
Teratology Swiss Mice Gavage Copper gluconate 0, 0.1, 3 or 30 mg/kg/day	No maternal effects. Litter parameters were not adversely affected by treatment.	No GLP	De la Iglesia F. W. *et al* (1972b)

		Deviations: The treatment duration is too short, the methodology suffers of insufficiencies, and there was no information in the summary on examination for visceral and skeletal defects. Size of the groups not given	
Teratology Cu^{2+} as copper wire (Intra uterine device) Rat Wistar Developing foetuses were exposed to intrauterine copper from days 9 to 21 of pregnancy	There was no significant increase in the incidence of congenital malformations or growth retardation in foetuses from uterine horns containing copper coils, when compared with those from unoperated horns, sham-operated horns, or horns containing stainless-steel coils. But there were significant increases in fetal brain, fetal liver, placenta and uterine copper levels in comparison with rats containing steel coils or no coils.	No guideline No GLP Purity = 99.9% Investigation of the effects of intrauterine exposure to copper IUDs and prenatal development in the rat	Barlow, S.M., Knight, A.F. and House, I. (1981)
Teratology Cu^{2+} as copper wire (Intra uterine device) **Rat:** Holtzman strain. **Hamster:** Not stated. **Rabbit:** New Zealand White. **Rat and Hamster:** approximately 2.75 µg per day **Rabbit:** approximately 5.50 µg per day **Rat/hamster:** From day 6 of pregnancy until sacrifice of parent **Rabbit:** From day 7 of pregnancy until sacrifice of parent	No adverse effects (teratogenicity or growth and development) attributable to the exposure of parent females to copper were seen in F_1 or F_2 animals.	No guideline No GLP Purity = 99.9% Investigation of the effects of intrauterine exposure to copper IUDs and prenatal development	Chang, C.C. And Tatum, H.J. (1973)

Teratology Wistar Rat 14 mated females/group Copper acetate Oral (drinking water) 0.185% w/v (approximately 65 mg Cu/kg body weight per day). Duration: 7 weeks immediately prior to mating	Histology of maternal liver and kidney showed changed consistent with toxicity. Foetal liver and kidney histologically normal, with some delays to ossification of skeleton.	No guideline (published paper) No GLP	Haddad, D.S., Al-Alousi, L.A. and Kantarjian, A.H. (1991)

4.10.1 Effects on fertility

4.10.1.1 Non-human information

Reference: Mylchreest, E. (2005)
Guideline: OECD 416
GLP: Yes
Deviations: Yes

- Testicular histopathological examinations are not fully described

Copper sulphate pentahydrate was selected as a representative form of copper for investigation of gonadal function, effects of conception, parturition and growth/development of rats over two generations. One set of litters were produced in each generation. Five groups of 30 male and 30 female Sprague-Dawley (Crl:CD (SD)IGS) rats were given copper sulphate pentahydrate in diet (by direct admixture – no vehicle included in test substance/diet mixture) at dose concentrations of 0, 100, 500, 1000 or 1500 ppm (equivalent to 0, 1.53-2.65, 7.7-13.3, 15.2-26.7, 23.6-43.8 mg/kg body weight/day). Animals in the P1 generation were dosed for at least 70 days prior to mating, continuing through to sacrifice on test day 109-113 (males) or day 21 postpartum (females). The F1 generation were given treated diet at same test substance concentrations from day 21, for at least 70 days prior to mating and then continuing to sacrifice on test day 119 (F1 males) or day 21 post-partum (F1 female dams) or the day of weaning for F1 or F2 pups.

Fresh treated diet was prepared for each group at weekly intervals throughout the study. The untreated diet, fed to controls, was a standard rodent breeder diet – certified Rodent LabDiet 5002. Diets were sampled for assessment of homogeneity and stability at room temperature for 7 or 14 days, and under refrigerated and/or frozen storage for periods of 7, 14 or 21 days. Drinking water and standard diet were sampled for analysis of copper concentration.

For the P1 generation, 165 male and 165 female rats were obtained at approximately 8 weeks of age and in a weight range of 262-332g (males) or 166-231g (females). The rats were non-siblings. The rats were housed individually in suspended stainless steel mesh cages except for mating when males and females were housed as breeding pairs. After completion of cohabitation phase the females were individually housed in polycarbonate pans (if no evidence of copulation), or, if pregnant, returned to stainless steel mesh cages for gestation and then transferred to polycarbonate pans from day 20 of gestation and through lactation. Food and water were provided ad libitum throughout the study.

For the P1 generation, the obtained rats were ranked by weight after a suitable acclimation period and then allocated to study groups using a stratified randomisation procedure to ensure group mean initial bodyweights were not statistically different. For the F1 litters, offspring were randomly selected on day 21 postpartum, one rat/sex/litter where possible, for allocation as parents for the F2 generation.

During the study cageside observations for assessment of clinical signs or evidence of moribundity/death were completed at least once daily and a full clinical examination (including handling and examination for abnormal appearance and/or behaviour) was completed weekly during pre-mating, gestation and lactation phases. Bodyweights were recorded at weekly intervals, pre-mating, and weekly thereafter for males and for females without evidence of copulation, or that did not deliver a litter. For the F1 generation, additional weights were recorded on achievement of developmental landmarks (vaginal patency or preputial separation). During gestation and lactation the dams were weighed on days 0, 7, 14 and day 21. Food consumption was recorded, and reported weekly during the 70 day pre-mating phase for both P1 and F1 generations (values for food efficiency and daily test substance intake were derived from food consumption during this phase of the study).

Food consumption was also recorded for pregnant P1 and F1 dams on days 0, 7, 14 and 21 of gestation and 0, 7 and 14 of lactation.

After approximately 10 weeks exposure to treated diet, the rats were pair-housed for breeding (1:1 with non-sibling mate), remaining together for up to two weeks or until evidence of copulation was observed. Vaginal lavage samples were analysed for oestrous cycling from all females beginning 3 weeks prior to mating period up to end of cohabitation or time of mating. Additional samples were collected at terminal sacrifice. Sperm parameters (number of motile sperm and abnormal sperm per 200 cells per animal, sperm count per cauda epididymis and per gram epididymis, spermatid count per testis and per gram testis) were evaluated from the left testis for males of both parental generations at terminal sacrifice. The right testis was preserved in Bouin's fluid for traditional histopathology.

From Day 20 of gestation, after transferring dams to polycarbonate pans, the females were examined twice daily for signs of delivery/offspring. During lactation, on days 0, 4, 7, 14 and 21, pups were handled and examined for abnormal behaviour and appearance. On day 0, live and dead pups were counted and live pups were sexed and weighed. Litters were culled to 4/sex (where possible) on day 4 when pups were again weighed and counted. Pups were weighed again on days 7, 14 and 21.

For the F1 generation, offspring (1 rat/sex/litter) from the F1 litters were selected. Developmental landmarks (vaginal patency, preputial separation) were checked.

Terminal procedures for all P1 and F1 parents involved macroscopic examination and examination of uteri for presence and number of implantation sites. Blood samples were collected from ten animals of each group. Tissues (males: testis, epididymides, prostate, seminal vesicles, coagulating glands; females: ovaries, uterus, vagina, cervix; both sexes: brain, liver, gross abnormalities, kidneys, pancreas, femur, intestines, heart.) were collected from each adult and preserved for possible histopathology.

Pups found dead during lactation and those surviving to termination were subject to gross pathological examination and the carcass preserved. From the pups culled on lactation day 4, six of each sex were selected per group and samples of brain and liver collected and stored deep frozen.

For the F1 and F2 weanlings - all showing gross abnormalities or clinical signs were subject to gross pathological examination; one pup/sex/litter was also subject to necropsy. Gross lesions and tissue from potential target organs (brain and liver) were preserved and microscopic examination of these tissues completed for F1 and F2 high dose and control pups. Blood samples were collected from ten rats of each sex from F1 and F2 males and females. Tissue samples (brain, liver, kidney, pancreas, femur, intestine and heart) were collected from the same pups and stored, after freezing in liquid nitrogen, for possible chemical analysis or microscopic evaluation.

Organ weights were collected for P1 and F1 adults males: testes, epididymides, right cauda epididymis, seminal vesicles, prostate; females: ovaries, uterus; both sexes: liver, brain, kidneys, spleen, adrenal, pituitary and thyroid. Final bodyweight data were used for calculation of relevant organ/weight ratios. No organ weights were recorded for nursing pups but liver, brain, spleen and thymus weights were recorded for one pup/sex/litter for F1 and F2 weanlings.

Tissues designated for histopathological examination included: reproductive organs, gross abnormalities, liver and brain for P1 and F1 adults – only high dose and control groups examined. In addition, reproductive organs were examined for all mated animals failing to produce a litter.

No microscopic examinations were completed for nursing offspring.
Liver, brain and gross abnormalities were examined from one pup/sex/litter for F1 and F2 weanlings of control and high dose groups only.
Quantitative assessment of primordial and growing ovarian follicles was completed for ten lactating F1 females from control and high dose groups only.

Analytical findings:

Stability evaluation indicated the test substance was stable in diet for the study duration. The test substance stability analysis indicated the test material was stable for the duration of the assay.
Homogeneity analyses indicated that the mixing procedures were adequate for the study.
Concentration assessment indicated that the nominal target dose levels had been achieved. The mean copper content of control diet was 13.7 ppm. The mean copper concentration added to test diet diets was in the range of 25 to 382 ppm (100 to 1500 ppm copper sulphate pentahydrate). Copper concentration in drinking water, analysed on two occasions during the study were 0.014 and 0.024 ppm.
Test substance achieved intake is tabulated in table 42, for the various phases of the study and for each generation.

Table 42: Summary of achieved test substance intake (mg/kg bw/day)

Group/study phase:	**Dose level (nominal ppm concentration)**			
	100	500	1000	1500
P1 males – pre-mating	1.53	7.7	15.2	23.6
P1 females – pre-mating	1.92	9.6	19.1	29.5
P1 females – gestation	1.67	8.6	17.0	26.2
P1 females – first two weeks of lactation	3.39	17.7	33.8	55.7
F1 males – pre-mating	2.25	11.5	23.5	36.1
F1 females – pre-mating	2.65	13.3	26.7	43.8
F1 females – gestation	1.69	8.5	17.1	26.5
F1 females – first two weeks of lactation	3.27	17.6	35.2	55.4

There were no clinical reactions to treatment throughout the study for the P1 male rats. The P1 females showed no clinical reaction to treatment during pre-mating, gestation or lactation at any of the four dose concentrations. Similarly there were no clinical signs of reaction to treatment for the F1 males or F1 females at any dose level or at any stage of the study.

There were no effects, considered attributable to treatment with copper sulphate pentahydrate, on either body weight or body weight gain in comparison with controls, for the males and females of the P1 generation. Occasional statistically significant increases (males) or decreases (females) were small in magnitude, of sporadic occurrence or showing no dose relationship and were considered spurious findings.

Similarly, for the F1 generation adults, there were no treatment related effects on bodyweight or weight gain in either sex at any of the dose concentrations.

While there were occasional statistically significant differences in food consumption and food utilisation efficiency (tables 43 and 44) between treated and control groups in both sexes in the P1 and F1 adult groups, these were either small in magnitude or showed no dose relationship. In summary, there were no consistent effects on food consumption of food conversion efficiency to indicate an effect of treatment for the males in either generation nor for the females, either pre-mating or during gestation/lactation.

Table 43: Food consumption P1 adults

Week	Males (ppm)					Females (ppm)				
	0	100	500	1000	1500	0	100	500	1000	1500
Pre-mating (g/day)										
0-7	25.3 [0.235]	26.5 [0.233]	25.4 [0.243]	25.5 [0.241]	27.1 [0.206] *	18.6 [0.174]	20.0* [0.143]	19.4 [0.154]	19.7 [0.147]	19.7 [0.154]
7-14	25.2 [0.190]	25.9 [0.190]	25.8 [0.205]	25.3 [0.206]	26.4 [0.169]	18.7 [0.109]	19.9 [0.103]	19.4 [0.097]	18.4 [0.089]	18.6 [0.075]
14-21	25.7 [0.180]	26.2 [0.161]	26.8 [0.166]	26.7 [0.165]	26.8 [0.161]	19.1 [0.064]	20.2 [0.079]	20.6* [0.072]	19.8 [0.099]	19.5 [0.094]
21-28	27.0 [0.156]	26.6 [0.140]	27.1 [0.148]	27.0 [0.158]	26.6 [0.149]	20.4 [0.107]	21.0 [0.109]	20.6 [0.072]	19.8 [0.10]	20.0 [0.083]
28-35	27.4 [0.136]	26.9 [0.126]	28.0 [0.139]	27.4 [0.131]	27.7 [0.121]	19.9 [0.053]	20.6 [0.053]	20.6 [0.084]	20.2 [0.067]	20.7 [0.074]
35-42	27.6 [0.108]	27.1 [0.114]	27.5 [0.115]	26.9 [0.109]	26.7 [0.113]	19.6 [0.047]	20.3 [0.031]	19.2 [0.002]*	19.4 [0.041]	20.1 [0.027]
42-49	27.4 [0.106]	26.9 [0.104]	27.3 [0.111]	26.6 [0.102]	26.3 [0.115]	18.6 [0.051]	19.3 [0.062]	18.5 [0.079]	18.6 [0.044]	19.4 [0.064]
49-56	27.2 [0.074]	27.1 [0.075]	27.3 [0.067]	26.9 [0.069]	27.2 [0.062]	18.7 [0.037]	19.5 [0.063]	18.9 [0.045]	19.1 [0.043]	19.4 [0.018]
56-63	26.4 [0.074]	27.6 [0.087]	28.2* [0.087]	27.4 [0.077]	27.3 [0.039]	18.6 [0.043]	18.9 [0.052]	18.9 [0.048]	19.5 [0.062]	19.8 [0.067]
63-70	26.3 [0.059]	27.0 [0.061]	28.0* [0.062]	27.8* [0.073]	27.6 [0.070]	18.8 [0.042]	19.6 [0.047]	19.5 [0.023]	20.2 [0.043]	20.3* [0.026]
During gestation (g/day)										
0-7						23.1 [0.218]	23.7 [0.214]	23.9 [0.212]	23.3 [0.215]	24.7 [0.209]
7-14						24.1 [0.170]	25.4 [0.160]	25.8 [0.170]	26.0 [0.175]	25.6 [0.171]
14-21						23.6 [0.428]	23.9 [0.400]	25.0 [0.409]	24.4 [0.413]	25.4 [0.428]
0-21						23.5 [0.272]	24.3 [0.257]	24.9 [0.264]	24.6 [0.265]	25.2* [0.270]
During lactation (g/day)										
0-7						35.9 [0.059]	38.3 [0.071]	40.2 [0.079]	37.8 [0.045]	42.7* [0.059]
7-14						49.7 [-0.002]	53.5* [0.008]	56.8* [0.009]	53.1 [0.007]	58.7* [0.006]
0-14						42.8 [0.025]	45.9 [0.035]	48.5* [0.037]	45.5 [0.025]	50.7* [0.028]

[ ] food conversion efficiency {grams weight gain/grams food consumed}
* Statistically significantly different from controls $p < 0.05$

Table 44: Food consumption F1 adults

Week	Males (ppm)					Females (ppm)				
	0	100	500	1000	1500	0	100	500	1000	1500
Pre-mating (g/day)										
0-7	14.7 [0.427]	14.9 [0.434]	15.8 [0.413]	14.9 [0.410]	15.3 [0.400]	14.1 [0.384]	13.6 [0.397]	14.1 [0.392]	13.4 [0.380]	14.1 [0.360]

7-14	19.9 [0.401]	20.8 [0.394]	22.1* [0.379]	21.1 [0.397]	21.9 [0.377]	18.1 [0.325]	18.3 [0.319]	19.5 [0.310]	19.3 [0.315]	20.8 [0.287]*
14-21	23.1 [0.362]	24.2 [0.352]	25.3* [0.333]*	24.3 [0.352]	25.3* [0.333]*	19.7 [0.239]	21.1 [0.221]	22.4* [0.219]	21.0 [0.243]	21.8* [0.221]
21-28	25.4 [0.344]	26.4 [0.336]	26.8 [0.335]	27.2 [0.326]	26.9 [0.309]*	19.7 [0.178]	20.3 [0.159]	20.4 [0.158]	20.6 [0.175]	22.2* [0.150]
28-35	27.2 [0.295]	28.5 [0.282]	28.6 [0.272]*	28.8 [0.258] *	29.6 [0.253]*	19.4 [0.153]	21.5 [0.153]	21.8* [0.142]	21.4 [0.151]	22.0* [0.127]
35-42	27.3 [0.240]	29.1 [0.230]	28.7 [0.228]	28.8 [0.228]	29.9 [0.204]	20.2 [0.124]	21.8 [0.122]	21.5 [0.132]	21.5 [0.123]	23.4 [0.114]
42-49	28.8 [0.186]	29.0 [0.189]	29.2 [0.183]	29.5 [0.171]	29.3 [0.177]	20.8 [0.117]	22.2 [0.099]	21.4 [0.099]	22.3 [0.094]	23.4 [0.090]
49-56	28.5 [0.156]	28.9 [0.162]	28.9 [0.148]	30.2 [0.160]	29.0 [0.145]	20.8 [0.079]	21.8 [0.105]	21.4 [0.089]	21.1 [0.075]	21.8 [0.069]
56-63	28.0 [0.124]	29.1 [0.140]	28.5 [0.126]	29.1 [0.120]	29.3 [0.128]	21.4 [0.083]	21.4 [0.063]	22.0 [0.080]	23.9 [0.089]	24.0 [0.066]
63-70	27.6 [0.112]	28.8 [0.126]	28.5 [0.122]	29.3 [0.117]	28.9 [0.119]	21.4 [0.069]	21.3 [0.072]	20.2 [0.060]	20.6 [0.054]	21.1 [0.057]
<u>During gestation (g/day)</u>										
0-7						23.1 [0.229]	23.5 [0.213]	23.9 [0.212]	23.4 [0.223]	23.8 [0.219]
7-14						24.3 [0.176]	24.3 [0.164]	24.6 [0.162]	25.5 [0.182]	25.0 [0.165]
14-21						25.0 [0.430]	24.1 [0.458]	25.1 [0.445]	24.4 [0.422]	24.5 [0.447]
0-21						24.1 [0.280]	23.9 [0.278]	24.5 [0.270]	24.4 [0.276]	24.4 [0.277]
<u>During lactation (g/day)</u>										
0-7						35.8 [0.047]	37.3 [0.078]	42.4* [0.070]	40.5 [0.060]	45.9* [0.062]
7-14						52.0 [0.019]	50.3 [-0.029]	54.7 [-0.007]	54.7 [-0.027]	54.8 [-0.018]
0-14						43.9 [0.032]	43.8 [0.018]	48.5* [0.028]	47.6 [0.014]	50.3* [0.026]

[ ] food conversion efficiency {grams weight gain/grams food consumed}
* Statistically significantly different from controls $p < 0.05$

There were no treatment-related effects on any of the sperm parameters investigated for males in either the P1 or F1 generation.

The mean percent number of days in oestrus, dioestrus or proestrus were unaffected in either the P1 or F1 generations. The total mean cycle length was similarly unaffected by treatment with copper sulphate pentahydrate. The total number of days spent in oestrus was slightly higher for the P1 females dosed at 1000 or 1500 ppm (47 and 40% respectively) in comparison with controls (30%) but since there were no effects on mean oestrous cycle length nor any adverse reproductive changes, this minor change was not considered to be biologically significant.

At termination the distribution of oestrous cycle stages was similar for P1 and F1 females and no treatment effect was postulated.

For the P1 and F1 generations there were no treatment-related effects on any of the reproductive indices investigated at any of the four dose concentrations (tables 45 and 46). These included precoital interval length, mating and fertility indices, gestation length, the number of implantation sites and the implantation efficiency.

Table 45: P1 adult reproductive performance

Group	**1**	**2**	**3**	**4**	**5**
Treatment (ppm)	**Control**	**100**	**500**	**1000**	**1500**
Males: n	30	30	30	30	30
Number mating	27	30	28	28	29
Mating index%	90.0	100	93.3	93.3	96.7
Females: n	30	30	30	30	30
Number pregnant	25	29	27	25	27
Fertility index (%)	92.6	96.7	96.4	89.3	93.1
Mean gestation length (days)	22.2	22.4	22.3	22.3	22.4
Total resorption	0	0	0	0	0
Mean number of implantation sites per pregnant female	14.5	14.1	13.9	14.0	13.8
Number of pregnant females	25	29	27	25	27
Implantation efficiency (%)	93.3	92.4	91.6	93.2	91.7
Mean number of pups born per litter	13.6	13.2	13.0	13.1	13.6

Table 46: F1 adult reproductive performance

Group	**1**	**2**	**3**	**4**	**5**
Treatment (ppm)	**Control**	**100**	**500**	**1000**	**1500**
Males: n	30	30	30	29	30
Number mating	29	30	30	29	30
Mating index%	96.7	100	100	100	100
Females: n	30	30	30	29	30
Number pregnant	28	30	28	25	30
Fertility index (%)	96.6	100	93.3	86.2	100
Mean gestation length (days)	22.2	22.2	22.2	22.2	22.3
Total resorption	0	0	0	0	0
Mean number of implantation sites per pregnant female	15.0	14.5	14.7	14.0	14.2
Number of pregnant females	28	30	27	25	30
Implantation efficiency (%)	94.7	95.8	93.8	95.6	91.7
Mean number of pups born per litter	14.2	13.9	13.8	13.3	13.2

Treatment with copper sulphate pentahydrate had no effect on the number of pups born, the number of liveborn pups or the numbers of pups surviving to 4, 7, 14 or 21 days post-partum (tables 47 and 48). In either generation, F1 or F2 offspring, there were any treatment-related effects on the sex ratio within litters, or survival indices during lactation at any of the dose concentrations tested.

Table 47: Litter data for F1 pups

Group	**1**	**2**	**3**	**4**	**5**
Treatment (ppm)	**Control**	**100**	**500**	**1000**	**1500**
Number of pregnant females	25	29	27	25	26
Mean litter size – birth	13.6	13.2	13.0	13.1	13.6
Mean number live born	13.6	13.1	12.7	12.9	13.5
Mean number of pups pre-culling on day 4	13.4	12.9	13.2	12.8	13.4
Mean number of pups per litter post day 4 culling	7.8	7.8	7.9	7.9	8.0
Mean number of pups per litter on day 7	7.8	7.8	7.9	7.9	8.0
Mean number of pups per litter on day14	7.8	7.8	7.9	7.9	8.0
Mean number of pups per litter on day 21	7.8	7.8	7.9	7.9	8.0
Sex ratio (% males)	52	48	53	49	50
Gestation index (% litters with at least one live pup)	100	100	100	100	100
Mean percent born alive	99.5	98.9	95.2	98.9	99.5
Viability Day 0-4 (%)	98.6	98.8	99.5	98.9	99.2
Lactation index	99.5	100	99.5	100	100
Litter survival (% litters with at least one pup alive at day 21)	100	100	100	100	100
Mean pup weight (g) –					
Day 0	6.6	6.7	6.7	6.5	6.7
Day 4 pre-culling	10.7	11.3	11.0	10.7	11.1
Day 4 post-culling	10.7	11.3	11.0	10.8	11.1
Day 7	17.3	18.5	18.0	17.2	17.8
Day 14	34.8	36.4	36.2	34.7	35.7
Day 21	57.8	59.5	59.0	55.7	57.0

Table 48: Litter data for F2 pups

Group	**1**	**2**	**3**	**4**	**5**
Treatment (ppm)	**Control**	**100**	**500**	**1000**	**1500**
Number of pregnant females	28	30	27	24	30
Mean litter size – birth	14.2	13.9	13.8	13.3	13.2
Mean number live born	14.1	13.7	13.7	13.2	13.1
Mean number of pups pre-culling on day 4	13.9	13.6	13.3	13.0	13.0
Mean number of pups per litter post day 4 culling	8.0	8.0	7.8	8.0	7.8
Mean number of pups per litter on day 7	8.0	8.0	7.8	8.0	7.8
Mean number of pups per litter on day14	7.9	8.0	7.8	8.0	7.8
Mean number of pups per litter on day 21	7.9	7.9	7.8	8.0	7.7
Sex ratio (% males)	49	53	56	49	51
Gestation index (% litters with at least one live pup)	100	100	100	100	100
Mean percent born alive	99.5	98.9	99.7	99.0	99.3

Viability Day 0-4 (%)	98.3	99.3	96.2	98.5	99.4
Lactation index	99.6	99.2	100	99.5	99.6
Litter survival (% litters with at least one pup alive at day 21)	100	100	100	96.0	100
Mean pup weight (g) –					
Day 0	6.3	6.4	6.5	6.4	6.6
Day 4 pre-culling	10.2	10.9	10.7	11.0	10.8
Day 4 post-culling	10.2	10.9	10.6	10.9	10.9
Day 7	16.8	17.7	17.6	17.7	17.5
Day 14	34.2	35.3	36.5	35.1	35.4
Day 21	56.0	58.1	58.4	57.3	56.7

Clinical signs were noted among the pups of the F1 or F2 generation but at low incidence and showing no dose-relationship. The clinical observations were not considered to be treatment-related or toxicologically significant.

An increase in mean pup weight in F1 litters dosed at 100 ppm (low dose) on lactation day 7 was not treatment-related since there were no other dose correlations. There were no treatment-related effects on pup weight at any dose levels for the F1 or F2 offspring.

There were no treatment-related effects on preputial separation for F1 males at any dose level. For the F1 females the mean age at vaginal opening was increased for the high dose group (1500 ppm) in comparison with concurrent controls – 33.6 versus 32.1 days. However, the historical control data for this parameter indicates a mean vaginal opening time of 32.3 days and a minimum and maximum range of 31.3 to 33.9 days. Hence the difference was small (1.5 days) and well within the range of historical control data. The apparent slight delay in vaginal opening was not considered an effect of treatment with copper sulphate pentahydrate.

Pathology findings:

There were no significant differences between the high dose (1500 ppm) and control groups in respect of total numbers for primordial and pre-antral follicles.

There were no test-substance related deaths during the course of the study. Of the 120 P1 and 120 F1 males, only one was sacrificed *in extremis* with a fractured nose (killed on day 14). From the same number of P1 and F1 females, only three rats died during the study. One was sacrificed *in extremis* on day 119 due to dystocia; one was found dead on day 17 – the cause of death being pyelonephritis and one was sacrificed *in extremis* on day 119 but the cause of morbidity was not established.

For the adult P1 rats there was a small decrease in mean absolute and relative spleen weight (circa 9% reduction compare with controls) in the high dose group (1500 ppm). The effect was statistically significant for females. While there were no significant differences among the males, the trend for a slight reduction in spleen weight at higher doses was evident. None of the other organs weighed for P1 animals showed any effect of treatment. Results are summarised in table 49.

For the F1 weanlings of the high dose group (1500 ppm), small decreases in absolute (9%) and relative (10-11 %) spleen weight were apparent in comparison with controls. None of the other organs weighed for F1 weanlings showed any effect of treatment. Results are summarised in table below.
There were no changes in organ weight among the F1 adults that were considered attributable to treatment

For the F2 weanlings of the high dose group (1500 ppm), small decreases in absolute (10% males, 15% females) and relative (10% males, 15% females) spleen weight were apparent in comparison with controls. The high dose group effects were significantly lower than the controls. None of the other organs weighed for F2 weanlings showed any effect of treatment. Results are summarised in table below.

Table 49: Summary of spleen weights for males and females in P1, and for F1 and F2 weanlings

	Males					**Females**				
Dose concentration (ppm)	**0**	**100**	**500**	**1000**	**1500**	**0**	**100**	**500**	**1000**	**1500**
P1 adults										
Final body weight	595.4	600.1	603.9	599.5	586.8	328.8	332.2	335.8	333.3	331.9
Absolute spleen weight [g]	0.866	0.887	0.892	0.881	0.841#	0.643	0.629	0.639	0.605	0.586#
Relative spleen weight [g/100g bw]	0.146	0.148	0.148	0.147	0.143	0.195	0.190	0.190	0.182	0.177*
F1 adults										
Final body weight	593.5	619.2	600.0	598.5	584.7	326.0	328.2	335.0	332.9	329.2
Absolute spleen weight [g]	0.897	0.887	0.867	0.900	0.841	0.624	0.641	0.632	0.642	0.612
Relative spleen weight [g/100g bw]	0.151	0.143	0.145	0.150	0.145	0.192	0.195	0.189	0.193	0.186
F1 weanlings										
Final body weight	58.3	60.1	60.9	56.6	58.7	54.5	56.8	56.2	53.5	55.3
Absolute spleen weight [g]	0.256	0.290	0.280	0.238	0.232#	0.245	0.283*	0.265	0.236	0.223#
Relative spleen weight [g/100g bw]	0.439	0.477	0.460	0.417	0.394	0.449	0.498*	0.470	0.429	0.401
F2 weanlings										
Final body weight	56.9	59.3	59.2	59.8	57.3	54.6	56.8	56.8	55.3	54.7
Absolute spleen weight [g]	0.253	0.269	0.254	0.252	0.227#	0.254	0.265	0.252	0.243	0.217*
Relative spleen weight [g/100g bw]	0.440	0.451	0.430	0.421	0.397*	0.462	0.465	0.444	0.440	0.396*

considered to be a treatment-related effect (decreased weight).

* Statistically significantly different from controls $p < 0.05$

The small decrease in spleen weight for F1 and F2 weanlings was considered an effect of treatment although weanling spleen weights are highly variable (e.g statistically significant increase in absolute weight (+16 %) for the low dose F1 females). The effect could be considered adverse, in the absence of any confirmatory microscopic examinations. However, the effect may reflect a transient physiological change such as a marginal decrease in sinusoidal dilatation. The pathologist's review of data indicated the ranges for the high dose spleen weights were similar to control ranges. Of 111 weanlings in the high dose group, only 4 had spleen weights that were lower than the control range. There were no treatment-related effects on thymus weight to indicate a test substance related effect on the lymphoid system. Extramedullary haematopoiesis in the livers of control and high dose weanlings was normal suggesting the haematopoietic system was unaffected by treatment.

The other ogan weights showed no changes that were considered attributable to treatment with copper sulphate pentahydrate.

There were no treatment-related changes apparent during necropsy of the P1 adult rats, F1 adults or F1 and F2 weanlings or F1 and F2 pups.
All macroscopic observations in the adult rats, P1 or F1, were within the range of normal background lesions. Among the F1 and F2 weanlings the incidence of gross lesions was low and observations were randomly distributed across control and treated groups. For the F1 and F2 pups, the observations of non-expanded lungs or no milk spot in stomach were considered non-specific lesions that are commonly observed among stillborn pups and were therefore not considered to be an effect of treatment with copper sulphate pentahydrate.

All microscopic findings seen in the P1 adults, F1 adults or F1 and F2 weanlings were considered to be incidental and common background lesions for the strain of rat used in the study. There were no treatment-related histopathological changes in liver, brain or reproductive organs.

Eighteen P1 and nine F1 pairs failed to produce litters. The cause of reproductive failure in 22 of these pairs was not determined. One F1 female had dystocia and in three of the P1 females there was an absence of recent corpora lutea in the ovaries. None of the breeding failures were considered attributable to test substance administration.

Tissue metal concentrations:

Specifically assessments of copper, iron, manganese and zinc concentrations were investigated in liver and brain and plasma for each subset of animals. Results were as follows.

For the P1 males there were no test-substance related changes in copper, iron, manganese and zinc concentrations at any dose level. Plasma samples were not obtained for these animals. There was a decreased in liver iron concentration in the high dose group but this was not considered a treatment effect due to high inter-individual variability and a lack of consistency with the female response and an absence of any dose relationship.

The P1 females dosed at 1500 ppm had a treatment-related increase in liver copper concentration and a decrease in liver iron concentration. Copper and iron levels in the brain were unaffected and there were no changes in manganese or zinc concentrations in liver, brain or plasma.

For the F1 adult males, liver copper concentration was increased in groups dosed at 1000 or 1500 ppm. Compared with the magnitude of similar changes seen in the P1 generation, the effects in F1 males were small but in comparison with controls, some individuals showed a 2-3 fold increase and the effect was considered attributable to treatment. Copper concentrations in brain or plasma were unaffected by treatment at any dose level. There were no treatment-related changes in iron, manganese or zinc concentrations in liver, brain or plasma at any dose level.

For the F1 adult females, liver and brain copper concentrations were increased at 1500 ppm. Copper concentration in plasma was not affected at any dose level. There were no treatment-related changes in iron, manganese or zinc concentrations in liver, brain or plasma at any dose level.

For the F1 and F2 weanlings, there was a treatment-related increase in liver copper concentration for males and females dosed at 1000 and 1500 ppm in each generation. Brain copper concentrations were slightly increased for the males (but not females) dosed at 1500 ppm in each generation. No plasma data were available for the F1 weanlings and there were no changes in plasma copper concentration for the F2 weanlings. A treatment-related decrease in plasma iron concentration was evident for the male and female F2 weanlings dosed at 1500 ppm. Changes in manganese and zinc concentrations in

liver, brain or plasma were all considered to be spurious since they showed no dose relationship, had high inter-individual variability or the changes were small in magnitude.

In summary, (tables 50, 51, 52 and 53), the concentration of copper in the liver of F1 males and F1 and F2 male and female weanlings dosed at 1000 and 1500 ppm was increased. The concentration of copper in the liver of P1 and F1 females dosed at 1500 ppm was also increased. Copper concentrations in the brain were increased for F1 females and F1 and F2 male weanlings dosed at 1500 ppm. The concentration of iron in the liver of P1 females dosed at 1500 ppm was decreased and plasma iron concentration was decreased in F2 male and female weanlings in the 1500 ppm dose group.

Table 50: Summary of tissue concentrations of copper, iron, manganese and zinc in brain, liver or plasma for males and females P1

	Males – P1					**Females – P1**				
Dose concentration (ppm)	**0**	**100**	**500**	**1000**	**1500**	**0**	**100**	**500**	**1000**	**1500**
Copper (ppm)										
Liver	6.44	4.47	5.20	5.60	5.98	4.76	5.30	5.46	5.67	8.73*
Plasma	--	--	--	--	--	1.43	1.36	1.35	1.48	1.38
Brain	3.27	3.46	431	4.98	3.26	3.17	3.41	3.58	2.93	3.38
Iron (ppm)										
Liver	158	143	155	143	128*	150	151	138	150	107*
Plasma	--	--	--	--	--	2.96	2.90	3.24	3.17	3.32
Brain	22.4	24.4	26.2	22.8	24.9	20.4	21.0	20.8	19.9	18.9
Manganese (ppm)										
Liver	2.45	2.26	2.62	2.75	2.34	3.46	3.49	3.20	3.52	3.56
Plasma	--	--	--	--	--	--	--	--	--	--
Brain	0.451	0.525	0.534	0.459	0.573	0.419	0.438	0.411	0.433	0.422
Zinc (ppm)										
Liver	33.1	31.6	33.8	32.4	28.7	28.8	28.5	29.4	30.5	29.2
Plasma	--	--	--	--	--	1.94	1.90	1.93	1.86	1.72
Brain	17.5	16.6	16.8	16.3	16.6	14.7	14.1	14.7	15.4	13.4

* Statistically significantly different from controls $p < 0.05$

Table 51: Summary of tissue concentrations of copper, iron, manganese and zinc in brain, liver or plasma for males and females F1 adults

	Males – F1 adults					**Females – F1 adults**				
Dose concentration (ppm)	**0**	**100**	**500**	**1000**	**1500**	**0**	**100**	**500**	**1000**	**1500**
Copper (ppm)										
Liver	4.56	4.87	6.16	7.36*	7.53*	5.70	5.16	5.36	5.35	15.3*
Plasma	1.24	1.38	1.28	1.51	1.44*	1.49	1.52	1.34	1.50	1.37
Brain	2.59	2.64	2.83	3.11*	2.80	2.89	2.93	3.00	3.23	3.49*
Iron (ppm)										
Liver	121	133	124	143	110	149	149	163	116	133
Plasma	2.41	2.27	2.72	2.35	2.88	3.46	4.00	4.01	3.56	4.12
Brain	18.5	18.4	16.5	17.2	17.5	18.5	18.4	21.2	20.6	19.9
Manganese (ppm)										
Liver	1.93	2.00	2.20	2.14	1.82	3.46	3.18	3.28	3.06	3.64
Plasma	--	--	--	--	--	--	--	--	--	--

Brain	0.355	0.350	0.343	0.376	0.319	0.368	0.394	0.405	0.412	0.438*
Zinc (ppm)										
Liver	26.7	27.8	32.4*	28.3	25.8	31.9	30.7	32.6	27.9	32.2
Plasma	0.971	0.916	0.989	1.019	1.080	1.93	1.87	2.03	1.54*	1.80
Brain	13.3	13.4	14.1	14.0	12.1	14.6	15.1	15.0	15.1	15.4

* Statistically significantly different from controls $p < 0.05$

Table 52: Summary of tissue concentrations of copper, iron, manganese and zinc in brain, liver or plasma for males and females F1 weanlings

	Males – F1 weanlings					**Females – F1 weanlings**				
Dose concentration (ppm)	**0**	**100**	**500**	**1000**	**1500**	**0**	**100**	**500**	**1000**	**1500**
Copper (ppm)										
Liver	14.7	24.2	25.2	50.0*	82.7*	21.5	22.8	23.5	53.1*	86.8*
Plasma	--	--	--	--	--	--	--	--	--	--
Brain	2.26	2.27	2.28	2.44	2.59*	2.43	2.35	2.43	2.40	2.60
Iron (ppm)										
Liver	33.9	33.0	32.0	37.4	36.1	33.6	36.1	38.3	39.5	37.4
Plasma	--	--	--	--	--	--	--	--	--	--
Brain	11.1	15..3	13.1	11.5	11.0	16.6	12.4	13.7	11.3	12.8
Manganese (ppm)										
Liver	2.01	1.95	2.01	2.08	2.27	2.08	2.13	1.96	2.18	2.23
Plasma	--	--	--	--	--	--	--	--	--	--
Brain	0.500	0.503	0.527	0.561	0.565	0.562	0.505	0.522	0.539	0.629
Zinc (ppm)										
Liver	31.4	31.5	35.2	37.4*	36.8*	32.5	31.8	34.3	39.7*	37.3*
Plasma	--	--	--	--	--	--	--	--	--	--
Brain	13.8	13.6	14.1	14.2	14.5	14.1	13.9	14.5	15.4	14.4

* Statistically significantly different from controls $p < 0.05$

Table 53: Summary of tissue concentrations of copper, iron, manganese and zinc in brain, liver or plasma for males and females F2 weanlings

	Males – F2 weanlings					**Females – F2 weanlings**				
Dose concentration (ppm)	**0**	**100**	**500**	**1000**	**1500**	**0**	**100**	**500**	**1000**	**1500**
Copper (ppm)										
Liver	16.4	30.2	28.0	47.6*	64.3*	24.9	21.5	27.9	38.8*	53.5*
Plasma	0.526	0.533	0.582	0.543	0.554	0.581	0.550	0.587	0.573	0.543
Brain	2.55	3.12	2.35	2.49	3.24*	2.59	2.63	2.52	2.41	2.78
Iron (ppm)										
Liver	33.8	37.0	37.1	36.8	29.8	41.8	38.1	39.1	42.2	35.2
Plasma	3.20	3.98	2.78	2.73	1.55	3.21	3.54	3.19	2.54	1.41*
Brain	11.1	11.4	11.8	11.0	10.0	11.6	11.1	12.5	11.4	10.7
Manganese (ppm)										
Liver	2.04	2.03	2.03	2.06	2.24	2.12	1.92	2.21	2.03	2.30
Plasma	--	--	--	--	--	--	--	--	--	--
Brain	0.490	0.535	0.465	0.510	0.570*	0.479	0.555	0.524	0.521	0.570*
Zinc (ppm)										
Liver	30.9	30.3	33.3	29.8	31.2	34.2	27.3*	31.6	33.2	31.7
Plasma	2.07	2.30	2.07	2.42	2.04	2.38	2.19	2.15	1.95	2.18

Brain	14.7	15.2	14.8	14.7	16.5*	15.6	15.1	15.1	15.0	14.5

* Statistically significantly different from controls $p < 0.05$

Overall summary of findings.

There were no effects considered to be related to copper sulphate treatment on the following parameters at any concentration (100 to 1500 ppm):

- Mortality and clinical signs of toxicity in P1 and F1 males and females
- Body weights, weight gain, food consumption, food efficiency in P1 and F1 males and females
- Sperm and estrous cycle parameters in P1 and F1 males and females
- Mating, precoital interval, fertility, gestation length, number of implantation sites, and implantation efficiency in the P1 and F1 generations
- Number of pups born, born alive, alive on day 4, 7, 14, or 21, sex ratio, and survival indices during the lactation period in F1 and F2 litters
- Body weights and clinical observations in F1 and F2 litters during lactation
- Age at preputial separation in F1 males and vaginal opening in F1 females
- Ovarian follicle counts in F1 females
- Weight of testes, epididymides, right cauda epididymis, seminal vesicles, prostate, ovaries, uterus, thyroid gland, brain, liver, adrenal glands, kidneys and pituitary in P1 and F1 males and females; Weight of liver, brain and thymus in F1 and F2 weanlings; Weight of the spleen in P1 males and F1 males and females
- Gross observations in P1 and F1 adults and F1 and F2 weanlings
- Microscopic observations in the liver, brain and reproductive organs in P1 and F1 adults
- Microscopic observations in the liver and brain in F1 and F2 weanlings.

Potentially adverse effects considered to be related to copper sulphate treatment were limited to the 1500 ppm groups and were comprised of:

- Decreased spleen weight in P1 adult females, and F1 and F2 male and female weanlings.

Under the conditions of this study there were no treatment-related effects in either generation (P1 and F1 adults or F1 and F2 offspring) on reproduction parameters or indications of systemic toxicity at any of the dose concentrations used (doses of 100 to 1500 ppm). There were no adverse effects of treatment at up to 1500 ppm on fertility, general reproductive performance or offspring viability and growth at any dose level (dietary levels 0, 100, 500, 1000 and 1500 ppm $CuSO_4$). Dietary intake varied with stage of maturation and effects observed at the high dose may reflect changes in food intake and test substance consumption for the different populations within the study. Actual dosed values were 1.53-2.65, 7.7-13.3, 15.2-26.7 and 23.6-43.8 mg/kg body weight/day, for the 100, 500, 1000 and 1500 ppm groups, respectively. However since young rats consume more diet the mg/kg bw/day exposure is greater at weaning and at the beginning of each maturation phase. Pregnant and lactating females also consume more diet and are subject to a greater mg/kg bw/day exposure.
The concentration of copper was increased in the liver of F1 males and F1 and F2 male and female weanlings at 1000 and 1500 ppm and in P1 and F1 females at 1500 ppm. Brain copper concentration was increased in F1 females and F1 and F2 male weanlings at 1500 ppm. The concentration of liver iron was decreased in P1 females at 1500 ppm. The concentration of plasma iron was decreased in F2 male and female weanlings at 1500 ppm. There were decreased spleen weight in P1 adult females, and F1 and F2 male and female weanlings.
The majority of effects are reported in weanlings and in dams at the end of lactation - the food intake and compound consumption data show that both of these “populations” were consuming significantly

higher amounts of diet than towards the end of the pre-mating maturation periods (the food intake of the weanlings is virtually the same as in the first week of the F1 maturation period, when compound consumption of F1 males is 58 mg Cu/kg/day, for example, and during lactation when the adult females are eating lots to feed their young), but that the spleen effects are not seen in males at termination, when compound consumption is much lower (22.9 mg Cu/kg/day in F1 males). From this it may be concluded that the spleen effects may be transient even at high doses, and that when the dietary intake i.e. dose level is reduced, the spleen effect diminishes. However, while the iron effects and the brain copper effects at 1500 ppm are also probably temporary and related to high dietary intakes (in that the male weanlings showed the finding, but when those weanlings grew older they did not), there is insufficient evidence to support 1500 ppm as a NOAEL.

From these results, the no-observed-effect level (NOEL) for reproductive toxicity was 1500 ppm, the highest concentration tested. The systemic NOEL for P1 and F1 rats and F1 and F2 offspring during lactation was 1000 ppm, based on reduced spleen weight in P1 adult females, and F1 and F2 male and female weanlings at 1500 ppm. The dietary concentration of 1000 ppm was equivalent to mean daily intakes of copper of 15.2 - 23.5 mg/kg body weight/day for male rats during pre-mating and 17.0 - 26.7 mg/kg body weight/day for female rats during pre-mating and gestation.

Reference: De la Iglesia F. W. (1973)
Guideline: No. Cross-mating fertility study
GLP: No

Three groups of 20 female Wistar rats were given copper gluconate orally by gavage at 0, 3 or 30 mg/kg/day for two weeks prior to mating through to either day 20 of pregnancy or day 21 post partum. Females were paired (1m:2f) with untreated males. In a parallel study, two groups of 10 males received copper gluconate at 3 mg/kg/day for 60 days prior to pairing (1m:2f) with either untreated females or females that had received copper gluconate at 3 mg/kg/day for 60 days prior to mating. A further group of 10 males and 20 females were maintained untreated for 60 days and allowed to mate. Parameters investigated included pregnancy rate (percentage of pregnancies), day 20 litter parameters including implantations, resorptions, live foetuses, gross foetal anomalies; litter parameters included duration of gestation, litter size, number of live young, gross anomalies, litter and mean pup weights through to weaning.

There were no significant differences between treated and control groups in any of the parameters studied.

Copper gluconate did not affect the fertility of either the male or female rat, following oral administration. This is discussed further at the end of this section.

Reference: Lecyk, M. (1980)
Guideline: No
GLP: No
Deviations: Yes (from OECD 414)

- Housing and feeding conditions of test animals,
- information on the age and weight of test animals,
- no detail given on the size of the groups,

- in several dose groups, the number of pregnant animals was smaller than recommended by the guideline (16 animals).
- in the absence of information on the weight of test animals and the weight of treated diet consumed, it was not possible to accurately determine the dose received on a mg/kg bodyweight basis,
- no information on maternal toxicity was presented in the report,
- no post-mortem information was presented in the report for dams,
- no information was presented on: the weight of gravid uteri; the number of corpora lutea; degrees of resorption of dead foetuses,
- the sex ratio of live foetuses was not reported,
- no justification is provided for use of mouse whereas the preferred rodent species is rat for this study,
- males were fed the appropriate test diet prior to mating and no information on male toxicity was then reported,
- the study did not measure maternal bw gains or maternal liver histology or copper content.

Copper sulphate was administered to groups of male and female mice, strains C57BL and DBA, by admixing the aqueous solution with the diet at dose levels of 0, 500, 1,000, 1,500, 2,000, 3,000 and 4,000 ppm corresponding approximately to 0, 71, 142, 214, 285, 427 and 570 mg/kg bw/day . The feed was granulated and dried before administering to the animals. The males and females were paired after one month of treatment and the day of mating (appearance of a vaginal plug) was designated Day 0 of gestation. On Day 19 of gestation the females were killed and foetuses (living and dead) were counted and weighed. One half of the foetuses in each group was examined for visceral abnormalities (Wilson technique) and the other half was cleared and stained with alizarin for skeletal examination.

Although the paper does not give details of group size and pregnancy rate, from the numbers of pregnant females (particularly at 4000, 3000 ppm), pregnancy rate was not adversely affected by dietary administration of copper at up to 4000 ppm for one month prior to mating (table below).

In both strains of mice, there was no effect on the embryonic growth at the lower doses, 2,000 ppm and below. The authors claimed a slight stimulation indicated by lower % foetal mortality and slightly higher weights of the foetuses than the controls at doses up to 2000 ppm. A treatment-related effect was noted at higher levels, at 3,000 and 4,000 ppm, where decreased foetal weights and a higher mortality were recorded (table 54). It should be noted that mean litter size was smaller than normal for the mouse in all groups. Various development malformations were observed in both these groups in both strains, although there was no consistent pattern of type. Abnormalities classed by the authors as malformations at 3000 ppm (3 foetuses in total) were last lumbar vertebra included in sacrum (one foetus) and unilateral fused rib (two foetuses); at 4000 ppm, hernia of the thoracic wall, hydrocephalus and fusion of thoracic ribs and vertebrae, (each one foetus, two foetuses with encephalocoel and two foetuses with (last lumbar) hemivertebra as part of sacrum. However, as no information was presented in this study regarded maternal toxicity, the possibility that the effects on embryonic development were secondary to maternal toxicity cannot be excluded.

Table 54: Mouse embryonic development

	Dose level (ppm)						
	0	**500**	**1000**	**1500**	**2000**	**3000**	**4000**
C57BL mice							
Number of pregnant females	21	10	18	7	10	22	18
Number of live foetuses (%)	65 (83.1)	46 (89.2)	81 (86.5)	31 (87.1)	42 (78.6)	55 (72.8)	35 (71.5)
Number of dead foetuses (%)	11 (16.9)	5 (10.8)	11 (13.5)	4 (12.9)	9 (21.4)	15 (27.2)	10 (28.5)
Mean litter size	3.09	4.60	4.50	4.42	4.20	2.50	1.94
Mean foetal weight (g)	1.10	1.35	1.22	1.14	1.25	1.00	0.99
Abnormal foetuses (%)	-	-	-	-	-	1 (1.8)	3 (8.5)
DBA mice							
Number of pregnant females	17	10	10	14	10	18	20
Number of live foetuses (%)	76 (84.3)	54 (90.8)	51 (88.3)	58 (82.8)	41 (83.0)	56 (75.0)	45 (70.4)
Number of dead foetuses (%)	12 (15.7)	5 (9.2)	6 (11.7)	10 (17.2)	7 (17.0)	14 (25.0)	16 (29.6)
Mean litter size	4.47	5.40	5.10	4.14	4.10	3.11	2.70
Mean foetal weight (g)	0.96	1.24	1.19	1.17	1.13	1.11	1.09
Abnormal foetuses (%)	-	-	-	-	-	2 (3.7)	4 (7.4)

Dietary administration of 3,000 and 4,000 ppm copper as sulphate (approximately equivalent to dose levels of 430 and 570 mg/kg bw/day, using the US FDA conversion factor of 7 for mice) for one month prior to pairing did not adversely affect mating performance or pregnancy rate but caused an increase in foetal mortality, decreases in foetal weights and slight increase in incidence of malformations.. It should be noted that the study did not measure maternal bodyweight gains, or maternal liver histology or copper content. The NOEL for fertility effects was greater than 4,000 ppm (approximately 570 mg/kg bw/day) and the NOEL for foetal effects was 2,000 ppm (approximately 285 mg/kg bw/day).

4.10.1.2 Human information

Reference: Ralph, A. and McArdle, H. (2001)
Guideline: No
GCP: No

The publication is a review of data on copper metabolism and toxicity during pregnancy and lactation, with emphasis on the human.

The review considers the following aspects:

Fertilisation: Copper metal is known to interrupt implantation and development of the blastocyst when present in the uterus as an intra-uterine contraceptive device (IUD), but once implantation has taken place, IUDs do not show adverse effects on maintenance of pregnancy.

Maternal serum copper levels and ceruloplasmin levels rise steadily throughout pregnancy, and fall significantly at parturition. The concentration in the mother is higher than in the foetus, which establishes a concentration gradient from the mother to the foetus. The rise in plasma concentration may be due to either enhanced uptake from food or decreased biliary excretion. It is induced by oestrogen. Various studies have shown that copper requirements of pregnant humans are up to one third greater than non-pregnant human females. Copper and ceruloplasmin are present in amniotic fluid, but uptake from amniotic fluid by the foetus is small. The placenta has been shown to take copper from the maternal blood as both ceruloplasmin and by lower-weight complexes (albumin, histidine), but that delivery by ceruloplasmin is more efficient. Ceruloplasmin is not itself passed across the placenta, but ceruloplasmin and histidine may deliver copper to the placental cells via specific cell surface receptors. The placenta has a regulatory role on the transfer of copper from mother to baby, as infant serum concentrations of copper do not correlate with those of the mother. This has been demonstrated in both human and rat. Women with Wilson's disease can give birth to healthy babies if the condition is well managed (zinc sulphate therapy). Pregnant women with untreated Wilson's disease tend to have spontaneous abortions. In the Brewer study (2000), of 26 pregnancies in 19 women who were on zinc therapy throughout their pregnancy, 24 new-borns were normal, one had a heart defect (corrected by surgery) and another showed anencephaly. Anencephaly has also been associated with very low maternal copper serum levels, and there have been two reported cases of anencephaly where an IUD was used (Graham et al. 1980).

Foetal development: copper accumulates in the placental layers and is transferred to the foetus by an active process driven by foetal needs; it is thought to be incorporated in the foetal liver into foetally synthesised ceruloplasmin. Copper is present in the foetal circulation in ceruloplasmin, albumin, α-fetoprotein, transcuprein and low molecular weight ligands. The human foetus accumulates copper at a rate of 50 μg/kg/day during the latter half of pregnancy, and 50% of it is stored as metallothionein in the liver. The ratio of copper in the liver of newborn infants to adults is 15:4. There are no reports of adverse effects of acute toxicity of copper in human pregnancy. Foetal copper accumulation occurs in the third trimester, and premature and low-weight babies are at risk of copper deficiency. Studies indicate that the capacity of pre-term infants to utilise copper from the diet is limited; most of the ingested copper is present in the stool, indicating either ineffective absorption or limited ability to retain and store copper.

Parturition. Serum maternal plasma level returns to normal in the human within two to five weeks. The timing of the return to normal may be influenced by the duration of breast-feeding.

Lactation. Ceruloplasmin occurs in the milk of humans and other mammals, concentrations being higher in the early stages of lactation. Approximately 20-25% of copper in human milk is present as ceruloplasmin. Breast-feeding supplies up to 60 μg/kg/day, and is approximately 24% bioavailable. Maternal copper blood levels are under hormonal control (e.g. oestrogen, see above), but alterations in maternal copper intake through dietary supplementation, or elevated blood levels through other factors, such as severe infections, and even Wilson's disease, do not alter copper content of breast milk. It is likely that there are homeostatic mechanisms that regulate mammary gland uptake of copper and its secretion in milk, but these have not been explained. In human breast milk, approximately 75% of the copper is in the whey, bound to soluble albumin or low molecular weight ligands. Another 15-20% is in lipids, bound to the outer fat globule membrane, and about 5% is in insoluble form, possibly bound to casein. Differences in composition of other milks (cow, soy) affect the bioavailability to the human baby. Absorption and retention rates from formula milks are very low, although toxicity has been observed where infants have been given substantial amounts of cow's milk boiled in untinned copper vessels. Awareness of the disease in India [Indian Childhood Cirrhosis] and Austria [Idiopathic Copper Toxicosis] has resulted in use of other containers, and the incidence has fallen. Human milk, unsurprisingly, contains the most bioavailable copper for the human baby.

Healthy infants fed exclusively on cow's milk for 6 months became copper deficient, but the condition reversed on weaning to solid foods.

Growth and development. Neonatal humans have high concentrations of copper in the liver and low concentrations of serum copper and ceruloplasmin. Newborn humans also show high concentrations of metallothionein that decrease after birth. Copper in the new-born's liver appears to provide much of the copper requirements of the infant while it is breast-fed, until weaning at 4-6 months. However, milk must provide a significant contribution, as mice showing 'toxic milk mutation' die if they are kept on mother's milk, because the mother cannot secrete the normal amounts of copper into the milk, and the pups die of copper deficiency. Premature birth restricts the hepatic storage of copper (as the mother's supply via the placenta is no longer available), and milk formulae for premature infants contains additional copper to compensate for this. Low copper levels at this time may have neurological implications during the critical period of brain growth. Excess copper in drinking water at concentrations of approximately 8 mg/L showed chronic toxicity in adults but not in children under 6 years of age. As the infant grows, levels of ceruloplasmin increase. Studies in rats show that copper absorption is high during the neonatal period, but decreases by weaning, as more is retained in the intestinal mucosa. With increasing postnatal age, more is transported to the liver and less is bound to the intestine. There is evidence in rats that during lactation, intestinal copper absorption occurs by diffusion and solvent drag, and only after weaning does a saturable (adult, see Section B.6.1) copper transport system become evident. Children require higher levels of copper in the diet than adults, especially during periods of rapid growth. Girls aged 6-10 were fed on diets of copper ranging from 1.1 to 3.8 mg/day. At intakes under 2 mg/day, copper balance was negative. A positive copper balance was achieved on a vegetarian diet with a copper intake of over 2.8 mg/day. It was suggested that an intake of 1.3 mg/day was sufficient for equilibrium, but that 2.5 mg/day was necessary for growth. Serum of normal children reaches a peak of 1.57 mg/L between 6 and 11 years and falls to 1.1 mg/L in adults between 22 and 75 years.

Intake: the review found no evidence of copper toxicity from customary dietary intake, unless the food had been accidentally contaminated with copper during preparation e.g. acid fruit such as apples, were stewed in a copper vessel, or there was repeated ingestion of milk heated in copper vessels. A study of three cities in the US state of Massachusetts showed no incidence of ill-health in adults or children under 6 years of age, despite drinking water concentrations of over 8 mg/L. Most dietary intakes are below the 10-12 mg/adult/day set by international organisations.

There is no evidence for adverse effects of oral exposure through customary diets worldwide (which includes countries where copper is used in agriculture) for any adverse effects of copper on pregnancy, parturition, lactation or growth and development in the human. There is evidence of toxicity particularly to neonates repeatedly exposed to milk heated in copper vessels, or exposure to acid fruit stewed in copper vessels.

4.10.2 Developmental toxicity

4.10.2.1 Non-human information

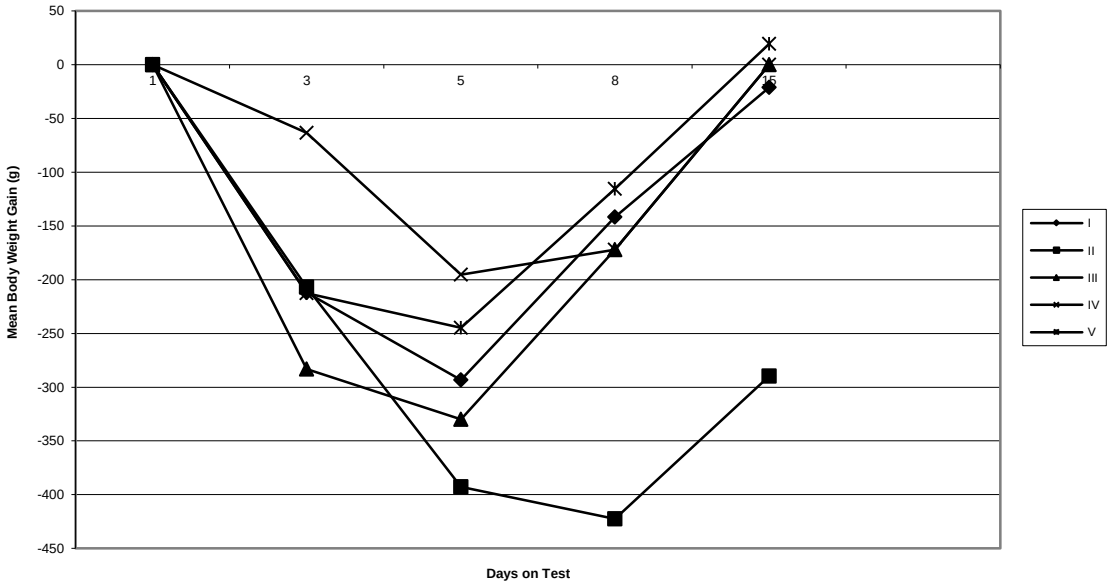
Reference: Munley S. M. (2003a)
Guideline: No. Range-finding study designed to assess relative tolerance of five technical copper substances in the rabbit.
GLP: Yes

Five technical materials, copper hydroxide (batch number 380-71-05, copper content 60.1% w/w), copper oxychloride (batch number 27003B, copper content 57% w/w), Bordeaux Mixture (batch

number 1/170), copper content 26.38% w/w), tribasic copper sulphate (batch number 471/2002, copper content 31.12% w/w), and copper (I) oxide, (batch number 280802, copper content 87% w/w) were given orally by gavage as suspension in 0.5% aqueous methyl cellulose in deionised water to non-pregnant female Hra:(NZW)SPF rabbits. The animals were approximately 6 to 6.5 months old and weighed from 3382 g to 4116 g on the day after arrival. Animals were quarantined for at least 5 days before dosing. Rabbits were housed individually, and were given 150 g laboratory rabbit diet each day, and tap water *ad libitum*. Food consumption and bodyweight were recorded daily, clinical observations were recorded daily and post dosing. Dose formulations were prepared daily at a dose volume of 1 mL/kg bw, based on that day's bodyweight. Any animals found dead were necropsied. At termination, all animals were given a gross external and visceral examination. Lesions were retained in an appropriate fixative. In the first part of the study, groups of two rabbits were dosed with each technical material for up to 14 days. Concentrations were calculated to give 30 mg as copper /kg bw/day. In view of the moderate toxicity seen at 30 mg Cu/kg bw/day, doses of 50 mg Cu/kg bw/day were given to a further group of 2 rabbits per technical substance, to assess tolerance to a higher dose. Mortalities occurred after the first dose and surviving rabbits were given 40 mg Cu/kg bw/day for the remaining six days of administration.

Animals at 30 mg Cu/kg bw/day showed bodyweight loss during the first half of the treatment period, followed by recovery during the second week of treatment (Figure 6.7.3.1.1.). There were no marked differences between the five technical substances. Food consumption reflected bodyweight changes; during the first week of dosing animals showed marked reductions in food consumption, and in the second week the animals generally resumed eating.

Figure 1: Bodyweight change with five forms of copper at 30 mg/kg/day



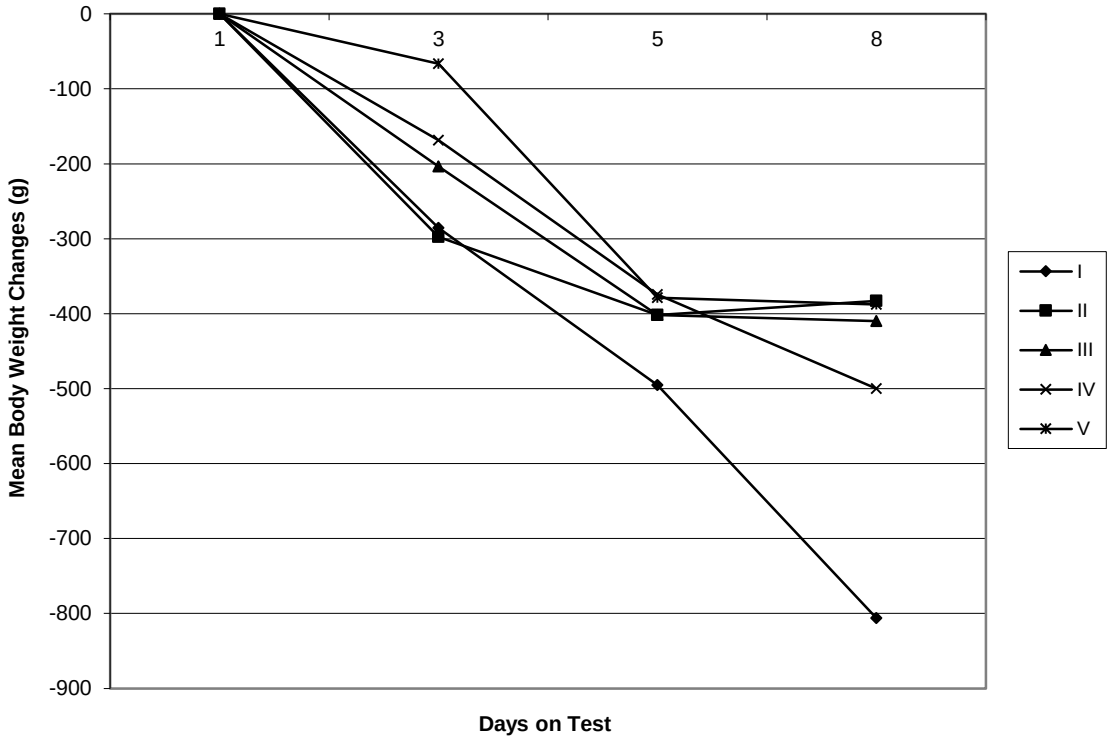
I: copper hydroxide; II: copper (I) oxide; III: copper oxychloride; IV: tribasic copper sulphate; V: Bordeaux mixture

There were no deaths among animals treated with copper hydroxide, Bordeaux mixture, tribasic copper sulphate or copper oxide. One animal dosed with copper oxychloride was found dead on day 2. There were no indications of any adverse effects of treatment, or of dosing error, and this animal

was replaced by a similar animal from the same batch. A second animal dosed with oxychloride died on day 11. The animal showed no remarkable necropsy findings other than fur staining. During the study, it was discovered that the two animals dosed with tribasic copper sulphate were underdosed by approximately 40%, because of a calculation error. These animals were also replaced by two similar animals, and the food/bodyweight data from the underdosed animals was not used in the comparison of the five substances. Three animals were inadvertently sacrificed prematurely in the second week of treatment. Necropsy revealed various stomach findings, including ulceration, red or dark discolouration, and haemorrhagic areas in one animal dosed with copper hydroxide, both animals dosed with copper oxide, and three of the four animals dosed with tribasic copper sulphate.

At 50 mg Cu/kg bw/day, one of the two animals died after the first dose in each group except tribasic copper sulphate. The Study Director immediately reduced the dose concentration to 40 mg Cu/kg bw/day (i.e. from day 2) and there were no further deaths. All decedents showed either stomach ulceration or dark discolouration and thickening of the non-glandular portion of the stomach. Survivors at 40 mg Cu/kg bw/day showed weight loss (Figure 6.7.3.1.2) and reduced food consumption. At termination, all survivors showed stomach ulcerations.

Figure 2: Bodyweight change with five forms of copper at 50/40 mg/kg/day



I: copper hydroxide; II: copper (I) oxide; III: copper oxychloride; IV: tribasic copper sulphate; V: Bordeaux Mixture

The general pattern and degree of inappetance and weight loss followed by recovery, and the observation of stomach ulceration at necropsy was considered sufficient to show that there were no major differences in the sensitivity of the rabbit to the five copper substances. Doses greater than 30 mg Cu/kg bw/day were considered unsustainable for repeat dosing studies. As there were no major differences between the five substances, further preliminary investigations would be performed on only one substance, copper hydroxide.

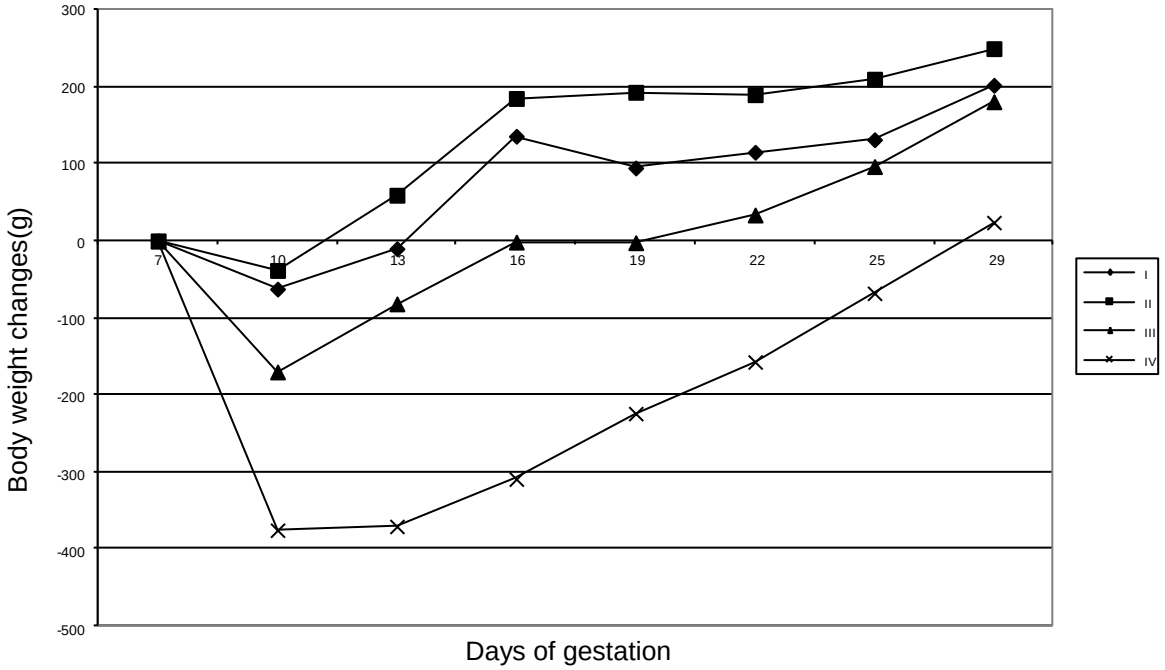
Reference: Munley S. M. (2003 b)
Guideline No. Range-finding study designed to assess effect of treatment equal in duration to a teratology study in the non-pregnant rabbit.
GLP: Yes

Technical copper hydroxide (batch number 380-71-05, copper content 60.1% w/w) was given orally by gavage as suspension in 0.5% aqueous methyl cellulose in deionised water to groups of five non-pregnant female Hra:(NZW)SPF rabbits for 23 consecutive days. Dose levels were 0, 7.5, 15 or 30 mg Cu/kg bw/day. Analysis of dose formulations confirmed stability, homogeneity and verified the accuracy of formulation. The animals were approximately 6 to 6.5 months old and weighed from 2936 g to 3748 g on the first day of dosing. Animals were quarantined for at least 5 days before dosing. Rabbits were housed individually, and were given 150 g laboratory rabbit diet each day, and tap water *ad libitum*. Food consumption and bodyweight were recorded daily, clinical observations were recorded daily and post dosing. Dose formulations were prepared daily at a dose volume of 1 mL/kg bw, based on that day's bodyweight. Any animals found dead were necropsied. At termination on day 24, all animals were given a gross external and visceral examination. Lesions were retained in an appropriate fixative.

There were two deaths at 30 mg Cu/kg bw/day, on day 2 and 3 respectively. The latter animal showed lethargy, weakness and abnormal gait or mobility prior to death. Both decedents showed haemorrhages and/or discolouration of the stomach lining. There were no deaths at 15 mg Cu/kg bw/day. Two animals at 7.5 mg Cu/kg/bw/day died on days 2 or 5 due to intubation errors. Necropsy findings included punctured lung tissue.

Bodyweights and food consumption at 30 and 15 mg Cu/kg bw/day were lower than controls from the start of treatment. There was a group mean bodyweight loss during the first week of treatment, with recovery to initial mean values by day 19 in both groups (Figure 6.7.3.2.1). Food consumption and bodyweight gains at 7.5 mg Cu/kg bw/day were not adversely affected by treatment.

Figure 3: Bodyweight change of females



I: Control; II: 7.5 mg/kg/day; III: 15 mg/kg/day; IV: 30 mg/kg/day

Necropsy findings in animals surviving to termination were limited to haemorrhages and/or discolouration of the stomach lining in one animal at 30 mg Cu/kg bw/day.

Treatment at 30 and 15 mg Cu/kg bw/day was associated with initial inappetance and bodyweight loss, followed by recovery. There were two deaths at 30 mg Cu/kg bw/day. Necropsy findings in decedents and one animal at 30 mg Cu/kg bw/day included to haemorrhages and/or discolouration of the stomach lining. There were no adverse effects of treatment at 7.5 mg Cu/kg bw/day.

Reference: Munley S. M. (2003c)
Guideline: No. Range-finding study in the pregnant rabbit.
GLP: Yes

Technical copper hydroxide (batch number 021121/1, copper content 61.14% w/w) was given orally by gavage as suspension in 0.5% aqueous methyl cellulose in deionised water to groups of five time-mated female Hra:(NZW)SPF rabbits during days 7 to 28 of pregnancy. Dose levels were 0, 7.5, 15 or 30 mg Cu/kg bw/day. Analysis of dose formulations confirmed stability, homogeneity and verified the accuracy of formulation. The animals were approximately 6 to 6.5 months old and weighed from 2885 g to 4330 g on the day of mating, which was defined as day 0 of pregnancy. Initial group size was five, but intubation errors resulted in deaths in treated groups; the dead animals were replaced with similar time-mated does from the same supplier. Group sizes were thus 5, 8, 9 and 8. Animals were quarantined for at least 5 days before dosing. Rabbits were housed individually, and were given 150 g laboratory rabbit diet each day, and tap water *ad libitum*. Food consumption and bodyweight were recorded daily from day 4. Clinical observations were recorded daily and post dosing. Dose formulations were prepared daily at a dose volume of 1 mL/kg bw, based on that day's bodyweight. Any dams dying prior to planned termination were necropsied and pregnancy status was assessed. At termination on day 29, all surviving animals were given a gross external and visceral examination.

Lesions were retained in an appropriate fixative. The gravid uterus was weighed, and corpora lutea were counted. Numbers of live and dead foetuses, early and late resorptions were recorded. Live foetuses were euthanased, weighed and examined externally. Any dams dying prior to planned termination were necropsied and pregnancy status was assessed.

There were two deaths at 30 mg Cu/kg/ bw/day. One dam was sacrificed *in extremis* on day 9. Necropsy revealed stomach haemorrhages. Subsequent histopathology indicated a haemolytic event that resulted in haemoglobin nephropathy and probable renal failure, consistent with acute copper toxicity. The second female was found dead on day 26; necropsy revealed a small liver and moderate autolysis.

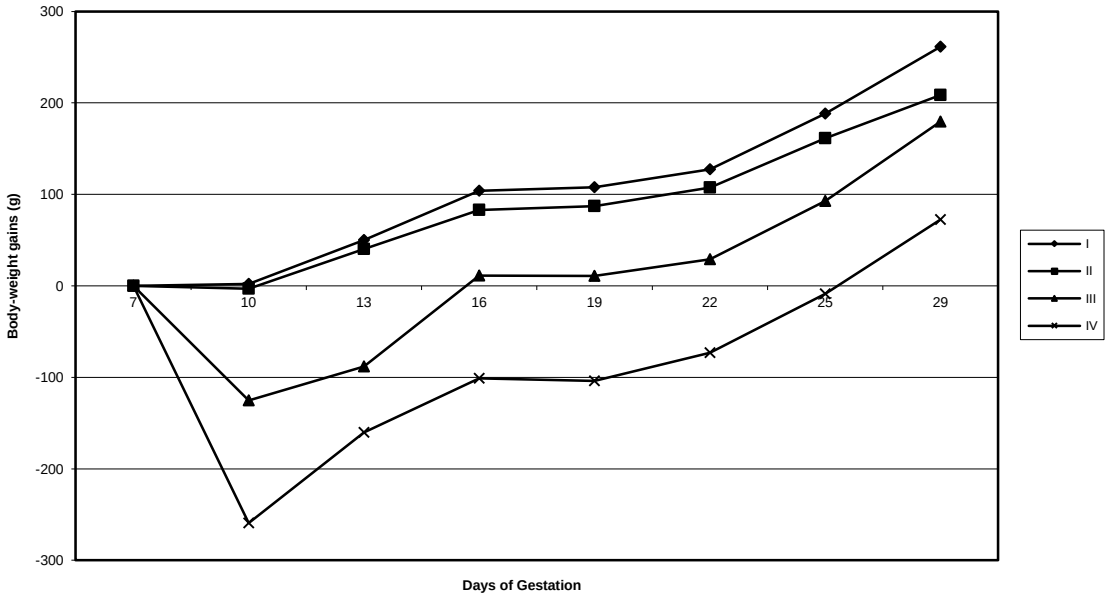
Five other animals (two each at 7.5 and 15 mg Cu/kg/ bw/day, and one at 30 mg Cu/kg/ bw/day) were either accidentally killed or were found dead as a result of intubation injuries. These deaths were not considered treatment-related. These animals were replaced on study with similar time-mated does from the same supplier.

Clinical observations were limited to low incidence of diarrhoea that was considered not to be related to treatment.

There were clear bodyweight losses and reduced food consumption at 15 and 30 mg Cu/kg/ bw/day (Figure 6.7.3.3.1). At 30 mg Cu/kg/ bw/day, overall bodyweight gain during the treatment period was reduced by 88% relative to the control group. Food consumption was also markedly reduced, being 44% lower than controls. At termination, mean bodyweight was 9% lower than controls. Similar but less pronounced effects were noted at 15 mg Cu/kg/ bw/day, where overall bodyweight gain and food consumption were 11% and 22% lower than controls, respectively.

Bodyweight gain and food consumption at 7.5 mg Cu/kg/ bw/day were not adversely affected by treatment.

Figure 4: Bodyweight change of dams



I: Control; II: 7.5 mg/kg/day; III: 15 mg/kg/day; IV: 30 mg/kg/day

Mean foetal weight at 30 mg Cu/kg/ bw/day was reduced by 12% relative to the control group.

Incidence of total resorptions was slightly increased (1.3 ±0.5 versus 0.3 ±0.5 in controls) and there were four foetuses (2 from 2 litters) with onphalocoele. These foetuses tended to be very low weight and immature (e.g. the two foetuses from one dam weighed only 28.95 g and 20.86 g respectively, compared to mean control foetal weight of 41.28 g). Omphalocoele is protrusion of the intestines at the umbilicus. During development, the intestines are contained within the membranes of the peritoneum and amnion. As the foetus matures, the body wall gradually encloses the abdominal cavity and the membrane-bound intestines effectively withdraw into the body, until by late gestation, the body wall has reached the umbilicus. Omphalocoele can occur in low-weight foetuses as a consequence of foetal immaturity secondary to marked maternal weight loss.
Litter parameters at 15 mg Cu/kg/ bw/day were similar to controls, and there were no malformed foetuses. There was one foetus at 7.5 mg Cu/kg/ bw/day with anasarca, domed head and short tail.

Treatment at 30 mg Cu/kg/ bw/day was associated with death and necropsy findings consistent with acute copper toxicity, marked maternal bodyweight loss and reduced food consumption, reduced mean foetal weight and foetal defects consistent with immaturity. Treatment at 15 mg Cu/kg bw/day was also associated with maternal bodyweight loss and reduced food consumption, but litter parameters were not adversely affected by maternal treatment. There were no adverse effects of treatment at 7.5 mg Cu/kg bw/day.

Reference: Munley S. M. (2003d)
Guideline: OECD 414
GLP: Yes
Deviations None

Technical copper hydroxide (batch number 021121/1, copper content 61.14% w/w) was given orally by gavage as suspension in 0.5% aqueous methyl cellulose in deionised water to groups of 22 time-mated female Hra:(NZW)SPF rabbits during days 7 to 28 of pregnancy. Dose levels were 0, 6, 9 or 18 mg Cu/kg bw/day. Analysis of dose formulations confirmed stability, homogeneity and verified the accuracy of formulation. The animals were approximately 5 months old and weighed from 2988 g to 4412 g on the day of mating, which was defined as day 0 of pregnancy. Animals were quarantined for at least 5 days before dosing. Rabbits were housed individually, and were given 150 g laboratory rabbit diet each day, and tap water *ad libitum*. Food consumption and bodyweight were recorded daily from day 4, clinical observations were recorded daily and post dosing. Dose formulations were prepared daily at a dose volume of 1 mL/kg bw, based on that day's bodyweight. Any dams dying prior to planned termination were necropsied and pregnancy status was assessed. At termination on day 29, all surviving animals were given a gross external and visceral examination. Lesions were retained in an appropriate fixative. The gravid uterus was weighed, and corpora lutea were counted. Numbers of live and dead foetuses, early and late resorptions were recorded. Live foetuses were euthanased, weighed and examined for external and visceral alterations. The eyelids of each foetus were removed to allow examination of the eyes. Foetal sex was recorded during visceral examination. The skull was part-sectioned between the parietal and frontal bones to allow inspection of the brain. After examination, foetuses were eviscerated, fixed in alcohol and stained with Alizarin red S for skeletal examination.

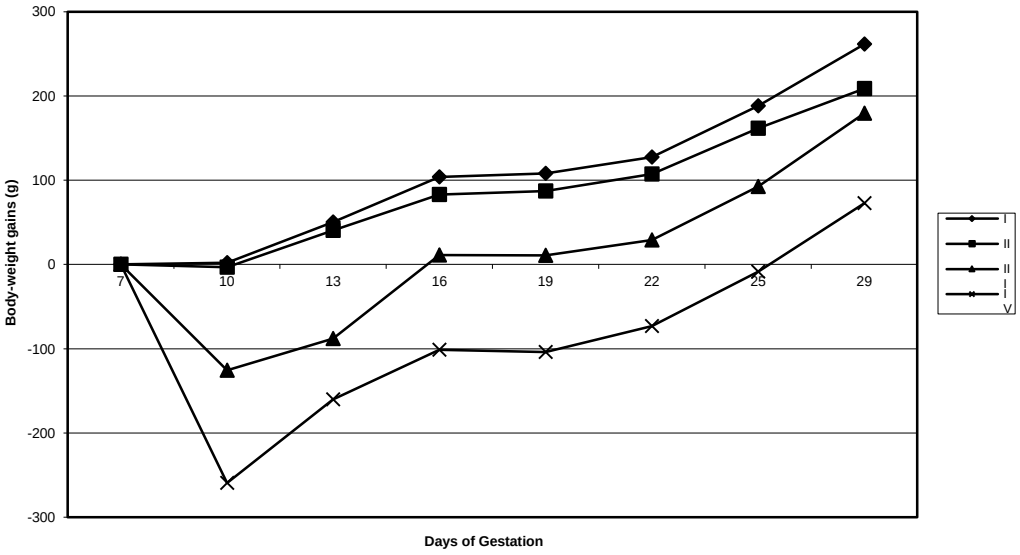
There were three deaths and two females with abortion (subsequently sacrificed) at 18 mg/kg bw/day. The dead animals were found on days 9, 10 and 16, and the aborted animals were killed on day 22 of pregnancy. One of the animals found dead showed diarrhoea, red staining of under-cage board, weakness and irregular respiration prior to death. The other two animals appeared normal prior to death, but all three showed necropsy findings including stomach haemorrhage and/or ulceration, dark discolouration or mottling o flung tissue, pale liver, gelatinous tan rectal discharge and brown liquid in the chest cavity. One of the animals showing abortion had diarrhoea. Necropsy of the other aborted

animal showed red discoloured stomach lining. Abortion in mid to late pregnancy is observed in rabbits that show marked inappetance and weight loss. One other female at 18 mg/kg bw/day was killed following intubation injury on day 15 of pregnancy. Necropsy findings included stomach haemorrhage and evidence of intubation injury to lung tissue.

There were no substance-related deaths among animals dosed at 9 or 6 mg/kg bw/day. One female at 6 mg/kg bw/day aborted on day 27of pregnancy. This was not considered to be related to treatment, as there were no abortions at 9 mg/kg bw/day, and the abortion occurred later than those at 18 mg/kg bw/day. Single instances of abortion in late pregnancy are not uncommon in groups of pregnant rabbits.In addition to clinical observations noted previously for decedents, occasional animals in all groups showed alopecia. This was not considered treatment-related. One control animal, and 6, 2 and 7 animals at 6, 9 and 18 mg/kg bw/day showed one or more daily records of diarrhoea.

Group mean bodyweight data showed marked initial weight losses at 18 and 9 mg/kg bw/day during the initial part of the treatment period, followed by part-recovery during middle and late pregnancy (Figure 6.7.3.4.1.). At termination, mean weight gain of animals at 9 mg/kg bw/day was 31% lower than controls, and mean weight gain of animals at 18 mg/kg bw/day was 72% lower than controls. Group mean bodyweight gains at 6 mg/kg bw/day were marginally lower than controls.

Figure 5: Bodyweight change of dams



I: Control; II: 6 mg/kg/day; III: 9 mg/kg/day; IV: 18 mg/kg/day

Group mean food consumption was consistent with bodyweight data: animals at 9 and 18 mg/kg bw/day showed marked inappetance during the initial part of the treatment period. At 18 mg/kg bw/day animals showed reduced food consumption throughout the remainder of the study, but at 9 mg/kg bw/day, food consumption during the latter half of the study was only slightly below controls. Total food consumption at 9 and 18 mg/kg bw/day was 17 and 30% lower than in the control group. Food intake at 6 mg/kg bw/day was marginally lower than controls.

Pregnancy rate was high. The number of litters available for examination was lower at 18 mg/kg bw/day because of the deaths and animals with abortion. One female at 6 mg/kg bw/day showed total resorption, but as this was a single incidence, and there were no similar findings at higher dose levels, this is considered unrelated to treatment (table below).

Table 55: Summary of adult performance

Number of females:	Dose level (mg/kg bw/day)			
	0	**6**	**9**	**18**
Mated	22	22	22	21
Pregnant	21	21	21	21
Aborted (killed)	0	1	0	2
Found dead	0	0	0	3
Intubation error (killed)	0	0	0	1
Total resorptions	0	1	0	0
With live young	21	19	21	15

The number of foetuses, and numbers of early and late embryonic deaths were not adversely affected by maternal treatment (table below). Mean foetal weight was slightly lower at 18 mg/kg bw/day (9% lower than controls). The difference from control was considered treatment-related, but it was not statistically significant.

Table 56: Group mean litter data

Group Mean Litter parameter	Dose level (mg/kg bw/day)			
	0	**6**	**9**	**18**
Corpora lutea	10.0	10.2	9.1	10.1
Number of implantations	8.8	9.0	7.8	9.0
Early embryonic death	0.8	0.7	0.2	0.4
Late embryonic death	0.1	0.3	0.0	0.2
Total embryonic death	1.0	1.0	0.2	0.6
Number of live young	7.9	8.0	7.6	8.4
Percent males in litter	48	58	50	48
Mean foetal weight (g)	42.95	41.71	43.93	38.91
Number with malformations	1	1	0	2

There was a total of four foetuses with malformations: one control foetus showed fused ribs, one foetus at 6 mg/kg bw/day showed ectopic kidney, and two foetuses (from separate litters) at 18 mg/kg bw/day showed hemivertebra (table below). These malformations were considered spontaneous and unrelated to treatment.

Table 57: Incidence of foetal variations

Variation	Dose level (mg/kg bw/day)			
	0	**6**	**9**	**18**
Number examined	165	152	159	126
Developmental				
External	0	0	0	0
Visceral	0	0	0	0
Head	0	0	0	0
Extra rib (%)	105 (64)	102 (67)	127 (80)	110 (87)
Fused sternebrae	1	2	0	0
Retardation				
Kidney small papilla	2	6	6	2
Ossification mandible	0	0	0	1
Ossification pelvis	0	1	1	2
Ossification skull	0	0	1	5

Ossification sternebrae	65	60	76	51
Ossification vertebrae	1	0	0	0
Total (%) with retardation	68 (41)	67 (44)	80 (50)	57 (45)
Total (%) with variations	125 (76)	124 (82)	143 (90)	118 (94)

Percentage values calculated from group totals, not from means of individual litter percentages

There was a slight increase in incidence of foetuses at 18 mg/kg bw/day with retarded ossification of skull and pelvic bones. However, there was no correlation with foetal weight, and the biological significance of such a slight increase is uncertain, as there was no increase in the incidence of retarded sternebral ossification. Retarded sternebral ossification is a more common indicator of foetal immaturity. Rib alterations occurred at a very high incidence across all groups in this study; almost all litters were affected. The biological significance of an increase in incidence of a very common finding is uncertain.

Administration of copper to pregnant rabbits at 18mg/kg bw/day was associated with marked initial bodyweight loss, inappetance, abortion and death. Pups in litters from surviving dams showed slightly lower mean foetal weight, and slightly increased incidence of a common skeletal variant. Maternal treatment at 9 mg/kg bw/day was associated with initial bodyweight loss and inappetance; pups also showed slightly increased incidence of a common skeletal variant, but mean foetal weights were not adversely affected. Maternal administration of copper hydroxide was not associated with increased incidence of foetal malformations, pre-implantation losses, or foetal (embryonic) deaths. The maternal and foetal no observed effect level was 6 mg/kg bw/day, based on maternal weight loss, inappetance, and an increased incidence of a common skeletal variant in foetuses at 9 mg/kg bw/day.

Comments:

- Copper hydroxyde appears to be more toxic in rabbits than in any other animal species.
- The study suffered of some events, including errors of intubation.
- The nutrition of the rabbit depends on bacterial digestion of cellulose, where the vegetation which forms the bulk of the diet is broken down by bacteria in the caecum to form sugars. These are ejested as soft faeces, and immediately eaten, a process known as refection or copography. Copper is known to have bacteriostatic/bactericidal activity, and oral administration of copper will affect the activity of the caecal bacteria, compromising the efficiency of the digestive process and effectively reducing the calorific intake of the rabbits, resulting in nutritional impairment. Metabolism studies show that copper is excreted in the bile, and copper from biliary excretion and any unabsorbed copper are excreted in faeces. Copper is an element; it is stable. Copper present in faeces will be taken in orally during coprophagy , so that the rabbit will have an extra dose of a stable material such as copper from its own faeces.

It is therefore impossible to quantify the actual daily oral dose, because the total oral intake is significantly more than the administered dose, and the study NOEL is too conservative. It can be concluded that the rabbit NOEL is not appropriate for establishing human risk assessment endpoints for copper.

Reference: De la Iglesia F. W. et al. (1972a)
Guideline: No
GLP: No
Deviations: Yes

- Partial summary,
- treatment duration too short (day5-15 of pregnancy),
- size of the groups not given.

Three groups of pregnant female Wistar rats were given copper gluconate orally by gavage at 0, 0.1, 3 or 30 mg/kg/day from days 5 to 15 of pregnancy. Bodyweight and food intake were recorded weekly. Day of sacrifice not stated in FAO summary, but presumed day 20. Litter parameters (corpora lutea, implantation sites, implantation losses, resorptions, numbers of live foetuses, foetal weight, crown-rump length) were recorded. Foetuses were examined for visceral and skeletal defects.

Maternal body weights and food intake were similar in all groups. Litter parameters were not adversely affected by treatment The incidence of skeletal and visceral abnormalities was not affected by maternal treatment. The NOEL was 30 mg/kg/day.

Copper as gluconate was stated to be not embryotoxic or teratogenic when administered orally to rats during the period of organogenesis.

Reference: De la Iglesia F. W. et al. (1972 b)
Guideline: No
GLP: No
Deviations: Yes

- The treatment duration is too short, the methodology suffers of insufficiencies, and there was no information in the summary on examination for visceral and skeletal defects,
- size of the groups not given.

Three groups of pregnant female Swiss mice were given copper gluconate orally by gavage at 0, 0.1, 3 or 30 mg/kg/day from days 6 to 14 of pregnancy. Bodyweight and food intake were recorded weekly. Day of sacrifice not stated in FAO summary, but presumed day 20. Litter parameters (corpora lutea, implantation sites, implantation losses, resorptions, numbers of live foetuses, foetal weight, crown-rump length) were recorded. There was no information in the summary on examination for visceral and skeletal defects.

Maternal body weights and food intake were similar in all groups. Litter parameters were not adversely affected by treatment. The NOEL was 30 mg/kg/day.

Copper as gluconate was stated to be not embryotoxic or teratogenic when administered orally to mice during the period of organogenesis.

Reference: Barlow, S.M., Knight, A.F. and House, I. (1981).
Guideline: No
GLP: No
Deviations: Yes

- IUDs were implanted on Day 9, and not prior to implantation
- Group sizes are smaller than recommended by the guideline
- The number of dose levels is fewer than recommended
- Levels of food consumption are not reported.
- Foetal sex is not reported.

These deficiencies do not, however, compromise the validity of the data reported.

A study was carried out to investigate the potential for intrauterine copper IUDs to affect prenatal development in the rat.

Female Wistar rats aged about 12 weeks and weighing 200-250 g were used in this study. For 2 weeks before mating and throughout the experiment they were held at 21-24°C under reversed

lighting conditions (12 h red light, 12 h white light). Food and water were fed *ad libitum*. At the beginning of the experimental period, female rats were housed in groups of 3 and a male was introduced into each cage in the morning. Males were removed in the evening and vaginal smears taken. The day on which spermatozoa were found in the smear was designated Day 1 of pregnancy. Rats were weighed daily from Days 1 to 21 of pregnancy.

On Day 9 of pregnancy, rats were assigned randomly to treatment groups. Animals receiving IUDs were anaesthetized and one uterine horn exposed through an incision in the flank. A coil was inserted between each implantation site by making an incision in the uterus with an intravenous cannula with cutting needle. The other horn was left unoperated as a control. To control for the physical presence of devices in the uterus, some animals had similar-sized coils of stainless-steel wire inserted into one horn, leaving the other unoperated. To control for the stress of the operation and other factors such as loss of uterine fluid, other animals were sham-operated, with no IUDs inserted. Animals in another group were left unoperated. Rats were returned to the animal room until sacrifice on Day 21.

This study was reported in terms of three separate experiments. The details of Experiments 1 and 2 are shown in the following table:

Table 58: Details of experiment 1 and 2

Experiment	Group	No. of animals	Uterine horn*
1	1 (COPPER IUD)	9	A OPERATED (9) B unoperated (8)
	2 (SHAM-OPERATED)	10	A operated (10) B unoperated (9)
	3 (No operation)	10	Unoperated (20)
2	4 (Copper IUD)	13	A operated (13) B unoperated (13)
	5 (Steel IUD)	14	A operated (14) B unoperated (13)
	6 (No operation)	7	Unoperated (14)

* Figures in parentheses indicate number of horns containing implantation sites.

Experiment 3 was carried out to determine whether copper released from IUDs penetrated into foetuses. Pregnant rats were treated as follows: on Day 9 of pregnancy, copper IUDs were inserted between each embryo in both uterine horns of 2 rats (Group 7). In another 2 rats, steel IUDs were inserted in both horns (Group 8). One rat was left as an unoperated control. Test animals were killed on Day 22 of pregnancy, and samples of maternal liver and uterus, all foetal brains, foetal livers and placentae were removed for copper analysis.

Rats were anaesthetised on Day 21 of pregnancy and a maternal blood sample taken for copper analysis. After sacrifice, the uterus was exposed and opened up. In IUD-bearing animals, copper or

steel coils were removed, washed and weighed. The number and position of live and full-term dead foetuses (no signs of maceration), late resorptions (maceration, death occurring at the foetal stage), and early resorptions (death occurring at the embryonic stage) were noted. Numbers of corpora lutea in each ovary were also noted. Foetuses were weighed and examined for gross external abnormalities. They were then either fixed in Bouin's fluid for examination of soft tissues by the slicing technique of Wilson or in alcohol and stained with Alizarin red S for skeletal examination.

Experiment 1 results: Gravimetric analysis of the IUDs before insertion on Day 9 and after removal on Day 21 of pregnancy showed a mean ± s.e.m copper loss of 48 ± 3 µg (about 4 µg/coil/day). Maternal plasma copper levels (mean ± s.e.m.) on Day 21 of pregnancy were 203 ± 5 (*n* = 9), 208 ± 12 (*n* = 10) and 200 ± 5 (*n* = 10) µg/100 ml in Groups 1, 2 and 3 respectively. Differences between the groups were not significant. Two rats had unilateral pregnancies, the remainder were bilateral. The only significant differences in comparisons of the 5 sub-groups of uterine horns were between resorptions in Group lA and Group 2A or 2B ($P < 0.015$) and between Group lA and Group 3 ($P = 0.03$). There were no significant differences between the sub-groups in either overall incidence of abnormal foetuses or specific abnormalities and anomalies.

Table 59: Outcome of pregnancy in rats carrying coiled copper IUDs from days 9 to 21 of pregnancy (experiment 1)

Group	No. of rats	Uterine horn	No. of implantation sites	Fetuses		Resorptions		Mean ± s.e.m. fetal wt (g)
				Live	Dead	Early	Late	
1 (copper IUD)	9	A Operated (9)	63	51	0	9	3	2·95 ± 0·12
		B Unoperated (8)	42	39	0	2	1	3·02 ± 0·10
2 (sham-operated)	10	A Operated (10)	57	55	0	1	1	2·97 ± 0·10
		B Unoperated (9)	47	47	0	0	0	2·85 ± 0·07
3 (no operation)	10	Unoperated (20)	126	117	0	9	0	3·12 ± 0·05

Figures in parentheses indicate number of horns containing implantation sites.

Experiment 2 results: Gravimetric analysis of the IUDs before insertion on Day 9 and after removal on Day 21 of pregnancy showed a mean ± s.e.m copper loss/coil of 74 ± 4 µg, i.e. about 6 µg/coil/day. No significant reduction in weight of the steel coils was found between insertion and removal. Mean ± s.e.m. copper levels in maternal plasma on Day 21 of pregnancy were 207 ± 6 (n = 13), 194 ± 9 (n = 12) and 208 ± 14 (n = 7) µg/100 ml in Groups 4, 5 and 6, respectively. The differences are not significant. There was a significant increase in the incidence of resorptions in Groups 4A and 5A in comparison with Groups 4B and 5B ($P < 0.005$). There was no significant difference between Groups 4A and 5A. There were no significant differences in the overall incidence of foetal abnormalities. The only significant difference in the incidence of specific soft tissue abnormality was an excess of tracheobronchomegaly in Group 4A compared with Group 4B ($P < 0.02$). However, the difference between Group 4A and Group 6 was not significant. The only significant difference in the incidence of skeletal anomalies was a slight excess of extra 14th rib in foetuses from Group 4B in comparison with Group 6 ($P < 0.05$).

Table 60: Outcome of pregnancy in rats carrying coiled copper IUDs from days 9 to 21 of pregnancy (experiment 2)

Group	No. of animals	Uterine horn	No. of implantation sites	Fetuses		Resorptions		Mean ± s.e.m. fetal wt (g)
				Live	Dead	Early	Late	
4 (copper IUD)	13	A Operated (13)	75	57	0	16	2	2·96 ± 0·08
		B Unoperated (13)	95	91	0	4	0	3·04 ± 0·07
5 (steel IUD)	14	A Operated (14)	98	75	0	13	10	2·83 ± 0·08
		B Unoperated (13)	110	108	0	2	0	2·86 ± 0·07
6 (no operation)	7	Unoperated (14)	102	102	0	0	0	2·79 ± 0·11

Figures in parentheses indicate number of horns containing implantation sites.

Experiment 3 results: Foetal brain and liver and placental copper levels were significantly elevated in Group 7 animals, compared with those from Group 8 or the unoperated control. Variance in foetal copper levels in Group 7 was low, suggesting relatively uniform exposure of embryos and foetuses. Maternal liver levels of copper were not elevated in Group 7 (5.0 and 6.8 µg/g) compared with Group 8 (4.9 and 5.2 µg/g) or the unoperated control (4.5 µg/g). Uterine copper levels were considerably elevated in Group 7 (33.1 and 21.3 µg/g) compared with values in Group 8 (2.0 and 2.1 µg/g) and the control animal (1.8 µg/g).

Examination of the offspring for structural abnormalities confirmed that copper had no significant teratogenic or growth-retarding effect in the rat. The incidence of major malformations was low in all groups and the minor disturbances that were seen in all groups are known to be common spontaneous malformations in the strain of rat used. The copper ions released from intrauterine wire were insufficient to elevate maternal plasma copper levels. Copper levels in the rat maternal liver were not elevated, but the copper released from the IUDs did penetrate the foetus. Foetal brain copper levels were increased by 65% and foetal liver levels by more than 100% in copper-exposed offspring compared with those from mothers with steel IUDs or no IUDs. The lack of teratogenicity of copper released from IUDs cannot therefore be attributed to lack of exposure of the conceptuses. Moreover, the embryos were exposed to copper throughout organogenesis. The IUDs were inserted on the morning of Day 9 of pregnancy, which corresponds to the primitive-streak stage marking the onset of organogenesis, and is well before the time of neural tube closure on Days 10-11.

Intrauterine mortality rates of 19 and 24% in copper IUD horns were significantly higher than in sham-operated (4%) or untreated controls (0 and 8%), but were no higher than in horns carrying inert steel IUDs (25%). These results suggest that the deaths were probably due to trauma from the insertion and the physical presence of devices in the uterus, rather than to any specific effect of copper.

Reference: Chang, C.C. and Tatum, H.J. (1973).
Guideline: No
GLP: No
Deviations: Yes (from OECD 414)

- The toxicity of copper to reproduction / teratogenicity was assessed only after implantation of embryos in the Parent females. No copper was administered to males,
- only a single 'dose level' was used. The dose received by parent females was estimated, not measured,
- F_1 and F_2 generations were not exposed to copper during their growth, mating and reproduction,
- test and control groups generally contain fewer animals than recommended,
- effects on the oestrus cycle were not assessed,
- sperm parameters were not assessed.

These deficiencies do not, however, necessarily compromise the validity of the data generated.

A study was carried out to determine whether copper wire, placed within the uterus after implantation and kept in situ throughout pregnancy, produced any teratogenic effects on the embryo, or altered in any way the development and subsequent growth of the offspring of rats, hamsters and rabbits. The potential for adverse effects on the fertility of treated animals was also assessed.
Nulliparous female rats of the Holtzman strain, adult cycling female hamsters and adult albino New Zealand female rabbits were used.

In rats and hamsters, positive matings were verified by the presence of sperm in vaginal smears. The day of insemination was designated as Day 1 of pregnancy. In rabbits, visual observation only was used to confirm copulation, and that day was designated as Day 0 of pregnancy.

Copper wire (99.9% pure) was inserted into the endometrial cavities of both uterine horns of rats and hamsters on Day 6 of pregnancy. It was estimated that the rate of dissolution of the wire used in the cycling rat was approximately 2.75 µg per day.

In rabbits, the wire was inserted into the uterine horns on Day 7 of pregnancy. The amount of copper released in 24 hrs from the wire was estimated to be approximately 5.50 µg on the assumption that the rate of dissolution of the wire used in the rabbit is similar to that in the rat.

The wire was left *in situ* during pregnancy and lactation, and the gestation period was recorded. The mothers were sacrificed at the time of weaning and the ovaries, uteri and adrenals were fixed with Bouin's solution for histological examination. The number and sex of the pups of rats and hamsters were recorded at birth and the offspring were observed for gross abnormalities. The body weight of F_1 generation rats was recorded at 5-day intervals from the age of 5 days through 60 days. The offspring of rats and hamsters were weaned at the age of 25 days and the number of surviving F_1 generation was recorded. In the meantime, the females were separated from the males and maintained in separate cages to raise F_2 and F_3 generations. In rabbits, laparotomy was done on Day 15 of pregnancy and the number of implantation sites was recorded. The offspring were weaned when 30-35 days of age. Some of the F_1 generation rabbits were sacrificed at the age of either 3 or 6 months.

When the F_1 generation rat and hamster females reached the age of 90 days and the males 120 days, each female was cohabited with one fertile male and each male with 2 virgin cycling females for 10 days. The fertility of the F_1 generation animals was evaluated by the following regimens: a) the ratio of the animals mated over the animals used and b) the number of implantation sites or the number of pups delivered. Some of the animals delivered by the F_1 generation were eliminated at the time of weaning and examined for gross malformations. The remaining animals were used for fertility testing when they reached maturity. The fertility of F_2 generation animals was tested in a manner similar to that described for the F_1 generation.

At autopsy, the body weight and the weights of the following organs were determined: ovaries, uteri and adrenals in the females; testes, seminal vesicles, epididymus (in the rabbit only), ventral prostate and adrenals in the males.

Rats: There was no difference in gestation periods between the mothers bearing the wire in the uterus and controls. All copper wire treated and control mothers delivered normally. However, a comparison of the average number of pups delivered from treated mothers to those from untreated rats showed that the copper wire treated females delivered 6.5 ± 0.7 pups, a number significantly lower than that of the untreated controls (8.6 ± 0.6) at the 5% confidence level. It is considered likely that the incidence of fewer pups in the treated group was due to manipulation of the uterus and damage to the embryos at or near the site when the copper wire was inserted.

No teratogenic effects were evident in offspring. No abnormalities were observed at birth, at weaning or at the time of the fertility test. There was no effect of copper wire on survival rates of the F_l generation animals at the time of weaning. Survival rates of the descendants of treated and untreated mothers indicates that lactation was not interrupted by the wire. F_l generation animals of both sexes grew normally, as evidenced by the increases in body weight. There were no significant differences in fertility of offspring of copper treated and untreated mothers of either sex in the F_1 generation. There were no significant differences in organ weights of offspring of copper wire treated and untreated mothers in either sex of the F_1 generation.

There were no significant differences in fertility among F_2 generation descendants of copper wire treated and untreated animals. There were no significant differences in body weights or organ weights in either sex of the F_2 generation.

At autopsy, there were no gross anatomical deformities noted in Parent, F_1 or F_2 generations. Histological examination of the ovaries, uteri and adrenals of Parent females, and of female and male tissues of F_1 and F_2 generations did not show deviations from normal.

Hamsters: There was no difference in the average number of pups born between the group bearing copper wire and the control group. The gestation period for treated animals was not different from the controls. Lactation in treated mothers was considered to be normal, based on the average body weights of pups and the percentage lost at weaning. No teratogenic effects were observed in the F_1 generation animals at birth and at weaning. Histological examination of the ovaries, uteri and adrenals of mothers with copper wire showed no deviation from normal.

There was no apparent effect on the fertility of offspring of treated and untreated mothers in either sex of the F1 generation. There were no significant differences in organ weights of offspring of copper wire treated and untreated mothers in either sex of the F1 generation. There were no significant differences in fertility among F2 generation descendants of copper wire treated and untreated animals. However, the average number of pups delivered in BB females (descendants of control Parent) and AA males (descendants of copper treated Parent) was significantly lower than that of normal animals (2.0±1.0 vs 7.8±0.9) for female parents, 3.1±0.6 vs 7.9±0.8 for mal parents). The cause for this difference in the F2 generation is not known.There were no significant differences in body weights or organ weights in either sex of the F2 generation, other than an unexplained increase in the adrenal weights of control males. At autopsy, there were no gross anatomical deformities noted in Parent, F1 or F2 generations. Histological examination of the ovaries, uteri and adrenals of Parent females, and of female and male tissues of F1 and F2 generations did not show deviations from normal.

Rabbits: At the time of insertion of the copper wire (Day 7 of pregnancy), there was no difference in the average number of implantation sites between the animals which were to be exposed to copper wire and the controls. However, at laparotomy on Day 15 of pregnancy, the number of implantation sites was significantly less than that observed on Day 7 of pregnancy. The number of pups subsequently delivered from these animals was reduced as compared to that in the control animals. This difference was thought to be due to manipulation of the uterus at the time of insertion of the copper wire.

There were no gross anatomical deformities noted in Fl generation at birth, at weaning or at autopsy.

The fertility of F1 generation was normal.

The Parent females were autopsied after weaning. Histological examination of the ovaries, uteri and adrenals showed no deviations from normal.

No teratogenic effects were observed in F1 generation animals and their growth rate was normal. There were no significant differences in body weight and organ weights between the Fl generation animals of either sex from copper treated and untreated mothers. Histological examination of the female and male reproductive tissues of Fl generation animals showed no deviations from normal.

Reference: Haddad *et al.(*1991)
Guideline: No
GLP: No.

Water loaded with copper acetate was administered to Wistar albino rats at increasing stepwise concentration of the copper acetate to 0.185% over a period of seven weeks (approximately 65 mg Cu/kg body weight per day). A group of control animals received demineralised water. At the end of seven weeks 7 rats from each group were sacrificed to serve as non-pregnant controls. The remaining rats were mated singly. The pregnant females were divided into three groups. The first group with 7 controls and 14 experimental rats were sacrificed at 11.5 days of gestation; the second group with 7 controls and 14 experimental rats were sacrificed at 21.5 days of gestation and the third group with 7 controls and 14 experimental rats were allowed to litter. Blood samples were collected for the measurement of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase levels.

Histopathology was performed on liver and kidneys, including staining for copper and iron. Samples of liver were subjected to atomic absorption spectrophotometry for copper levels. Embryos from the dams killed after 11.5 days were examined for growth and development and 21.5 day foetuses and newborn pups were counted, weighed and examined for external malformations. Two foetuses and two newborn pups (from each litter) were processed and examined for visceral malformations. Histopathological examination was performed on sections of liver and kidney from one foetus and one newborn pup. The remaining foetuses and newborn pups were processed for skeletal assessment. Statistical analyses were performed.

General observations: There were no treatment related clinical signs throughout dosing and maternal weight gains for the treated animals were similar to those in the controls. Pregnancy rate was not adversely affected by maternal treatment.

Duration of gestation: There was no difference in the duration of gestation between the controls and the copper loaded group.

Clinical chemistry: There were no differences in the serum AST, ALT and alkaline phosphatase activities between the control and the copper loaded groups (Table below).

Table 61: Clinical chemistry parameters

Parameter	**Controls**	**Copper loaded**
AST (IU/L)	27.3	25.9
ALT (IU/L)	14.2	17.3
Alkaline phosphatase (IU/L)	11.8	9.6

Histopathology: Liver and kidney sections from the control animals showed normal histology with no copper deposits. Liver sections from the copper loaded rats showed copper deposition in the hepatocytes and to a lesser extent in the Kupffer cells; copper was present as clusters or granules in the cytoplasm. Analysis of copper content showed that copper levels of treated rats was higher than controls (207.7 μg/g dry weight in treated compared to 23.4 μg/g dry weight in controls). Lesions included hypertrophy of the hepatocytes with cloudy eosinophilic cytoplasm, areas of focal necrosis surrounded by inflammatory foci of polymorph and lymphocyte infiltration, the presence of sinusoidal dilatation and the appearance of cytoplasmic vacuolation. In the kidneys, copper deposition was present in the proximal convoluted tubules. Lesions were confined to the proximal convoluted tubules, characterised as cloudy swelling due to hydropic degeneration and obliteration of the lumen with occasional desquamation of the epithelial cells. Liver and kidney sections stained for iron showed no deposits. These findings are similar to those seen in papers by Haywood et.al (Section B.6.5.2.3 –B.6.5.2.5), where liver damage and kidney changes were recorded at high levels of dietary copper administration. The histological changes indicated that the levels of copper loading were in excess of the maximum tolerated dose, although actual analysed liver levels of copper were lower than where analysed by Haywood (Section B.6.5.2.4). Foetal and newborn liver and kidney sections showed a normal histological pattern with no copper deposits.

Foetal and newborn examinations: At 11.5 days gestation, overt embryonic development was similar in most parameters analysed. However, there were minor changes in mean somite number, mean crown-rump length and mean yolk sac diameter were slightly decreased when compared with the controls (Table below). These changes indicated a slight delay in development for time of gestation, although the small sample size, and the imprecise nature of the parameters measured must be taken into account.

Table 62: Growth and development of 11.5 day old embryos

Parameter	**Controls**	**Copper loaded**
Number of dams examined	14	6
Number of embryos examined	56	95
Number (%) of embryos showing:		
Presence of heart beat	56 (100)	95 (100)
Presence of fused allantois	56 (100)	94 (99)
Normally closed anterior neuropore	56 (100)	92 (96)
Normally closed posterior neuropore	53 (94)	80 (84)
Presence of normal turning	54 (96)	87 (91)
Presence of forelimb buds	56 (100	95 (100)
Presence of normal optic vesicle	56 (100)	92 (96)
Presence of normal otic vessel	56 (100)	93 (97)
Mean somite number	23.48	22.03*
Mean crown-rump length in mm	2.98	2.71*
Mean yolk sac diameter in mm	4.56	3.98*

* $P < 0.005$

The number of offspring per litter and the mean foetal weights of the treated animals were stated to be similar to controls. Similarly, external and visceral examination revealed no differences. Skeletal

examination showed reduction in the number of ossified centres in almost all the ossification centres examined, which was significant generally in 21.5 day old foetuses but significant only in cervical vertebrae, caudal vertebrae and hindlimb phalanges in newborn pups (Table below). These ossification findings are generally considered transient, in that they reflect the stage of the ossification process, and it is significant that the incidence was much lower in the new-born pups than in the day 20.5 foetuses. It should be noted the presence or absence of an ossification centre is not the same as the presence or absence of the feature itself; absence means that the feature has not yet ossified i.e. it is still cartilage. The differences may reflect maternal copper-calcium balance, leading to slightly reduced availability of calcium to the foetus.

4.10.3 Other relevant information

Table 63: Summary of investigative studies (data from literature)

Method Species	Exposure conditions and doses	Observations and remarks
Pregnancy Marois (1972) No GLP Rat and Rabbit	Sub cutaneous (rat) ; i.v. (rabbit) Copper acetate 10 or 15 mg/kg with or without progesterone to rat, 8 mg/kg copper acetate only to rabbit	Copper acetate alone terminated pregnancy in 3/6 rats; copper acetate + progesterone did not. Authors state that copper interrupted CNS control of pregnancy in rat.
Post-implantation embryo *in vivo* and *in vitro* O'Shea (1979) No GLP Mouse 6 mated females/group	i.v. *in vitro* phase in culture bath Copper sulphate 4 mg Cu/kg bw i.v. *In vitro* phase 0.332, 1.60 or 3.2 µg copper/mL culture bath	Injection on early day 7 of pregnancy killed all embryos. Injection on day 8 showed a high incidence of neural tube, cranial and heart defects, injection on day 9 showed fewer anomalies. Embryoculture showed similar malformations
Fertility Auerlich et al. (1982) No GLP Mink 12/sex/group	Diet Copper sulphate 0, 25, 50, 100 or 200 ppm (approximately 3, 6, 12 or 24 mg Cu/kg body weight per day) Duration: 9 months before mating and 3 months after mating	Reduced offspring survival at 100 and 200 ppm. Reduce kit weight was observed at and above 100 ppm. Elevated copper levels in liver and plasma in mink. No information was provided on developmental malformations.
Post implantation embryo development Ferm (1974) No GLP Golden Hamster 10 mated females/ groups.	i.v. Copper sulphate 2.13, 4.25, 7.50 or 10.0 mg Cu/kg bw or copper citrate complex 0.25-1.50, 1.80, 2.20, or 4.9 mg Cu/kg bw	High doses maternally lethal, near-lethal doses increased resorption and embryos with neural tube, cranial, tail, thoracic wall and heart malformation. Sulphate much better tolerated than citrate complex.
Post implantation embryo development Di Carlo(1979) No GLP Golden Hamster 12 -17 mated females/groups	i.p. Copper citrate complex 2.7 mg Cu/kg bw	5% of embryos with heart defects
Antitesticular effect Kamboj (1963) No guideline applicable Swiss albino rats	Cupric sulphate Rat single intratesticular injection to the left testis of 0.02, 0.04 and 0.08 mM/kg	Intratesticular injection in rats caused dose-related degrees of degeneration of the seminiferous epithelium and the interstitium. Single subcutaneous injection was ineffective.

6 males/group Swiss albino mice 3 males/group	Rat and mice subcutaneous injection 0.08 mM/kg Killed after 2-7 days in rats And after 30 days in mice Note: the route of administration is not appropriate for risk assessment purposes.	Continuous administration to mice caused slight weight change but no histopathological changes. There were no changes following subcutaneous administration.

In these studies single high doses of copper have been administered parentally, via intravenous, sub cutaneous or intra peritoneal injection. These studies appear to have been performed in order to study induction of foetal malformations, and the routes of administration were chosen because it is not possible to engender similar malformations by oral administration. The studies are not strictly relevant to the classification of copper hydroxide but they are known to the regulatory authorities.

4.10.4 Summary and discussion of reproductive toxicity

- Non human data

Fertility

Effects on fertility were investigates in a two generation study in rat. In this study, no treatment related effects were observed on reproduction parameters or systemic toxicity.

In the four other fertility studies (non guideline, not GLP), there were no differences from controls in any of the parameters studied.

Development

Developmental toxicity of copper has been investigated in a well-conducted GLP study in rabbit. In this study, no malformation of concern was noted and the observed developmental effects were considered to be secondary non specific consequence of the maternal toxicity and not a direct effect on development.

Moreover the extensive data on the absorption and excretion of copper in the human, in livestock and in laboratory animals, show that there is no potential for any cumulative effect over several generations.

- Human data

There is a comprehensive review of copper in pregnancy and childhood in the human. This review identified risk to neonates fed cows milk boiled in untinned copper vessels. In most of the reported studies, there were no indications of any adverse effects on pregnancy, birth or growth that were associated with exposure to copper.

However, Graham *et al.* (1980) reported two cases of anencephaly in women where an intra-uterine contraceptive device (IUD) was used. Anyway, copper released from these devices significantly increases copper concentrations only in the intrauterine fluid in the first 12 months of utilization, but it do not increase serum copper or caeroplasmin concentrations (Gosden et al., 1977). In addition, the mean daily release of copper by the IUD corresponds at only 1% of the mean daily copper intake by the alimentation. Moreover, others more recent studies reported that the IUD does not increase the risk of congenital abnormalities (Pasquale 1996; Weissmann-Brenner et al. 2007).

4.10.5 Comparison with criteria

Reprotoxic substances can be toxic to the development of the unborn child or can cause impairment of fertility in male and female subjects.

Reprotoxic substances are divided into 2 groups;

- Effects on male or female fertility, including adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response.
- Developmental toxicity, including any effect interfering with normal development before and after birth.

1) Criteria in the CLP classification :

- Fertility and developmental toxicity

Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

2) Comparison with criteria:

⇨ As in a rat two-generation study (guideline and GLP) and in four other fertility studies (non guideline, not GLP) there were no differences from controls in any of the parameters studied, no classification is proposed for copper concerning fertility and reproduction

⇨ As no malformation of concern was noted in a well-conducted GLP study in rabbit, no classification is proposed for copper concerning developmental toxicity reproduction

4.10.6 Conclusions on classification and labelling

Based on all the available data and the weight of evidence on the impact of copper on developmental toxicity, no classification is required for copper compounds concerning reproductive and developmental effects.

RAC evaluation of reproductive toxicity
Summary of the Dossier Submitter's proposal No data on granulated copper were provided in the CLH report. However, in light of the proposal to read-across between the different copper compounds for systemic endpoints, (see section "RAC general comment" above), the dossier submitter included in the CLH report some human data, as well as several animal studies investigating the reproductive toxicity of other copper compounds.

Fertility

Effects of copper sulphate pentahydrate on fertility were examined in a 2-generation study conducted according to OECD TG 416 (Mylchreest, 2005). No treatment-related effects were seen on any of the fertility and litter parameters investigated. Two other non GLP studies conducted with copper gluconate (De la Iglesia *et al.*, 1973) and copper sulphate (Lecyk, 1980), included as supporting evidence, also showed no effects on fertility.

Development

An OECD TG 414 compliant rabbit developmental toxicity study conducted with copper dihydroxide (Munley, 2003d) showed some slightly increased incidences in common skeletal variants that were considered secondary non-specific consequences of maternal toxicity. Two other non-guideline studies exposing rats and mice to copper gluconate via gavage (De la Iglesia *et al.*, 1972) did not reveal treatment-related effects on developmental parameters. Another non-guideline compliant study with copper acetate administered to rats via drinking water (Haddad *et al.*, 1991) showed some delayed ossification in foetuses but not in new-borns. In addition, two studies exposing pregnant rats, rabbits and hamsters to intra-uterine copper wire (to mimic exposure to intra-uterine contraceptive device (IUD)) showed no teratogenic or growth-retarding effects in the offspring (Barlow *et al.*, 1981; Chang & Tatum, 1973).

Human exposure

Copper in the uterus (as IUD) is known to prevent implantation of the blastocyst, but once implantation takes place the foetus develops normally. The CLH report mentions that although two cases of anencephaly after use of IUD have been reported (Graham *et al.*, 1980), more recent reports indicated that IUD did not increase the risk of congenital abnormalities (Pasquale, 1996; Weissmann-Brenner *et al.*, 2007). No further details on any of these publications were however presented. Dietary exposure to copper does not appear to result in adverse effects on pregnancy, birth or growth and development (Ralph & McArdle, 2001).

Based on the available data and the weight of evidence assessment, the dossier submitter concluded that no classification for reproductive and developmental effects is warranted for copper compounds, including granulated copper.

Comments received during public consultation

No comments were received.

Assessment and comparison with the classification criteria

RAC notes that no data on granulated copper are available. The CLH report contains data on other copper compounds (among which copper sulphate pentahydrate), from which the dossier submitter proposed to read-across to granulated copper. In view of the considerations presented in the section "RAC general comment", RAC has not pursued the aspect of grouping any further. RAC concludes that **in the absence of relevant data no proposal for classification for reproductive toxicity can be made for granulated copper**.

4.11 Other effects

In the review of copper toxicity database of Stern et al. (2007), no new information was available in human or animals.

4.11.1 Non-human information

4.11.1.1 Neurotoxicity

Table 64: Neurotoxicity study results

Method Species	Exposure conditions and doses	Observations and remarks
Malhotra (1982) No guideline applicable No GLP 12 male/groups treated for 21 days	Group 1: lead acetate 100mg/Pb/L Group2: i.p cupric chloride at 2 mg cu/kg Group 3: lead acetate (100mg/L) in dreaking water + cupric chloride (2 mg cu/kg) i.p. Group 4: control: sodium acetate 100mg/L in drinking water	Copper treatment alone had no effect on the levels of copper in the brain mitochondria, and did not affect enzyme activities in mitochondria. The presence of copper reduced the levels of lead and the adverse effects of lead.
Murthy *et al.*, (1981) No guideline applicable No GLP 6 male rats	Dietary administration of 250 mg/kg Cu (as copper (II) sulphate in pentahydrate) for 30 days, equivalent to about 20 mg/kg bw/day	No affect their locomotor activity, learning ability or re-learning capacity and memory. But analysis of biogenic amines in the brain revealed an increase in the dopamine and norepinephrine levels of animals receiving the protein-adequate diet. Furthermore, the administration of Cu was associated with decreased levels of calcium and zinc in the brains of rats fed both the low- and high-protein diets. The Cu content of the brain was elevated in all Cu-treated animals (+174 and +172% for low- and high-protein diets, respectively). The neurotoxicological significance of these findings is unclear, given that there were no associated effects on behaviour.

Summary and discussion of neurotoxicity

The limited amount of evidence indicates that excess copper does not adversely affect function of brain mitochondria. In the many toxicity studies on animals, there have been no indications that copper is selectively neurotoxic. However, in humans with the genetic condition Wilson's disease, where the copper transporter WND protein is inactive, copper progressively accumulates in the liver and in the brain, and subjects in the later stages of the disease, which is fatal through liver failure if not treated, show signs of neurotoxicity. In genetically normal humans, and in normal laboratory animals, the natural homeostatic mechanisms that regulate copper prevent any accumulation in brain and neural tissues, such that copper is never neurotoxic.

Acute, short term and long term studies where copper has been administered in the diet to laboratory animals have not shown any neurotoxic signs, and histopathology of neural tissues have not shown any adverse effects associated with copper administration.

4.11.1.2 Immunotoxicity

Table 65: Immunotoxicity study results

Method **Species**	**Exposure conditions and doses**	**Observations and remarks**
Immune response Pocino, M (1991) No standard guideline C57BL/6 mice Males and females	$CuSO_4$ Drinking water 50, 100 and 200 ppm 3-10 weeks	100 or 200 ppm: depressed levels of all of the four immunological parameters investigated, including both cellular and humoral immune responses. Other studies in humans have shown that the nausea threshold for copper as sulphate in drinking water is 6 – 8 μg/L.

4.11.1.3 Specific investigations: other studies

Table 66: Complementary studies

Method **Species**	**Exposure conditions and doses**	**Observations and remarks**
Johansson, A (1983&1984) No standard guideline 8 male rabbits	$CuCl_2$ Inhalation 0.6mg/m^3	No effects on alveolar type II cells, alveolar macrophages, and no increased pulmonary phospholipids
Acute toxicity of copper to mink Auerlich (1982) No guideline applicable dark mink 6animals groups	Copper sulphate intraperitoneal injection 3.1, 6.2, 9.4, 12.5 and 25.0 mg/kg Copper acetate 5, 10 and 20 mg/kg	LD_{50} of copper sulphate was 7.5 mg/kg, and the LD_{50} of copper acetate was 5.0 mg/kg.

4.11.1.4 Human information

No data available.

4.11.2 Summary and discussion

Neurotoxicity

The limited amount of evidence indicates that excess copper does not adversely affect function of brain mitochondria. In many toxicity studies on animals, there have been no indications that copper is selectively neurotoxic.

Immunotoxicity

In a drinking water study in mice, concentrations of copper sulphate as high as 200 ppm were associated with inhibition of the immune response, although the authors indicated that the effects were similar to zinc deficiency immune inhibition, as excess copper can cause zinc deficiency through induction of metallothionein, which removes both metals. A NOAEL of 50 ppm was demonstrated.No data on food or water consumption or on bodyweights were present in this paper, so it was impossible to assess either the dose administered or to quantify a NOEL in terms of actual intake of copper

Other studies

A series of studies was performed on salts of copper, cadmium and cobalt, to determine if rabbit alveolar type II cells and alveolar macrophages showed similar changes to those induced in earlier studies with nickel. Rabbits were exposed 6 hours/day, daily for 4 to 6 weeks to 0.6mg Cu/m3 as CuCl2 but findings were generally similar to controls.

In an acute intra peritoneal study on mink, the LD50 of copper sulphate was 7.5 mg/kg and the LD50 of copper acetate was 5.0 mg/kg.

4.11.3 Comparison with criteria

In many toxicity studies on animals, there have been no indications that copper is selectively neurotoxic. No classification under Regulation (EC) 1272/2008 is proposed. No classification or SCLs are considered necessary.

Excess copper is associated with inhibition of the immune response in mice. However, this may be an indirect effect of copper-induced zinc deficiency rather than a direct effect of copper. Thus, immune system is not a primary target organ of toxicity for copper.

4.11.4 Conclusions on classification and labelling

Copper compounds should not be classified. No SCL is considering necessary.

5 ENVIRONMENTAL HAZARD ASSESSMENT

The environmental hazard assessment of granulated copper is based on the information provided in the EU Voluntary Risk Assessment (2008) (which was already included in previous classification dossiers for copper flakes and nine copper compounds). These data have been completed by the data submitted in the updated REACH registration dossier of 18 January 2017 and presented in details in two reports of Heijerick and Van Sprang, 2016a and b. The two reports, presented the updated aquatic ecotoxicological dataset including data submitted during the commenting period of the CLH reports for the previous dossiers copper flakes and copper compounds (detailed in the report of Van Sprang and Delbeke, 2010) and also new data generated and published up to the beginning of 2015. Furthermore, the conclusions drawn by the RAC in the RAC opinions on copper flakes and nine copper compounds adopted in December 2014 were also taken into account[3]. In addition, the REACH registration dossier updated in January 2017 included two studies of 7/28 days transformation/dissolution on granulated copper which were included in this CLH report.

The environmental fate properties assessment of copper is based on the reports presented above, and on the final Assessment Report for Granulated Copper for Biocidal Product PT08 (2016).

5.1 Degradation

In soil

Metals are natural elements and are therefore, by definition, not degradable. It is therefore not possible and not relevant to define a route and a rate of degradation in soils as usually made for organic compounds.

However, copper can be present under different forms, most of which are strongly bound to inorganic and organic ligands contained within soil and sediments; while a marginal fraction of copper is present as various species in the soil solution. The fate and behaviour of copper, as its bio availability, strongly depend on the distribution of these different forms.

The distribution and equilibrium between the different forms of copper in soil depend on many factors, such as soil pH, texture and organic matter content. If the mobile, active and toxicologically significant substance is mainly the free copper ions Cu^{2+} present in the soil solution, it is not possible to predict how much this form will represent from the total applied amount of copper. The activity of the free copper ion will steadily increase with decreasing pH for instance, while the contribution of complex species will decrease. The binding affinities of Cu^{2+} with organic or inorganic matter are also dependent on the presence of competing metal ions and inorganic anions.

In water

In water, copper cannot be transformed into related metabolites or degradation products and consequently hydrolysis and biodegradation processes in water will have no action on copper in this respect. Although unable to degrade, copper is subject to chemical transformation processes with a wide array of materials so that the vast majority of copper in aquatic systems is rapidly bound to mineral particles, precipitated as insoluble inorganic salts, or bound to organic matter. In pure water very low levels of the free Cu^{2+} ion are present in solution, with amounts governed by the propensity of the metal cation to hydrolysis in water, as shown in the following equation:

[3] http://echa.europa.eu/web/guest/opinions-of-the-committee-for-risk-assessment-on-proposals-for-harmonised-classification-and-labelling

$Cu(H_2O)_6^{2+} + H_2O \leftrightarrow CuOH(H_2O)_5^{+} + H_3O^{+}$

The reaction is pH dependent with a distribution constant equal to 6.8. Therefore, below pH 5.8 the predominant form will be $Cu(H_2O)_6^{2+}$, whilst above pH 7.8, the predominant form will be $CuOH(H_2O)_5^{+}$. This latter form of copper is an inorganic complex for which a wide range of other possible types could be formed in natural water, with either cupric or cuprous ions and a range of inorganic ligands (e.g. OH^-, HCO_3^-/CO_3^{2-}, $H_2PO_4^-/HPO_4^{2-}$, Cl^-, SO_4^{2-} and S^{2-}) and organic ligands (e.g. humic and fulvic acids) associated with dissolved organic matter. In natural water, the solubility of copper is regulated primarily by the formation of malachite ($Cu_2(OH)_2CO_3$) at pH < 7 and by tenorite (CuO) at pH > 7. The concentration of Cu^{2+} ions in solution will be higher at low pH, however, the exact concentration will depend considerably on the type and concentration of ligands present in water.

Copper entering a water body is rapidly bound to material in the water phase resulting in very low levels of free Cu^{2+} ion in solution. In a water-sediment system, total copper was re-distributed from the surface water to the sediment, at a worst case dissipation rate of 30.5 days (considered as a DT_{50} for the water column), calculated using first-order kinetics. The majority of the applied copper in the sediment is bound to solid matter. Therefore, in a complex environment, total or even dissolved copper levels are not appropriate to assess bio-available copper exposure. Within the soluble water phase, complexation process reduces the actual amount of copper, available for uptake by biological organisms.

In the Guidance on the Application of the CLP criteria (version 4.1, June 2015), section IV.3, it is stated that:
"*Environmental transformation of one species of a metal to another species of the same metal does not constitute 'degradation' as applied to organic compounds and may increase or decrease the availability and bioavailability of the toxic species. In addition naturally occurring geochemical processes can partition metal ions from the water column while also other processes may remove metal ions from the water column (e.g. by precipitation and speciation). Data on water column residence time, the processes involved at the water – sediment interface (i.e. deposition and re-mobilisation) are fairly extensive for metals. Using the principles and assumptions discussed above in Section IV.1 of this document, it may be possible to incorporate this approach into classification. Such assessments are difficult to give guidance for and will normally be addressed on a case-by-case approach. However, the following may be taken into account:*

a. Changes in speciation if they are to non-available forms, however, the potential for the reverse change to occur must also be considered;

b. Changes to a metal compound which is considerably less soluble than that of the metal compound being considered."

In the sediment compartment, copper binds to the sediment organic carbon (particulate and dissolved) and to anaerobic sulphides, resulting in the formation of CuS. CuS has a very low stability constant/solubility limit (LogK=-41 (Di Toro *et al.,* 1990) – see section 5.2.1 *Adsorption/Desorption* of this report) and therefore the 'insoluble' CuS keeps copper in the anaerobic sediment layers, limiting the potential for remobilisation of Cu-ions into the water column Simpson et al (1998) and Sundelin and Erikson (2001).

In order to demonstrate removal from the water column to assess the "persistence" or lack of degradation of metal ions, responsible for the toxicity of metals and metal compounds (> 70% removal within 28 days), the registrants provided the study of Rader *et al*., 2013. The summary of this study is not detailed in this dossier considering the conclusion of the RAC opinion:

"RAC considers that the TICKET-Unit World Model (UWM) [which describes partitioning to dissolved organic carbon, particulates, etc., deposition and transformation to sulfides in sediment] *provides a useful insight into key fate pathways for metal ions including copper in a model shallow lake system. This generic approach allows systematic comparisons to be made between metals. However, the choice of model defaults is open to question, especially as some properties are likely to vary spatially and temporally. For example, comparison with monitoring data in the CLH dossier suggests that the model may overestimate the extent to which copper binds to particles, and may use a settling velocity that is higher than observed in reality. In addition, post-loading simulations for one field study that was claimed to be "more representative of a worst case scenario" (on the basis of settling velocity, distribution coefficient and a relatively low suspended solids concentration compared to model defaults) did not predict 70% removal from the water column after 28 days. As this was a natural lake, RAC does not agree that it should be dismissed as a "worst case". Since the concept of rapid degradation for organic substances is conservative and does not include sequestration by particulate matter (or other fate pathways such as volatility), it seems inconsistent to apply such approaches to metals.*

The proposal also relies heavily on the premise that copper (II) ions will partition rapidly to sediment, where they will be transformed at the surface to insoluble minerals (especially copper (II) sulfide) over a relatively short timescale so that binding to sediment is effectively irreversible. RAC notes that the proposal does not describe the behaviour of copper (II) ions in aquatic systems with little or no sediment (e.g. rivers or lakes with sand or gravel substrates), high turbulence or sediment at depths substantially in excess of 3 metres. Even where sediment is present, the oxidation state of surface layers may not always favour sulfide formation, and the situation may also be complicated if there is a high level of existing metal contamination. RAC therefore does not consider that a convincing case has been made that copper (II) ions will always rapidly speciate to non-available forms, or that this process has been demonstrated to be irreversible under all relevant circumstances.

In conclusion, RAC considers that copper (II) ions are not subject to rapid environmental transformation for the purposes of classification and labelling."

This conclusion could also be applied to granulated copper.

In their updated Copper REACH registration dossier (dated 18/01/2017), the applicants consider that newly available evidence, part of which has not been considered by RAC in the previous discussion of copper and copper compounds, demonstrates that under "environmentally relevant" conditions, more than 70 % of dissolved copper is removed within 28 days. Copper is transformed to sulfide complexes (Cu-S) which are stable. Remobilisation of Cu to the water-column is not likely to occur. Copper is therefore considered rapidly removed, conceptually equivalent to "rapid degradation" for organic substances.
As there is no new guidance available about the "rapid removal concept" for metal compounds, these new considerations were not further considered in this dossier.

Summary and discussion of degradation

Considering the fate and behavior of copper in soil and water compartments, 'degradation' of copper isa complex processes (bioavailability depending on distribution and equilibrium). The granulated copper could not be subject to rapid environmental transformation for the purpose of classification and labelling.

5.2 Environmental distribution

5.2.1 Adsorption/Desorption

In the EU RAR (2008), it was stated that adsorption of copper to soil, sediment, colloids and suspended particles plays an important role for the behaviour of copper in the environment. Inorganic particles such as clay minerals and iron, manganese and aluminium oxides, as well as organic materials, constitute the principal adsorbents for copper in water, sediment and soil (Landner and Lindeström, 1999).

pH and organic matter are the most important abiotic factors affecting the adsorption of copper. Copper adsorption increases with pH. Organic matter restricts heavy metal movement and availability, even under very acidic conditions (Tyler and McBride 1982).

5.2.2 Volatilisation

Not relevant for granulated copper.

5.2.3 Distribution modelling

According to the EU RAR (2008), the most important parameters determining the distribution of copper in the aquatic and soil compartments is adsorption onto solid materials and therefore the copper partitioning coefficients. From the literature overview, the following partitioning coefficients have been derived for Cu metal and Cu compounds:

Partition coefficient in suspended matter

Kpsusp = 30,246 l/kg (log Kp (pm/w) = 4.48) (50^{th} percentile)

Partition coefficient in sediment

Kpsed = 24,409 l/kg (log Kp(sed/w) = 4.39) (50^{th} percentile)

Partition coefficient in soil

Kpsoil = 2 120 l/kg (log Kp (soil/w) = 3.33) (50^{th} percentile)

5.3 Aquatic Bioaccumulation

5.3.1 Aquatic bioaccumulation

5.3.1.1 Bioaccumulation estimation

Based on its log Pow of 0.44, no concern over any potential for bioaccumulation could be concluded for copper compounds. No study is therefore available to determine bioconcentration factors in fish.

Because of homeostasis of metals in vertebrates, BCF values are not indicative of potential bioaccumulation.

The EU RAR (2008) provided detailed information on (1) the essentiality of copper; (2) the homeostatic control of copper; (3) the mechanisms of action of copper-ions; (4) the comparison between copper toxicity from dietary versus waterborne exposures.

These data demonstrate that:

- Copper is an essential nutrient for all living organisms
- Copper ions are homeostatically controlled in all organisms and the control efficiencies increase with trophic chain.

  As a consequence:
  - copper BCF/BAF values
    - decrease with increasing exposure concentrations (water and food)
    - vary depending on nutritional needs (seasonal, life stage, species dependent)
    - vary pending on "internal detoxification" mechanisms
  - Copper BMFs values are < 1

Water-borne exposure (not diet borne exposure) is the exposure route critical to copper toxicity.

In the RAC opinions on copper flakes and nine copper compounds adopted in December 2014, it is stated that "*The bioaccumulation behavior of copper (II) ions is complicated by essentiality and homeostatic mechanisms in organism. […]. However, in view of the degradability conclusion, this end-point does not influence the determination of the chronic M-factor and so is not considered further*."

5.3.1.2 Measured bioaccumulation data

5.3.2 Summary and discussion of aquatic bioaccumulation

Taking into account the homeostasis phenomenon, neither bioaccumulation nor biomagnification are expected for copper compounds.

5.4 Aquatic toxicity

A large copper database was taken into account to determine the classification proposal. All available ecotoxicity data on soluble copper compounds were compiled and the results (EC_{50}, $NOEC/EC_{10}$ values) are expressed as soluble Cu^{2+}. All data used for classification purposes are presented in the annex at the end of this document. Only new data submitted after the EU RAR (2008) publication are described.

The reliability of data coming from the EU RAR (2008) were evaluated in depth by the relevant industry experts and reviewed by the pre-REACH CAs[4].

Data submitted in the reports of Heijerick and Van Sprang, 2016a and Heijerick and Van Sprang, 2016b were also evaluated in depth by the relevant industry experts and the new data issued from these reports are presented in this CLH dossier.

In accordance to the CLP guidance, Heijerick and Van Sprang, 2016a and 2016b, have proposed to use data from standardized methods. Nevertheless, valid data on other species at the same trophic level have also been considered. Considering the difficulty in assessing "equivalence" and recognizing the high number of copper ecotoxicity data available on standard species only the "standard species" – representing three main trophic levels - and endpoints are considered when

[4] Italy has been acting as a reviewing Member State for the substance and the risk assessment report has been reviewed by the Technical Committee on New and Existing Substances (TC NES) according to standard operational procedures of the Committee

deriving ERVs. This approach was also followed for earlier copper classification and is also supported in this dossier.

The general methodology followed by Heijerick and Van Sprang, 2016a and 2016b, are described in their reports. Only a summary is presented below.

- Species selection and test duration:

For acute database, the following test guidelines and standard species/endpoints are considered relevant for classification purposes:

- for fish: 96h LC_{50} values generated according to OECD 203 conducted with juvenile fish (0.1 – 5g) following the EU CLP recommendations. Moreover, as the RAC committee has retained *P. promelas* ELS studies at pH6 for classification of copper flake and copper compounds, the acceptance of the larvae data was considered to be compliant with the OECD 236, and the new proposed database includes all life stages.

- For daphnids, 48 h EC50 values determined according to OECD TG 202 Part I are recommended and are used in this database.
Acute toxicity data for the invertebrate *Ceriodaphnia dubia*, which is not a standard species under CLP but is widely used in the United States, are also included in this assessment.

- For aquatic plants, algal growth inhibition tests consistent with OECD TG 201 and providing 72 or 96 h EC_{50} values are included in the database. Only the growth rate reduction endpoint (i.e. ErC_{50}) is retained.

- For vascular plants (e.g. *Lemna* sp), EC_{50} values obtained from tests consistent with OECD TG 221 and US EPA 850:4400 are retained for the database. The observational acute endpoint is the change (50% effect) in the number of fronds produced and the test duration is 7 days.

For chronic database, the following test guidelines and standard species/endpoints are considered relevant for classification purposes:

- for fish: chronic or long-term tests with fish were initiated with fertilized eggs, embryos, juveniles, or reproductively active adults. Tests consistent with OECD TG 210 (Fish Early Life Stage), the fish life-cycle test (US EPA 850.1500), or equivalent can were used. Durations varied anywhere from 7 days to 330 days. Observational endpoints include hatching success, growth (length and weight changes of the surviving fish), spawning success, and survival.
The data from 7 days *P. promelas* tests were also retained in the chronic data-base if carried out with a sensitive life stage. The use of 7 days *P. promelas* tests with sensitive life stages is justified from Nordberg et al., 1985. They compared the sensitivity of *P. promelas* (tested in similar test waters) to copper, zinc and Dursban (an organic chemical) from 7 days sub-chronic larvae tests, 32 days Early life stage tests and a 327 days full life cycle and concluded that the 7 days *P. promelas* larvae Maximum Acceptable Tolerable Concentrations (MATC) were similar. For copper the MATCs varied between 14 and 19 µg Cu/L.

- For daphnids, tests, consistent with OECD TG 211 (duration: 21 days) and/or OECD TG 202 Part II (duration: 14 days) are recommended and retained. Regarding toxicity study on *Ceriodaphnia dubia*, the results obtain at 7 days of exposure were rejected in the Joint Research Centre (JRC) report (page 20) "*New criteria for environmental long-term aquatic hazard classification under the CLP Regulation (EC) N° 1272/2008 (2nd ATP) - Screening of Annex VI substances with harmonised classifications*". Moreover, only 21 days data are used in the CLP guidance. Therefore, even if the Copper Task Force proposes to include these data

in the current database as the 7 days test is widely used in the United States as typical test species for risk/hazard assessment purposes, these values (7days data) have not been included in the database for classification purposes.

- For algae, NOErC and ErC_{10} values derived from 72 or 96 hours with OECD TG 201 are included in the database.

- For vascular plants (e.g. *Lemna sp*), NOEC/EC_{10} values obtained from tests consistent with OECD TG 221 and US EPA 850:4400 were used for classification. In these international protocols, the observational endpoint is the change in the number of fronds produced and the test duration is 7 days.

- Quality criteria:

Tests have to be conducted with the above mentioned standard test species, endpoints, and guidelines. Sufficient information on the test (test design, test set-up, validity criteria) has to be available. Only test data that meet the criteria for a Klimisch scoring of 1 (reliable without restrictions) or 2 (reliable with restrictions) (Klimisch et al, 1997) have been used for the hazard assessment of copper. Reported adverse effect levels have to be expressed as measured, dissolved copper concentrations. Nominal data are not acceptable.

- Physico-chemical conditions of test media:

  - *Effect of pH:*

Considering the crucial importance of pH of the test media on the copper solubility and ecotoxicity, for the acute and chronic toxicity endpoints, 3 pH categories were distinguished within the acute and chronic ecotoxicity database: pH 5.5-6.5, >6.5-7.5 and >7.5-8.5. These pH categories have been defined in accordance to the Guidance on the Application of the CLP criteria (version 4.1, June 2015) section IV.2. to be in line with the UN GHS transformation/dissolution protocol (T/Dp) which specifies a pH range of 6-8.5 for the 7days test and 5.5 to 8.5 for the 28 days test. Thus, both T/Dp and ecotoxicity data could be compared at a similar pH since both parameters will vary with pH.

  - *Effect of dissolved organic carbon (DOC)*

The registrants have also investigated the impact of DOC on the derivation of the ERV. Their reports proposed a normalisation to a DOC level of 2 mg/L which was performed for all studies in the database for which DOC data were available or could reliably be estimated.

Therefore, two different ERVs for each pH group were derived:

- for acute classification:
  - ERV_{acute} based on all short-term data,
  - ERV_{acute} based on all toxicity data after normalization to a DOC of 2 mg/L, which is the limit value in the OECD 202 guideline;

- for chronic classification:
  - $ERV_{chronic}$ based on all long-term data,
  - $ERV_{chronic}$ based on all toxicity data after normalization to a DOC of 2 mg/L.

  - *Effect of water hardness*

- In the acute toxicity studies, the hardness of the test medium does not influence the sensitivity of algae to Cu and reduces the sensitivity of invertebrates and fish to Cu.

The table below summarizes the characteristics of the test-waters retained in this report. If the reported DOC values were below detection limit, then half of the detection limit was used.

Table 67: Median test water characteristics of the algae, invertebrates and fish tests in the acute copper ecotoxicity database.

Trophic level	Parameter	pH 5.5-6.5	pH 6.5-7.5	pH 7.5-8.5
Algae	DOC (mg/L)	7.7	10.2	6.1
	Hardness (mg CaCO3/L)	100	250	244
Invertebrates	DOC (mg/L)	2.6	3.2	2.5
	Hardness (mg CaCO3/L)	31	50	134
Fish	DOC (mg/L)	1.3	0.5	1.5
	Hardness (mg CaCO3/L)	48	54	102

For invertebrate tests, the reported median hardness is, for each pH class, below the OECD recommendations for *D. magna* (140-250 mg $CaCO_3$/L). The median hardness of the fish tests is, for each pH class, in the lower range of the OECD recommendations (10-250 mg $CaCO_3$/L). Therefore, the acute ecotoxicity data used in this report are generally conservative with regards to the hardness of the test media.

- In the chronic toxicity studies, the hardness of the test medium does not influence the sensitivity of algae to Cu, has little influence on the sensitivity of invertebrates to Cu and reduces the sensitivity of fish to Cu.

The table below summarized the characteristics of the test-waters retained in this report. If the reported DOC were below detection limit, then half of the detection limit was used

Table 68: Median values for the reported test-water characteristics of the chronic algae, invertebrates and fish tests.

Trophic level	Parameter	pH 5.5-6.5	pH 6.5-7.5	pH 7.5-8.5
Algae	DOC (mg/L)	9.8	10.3	6.1
	Hardness (mg CaCO3/L)	101	251	214
Invertebrates	DOC (mg/L)	2.5	1.8	5
	Hardness (mg CaCO3/L)	129	41.3	129
Fish	DOC (mg/L)	1.5	1	0.6
	Hardness (mg CaCO3/L)	12.6	38.5	137

The median hardness of the fish tests is, for each pH class, in the lower range of the OECD recommendations (10-250 mg CaCO3/L) and the Foregs database (http://weppi.gtk.fi/publ/foregsatlas/). The median hardness of the algal and invertebrate tests is somewhat higher but has no or little influence on the copper toxicity. Therefore, the chronic ecotoxicity data used in this report are generally conservative with regards to the hardness of the test media.

- Data treatment:

The registrants considered that taking into account the data-richness of the toxicity copper database, the split-up by pHs, the median low hardness measured for critical species (fish) and the normalizations to DOC, geometric mean values could be calculated, also when less than 4 acceptable L(E)C_{50} values are available for the same species. Indeed, the CLP guidance (version 4.1, p. 500-501, section 4.1.3.2.4.3) further mentions that geometric means can be used if four or more data points are available for a species. The registrants raised that the CLP guidance does not mention that there must be four data points within each pH band and therefore think that it is most appropriate to continue using geometric means after splitting up the database by pH band, as long as at least four data points are available across all pH values. Not using geometric means would lead to a double conservatism: the dataset is split-up according to pH because of data-richness, whereas within each pH band (less data-points), the lowest value is selected due to data scarcity.

Nevertheless, there is no reason to deviate from the CLP Guidance which indicates that the geometric mean have to be used if at least 4 data points on the same species and endpoint are available. However, to analyse the impact of the use of geomean or lowest value if less than 4 data points are available, both approach are presented in this CLH report.

5.4.1 Fish

5.4.1.1 Short-term toxicity to fish

According to the EU RAR (2008), 249 individual data points for fish were selected for 5 standard species (*Oncorhynchus mykiss, Pimephales promelas, Lepomis macrochirus, Brachydanio rerio* and *Cyprinus carpio).*

In the database submitted in the updated REACH registration dossier detailed in Heijerick and Van Sprang, 2016a, 94 new individual data points for fish were included for the 4 standard species (*Oncorhynchus mykiss, Pimephales promelas, Danio rerio* and *Cyprinus carpio).* These new data are presented below and details are given in the table 69.

- *Oncorhynchus mykiss*: Five different publications (Calfee et al, 2014; Ingersoll and Mebane, 2014; Little et al, 2012; Ng et al, 2010; Vardy et al, 2013) were identified until the EU RAR publication. A total of 28 reliable LC_{50} values for copper were reported in these studies, with acute effect levels situated between 5.9 and 63 µg Cu/L. New data were available for each of the three classification pH-classes. An LC_{50} of 19.4 µg/L determined by Ng et al (2010) was excluded of the database as it was obtained at pH 5. It should also be noted that the information on the testing methodology that is provided by Ng et al (2010) indicated that no real replicates were used and no acclimation was carried out to lower pHs; such a deficiency has an impact on the reliability of this study, but the generated acute effect levels in soft test water with hardness of 14-22 mg/L as $CaCO_3$ were taken into account for the derivation of acute ERVs. Another study by Dwyer et al (2005) reports an LC_{50} of 80 µg/L. This value was not included in the database, as it was based on nominal copper levels.

- *Cyprinus carpio*: One publication (Dehghani et al, 2012) was identified for the common carp *C.carpio* given an LC_{50} value for copper equal to 820 µg/L which is in the same range than the previous available dataset. The test was conducted at a pH of 7.6 and a hardness of 220 mg/L as $CaCO_3$.

- *Danio rerio*: One publication (Alsop and Wood, 2011) were identified for the zebra fish *D.rerio* given 96h-LC_{50} ranging between 11.7 and 212.1 µg/L. It was mentioned, in the Heijerick and Van Sprang report, that the lowest value was obtained in a very soft water test

medium (hardness below 10 mg/L as $CaCO_3$), resulting in an LC_{50} that was more than one order of magnitude lower than acute levels that were generated in test media with medium hardness (141 mg /L as $CaCO_3$). The difference in pH among those tests was less than 0.5 pH units. It can thus be assumed that the difference in hardness was responsible for the observed variation – either by decreased Cu-bioavailability (competition between Ca/Mg and Cu), or by a reduced overall health condition of the fish in very soft waters.

- *Pimephales promelas*: six new different publications (Johnson et al, 2008 ; Nimmo et al, 2006 ; Ryan et al, 2004 ; Van Genderen et al, 2007, 2008 ; Vardy et al, 2013) given 59 LC_{50} values ranged from 5.9 to 2034 µg/L have been included in the database. As for *O. mykiss*, another study by Dwyer et al (2005) reports an LC_{50} of 470 µg/L which was not included in the database, as it was based on nominal copper levels. The lowest pH-class (5.5-6.5) is poorly represented in the new literature data-set and questions were raised on increased sensitivity in the lower pH range. Therefore, new acute and chronic ELS toxicity tests with *P.promelas* at pH 6, 6.5 and 7 (OSU, 2016a) were included in the updated registration dossier. The effect on mortality after 96h of exposure was negligible (or well below 50%) in any of the highest test concentrations, resulting in unbounded LC_{50} values of >12.2 µg/L, >13.0 µg/L (pH 6.0) and > 24.4 µg/L (pH 6.5). Due to the limited number of data for this species and pH-class, the unbounded values were included in the database as a worst-case estimate for the actual LC_{50}. The OSU (2016) study also reported an unbounded 96h-LC_{50} of >55.1 µg/L at a pH of 7.0. However, as a large number of bounded LC_{50}-values are already available for this species and pH-category (n=46), this unbounded value was not retained.

Table 69: Overview of the E(L)C50 values for fish published or generated since the EU RAR (2008)

	E(L)C50 Value	E(L)C50 Normalised value		DOC	Hardness	Temperature			
Species	**µg/L**	**µg/L**	**pH**	**mg/L**	**mg/L as CaCO3**	**°C**	**Type of water**	**Reference**	**Lifestage**
Danio rerio	11,7	26	7,34	0,9	7,8	28	Soft water	Alsop and Wood, 2011	larvae
Danio rerio	148,4	94,7	7,8	3,5	141	28	Hard water from Lake Ontario	Alsop and Wood, 2011	larvae
Danio rerio	212,1	146,9	7,8	3,5	141	26,5	Hard water from Lake Ontario	Alsop and Wood, 2011	Adult
Oncorhynchus mykiss	5,9	8,4	6,2	1,4	14-22	n.r.	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
Oncorhynchus mykiss	9,2	13,1	7,1	1,4	14-22	n.r.	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
Oncorhynchus mykiss	21	19,1	7,5	2,2	57	13	Carbon & biofiltered city water	Vardy et al., 2013	swim-up larvae
Oncorhynchus mykiss	22	20	7,5	2,2	57	13	Carbon & biofiltered city water	Vardy et al., 2013	juvenile
Oncorhynchus mykiss	24	21,8	7,5	2,2	57	13	Carbon & biofiltered city water	Vardy et al., 2013	juvenile
Oncorhynchus mykiss	40	36,4	7,5	2,2	57	13	Carbon & biofiltered city water	Vardy et al., 2013	yolc sac age
Oncorhynchus mykiss	60	127,2	7,84	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	1d post-hatch
Oncorhynchus mykiss	56,6	120,5	7,97	0,4	104	12	well water + deionized water	Ingersoll & Mebane, 2014	18d post-hatch
Oncorhynchus mykiss	40,8	99,0	8	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	60d post-hatch
Oncorhynchus mykiss	42,4	101,2	8	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	60d post-hatch
Oncorhynchus mykiss	50,1	110,9	8,04	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
Oncorhynchus mykiss	59	122,4	8,04	0,4	103	12	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
Oncorhynchus mykiss	19,1	55,7	8,05	0,4	105	12	well water + deionized water	Ingersoll & Mebane, 2014	95d post-hatch
Oncorhynchus mykiss	60,6	112,7	8,05	0,4	105	12	well water + deionized water	Ingersoll & Mebane, 2014	74d post-hatch
Oncorhynchus mykiss	63	127,2	8,05	0,4	105	12	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
Oncorhynchus mykiss	8,5	13,1	8,1	1,3	14-22	n.r.	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
Oncorhynchus mykiss	30,9	70,1	8,1	0,5	108	12	Reconstituted water	Little et al., 2012	160d post-hatch
Oncorhynchus mykiss	36,5	78,9	8,1	0,5	107,7	12	Reconstituted water	Little et al., 2012	30d post-hatch
Oncorhynchus mykiss	59,9	122,4	8,1	0,4	105	12	well water + deionized water	Ingersoll & Mebane, 2014	32d post-hatch
Oncorhynchus mykiss	62,9	126,3	8,11	0,4	95	12	well water + deionized water	Ingersoll & Mebane, 2014	1d post-hatch

Oncorhynchus mykiss	6,7	8,9	8,5	1,5	14-22	n.r.	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
Oncorhynchus mykiss	56,6	107,8	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	18d post-hatch
Oncorhynchus mykiss	62,9	115,5	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	1d post-hatch
Oncorhynchus mykiss	59,9	111,8	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	32d post-hatch
Oncorhynchus mykiss	59	110,8	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	46d post-hatch
Oncorhynchus mykiss	42,4	90,1	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	60d post-hatch
Oncorhynchus mykiss	60,6	124,2	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	74d post-hatch
Oncorhynchus mykiss	19,1	64,6	8,0-8,1	0,4	95-108	12	Well water + deionized water	Calfee et al., 2014	95d post-hatch
Pimephales promelas	12,2	14,9	6	1,58	48	25	reconstituted water	OSU, 2016	larvae
Pimephales promelas	13	21,8	6	1,29	44	25	reconstituted water	OSU, 2016	larvae
Pimephales promelas	24,4	40,1	6,5	0,98	48	25	reconstituted water	OSU, 2016	larvae
Pimephales promelas	5,9	22,6	7,01	0,5	17,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	12,8	44,0	7,01	0,5	23,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	7,8	29,7	7,13	0,5	17,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	180	232,6	7,2	0,7	1213	n.r.	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	13,2	45,2	7,22	0,5	28,4	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	30,2	75,4	7,28	0,5	108,5	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	7,5	29,1	7,29	0,5	4,2	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	10,2	37,4	7,29	0,5	19,3	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	7,2	27,6	7,3	0,5	10,9	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	21,4	62,6	7,31	0,5	52,3	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	102	94,4	7,5	2,2	57	22	Carbon & biofiltered city water	Vardy et al., 2013	yolk sack stage
Pimephales promelas	15,3	50,7	7,58	0,5	19,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	17,4	52,8	7,6	<0,5	1245	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	31,7	82,2	7,8	0,5	97	25	Reconstituted water	Nimmo et al., 2006	larvae
Pimephales promelas	337,6	97,5	7,9	8,5	107	25	Natural water	Nimmo et al., 2006	larvae
Pimephales promelas	24,4	65,9	7,94	0,5	23,8	25	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	769	552,6	8	6,9	438	n.r.	Natural water	Van Genderen et al., 2007	larvae

Pimephales promelas	684	658,3	8,1	2,5	187	n.r.	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	1870	1669,4	8,1	4,4	66	n.r.	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	300	384,9	8,2	<0,5	287	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	906	550,2	8,2	9,8	288	n.r.	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	544	580,2	8,2	1,2	294	n.r.	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	1390	1185,1	8,2	5,4	794	n.r.	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	250	330,8	8,3	<0,5	156	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	262	341,7	8,3	<0,5	284	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	315	395,8	8,3	<0,5	767	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	197	273,8	8,4	<0,5	70	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	473	562,5	8,5	<0,5	445	n.r.	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	137	104,3	7,85-8,17	2,96	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	96,6	159,1	7,85-8,17	0,42	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	106,1	170,6	7,85-8,17	0,41	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	259,5	178,8	7,85-8,17	3,94	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	129	192,0	7,85-8,17	0,51	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	597,7	245,5	7,85-8,17	9,53	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	283,6	263,7	7,85-8,17	2,42	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	494,8	332,5	7,85-8,17	5,14	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	363,6	339,8	7,85-8,17	2,46	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	499,5	344,2	7,85-8,17	4,97	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	468,1	349,4	7,85-8,17	4,26	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	395,5	356,9	7,85-8,17	2,73	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	402,4	378,1	7,85-8,17	2,45	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	383,7	379,9	7,85-8,17	2,07	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae

Pimephales promelas	429	380,9	7,85-8,17	2,89	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	422,1	388,9	7,85-8,17	2,61	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	596,3	394,0	7,85-8,17	5,68	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	613,3	418,3	7,85-8,17	5,47	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	774,3	531,4	7,85-8,17	5,95	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	726,5	542,1	7,85-8,17	4,98	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	671,2	610,5	7,85-8,17	2,94	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	2034	825,5	7,85-8,17	18,23	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1405	843,9	7,85-8,17	9,56	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1526	903,0	7,85-8,17	10,16	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1564	957,0	7,85-8,17	9,77	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1681	1039,0	7,85-8,17	9,94	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1959	1192,1	7,85-8,17	10,94	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1514	1251,9	7,85-8,17	5,01	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	2013	1260,1	7,85-8,17	10,58	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1949	1327,5	7,85-8,17	8,93	84,5-91,1	25	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Cyprinus carpio	820		7,6		220	24	dechlorinated well water	Dehghani et al, 2012	Adult
Pimephales promelas	230		7,7		80-120	23	Reconstituted water	Johnson et al., 2008	< 24h old

Table 70: Summary of the acute toxicity data for fish for the three pH classes

Test organism	**L(E)C_{50} (µg Cu/L)**		
	pH: 5.51-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Oncorhynchus mykiss			
n	8	22	47
Min	4.2	2.8	6.7
Max	82.2	890	516
Geometric mean	24.2	47.4	63.6
Lowest value (only when data<4)	/	/	/
Danio rerio			
n	/	2	3
Min	/	11.7	148.4
Max	/	35	212.1
Geometric mean	/	20.3	167.4
Lowest value (only when data<4)		11.7	148.4
Cyprinus carpio			
n	/	/	3
Min	/	/	800
Max	/	/	820
Geometric mean	/	/	810
Lowest value (only when data<4)	/	/	800
Pimephales promelas			
n	5	46	207
Min	4.4	5.9	12.4
Max	24.4	1400	2034
Geometric mean	12.1	96.7	255.9
Lowest value (only when data<4)	/	/	/
Lepomis macrochirus			
n		2	3
Min	/	1000	4250
Max	/	1100	9150
Geometric mean	/	1049	5509
Lowest value (only when data<4)	/	1000	4250

Table 71: Summary of the acute toxicity data for fish for the three pH classes considering DOC normalisation at 2mg/L

Test organism	**L(E)C_{50} (µg Cu/L)**		
	pH: 5.51-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Oncorhynchus mykiss			
n	8	8	25
Min	6.28	13.1	8.9
Max	99.3	336.3	561.9
Geometric mean	40.6	45.9	94.7
Lowest value (only when data<4)	/	/	/
Danio rerio			

n	/	1	2
Min	/	26	94.7
Max	/	/	146.9
Geometric mean	/	/	117.9
Lowest value (only when data<4)		26	94.7
Pimephales promelas			
n	3	11	47
Min	14.9	22.6	50.7
Max	40.1	232.6	1669.4
Geometric mean	23.5	49.3	382.86
Lowest value (only when data<4)	14.9	/	/

It should be noted that in the Heijerick et al., 2016 reportndata points for juveniles has been added. Nevertheless, as the significant number of studies did not provide sufficient information on the life stage, the number of data points was limited. Moreover, with the available data, the lowest E(L)C50 retained for juveniles was in general somewhat higher than the overall geometric mean. Therefore, only data from all life stage are considered for ERV derivation.

As expected, an increased LC_{50} with increasing pH was noted for these fish species. It should be noted that the previous lowest geomean LC50 value 8.1 µg/L was recorded for *P. promelas* tested in ecotoxicity media with low pH (between 5.5 and 6.5) was challenged during the discussions on the previous copper CLH reports. These discussions are reflected in the opinion of the copper flakes (CLH-O-0000001412-86-30/F adopted 04 December 2014) and are reported here below. However, according to the improvement of database described before, **the current lowest LC50 value for fish was calculated to be 12.1 µg/L corresponding to the geomean also recorded for *P. promelas* at pH between 5.5 and 6.5.**

If the geomean is used whatever the number of available data, the lowest LC50 value for fish would be 11.7 µg/l recorded for *D. rerio* at pH between 6.5 and 7.5.If the normalisation with the DOC is taking into account, **the lowest LC50 value for fish was calculated to be 14.9 µg/L corresponding to the lowest value recorded for *P. promelas* at pH between 5.5 and 6.5.**

If the geomean is used whatever the number of available data, the lowest LC50 value for fish would be 23.5 µg/l recorded for *P. promelas* at pH between 6.5 and 7.5.

Extract from CLH-O-0000001412-86-30/F adopted 04 December 2014*:"The lowest geometric mean LC_{50} reported [in the CLH report] is 8.1 µg/L (as copper) for fathead minnow P. promelas at pH 5.5-6.5 (…). This is based on two values, both for larval fish, 15.0 µg/L and 4.4 µg/L. During PC (commenting period), industry indicated that the test medium in the study which resulted in the lowest EC_{50} (cited as Erickson et al., 1996) used a high flow-through rate, had low hardness (22 mg $CaCO_3$/L) and low DOC concentration (not stated), and used larvae that were less than 24 hours' old. Although not mentioned in the CLH report, in the original paper the lowest LC_{50} was determined at the minimum pH, i.e. 6.0. Industry therefore considered this test to represent a worst case, and suggested that the sensitivity of this species at pH 6 versus pH 7 was unexpected and may be related to insufficient adaptation to low pH conditions. The data were therefore not considered reliable and not used for classification in the REACH registrations as well as the vRAR. Nevertheless, RAC notes that other minimum acute fish LC_{50}s are of the same order of magnitude (e.g. O. mykiss at all pHs, and P. promelas at pH 6.5-7.5). The OECD TG 203 permits testing in waters with total hardness as low as 10 mg $CaCO_3$/L, and a preferred minimum pH of 6.0, so the conditions used in the Erickson (1996) study were within the validity criteria of the guidelines and cannot be considered a worst case. In addition, this species can tolerate poor conditions such as turbid, hot, poorly oxygenated, intermittent streams, which are unsuitable for most fishes*

(http://www.fishbase.org/Summary/speciesSummary.php?ID=4785&AT=fathead+minnow).
Further papers provided by industry stakeholders following public consultation (Mount, 1973 and Zischke et al., 1983) indicate that P. promelas can survive at pHs as low as 4.5, so that a pH of 6.0 does not appear to be intolerable over short exposures. RAC also notes that the replacement test for acute fish toxicity (OECD TG236) involves embryos, so the life stage argument was not considered relevant either. It is also unclear why the dossier submitter decided to include them in the CLH report if they had been previously rejected. RAC accepts that an acute toxicity test with fish larvae may be more sensitive 14 than one with older fish if they were not properly acclimated, but does not find the other reasons for rejection convincing.
Data for other species show trend of increasing acute fish toxicity with declining pH, presumably due to increasing bioavailability. The acute LC_{50} for Danio rerio at pH 6.5-7.5 (35 µg/L, n=3 so a geometric mean is not appropriate) is similar to that of O. mykiss at pH 5.5-6.5 (geometric mean 29 µg/L, based on n=6), implying that the sensitivity of D. rerio at the lower pH could be higher. Rather than ignoring the P. promelas data completely, the geometric mean LC_{50} of 8.1 µg/L is considered to be relevant for hazard classification as it takes account of uncertainties about the sensitivity of fish at acidic pH, although this is a conservative approach given the life stages that were tested (N.B. if the most sensitive value of 4.4 µg/L were used the consequence for classification would be the same for coated copper flakes). RAC has not considered how DOC or hardness affect the observed pattern in ecotoxicity data, as such an analysis was not presented in the CLH report.

5.4.1.2 Long-term toxicity to fish

According to the EU RAR (2008), 29 individual data points for fish were selected for 3 standard species (*Oncorhynchus mykiss, Pimephales promelas* and *Salvelinus fontinalis*).

In the updated database summaries by Heijerick and Van Sprang, 2016b, 25 new individual data points for fish were included for the 2 standard species (*Oncorhynchus mykiss and Pimephales promelas).* The paper also re-evaluated some endpoint on existing studies to derived EC10 instead of NOEC when possible. These new and re-evaluated data are presented below and details are given in the table 72.

- *Oncorhynchus mykiss:* three new publications (Besser et al., 2005 ; Ingersoll and Mebane, 2014 ; Ng et al., 2010) were identified until the EU RAR (2008) publication. A total of 12 reliable chronic endpoints were reported at pH varying between 6.2 and 8.3. The information on the testing methodology that is provided by Ng et al (2010) indicated that no real replicates were used and no acclimation was carried out to lower pHs; such a deficiency has an impact on the reliability of this study. There were also a number of tests (reported by Ng et al., 2010) that were conducted at a pH that fell outside the pH-range that is considered relevant for classification purposes (5.5-8.5). In addition, Ng et al. (2010) published EC_{10} values for survival and growth. No concentration-response relationship was found when growth (biomass/surviving fish) was used as parameter.

  However, to complete the database on chronic toxicity to fish at low pH values, Oregon State University (OSU, 2016) conducted a 56 days early life stage test (OECD 210, 2013) with the rainbow trout, *O. Mykiss*, at pH 6 and pH 7 which is in line with the OECD 210 guideline. The survival and growth (wet weight) endpoints of the OSU (2016b) study were retained. These endpoints result in EC_{10} values of 28.5 (survival) and 36.0 (wet weight) µg Cu/L at pH 6, and 49.3 (survival) and 47.3 (wet weight) µg/L at pH 7. This test was carried out in a test water similar to that of the Ng et al. (2010) study, but with replicates and corresponding statistical assessments, with organisms acclimated to low pH conditions, and with pre-equilibrated test waters. This OSU (2016) study may therefore replace the Ng et al., 2010 data.

A fourth publication by Welsh et al (2008) only reported chronic LC_{50} values (n=6). These values cannot be used for the chronic hazard assessment of copper, and were therefore not further considered.

Moreover, the Heijerick and Van Sprang, 2016b report proposed to replace some of the NOEC-values that have been used until now from the previous study published in Seim et al (1984) and Marr et al (1996) by EC_{10} values. This approach is agreed as EC_{10} values are generally considered more reliable than NOEC-values in the current hazard assessment. The data that were provided in Seim et al (1984) also allowed the derivation of an EC_{10} for an endpoint (survival) that had not been determined for this study.

Table 72: Proposed re-evaluated chronic data for the rainbow trout *O.mykiss*

Reference	**Testing conditions**	**Existing NOEC**	**New EC_{10}**	**Endpoint**
Marr et al, 1996	pH: 7.5 DOC: 0.2 mg/L	2.2 µg/L	 8.1 µg/L 3.3 µg/L	Growth Growth (length) Growth (weight)
Seim et al, 1984	pH: 7.65 DOC: 1.30 mg/L	16 µg/L	23.5 µg/L 53.3 µg/L	Growth (DW) Mortality (new)

- *Pimephales promelas*: a new publication (Besser et al, 2005) conducted a number of 30 days chronic tests (pH 8 class) with the fathead minnow *P. promelas*, and studied the long-term effects of copper exposure on mortality, weight-based growth of newly hatched larvae.

  To complete the database at pH 6-7, the registrants have conducted a chronic ELS toxicity test with *P.promelas* at pH 6, 6.5 and 7 (OSU, 2016) as available in the updated REACH registration dossier of January 2017. The data allowed to calculate reliable NOEC/L(E)C_{10} values for mortality and growth at pH 6, 6.5 and 7. The estimated EC10 values decrease from 22 µg/L at pH 7 to 10 µg/L at pH 6, i.e. an estimated factor 2. However, given that the uncertainty on these EC10 estimates is larger than a factor 2, these data cannot confirm nor exclude a pH effect on chronic copper toxicity to *P. promelas*. This study is considered reliable.

  Moreover, as for *O. mykiss*, the Heijerick and Van Sprang, 2016b has proposed to re-evaluate the existing studies with the fathead minnow (Scudder et al, 1988; Mount and Stephan, 1969; Pickering et al, 1977; Brungs et al, 1976; Spehar and Fiandt, 1985) resulted in the identification of some additional data points that has not been picked up in the original evaluation of these studies. Secondly, some of the NOEC-values that have been used until now for *P.promelas* can be replaced by EC_{10} values that were determined with available raw data.

Table 73: Proposed re-evaluation of chronic data for the fathead minnow *P.promelas (*Heijerick and Van Sprang, 2016b)

Reference	**Testing conditions**	**Existing NOEC**	**New EC_{10}**	**Endpoint**
Spehar & Fiandt, 1985	pH: 7.05 DOC: 1.0 mg/L	4.8 µg/L 4.8 µg/L 16 µg/L (new)	3.8 µg/L 5.9 µg/L ---	Growth Mortality Reproduction

Mount & Stephan, 1969	pH: 6.90 DOC: 0.55 mg/L	10.8 µg/L[(1)] 10.8 µg/L[(1)] 10.8 µg/L[(1)]	---[(5)] 13.1 µg/L 14.9 µg/L 16.7 µg/L	Reproduction[(2)] Mortality $Growth_{length}$ $Growth_{length,male}$ $Growth_{length,female}$
Pickering et al, 1977	pH: 7.85 DOC: 0.55 mg/L	37 µg/L (new) 22.5 µg/L 22.5 µg/L 25.5 µg/L 25.5 µg/L	 22.3 µg/L ---[(5)] 16.3 µg/L ---[(5)]	$Growth_{length}$ Reproduction[(3)] Reproduction[(4)] Reproduction[(3)] Reproduction[(4)]
Brungs et al, 1976	pH: 8.10 DOC: 5.9 mg/L	66 µg/L	47.6 µg/L	Reproduction[(3)]
Scudder et al, 1988	pH: 8.17 DOC: 1.30 mg/L		53.7 µg/L 30.3 µg/L ---[(5)]	Abnormalities $Growth_{weight}$ Mortality

[(1)]: the original value of 10.6 µg/L was the average of three analytical methods; the value that was based on AAS-measured Cu-concentrations was 10.8 µg/L
[(2)]: #spawnings/female
[(3)]: #eggs/female
[(4)]: #eggs/spawning
[(5)]: NOEC was retained as the range of the 95% Confidence Interval around the EC_{10} was more than one order of magnitude.

- *Salvenius fontinalis*: Heijerick and Van Sprang, 2016b has also re-evaluate the existing studies with the brook trout (Sauter et al., 1976). EC_{10} values which could be determined could replace the previously used NOEC values. The new values are presented in table 74.

Table 74: Proposed re-evaluation of chronic data for the brook trout *S.fontinalis (*Heijerick and Van Sprang, 2016b)

Reference	**Testing conditions**	**Existing NOEC**	**New EC_{10}**	**Endpoint**
Sauter et al, 1976	pH: 6.85[(1)] DOC: 1.3 mg/L	< 5 µg/L 13 µg/L 6.4 µg/L	11.2 µg/L 12.4 µg/L 6.4 µg/L	Growth Mortality Reproduction
	pH: 6.90[(2)] DOC: 1.3 mg/L	21 µg/L 21 µg/L 49 µg/L	44.4 µg/L 41.3 µg/L 36.4 µg/L	Growth Mortality Reproduction

[(1)]: 60d exposure ; [(2)]: 30d exposure

Table 75: Overview of the NOEC/EC10 values for fish published or generated since the EU RAR (2008) and re-evaluated chronic toxicity data for fish

Organism	Age/size of organisms	Exposure time	Endpoint	NOEC/EC10	Normalised	pH	DOC	Alk	Medium	Reference
				(µg/L)	(µg/L)		mg/L	mg/L as CaCO3		
Oncorhynchus mykiss	juveniles	51 d	Mortality	28,5	43.75	6	1,15	40	Laboratory water	OSU, 2016
Oncorhynchus mykiss	juveniles	51 d	Growth - wet biomass	28,2	43.33	6	1,15	40	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Mortality	10,1	17,1	6	1,10	12,0	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Mortality	11,9	14,6	6	1,59	48,5	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Mortality	13,8	23,5	6,5	1,14	15,0	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Growth - dw	9	15,3	6	1,10	12,0	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Growth - dw	11,9	14,6	6	1,59	48,5	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Growth - dw	13,8	23,5	6,5	1,14	15,0	Laboratory water	OSU, 2016
Oncorhynchus mykiss	juveniles	51 d	Growth - biomass	38,8	57.67	7	1,26	40	Laboratory water	OSU, 2016
Oncorhynchus mykiss	juveniles	51 d	Mortality	54,2		7	1,26	40	Laboratory water	OSU, 2016
Pimephales promelas	fry (10 - 20 mm)	327 d	Growth - length male	14,9	45,2	6,9	0,55	30,0	Spring+ deionised tap	Mount & Stephan, 1969
Pimephales promelas	fry (10 - 20 mm)	327 d	Growth - length female	16,7	49,3	6,9	0,55	30,0	Spring+ deionised tap	Mount & Stephan, 1969
Pimephales promelas	embryo-larval	32 d	Growth	3,8	7,5	7,05	1	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
Pimephales promelas	larvae	7 d	Growth - dw	22	31,5	7	1,37	32,5	Laboratory water	OSU, 2016
Pimephales promelas	fry (10 - 20 mm)	327 d	Mortality	13,1	40,9	6,9	0,55	30,0	Spring+ deionised tap	Mount & Stephan, 1969
Pimephales promelas	embryo-larval	32 d	Mortality	5,9	11,6	7,05	1	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
Pimephales promelas	larvae	7 d	Mortality	22,5	32,2	7	1,37	32,5	Laboratory water	OSU, 2016
Pimephales promelas	embryo-larval	32 d	Reproduction (hatching)	16	30,6	7,05	1	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
Pimephales promelas	fry (10 - 20 mm)	327 d	Reproduction	10,8	34,9	6,9	0,55	30,0	Spring+ deionised tap	Mount & Stephan, 1969
Salvelinus fontinalis	fry	60 d	Growth	11,2	17,11	6,85	1,3	27,8	Well	Sauter et al., 1976

Salvelinus fontinalis	fry	60 d	Mortality	12,4	18,91	6,85	1,3	27,8	Well	Sauter et al., 1976
Salvelinus fontinalis	fry	60 d	Reproduction	6,4	10,7	6,85	1,3	27,8	Well	Sauter et al., 1976
Oncorhynchus mykiss	fry (0.12 g; 2.6 cm)	60 d	Growth - length	8,1	52,7	7,5	0,2	27,7	Well + deionised water	Marr et al., 1996
Oncorhynchus mykiss	fry (0.12 g; 2.6 cm)	60 d	Growth - weight	3,3	28,5	7,5	0,2	27,7	Well + deionised water	Marr et al., 1996
Oncorhynchus mykiss	eggs	63 d	growth (dry wt)	23,5	34,02	7,65	1,3	126,0	Well	Seim et al., 1984
Oncorhynchus mykiss	larvae (26 days post hatch)	28 d	Growth (weight)	13	43,3	7,95	0,4	92,9	well water + deionized water	Ingersoll & Mebane, 2014
Oncorhynchus mykiss	eyed embryo	30 d	Growth (weight)	8,4	26,2	8,3	0,5	115,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Oncorhynchus mykiss	swim-up fry	30 d	Growth (weight)	12	35	8,3	0,5	115,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Oncorhynchus mykiss	eggs	63 d	Mortality	53,3	70,92	7,65	1,3	126,0	Well	Seim et al., 1984
Oncorhynchus mykiss	larvae (26 days post hatch)	21 d	Mortality	37	83,2	7,87	0,4	91,1	well water + deionized water	Ingersoll & Mebane, 2014
Oncorhynchus mykiss	larvae (1 day post hatch)	21 d	Mortality	41	88,4	7,92	0,4	91,1	well water + deionized water	Ingersoll & Mebane, 2014
Oncorhynchus mykiss	larvae (1 day post hatch)	52 d	Mortality	34	78,9	7,92	0,4	91,1	well water + deionized water	Ingersoll & Mebane, 2014
Oncorhynchus mykiss	larvae (26 days post hatch)	28 d	Mortality	34	78,9	7,95	0,4	91,1	well water + deionized water	Ingersoll & Mebane, 2014
Oncorhynchus mykiss	eyed embryo	30 d	Mortality	17	45,6	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Oncorhynchus mykiss	swim-up fry	30 d	Mortality	22	54,8	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	larvae	28 d	Abnormalities	53,7	69,9	8,17	1,3	211,9	Ground water	Scudder et al., 1988
Pimephales promelas	newly hatched larvae	30 d	Growth (weight)	10	31,4	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	newly hatched larvae	30 d	Growth (weight)	16	45	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	larvae	28 d	growth (weight)	30,3	41,79	8,17	1,3	211,9	Ground water	Scudder et al., 1988

Pimephales promelas	4 weeks old	187 d	growth/length	37	86,1	7,85	0,55	15,4	Spring+ deionised tap	Pickering et al., 1977
Pimephales promelas	newly hatched larvae	30 d	Mortality	19	51,3	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	newly hatched larvae	30 d	Mortality	19	51	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	newly hatched larvae	30 d	Mortality	24	59,8	8,3	0,5	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	larvae (4 weeks old)	187 d	reproduction (#eggs/female)	16,3	50,3	7,85	0,55	15,4	Spring+ deionised tap	Pickering et al., 1977
Pimephales promelas	larvae (7 month old)	7 d	reproduction (#eggs/female)	22,3	62,92	7,85	0,55	15,4	Spring+ deionised tap	Pickering et al., 1977
Pimephales promelas	juvenile (32 - 38 mm; 5 months old)	270 d	reproduction (#spawnings/female)	47,6	47,6	8,1	2	183,0	River	Brungs et al., 1976

Table 76: Summary of the chronic toxicity data for fish for the 3 pH classes

Test organism	**NOEC/EC10 (µg Cu/l)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Oncorhynchus mykiss(mortality)			
n	1	3	8
Min	28.5	24	7.6
Max	/	54.2	53.3
Geometric mean	28.5	33.2	28.2
Lowest value (only when data<4)	/	24	/
Oncorhynchus mykiss(growth)			
n	1	4	5
Min	28.2	3.3	8.4
Max	/	45	23.5
Geometric mean	/	14.7	12.9
Lowest value (only when data<4)	28.2	/	/
Pimephales promelas (Mortality)			
n	3	3	5
Min	10.1	5.9	19
Max	13.8	22.5	61
Geometric mean	11.8	12.02	28.1
Lowest value (only when data<4)	10.1	**5.9**	/
Pimephales promelas (Growth)			
n	3	4	6
Min	8.7	3.4	10
Max	13.8	22	59.5
Geometric mean	11.4	12	26.6
Lowest value (only when data<4)	8.7	/	/
Pimephales promelas (Reproduction)			
n	/	2	9
Min	/	10.8	14.5
Max	/	16	66
Geometric mean	/	17.3	25.8
Lowest value (only when data<4)	/	10.8	/
Salvelinus fontinalis (Mortality)			
n	/	4	1
Min	/	9.5	22.3
Max	/	44.4	/
Geometric mean	/	16.9	/
Lowest value (only when data<4)	/	/	22.3
Salvelinus fontinalis (Growth)			
n	/	4	1
Min	/	9.5	22.3
Max	/	41.3	/
Geometric mean	/	17.1	/
Lowest value (only when data<4)	/	/	22.3
Salvelinus fontinalis (Reproduction)			
n	/	3	/

Min	/	6.4	/
Max	/	36.4	/
Geometric mean	/	/	/
Lowest value (only when data<4)	/	6.4*	/

* No geomean could be apply as only 3 data are available considering all pH-categories

Table 77: Summary of the chronic toxicity data for fish for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	**NOEC/EC10 (µg Cu/l)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Oncorhynchus mykiss(mortality)			
n	1	2	8
Min	43.75	16.5	22.41
Max	/	19.3	88.4
Geometric mean	/	17.5	60.6
Lowest value (only when data<4)	43.75	16.5	/
Oncorhynchus mykiss(growth)			
n	1	4	5
Min	43.33	28.5	22.4
Max	/	57.67	43.3
Geometric mean	/	40.52	31.3
Lowest value (only when data<4)	43.33	/	/
Pimephales promelas (Mortality)			
n	3	3	5
Min	14.6	11.6	51
Max	23.5	40.9	78.2
Geometric mean	17.9	24.8	60.5
Lowest value (only when data<4)	14.6	11.6	/
Pimephales promelas (Growth)			
n	3	4	6
Min	14.6	7.5	31.4
Max	23.5	49.3	115.9
Geometric mean	17.3	27	63.8
Lowest value (only when data<4)	14.6	/	/
Pimephales promelas (Reproduction)			
n	/	2	9
Min	/	30.6	36.4
Max	/	34.9	68.5
Geometric mean	/	32.7	57
Lowest value (only when data<4)	/	30.6	/
Salvelinus fontinalis (Mortality)			
n	/	4	1
Min	/	18.7	42.3
Max	/	55.3	/
Geometric mean	/	28.4	/
Lowest value (only when data<4)	/	/	42.3
Salvelinus fontinalis (Growth)			
n	/	4	1

Min	/	17.1	42.3
Max	/	58.9	/
Geometric mean	/	28.2	/
Lowest value (only when data<4)	/	/	42.3
Salvelinus fontinalis (Reproduction)			
n	/	3	/
Min	/	10.7	/
Max	/	49.38	/
Geometric mean	/	/	/
Lowest value (only when data<4)	/	10.7*	/

* No geomean could be apply as only 3 data are available considering all pH-categories

The improvment of database allowed to obtain chronic toxicity values for fish at pH 5.5-6.5. These values show that no more toxicity for fish is expected for long term exposure at pH5.5-6.5 compared to toxicity observed at pH 6.5-7.5.

According to the improvement of database described before, **the current lowest NOEC/EC_{10} value for fish was calculated to be 5.9 µg/L corresponding to the lowest value recorded for *P.promelas* at pH between 6.5 and 7.5 for mortality.**

If the geomean is used whatever the number of available data, the lowest NOEC/EC_{10} value for fish would be 6.4 µg/l recorded for *S. fontinalis* at pH between 6.5 and 7.5.

If the normalisation with the DOC is taking into account, **the lowest NOEC/EC_{10} value for fish was calculated to be 10.7 µg/L corresponding to the lowest value recorded for *S. fontinalis* at pH between 6.5 and 7.5 for reproduction effect.**

If the geomean is used whatever the number of available data, .

5.4.2 Aquatic invertebrates

5.4.2.1 Short-term toxicity to aquatic invertebrates

According to the EU RAR (2008), 91 individual data points for aquatic invertebrates were selected for 2 standard species (*Ceriodaphnia dubia and Daphnia magna).*

In the updated database submitted by the registrants of the REACH registration dossier (as detailed in Heijerick and Van Sprang, 2016a), 239 new individual data points for invertebrates were included for the 2 standard species (*Daphnia magna* and *Ceriodaphnia magna).* These new data are presented below and details are given in the table 78.

- *Daphnia magna*: New acute data for *D.magna* available for each of the three classification pH-classes were identified in eleven different publications (Bossuyt et al, 2004 ; De Schamphelaere et al, 2004, 2007 ; Fulton and Meyer, 2014 ; Johnson et al, 2008 ; Kramer et al, 2004 ; Long et al, 2004 ; Rodriguez and Arbildua, 2012 ; Ryan et al, 2009; Villavicencio et al, 2005 ; Yim et al, 2006), resulting in 180 new reliable E(L)C_{50} values.

- *Ceriodaphnia dubia*: Eight different publications reported new information on the acute toxicity of copper for *C. dubia* (Cooper et al, 2009; De Schamphelaere et al, 2007 ; Hyne et al, 2005 ; Johnson et al, 2008 ; Markich et al, 2005 ; Nimmo et al, 2006 Van Genderen et al, 2007; Wang et al, 2011), resulting in 59 new, reliable and relevant E(L)C_{50} values. The data

from Cooper et al., 2009 were rejected because an Australian native strain was uses as test species, and the calculated EC_{10} (0.5 µg Cu/L)/NOEC (1.3 µg Cu/L) is below/at the copper concentration in the control (1 µg Cu/L).

Table 78: Overview of the E(L)C50 values for invertebrates published since the EU RAR (2008)

	E(L)C50Value	E(L)C50 Normalised value		DOC	Hardness	Temperature		
Species	**µg/L**	**µg/L**	**pH**	**mg/L**	**mg/L as CaCO3**	**°C**	**Type of water**	**Reference**
Ceriodaphnia dubia	9,0	7,4	6,1	2,5	375	25	Natural water	Markich et al., 2005
Ceriodaphnia dubia	12,0	9,8	6,1	2,5	140	25	Natural water	Markich et al., 2005
Ceriodaphnia dubia	1,6	16,4	6,5	0,1	44	25	Reconstituted soft water	Hyne et al., 2005
Ceriodaphnia dubia	1,6	11,6	6,5	0,1	374	25	Reconstituted soft water	Hyne et al., 2005
Ceriodaphnia dubia	73	14,3	6,5	10	44	25	Reconstituted soft water	Hyne et al., 2005
Ceriodaphnia dubia	23	18,4	7	2,5	25	25	Natural water	Hyne et al., 2005
Ceriodaphnia dubia	30	24,2	7	2,5	374	25	Natural water	Hyne et al., 2005
Ceriodaphnia dubia	32	25,7	7	2,5	140	25	Natural water	Hyne et al., 2005
Ceriodaphnia dubia	5,38	14,1	7,2	0,7	1213	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	50	4,9	7,2	15,5	6,3	25	Natural water	Nimmo et al., 2006
Ceriodaphnia dubia	2,2	27,7	7,5	0,1	44	25	Reconstituted soft water	Hyne et al., 2005
Ceriodaphnia dubia	2,8	32,3	7,5	0,1	44	25	Reconstituted soft water	Hyne et al., 2005
Ceriodaphnia dubia	5,02	17,9	7,6	0,5	1245	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	23,6	7,0	7,7	6,5	114	25	Natural water	Nimmo et al., 2006
Ceriodaphnia dubia	39,0	31,3	7,8	2,5	140	25	Natural water	Markich et al., 2005
Ceriodaphnia dubia	42	33,7	7,8	2,5	25	25	Natural water	Hyne et al., 2005
Ceriodaphnia dubia	44,0	35,5	7,8	2,5	375	25	Natural water	Markich et al., 2005
Ceriodaphnia dubia	14	3,1	7,9	8,5	110	25	Natural water	Nimmo et al., 2006
Ceriodaphnia dubia	42,2	9,5	7,9	8,5	107	25	Natural water	Nimmo et al., 2006
Ceriodaphnia dubia	77,4	28,0	8	5,8	159	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	148	55,2	8	6,4	268	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	279	131,5	8	6,9	438	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	257	100,5	8	7,7	349	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	10,4	31,2	8	0,5	158	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	6,5	48,0	8,1	0,1	44	25	Reconstituted soft water	Hyne et al., 2005

Ceriodaphnia dubia	44,1	36,4	8,1	2,5	187	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	131	68,8	8,1	4,4	66	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	79,2	26,4	8,1	6,3	149	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	17,7	44,5	8,1	0,5	305	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	36,3	51,4	8,2	1,2	294	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	78,5	49,4	8,2	3,5	223	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	207	105,0	8,2	5,4	509	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	302	91,5	8,2	9,8	288	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	23,1	53,0	8,2	0,5	287	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	26,2	58,6	8,2	0,5	220	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	147	55,8	8,3	5,8	235	25	natural well water	Wang et al., 2011
Ceriodaphnia dubia	157	60,6	8,3	5,8	238	25	natural well water	Wang et al., 2011
Ceriodaphnia dubia	115,0	23,4	8,3	9,8	198	20	Natural water	Bossuyt et al., 2004
Ceriodaphnia dubia	267	61,3	8,3	10	249	25	natural well water	Wang et al., 2011
Ceriodaphnia dubia	14,5	42,2	8,3	0,5	164	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	20,3	51,3	8,3	0,5	156	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	29,3	63,1	8,3	0,5	260	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	37	72,7	8,3	0,5	284	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	39,5	80,3	8,3	0,5	767	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	16	65,5	8,4	0,1	44	25	Reconstituted soft water	Hyne et al., 2005
Ceriodaphnia dubia	25	68,2	8,4	0,4	105	25	natural well water	Wang et al., 2011
Ceriodaphnia dubia	58,5	48,8	8,4	2,5	249	n.r.	Natural water	Van Genderen et al., 2007
Ceriodaphnia dubia	10,4	32,6	8,4	0,5	70	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	29,8	65,6	8,4	0,5	349	n.r.	Reconstituted water	Van Genderen et al., 2007
Ceriodaphnia dubia	30	77,8	8,5	0,3	174	25	Reconstitued water (ASTM hard water)	Wang et al., 2011
Ceriodaphnia dubia	24,8	55,6	8,5	0,5	445	n.r.	Reconstituted water	Van Genderen et al., 2007
Daphnia magna	1	2,3	5,96	0,8	19,8	25	Reconstituted water	Ryan et al., 2009
Daphnia magna	3,2	2,9	6,02	2,2	9,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	4,9	4,5	6,11	2,2	22,4	25	Reconstituted water with natural DOC	Ryan et al., 2009

Daphnia magna	0,5	1,6	6,16	0,6	10,6	25	Reconstituted water	Ryan et al., 2009
Daphnia magna	7	6,1	6,17	2,3	39,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	4	3,1	6,19	2,6	10,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	8,6	6,6	6,23	2,6	19,8	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	7,4	7,4	6,28	2	21	20		Rodriguez & Arbildua, 2012
Daphnia magna	29,1	29,1	6,28	2	394	20		Rodriguez & Arbildua, 2012
Daphnia magna	25,2	5,3	6,28	9	21,1	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	16,5	15,8	6,29	2,1	169	20		Rodriguez & Arbildua, 2012
Daphnia magna	8,3	6,4	6,29	2,6	42,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	25,9	5,6	6,3	8,7	9,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	22,8	5,3	6,33	8,4	44,9	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	18,8	4,0	6,33	8,7	10,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	26,6	5,8	6,33	8,7	22,4	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	1,6	5,9	6,34	0,5	42,2	25	Reconstituted water	Ryan et al., 2009
Daphnia magna	47,3	10,6	6,42	8,7	42,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	81,8	56,9	6,9	3,04	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	128	62,8	6,92	4,53	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	311	98,9	6,92	8,54	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	53,8	54,9	6,94	1,95	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	192	86,9	6,97	5,35	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	86,6	87,5	6,98	1,97	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	607	101,7	6,98	16,9	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	332	74,9	6,99	10,8	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	638	198,8	6,99	11,7	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	542	101,3	6,99	15,4	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	261	139,4	7	5,11	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004

Daphnia magna	101,8	22,2	7	9,01	40	20	Natural water	Villavicencio et al., 2005
Daphnia magna	129	130,8	7,01	1,95	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	275	71,0	7,03	9,22	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	60,6	48,1	7,06	2,58	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	212	31,7	7,07	13,7	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	50,6	48,8	7,08	2,08	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	9,2	16,6	7,1	1,1	81	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	372	44,7	7,1	17,8	250,0	20	Reconstituted water with natural DOC addition	De Schamphelaere et al., 2004
Daphnia magna	34,4	8,7	7,13	7,28	8	20	Natural water	Villavicencio et al., 2005
Daphnia magna	21	9,9	7,21	4,1	9	20	Natural water	Villavicencio et al., 2005
Daphnia magna	6,183	23,1	7,3	0,5	42	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
Daphnia magna	4,9	12,2	7,41	0,81	9	20	Natural water	Villavicencio et al., 2005
Daphnia magna	118,5	27,0	7,43	8,47	14	20	Natural water	Villavicencio et al., 2005
Daphnia magna	77,3	15,3	7,45	9,4	12	20	Natural water	Villavicencio et al., 2005
Daphnia magna	3,7	21,3	7,5	0,34	12	20	Natural water	Villavicencio et al., 2005
Daphnia magna	50	30,5	7,55	3,27	18	20	Natural water	Villavicencio et al., 2005
Daphnia magna	5,5	20,6	7,57	0,53	20	20	Natural water	Villavicencio et al., 2005
Daphnia magna	14	14,7	7,6	1,9	75	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	61,82	10,2	7,6	11,4	50	20	natural water	Fulton & Meyer, 2014
Daphnia magna	47	34,2	7,62	2,75	12	20	Natural water	Villavicencio et al., 2005
Daphnia magna	39	34,7	7,64	2,25	12	20	Natural water	Villavicencio et al., 2005
Daphnia magna	7,3	23,9	7,65	0,6	18	20	Natural water	Villavicencio et al., 2005
Daphnia magna	19	23,9	7,65	1,59	13	20	Natural water	Villavicencio et al., 2005
Daphnia magna	6,596	23,7	7,65	0,5	72	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
Daphnia magna	11,4	23,7	7,7	0,96	14	20	Natural water	Villavicencio et al., 2005
Daphnia magna	35,23	22,8	7,7	3,1	80	20	natural water	Fulton & Meyer, 2014
Daphnia magna	3,976	15,4	7,71	0,5	42	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014

Daphnia magna	3,8	20,1	7,78	0,37	14	20	Natural water	Villavicencio et al., 2005
Daphnia magna	30,0	83,4	7,8	0,38	250	20	Reconstituted water	Bossuyt et al., 2004
Daphnia magna	40,6	98,6	7,8	0,38	250	20	Reconstituted water	Bossuyt et al., 2004
Daphnia magna	53,2	115,3	7,8	0,38	250	20	Reconstituted water	Bossuyt et al., 2004
Daphnia magna	7,8	21,5	7,8	0,72	19	20	Natural water	Villavicencio et al., 2005
Daphnia magna	7,7	17,0	7,8	0,9	79	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	6,4	40,9	7,81	0,25	28	20	Natural water	Villavicencio et al., 2005
Daphnia magna	6,2	30,4	7,83	0,38	23	20	Natural water	Villavicencio et al., 2005
Daphnia magna	8,4	35,3	7,83	0,44	20	20	Natural water	Villavicencio et al., 2005
Daphnia magna	7,4	23,9	7,83	0,6	22	20	Natural water	Villavicencio et al., 2005
Daphnia magna	8,9	24,1	7,83	0,73	18	20	Natural water	Villavicencio et al., 2005
Daphnia magna	30,5	52,4	7,84	1,1	42,7	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	30,8	28,0	7,84	2,2	9,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	56,1	53,6	7,85	2,1	42,7	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	9,46	33,0	7,85	0,5	72	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
Daphnia magna	7,2	30,5	7,86	0,44	22	20	Natural water	Villavicencio et al., 2005
Daphnia magna	7,4	30,8	7,86	0,45	22	20	Natural water	Villavicencio et al., 2005
Daphnia magna	38,2	29,6	7,86	2,6	19,8	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	45,1	35,1	7,87	2,6	43,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	72	47,2	7,87	3,1	42,7	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	100	38,9	7,87	5,2	42,7	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	108,8	24,1	7,87	8,9	10,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	6,03	22,8	7,87	0,5	46	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
Daphnia magna	87,39	22,4	7,88	7,8	84	20	natural water	Fulton & Meyer, 2014
Daphnia magna	155,7	24,2	7,88	12,5	54	20	natural water	Fulton & Meyer, 2014
Daphnia magna	8,4	59,5	7,9	0,1	42,7	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	5,7	33,2	7,9	0,3	26	20	Natural water	Villavicencio et al., 2005
Daphnia magna	5,2	29,2	7,91	0,3	9,2	25	Reconstituted water	Ryan et al., 2009
Daphnia magna	88,8	43,0	7,91	4,2	42,7	20	Reconstituted water	Villavicencio et al., 2005

Daphnia magna	15,3	13,3	7,92	2,3	9,2	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	12,1	52,4	7,94	0,31	132	20	Natural water	Villavicencio et al., 2005
Daphnia magna	187,6	47,5	7,94	8,3	21,1	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	9,3	28,2	7,95	0,6	42,2	25	Reconstituted water	Ryan et al., 2009
Daphnia magna	16,7	13,9	7,95	2,4	21,1	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	106,4	24,7	7,95	8,5	10,6	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	130,5	31,3	7,96	8,4	21,1	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	2,6	15,6	7,97	0,3	18,5	25	Reconstituted water	Ryan et al., 2009
Daphnia magna	21,1	20,1	7,98	2,1	40,9	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	122,5	28,7	7,98	8,6	44,9	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	159	37,1	7,98	8,8	40,9	25	Reconstituted water with natural DOC	Ryan et al., 2009
Daphnia magna	208,0	41,0	8	10,4	198	20	Natural water	Bossuyt et al., 2004
Daphnia magna	68,45	23,4	8,02	5,8	42	20	natural water	Fulton & Meyer, 2014
Daphnia magna	19,28	53,8	8,02	0,5	100	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
Daphnia magna	8,3	40,8	8,03	0,31	61	20	Natural water	Villavicencio et al., 2005
Daphnia magna	68,31	12,5	8,03	10,5	60	20	natural water	Fulton & Meyer, 2014
Daphnia magna	96,23	25,0	8,06	7,8	106	20	natural water	Fulton & Meyer, 2014
Daphnia magna	304	49,5	8,1	14,7	229	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	27,8	45,1	8,11	1,18	38	20	Natural water	Villavicencio et al., 2005
Daphnia magna	156,1	76,9	8,11	4,65	85,2	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	9,2	57,9	8,12	0,14	32	20	Natural water	Villavicencio et al., 2005
Daphnia magna	104,5	61,7	8,12	3,63	85,2	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	19,24	54,3	8,13	0,5	96	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
Daphnia magna	12,1	46,5	8,15	0,38	88	20	Natural water	Villavicencio et al., 2005
Daphnia magna	32,3	67,8	8,16	0,71	270	20	Natural water	Villavicencio et al., 2005
Daphnia magna	24,3	54,4	8,17	0,76	68	20	Natural water	Villavicencio et al., 2005
Daphnia magna	141,6	28,4	8,19	10	54	20	natural water	Fulton & Meyer, 2014
Daphnia magna	116,3	21,6	8,19	10,7	90	20	natural water	Fulton & Meyer, 2014
Daphnia magna	25,3	84,8	8,2	0,1	85,2	20	Reconstituted water	Villavicencio et al., 2005

Daphnia magna	60,3	98,1	8,2	0,87	85,2	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	69	42,4	8,2	3,5	230	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	71	37,9	8,2	4	189	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	87	37,9	8,2	5	218	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	93	37,8	8,2	5,3	174	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	314	58,8	8,2	17,3	591	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	10,14	34,6	8,2	0,5	80	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
Daphnia magna	78,4	85,3	8,22	1,78	85,2	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	109,7	87,6	8,23	2,69	85,2	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	32	64,7	8,24	0,79	72	20	Natural water	Villavicencio et al., 2005
Daphnia magna	135,5	22,2	8,24	12,3	102	20	natural water	Fulton & Meyer, 2014
Daphnia magna	81,06	14,5	8,27	11	104	20	natural water	Fulton & Meyer, 2014
Daphnia magna	37,78	30,6	8,29	2,5	88	20	natural water	Fulton & Meyer, 2014
Daphnia magna	104	53,9	8,3	4,3	203	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	170,0	43,5	8,3	8,2	236	20	Natural water	Bossuyt et al., 2004
Daphnia magna	178,0	37,3	8,3	9,8	198	20	Natural water	Bossuyt et al., 2004
Daphnia magna	308	49,8	8,3	18	481	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	15,58	47,8	8,31	0,5	98	20	Reconstituted water (US EPA)	Fulton & Meyer, 2014
Daphnia magna	60	123,5	8,36	0,1	166,9	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	48,2	74,6	8,36	1,07	120	20	Natural water	Villavicencio et al., 2005
Daphnia magna	28,3	89,7	8,39	0,07	88	20	Natural water	Villavicencio et al., 2005
Daphnia magna	239,8	167,7	8,39	3,94	166,9	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	109	60,1	8,4	4,1	195	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	207	33,3	8,4	14,2	294	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	59,9	91,6	8,43	0,98	112	20	Natural water	Villavicencio et al., 2005
Daphnia magna	31,65	19,7	8,44	3,2	48	20	natural water	Fulton & Meyer, 2014
Daphnia magna	100	141,0	8,45	0,84	166,9	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	66,2	75,4	8,45	1,67	128	20	Natural water	Villavicencio et al., 2005
Daphnia magna	235,9	187,6	8,45	3,25	166,9	20	Reconstituted water	Villavicencio et al., 2005

Daphnia magna	125,2	140,4	8,46	1,57	166,9	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	206	193,1	8,46	2,33	166,9	20	Reconstituted water	Villavicencio et al., 2005
Daphnia magna	172,8	22,8	8,48	15,7	154	20	natural water	Fulton & Meyer, 2014
Daphnia magna	43,1	71,1	8,5	0,91	271	20	Natural water	Villavicencio et al., 2005
Daphnia magna	99	70,3	8,5	3,1	234	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	124	56,3	8,5	5	193	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	151	24,7	8,5	13,3	298	20	Filtered natural water	Kramer et al., 2004
Daphnia magna	354	58,1	8,5	15,1	186	20	Filtered natural water	Kramer et al., 2004
Ceriodaphnia dubia	1		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Ceriodaphnia dubia	6,7		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Ceriodaphnia dubia	9,7		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Ceriodaphnia dubia	11,3		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Ceriodaphnia dubia	12		7		112	25	Reconstituted water	Nimmo et al., 2006
Ceriodaphnia dubia	18		7,5		82,4	25,3	Reconstituted water (US EPA)	Cooper et al., 2009
Ceriodaphnia dubia	7,3		7,8		97	25	Reconstituted water	Nimmo et al., 2006
Ceriodaphnia dubia	42		8		80-110	23	Reconstituted water	Johnson et al., 2008
Daphnia magna	2		5,6		7,1	25	Reconstituted water	Long et al., 2004
Daphnia magna	2,5		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Daphnia magna	4,3		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Daphnia magna	17,8		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Daphnia magna	19,3		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Daphnia magna	21,3		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Daphnia magna	25,9		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Daphnia magna	28,9		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Daphnia magna	29,4		6,8		50	19-20	Deionized water with salt addition	De Schamphelaere et al., 2007
Daphnia magna	2,8		7		7,1	25	Reconstituted water	Long et al., 2004
Daphnia magna	7,4		7		20,6	25	Reconstituted water	Long et al., 2004
Daphnia magna	5		7,7		80-110	23	Reconstituted water	Johnson et al., 2008
Daphnia magna	4		7,8		44	25	Reconstituted water	Yim et al., 2006

Daphnia magna	12		8		150	25	Reconstituted water	Yim et al., 2006
Daphnia magna	21		8		211	20	Reconstituted water	Kramer et al., 2004
Daphnia magna	25		8		211	20	Reconstituted water	Kramer et al., 2004
Daphnia magna	34		8		211	20	Reconstituted water	Kramer et al., 2004
Daphnia magna	40		8		211	20	Reconstituted water	Kramer et al., 2004
Daphnia magna	2		8,2		11,1	25	Reconstituted water	Long et al., 2004
Daphnia magna	11,1		8,2		50,7	25	Reconstituted water	Long et al., 2004
Daphnia magna	2		8,3		7,9	25	Reconstituted water	Long et al., 2004
Daphnia magna	10		8,3		22,2	25	Reconstituted water	Long et al., 2004
Daphnia magna	6,5		8,5		20,6	25	Reconstituted water	Long et al., 2004
Daphnia magna	14,1		6,1-6,3		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
Daphnia magna	14,7		6,17-6,25		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
Daphnia magna	21,6		6,95-7,05		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
Daphnia magna	19,4		6,98-7,03		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
Daphnia magna	67,3		7,96-8,06		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004
Daphnia magna	68,4		7,99-8,02		250	20	Deionized water with salt addition	De Schamphelaere et al., 2004

Sufficient data for the 3 pH classes were found for these 2 invertebrate species.

Table 79: Summary of the acute toxicity data for invertebrates for the 3 pH classes

Test organism	**L(E)C_{50} (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Daphnia magna(mortality)			
n	29	57	196
Min	0.5	2.5	2
Max	465	1213	826
Geometric mean	16.3	66.2	45.4
Lowest value (only when data<4)	/	/	/
Ceriodaphnia dubia(mortality)			
n	9	17	54
Min	1.6	1	5.02
Max	73	84	302
Geometric mean	12.6	14	40
Lowest value (only when data<4)	/	/	/

Table 80: Summary of the acute toxicity data for invertebrates for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	**L(E)C_{50} (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Daphnia magna(mortality)			
n	26	39	130
Min	1.6	8.7	102
Max	204.2	418	398.4
Geometric mean	11	63	49.2
Lowest value (only when data<4)	/	/	/
Ceriodaphnia dubia(mortality)			
n	8	10	42
Min	7.4	4.9	3.1
Max	52	76	131.5
Geometric mean	16	24.1	43.9
Lowest value (only when data<4)	/	/	/

According to the improvement of database described before, **the current lowest E(L)C_{50} value for invertebrate was calculated to be 12.6 µg/L corresponding to the geomean recorded for *C. dubia* at pH between 5.5 and 6.5 for mortality.**

If the normalisation with the DOC is taking into account, **the lowest E(L)C_{50} value for invertebrate was calculated to be 11 µg/L corresponding to the geomean recorded for *D. magna* at pH between 5.5 and 6.5 for mortality.**

5.4.2.2 Long-term toxicity to aquatic invertebrates

According to the EU RAR (2008), 19 individual data points for aquatic invertebrates were selected for 2 standard species (*Ceriodaphnia dubia and Daphnia magna*).

In the updated database detailed in the Heijerick and Van Sprang, 2016b, 25 new individual data points for invertebrates were included for the 2 standard species (*Daphnia magna* and *Ceriodaphnia dubia).* These new data are presented below and details are given in the table 81.

- *Daphnia magna*: New reliable chronic data for the invertebrate *D. magna* were identified in five different publications (Adam et al, 2015; Bossuyt and Janssen, 2004 ; Van Regenmortel et al, 2013, 2015 ; Rodriguez and Arbildua, 2012), and a total of 14 new relevant EC_{10} or NOEC for reproduction situated between 4.7 and 300 µg/L were withheld for classification purposes. Villavicencio et al (2011) reported an extensive data set of chronic EC_{50} values (endpoint: reproduction), but as no EC10 could be derived from this study, it cannot be used for classification purposes.

- *Ceriodaphnia dubia*: Two publications (Wang et al, 2011 ; Cooper et al, 2009) reported on 12 different tests with *C. dubia*. Relevant endpoints were mortality (6 data points) and reproduction (6 data points). EC_{10} values were available for 11 tests, whereas a NOEC was given for the remaining test. Values ranged between 2.4 and 200 µg/L for mortality, and between 1.3 and 46 µg/L for reproduction. The data from Cooper et al., 2009 were rejected because the NOEC/calculated EC_{10} value for reproduction (1.3/0.5 µg Cu/L) was at or below the copper concentration in the control (1 µg Cu/L). Schwartz and Vigneault (2007) also reported a number of chronic data, but values represented an EC_{25} which could not be considered for classification.

  In addition, an extra EC_{10}-value of 14 µg/L (endpoint: reproduction) was calculated by Heijerick and Van Sprang, 2016b from the data that were published in Belanger et al (1989).

  Nevertheless, as mentioned before, in the section of the selection of data, results obtain at 7 days of exposure were rejected in the Joint Research Centre (JRC) in their Report "New criteria for environmental long-term aquatic hazard classification under the CLP Regulation (EC) N° 1272/2008 (2nd ATP) - Screening of Annex VI substances with harmonised classifications" (p.20). Therefore, these values are only presented in italic in the table 81 for information but not used for classification purposes.

Table 81: Overview of the NOEC/EC10 values for invertebrates published since the EU RAR (2008)

Organism	Age/size of organisms	Exposure time	Endpoint	NOEC/EC10 (µg/L)	Normalised (µg/L)	pH	DOC mg/L	Alk mg/L as CaCO3	Medium	Reference
Daphnia magna	Neonates	21 d	Reproduction	8,2	8,2	6,27	2	13,4	Reconstitued EPA water	Rodriguez & Arbildua, 2012
Daphnia magna	Neonates	21 d	Reproduction	5,9	5,9	6,28	2	13,4	Reconstitued EPA water	Rodriguez & Arbildua, 2012
Daphnia magna	Neonates	21 d	Reproduction	6,7	6,5	6,28	2,1	13,4	Reconstitued EPA water	Rodriguez & Arbildua, 2012
Daphnia magna	Neonates	21 d	Reproduction	32,9	7,3	6,41	6,1	96,2	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
Daphnia magna	Neonates	21 d	Reproduction	10,7	16,2	6,41	6,1	96,2	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Mortality*	*20*	*11,3*	*8,3*	*3*	*91,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Mortality*	*65*	*19,6*	*8,3*	*5,8*	*94,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Mortality*	*91*	*28,5*	*8,3*	*5,8*	*93,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Mortality*	*200*	*37,8*	*8,3*	*10*	*96,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Mortality*	*10*	*32,92*	*8,4*	*0,4*	*100,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Reproduction*	*34*	*19,95*	*8,3*	*3*	*91,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Reproduction*	*29*	*8,15*	*8,3*	*5,8*	*94,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Reproduction*	*46*	*13,4*	*8,3*	*5,8*	*93,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Reproduction*	*25*	*3,74*	*8,3*	*10*	*96,0*	*natural well water*	*Wang et al., 2011*
Ceriodaphnia dubia	*< 24 h*	*7 d**	*Reproduction*	*8*	*31,1*	*8,4*	*0,4*	*100,0*	*natural well water*	*Wang et al., 2011*
Daphnia magna	Neonates (< 24 h old)	21 d	Reproduction	56,1	22,4	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
Daphnia magna	Neonates (< 24 h old)	21 d	Reproduction	57,1	22,8	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
Daphnia magna	Neonates (< 24 h old)	21 d	Reproduction	70,5	28,2	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
Daphnia magna	Neonates (< 24 h old)	21 d	Reproduction	46,5	18,5	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
Daphnia magna	Neonates (< 24 h old)	21 d	Reproduction	50,2	20	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
Daphnia magna	Neonates (< 24 h old)	21 d	Reproduction	56,2	22,4	7,7	5	11,2	Reconstituted water (modified M4 medium)	Bossuyt & Janssen, 2004
Daphnia magna	< 24 h	21 d	Reproduction	17	54,12	7,92	0,5	18,9	Reconstituted water (ISO medium)	Adam et al., 2015

Daphnia magna	Neonates	21 d	Reproduction	76,9	5,5	8,44	6,1	186,4	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
Daphnia magna	Neonates	21 d	Reproduction	55,4	8	8,45	6,1	186,5	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
Ceriodaphnia dubia	*neonates (2-8 h)*	*7 d**	*Reproduction*	*14*	*7,66*	*8,31*	*3,7*	*140,0*	*River (Clinch River)*	*Belanger et al., 1989*

**in italics: not retained for classification purposes*

Table 82: Summary of the chronic toxicity data for invertebrates for the 3 pH classes

Test organism	**NOEC (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Daphnia magna (mortality)			
n	/	/	1
Min	/	/	36.8
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data<4)	/	/	36.8
Daphnia magna (reproduction)			
n	7	2	15
Min	5.9	181	17
Max	32.9	300	108
Geometric mean	13.2	233	55
Lowest value (only when data<4)	/	181	/
Daphnia magna (growth)			
n	/	/	1
Min	/	/	12.6
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data<4)	/	/	12.6

Table 83: Summary of the chronic toxicity data for invertebrates for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	**NOEC (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Daphnia magna (mortality)			
n	/	/	1
Min	/	/	36.8
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data<4)	/	/	36.8
Daphnia magna (reproduction)			
n	7	2	15
Min	5.9	16.5	5.5
Max	20.6	34.4	68.9
Geometric mean	10.5	23.8	24.8
Lowest value (only when data<4)	/	16.5	/
Daphnia magna (growth)			
n	/	/	1
Min	/	/	12.6
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data<4)	/	/	12.6

According to the improvement of database described before, **the current lowest NOEC/EC_{10} value for invertebrate was calculated to be 12.6 µg/L corresponding to the lowest value recorded for *D magna* at pH between 7.5 and 8.5 for growth effect.**

If the normalisation with the DOC is taking into account, **the lowest NOEC/EC_{10} value for invertebrate was calculated to be 10.5 µg/L corresponding to the lowest value recorded for *D. magna* at pH between 6.5 and 7.5 for reproduction effect.**

5.4.3 Algae and aquatic plants

According to the EU vRAR (2008), 17 individual acute data points for algae were selected for 1 standard species (*Pseudokirchneriella subcapitata).*

In the updated database detailed by Heijerick et al., 2016a, 38 new individual data points for algae were included for the standard species *Pseudokirchneriella subcapitata.* New individual data points are included for 2 other standard species *Chlamydomonas reinhardtii* and *Chlorella sp.* These new data are presented below and details are given in the table 84.

- *Pseudokirchneriella subcapitata*: new reliable acute data for green algae *P. subcapitata* were identified in 4 different publications (Heijerick et al, 2005; De Schamphelaere & Janssen, 2006; De Schamphelaere, 2005; De Schamphelaere et al, 2005). A total of 31 new relevant EbC50 and ErC50 between 16.5 and 824 mg/L. Levy et al (2009) also published two 72h-ErC50 values, but these tests did not meet the OECD-test guidelines and were therefore not retained for hazard classification purposes. As all data issued from Heijerick et al. (2005) publication related on biomass, therefore not retained for classification. Only data on ErC50 were retained for classification and were presented in the table below.

- *Chlamydomonas reinhardtii:* De Schamphelaere & Janssen (2006) reported 3 ErC50 varying between 146 µg/l and 380 µg/L at 3 different pH (6.02 ; 7.03 ; 8.11) which were included in the acute classification dataset.

- *Chlorella sp.*: Also in a publication of De Schamphelaere & Janssen (2006), 16 new ErC50 were reported and added in the acute classification dataset.

Table 84: Overview of the ErC50 values for algae/plants published since the EU RAR (2008)

		Value	Normalised value		DOC	Hardness	Temperature		
Species		**µg/L**	**µg/L**	**pH**	**mg/L**	**mg/L as CaCO3**	**°C**	**Type of water**	**Reference**
Chlamydomonas reinhardtii	ErC50	380	143,2	6,02	9,84	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlamydomonas reinhardtii	ErC50	315	80,4	7,03	9,84	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlamydomonas reinhardtii	ErC50	146	31,4	8,11	9,84	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	602	305,2	5,5	10,27	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	440	319,3	6,01	5,03	400	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	333	199,9	6,03	5,17	100	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	773	236,8	6,04	15,49	100	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	987	404,7	6,05	15,24	400	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	254	59,4	7,01	10,03	500	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	296	63,7	7,03	10,81	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	364	87,5	7,03	10,81	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	60	74,3	7,04	1,5	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	208	43,6	7,04	10,23	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	446	49,3	7,05	19,9	250	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	238	48,1	7,07	10,26	25	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	111	44,2	7,88	5,31	100	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	380	54,1	7,88	15,66	100	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	99	42,1	7,92	5,04	400	NA	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	ErC50	506	89,1	7,97	15,82	400	NA	field water	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	756	411,2	5,68	9,84	250	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	205	113,1	5,99	5,64	400	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	368	65,2	6,17	14,9	100	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	122	56,5	6,18	5,07	100	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	230	122,0	6,19	5,23	100	25	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	199	97,5	6,2	5,31	100	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	811	205,9	6,2	15,6	100	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006

Pseudokirchneriella subcapitata	ErC50	824	227,7	6,22	15,8	100	25	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	174	18,9	6,95	18,2	250	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	100	19,7	7,01	10,2	250	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	30	30,7	7,02	1,95	250	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	105	20,6	7,02	10,4	500	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	102	20,5	7,03	9,98	250	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	99	19,6	7,04	10,1	250	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	190	42,0	7,04	9,89	500	25	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	35	31,8	7,05	2,21	250	25	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	35	34,0	7,08	2,06	250	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	156	32,5	7,09	9,99	250	25	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	178	33,1	7,09	11,1	250	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	685	94,2	7,11	19,9	250	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	281	63,2	7,12	10,5	500	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	462	57,7	7,17	18,5	250	25	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	193	37,1	7,19	10,4	25	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	151	20,0	7,78	15,2	400	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	37	37	7,8	2			Reconstitued medium	De Schamphelaere et al, 2005
Pseudokirchneriella subcapitata	ErC50	51	19,2	7,92	5,46	400	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	93	32,7	7,92	5,99	400	25	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	268	38,8	8,01	15,1	400	25	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	59	22,2	8,02	5,42	100	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	209	27,9	8,05	15,3	100	25	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	346	48,7	8,05	16,1	400	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	92	34,0	8,07	5,75	400	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	161	33,8	8,25	10,3	250	25	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	ErC50	219	49,8	8,37	10,3	250	25	Synthetic-Bihain	De Schamphelaere, 2006
Pseudokirchneriella subcapitata	ErC50	18		8,5	<2		25	Reconstitued medium	De Schamphelaere et al, 2005
Pseudokirchneriella subcapitata	ErC50	46		5,9	<2		25	Reconstitued medium	De Schamphelaere et al, 2005

Table 85: Summary of the acute toxicity data for algae/plants for the 3 pH classes

Test organism	**ErC_{50} (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Pseudokirchneriella subcapitata			
Growth rate			
n	9	15	12
Min	46	30	18
Max	824	685	346
Geometric mean	277.6	131.6	104.9
Lowest value (only when data<4)	/	/	/
Chlamydomonas reinhardtii			
Growth rate			
n	1	1	1
Min	380	315	146
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data <4)	380	315	146
Chlorella vulgaris			
Growth rate			
n	5	7	4
Min	333	60	99
Max	987	446	506
Geometric mean	582.9	232.7	214.4
Lowest value (only when data<4)	/	/	/

Table 86: Summary of the acute toxicity data for algae/plants for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	**ErC_{50} (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Pseudokirchneriella subcapitata			
Growth rate			
n	8	15	11
Min	56	18.9	19.2
Max	411.2	94.2	19.8
Geometric mean	132.6	33	31.6
Lowest value (only when data<4)	/	/	/
Chlamydomonas reinhardtii			
Growth rate			
n	1	1	1
Min	143.2	80.4	31.4
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data<4)	143.2	80.4	31.4
Chlorella vulgaris			
Growth rate			
n	5	7	4
Min	199.9	43.6	42.1
Max	404.7	87.5	89.1
Geometric mean	284.6	59.2	54.7
Lowest value (only when data <4)	/	/	/

According to the improvement of database described before, **the current lowest ErC_{50} value for algae/plants was calculated to be 104.9 µg/L corresponding to the lowest geomean recorded for *P. subcapitata* at pH between 7.5 and 8.5.**

If the normalisation with the DOC is taking into account, **the lowest ErC_{50} value for algae/plants was calculated to be 31.4 µg/L corresponding to the lowest value recorded for *C. reinhardtii* at pH between 7.5 and 8.5.**

According to the EU vRAR (2008), 29 individual chronic data points for algae were selected for 3 standard species (*Pseudokirchneriella subcapitata*, *lemna minor* and *Chlorella vulgaris).*

In the updated database detailed by Heijerick and Van Sprang, 2016b, 37 new individual data points for algae/plants were included for the 2 standard species (*Pseudokirchneriella subcapitata and Chlamydomonas reinhardtii).*

- *Pseudokirchneriella subcapitata*: De Schamphelaere and Janssen (2006) reported 34 reliable E_rC_{10} values on copper for the green alga *P. subcapitata*. One additional value was obtained at a pH > 8.5, and was therefore not considered for hazard assessment purposes. The ErC_{10} values ranged from 16.7 to 337 µg/L. Only data based on growth rate are retained for chronic classification purposes.

  A test conducted by Levy et al (2009) was rejected for the chronic hazard assessment of copper as it was conducted in axenic conditions which is not in line with the OECD test protocol.

- *Chlamydomonas reinhardtii:* three new chronic data available for 3 different pH values are extracted from a publication of De Schamphelaere and Janssen (2006).

  These new data are presented in the table below.

Table 87: Overview of the ErC10 values for algae/plants published since the EU RAR (2008)

Organism	Age/size of organisms	Exposure time	Endpoint	NOEC/EC10 (µg/L)	Normalised (µg/L)	pH	DOC mg/L	Alk mg/L as CaCO3	Medium	Reference
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	3 d	growth	178	61,7	6,02	9,84	0,5	Reconstituted	De Schamphelaere et al., 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	161,6	50,6	5,68	9,84	0,3	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	49,4	20,5	5,99	5,64	0,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	148,4	24,5	5,99	15,3	0,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	155,9	22,8	6,17	14,9	1,0	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	52,3	21,9	6,18	5,07	1,0	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	110,3	49,1	6,19	5,23	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	97,7	41,2	6,2	5,31	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	336,9	53,7	6,2	15,6	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	337,0	56,1	6,22	15,8	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	3 d	Growth	108	27,4	7,03	9,84	9,9	Reconstituted	De Schamphelaere et al., 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	86,8	9,3	6,98	18,2	8,9	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	52,2	10,2	7,01	10,2	13,9	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	16,7	17,1	7,02	1,95	9,7	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	54,8	10,6	7,02	10,4	9,7	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	51,6	10,3	7,03	9,98	10,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	93,2	19,4	7,04	9,89	10,2	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	50,6	10,0	7,04	10,1	10,3	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006

Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	18,0	16,4	7,05	2,21	10,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	18,1	17,6	7,08	2,06	11,2	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	67,2	13,6	7,09	9,99	11,4	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	62,1	11,1	7,09	11,1	11,4	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	202,6	20,3	7,11	19,9	12,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	78,4	15,1	7,12	10,5	12,3	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	105,7	11,4	7,17	18,5	13,8	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	74,9	14,0	7,19	10,4	15,4	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	3 d	growth	96	24,7	8,11	9,84	92,5	Reconstituted	De Schamphelaere et al., 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	99,6	13,2	7,78	15,2	13,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	40,6	13,9	7,92	5,99	18,6	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	25,3	9,4	7,92	5,46	18,6	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	121,1	16,6	8,01	15,1	23,0	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	19,9	7,4	8,02	5,42	23,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	171,5	22,2	8,05	16,1	25,2	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	81,6	10,6	8,05	15,3	25,2	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	87,0	32,2	8,07	5,75	26,4	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	52,6	10,5	8,25	10,3	40,2	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	86,8	17,8	8,37	10,3	53,3	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006

Table 88: Summary of the chronic toxicity data for algae/plants for the 3 pH classes

Test organism	**NOErC (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Pseudokirchneriella subcapitata (growth rate)			
n	9	15	10
Min	49.4	16.7	19.9
Max	337	202.6	171.5
Geometric mean	131.6	56.1	65.1
Lowest value (only when data<4)	/	/	/
Chlamydomonas reinhardtii			
n	2	1	1
Min	22	108	96
Max	178	/	/
Geometric mean	62.6	/	/
Lowest value (only when data<4)	**22**	108	96
Chlorella vulgaris			
n	5	7	4
Min	108.3	36.4	31
Max	510.2	282.9	188
Geometric mean	279.6	106.3	86.5
Lowest value (only when data<4)	/	/	/
Lemna minor			
n	1	/	/
Min	30	/	/
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data<4)	30	/	/

Table 89: Summary of the chronic toxicity data for algae/plants for the 3 pH classes considering DOC normalisation at 2mg/L

Test organism	**NOErC (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Pseudokirchneriella subcapitata (growth rate)			
n	9	15	10
Min	20.5	9.3	7.4
Max	56.1	20.3	32.2
Geometric mean	34.9	**13.3**	14.1
Lowest value (only when data<4)	/	**/**	/
Chlamydomonas reinhardtii			
n	2	1	1
Min	61.7	27.4	24.7
Max	64.8	/	/
Geometric mean	63.2	/	/
Lowest value (only when data<4)	61.7	27.4	24.7
Chlorella vulgaris			
n	5	7	4
Min	60.2	19	14.3
Max	213	54.1	29.9
Geometric mean	124.4	30.3	24
Lowest value (only when data<4)	/	/	/
Lemna minor			
n	1	/	/
Min	75.8	/	/
Max	/	/	/
Geometric mean	/	/	/
Lowest value (only when data<4)	75.8	/	/

According to the improvement of database described before, **the current lowest NOErC value for algae/plants was calculated to be 22 µg/L corresponding to the lowest value recorded for *C. reinhardtii* at pH between 5.5 and 6.5.**

If the geomean is used whatever the number of available data, the lowest NOErC value for algae/plants would be 30 µg/l recorded for *L. minor* at pH between 5.5 and 6.5.

If the normalisation with the DOC is taking into account, **the lowest NOErC value for algae/plants was calculated to be 13.3 µg/L corresponding to the lowest geomean recorded for *P. subcapitata* at pH between 6.5 and 7.5 for growth rate effect.** The same value is retained if the geomean is used whatever the number of available data

5.4.4 Overall conclusion of aquatic toxicity and derivation of the relevant ERV

Based on the result presented in the section 5.4.1 to 5.4.3, the table 90 and 91 below summarised the lowest values retained to derive the ERV values for acute and chronic classification respectively.

Table 90: Overview of all acute toxicity data available at 3 pH-categories with or without normalisation at 2mg/L DOC

	L(E)C_{50} (µg Cu/L) (number of available data)					
	pH: 5.51-6.5		**pH: >6.5-7.5**		**pH: >7.5-8.5**	
	Non normalised	Normalised	Non normalised	Normalised	Non normalised	Normalised
Oncorhynchus mykiss Lowest value or geomean	24.4 (8)	40.6 (8)	47.4 (22)	45.9 (8)	63.6 (47)	94.7 (25)
Danio rerio Lowest value or geomean	/	/	11.7 (2)	26 (1)	148.4 (3)	94.7 (2)
Danio rerio Geomean	/	/	20.24 (2)	26 (1)	167.4 (3)	117.9 (2)
Cyprinus carpio Lowest value or geomean	/	/	/	/	800 (3)	/
Cyprinus carpio Geomean	/	/	/	/	810 (3)	/
Pimephales promelas Lowest value or geomean	12.1 (5)	14.9 (3)	96.7 (46)	49.3 (11)	255.9 (207)	382.86 (47)
Pimephales promelas Geomean	12.1 (5)	23.5 (3)	96.7 (46)	49.3 (11)	255.9 (207)	382.86 (47)
Lepomis macrochirus Lowest value or geomean	/	/	1000 (2)	/	4250 (3)	/
Lepomis macrochirus Geomean	/	/	1048 (2)	/	5509 (3)	/
Daphnia magna(mortality) Lowest value or geomean	16.3 (29)	11 (26)	66.2 (57)	63 (39)	45.4 (196)	49.2 (130)
Ceriodaphnia dubia(mortality) Lowest value or geomean	12.6 (9)	16 (8)	14 (17)	24.1 (10)	40 (54)	43.9 (42)
Pseudokirchneriella subcapitata (growth rate) Lowest value or geomean	277.6 (9)	132.6 (8)	131.6 (15)	33 (15)	104.9 (12)	31.6 (11)
Chlamydomonas reinhardtii (growth rate) Lowest value or geomean	380 (1)	143.2 (1)	315 (1)	80.4 (1)	146 (1)	31.4 (1)
Chlorella vulgaris(growth rate) Lowest value or geomean	582.9 (5)	284.6 (5)	232.7 (7)	59.2 (7)	214.4 (4)	54.7 (4)

In grey, values used for classification proposal.

Table 91: Overview of all chronic toxicity data available at 3 pH-categories with or without normalisation at 2mg/L DOC

	NOEC/EC10 (µg Cu/L) (number of available data)					
	pH: 5.51-6.5		**pH: >6.5-7.5**		**pH: >7.5-8.5**	
	Non normalised	Normalised	Non normalised	Normalised	Non normalised	Normalised
Oncorhynchus mykiss(mortality) Lowest value or geomean	28.5 (1)	/	24 (3)	16.5 (2)	28.2 (8)	60.6 (8)
Oncorhynchus mykiss(mortality) Geomean	28.5 (1)	/	33.2 (3)	17.5 (2)	28.2 (8)	60.6 (8)
Oncorhynchus mykiss(growth rate) Lowest value or geomean	28.2 (1)	/	14.7 (4)	40.52 (4)	12.9 (5)	31.3 (5)
Pimephales promelas (mortality) Lowest value or geomean	10.1 (3)	14.6 (3)	5.9 (3)	11.6 (3)	28.1 (5)	60.5 (5)
Pimephales promelas (mortality) Geomean	11.8 (3)	17.9 (3)	12 (3)	24.8 (3)	28.1 (5)	60.5 (5)
Pimephales promelas (growth) Lowest value or geomean	8.7 (3)	14.6 (3)	12 (4)	27 (4)	26.6 (6)	63.8 (6)
Pimephales promelas (growth) Geomean	11.4 (3)	17.3 (3)	12 (4)	27 (4)	26.6 (6)	63.8 (6)
Pimephales promelas (reproduction) Lowest value or geomean	/	/	10.8 (2)	30.6 (2)	25.8 (9)	57 (9)
Pimephales promelas (reproduction) Geomean	/	/	13.14 (2)	32.7 (2)	25.8 (9)	57 (9)
Salvelinus fontinalis (Mortality) Lowest value or geomean	/	/	16.9 (4)	28.4 (4)	22.3 (1)	42.3 (1)
Salvelinus fontinalis (Growth) Lowest value or geomean	/	/	17.1 (4)	28.2 (4)	22.3 (1)	42.3 (1)
Salvelinus fontinalis (Reproduction) Lowest value or geomean	/	/	6.4 (3)	10.7 (3)	/	/
Daphnia magna (mortality)	/	/	/	/	36.8 (1)	36.8 (1)

Lowest value or geomean						
Daphnia magna (reproduction) Lowest value or geomean	13.2 (7)	10.5 (7)	181 (2)	16.5 (2)	55 (15)	24.8 (15)
Daphnia magna (reproduction) Geomean	13.2 (7)	10.5 (7)	233 (2)	23.8 (2)	55 (15)	24.8 (15)
Daphnia magna (growth) Lowest value or geomean	/	/	/	/	12.6 (1)	12.6 (1)
Pseudokirchneriella subcapitata (growth rate) Lowest value or geomean	131.6 (9)	34.9 (9)	56.1 (15)	13.3 (15)	65.1 (10)	14.1 (10)
Chlamydomonas reinhardtii Lowest value or geomean	22 (2)	61.7 (2)	108 (1)	27.4 (1)	96 (1)	24.7 (1)
Chlamydomonas reinhardtii Geomean	62.6 (2)	63.2 (2)	108 (1)	27.4 (1)	96 (1)	24.7 (1)
Chlorella vulgaris Lowest value or geomean	279.6 (5)	124.4 (5)	106.3 (7)	30.3 (7)	86.5 (4)	24 (4)
Lemna minor Lowest value or geomean	30 (1)	75.8 (1)	/	/	/	/

In grey: values used for classification proposal.

Regarding the all data set presented in the sections above, the table below summarised the acute and chronic ERV retained for classification. These ERV correspond to the lowest EC50 and EC10/NOEC or geomean EC50 and EC10/NOEC for each pH-category.

Table 92: Acute and chronic ERV considering the geomean only when at least 4 data are available. If less than 4 data are available, the lowest data is retained.

Ecotoxicity Reference Values - µg dissolved Cu/L				
		pH: 5.51-6.5	**pH: >6.5-7.5**	**pH:>7.5-8.5**
Non normalised values				
Acute ERV	LC50	12.1 (*P. promelas* - 5)	11.7 (*D. rerio* -2)	40 (*C. dubia*- 54)
Chronic ERV	EC10/NOEC	8.7 (*P. promelas -3*)	5.9 (*P. promelas* -3)	12.6 (*D. magna* -1)
Values considering DOC normalisation to 2 mg/L				
Acute ERV	LC50	11 (*D. magna* -26)	24.1 (*C. dubia* -10)	31.4 (*C. reinhardtii* -1)
Chronic ERV	EC10/NOEC	10.5 (*D. magna* -7)	5.6 (*O. mykiss* -3)	12.6 (*D. magna* -11)

It could be noted that the normalisation of the EC50 and EC10/NOEC results in a similar ERV values than results without DOC normalisation. Therefore, only acute and chronic ERV without DOC normalisation are used for comparison with criteria for environmental classification.

Table 93: Acute and chronic ERV considering the geomean even when less 4 data are available

Ecotoxicity Reference Values - µg dissolved Cu/L				
		pH: 5.51-6.5	**pH: >6.5-7.5**	**pH:>7.5-8.5**
Non normalised values				
Acute ERV	LC50	12.1 (*P. promelas* - 5)	14 (*C. dubia* -17)	40 (*C. dubia*- 54)
Chronic ERV	EC10/NOEC	11.4 (*P. promelas* -3)	6.4* (*S. fontinalis* -3)	12.6 (*D. magna* -1)
Values considering DOC normalisation to 2 mg/L				
Acute ERV	LC50	11 (*D. magna* -26)	24.1 (*C. dubia* -10)	31.4 (*C. reinhardtii* -1)
Chronic ERV	EC10/NOEC	10.5 (*D. magna* -7)	10.7* (*S. fontinalis* -3)	12.6 (*D. magna* -11)

* No geomean could be apply as only 3 data are available considering all pH-categories

5.4.5 Other aquatic organisms (including sediment)

5.5 Comparison with criteria for environmental hazards (sections 5.1 – 5.4)

According to the data presented 5.1 and 5.2, granulated copper is not subject to rapid environmental transformation for the purposes of classification. This substance is also not subjected to bioaccumulation in organisms.

Based on the Guidance on the Application of the CLP criteria (2015) the classification strategy for metals is based on a comparison of acute and chronic ERVs (derived via testing of the soluble metal species) with the concentration of metal ions in solution after a period of 7 days (short-term test) and 28 days (long-term test), respectively, at different loadings and according to the Transformation/Dissolution protocol (T/Dp).

Two Transformation/Dissolution studies are performed using granulated copper at loading of 1 mg/L. A specific surface area defined for this compound is 25.6 cm^2/g (see Table 9 in section 1.2.2 of this report).

In the first study, the material was tested as such, without anti-abrasion measures (ECTX, 2016a). Only one particle of granulated copper was added to each vessel in order to attain the desired mass loading of 1 mg/L only at pH6. This experiment showed a copper release of 1.4 µg/L after 7 days at pH 6 and at a mass loading of 1 mg/L, and 6.0 µg/L after 28 days at pH 6 (coefficients of variation 23 and 27%). The high variability of these results could be explained by the limited number of particles (only 8) used in this study.
A second study (ECTX, 2016b) was performed with the granulated copper particles which were embedded in epoxy resin. This allows setting the exposed surface area more accurately, it avoids abrasion, and the surfaces were polished before exposure. These results had much higher reliability (coefficients of variation only 7—11%) and showed more Cu release than the first experiment. This second study is therefore retained for classification purpose of granulated copper.

The results of this second study are presented in the table below:

Table 94: Release from granulated copper at 1 mg/L issued from T/Dp

Transformation/dissolution		**Average copper concentration released (µg dissolved Cu/L)**			**Release per unit surface area (µg/mm²)**			**Release from granulated copper (µg Cu/L)***		
loading	Time	pH 6	pH 7	pH 8	pH 6	pH 7	pH 8	pH 6	pH 7	pH 8
1 mg/L	7 days	4.3	2.7	1.4	1.3	0.88	0.45	3.4	2.3	1.2
1 mg/L 0.1 mg/L**	28 days	16.1	10.4	5.9	5	3.3	1.9	13 1.3	8.6 0.86	4.9 0.49

*Considering the specific surface area of granulated copper of 2.56 mm^2/mg

** Extrapolated values

The releases of copper ions from granulated copper at different pH were compared to the acute and chronic ERV.

For acute classification, as indicated in the table below, the releases of copper ions after 7 days (1 mg/L loading) are below the acute ERVs of the dissolved form of copper, whatever the pH range. Therefore, granulated copper does not have acute aquatic classification.

Table 95: Comparison of acute ERV and release of copper ions after 7 days

Acute	**Acute ERV (µg/L)**	**Release of copper after 7 days at 1 mg/L loading (µg/L)**	**Classification Aquatic acute 1**
pH: 5.51-6.5	12.1	3.4	No
pH: >6.5-7.5	11.7	2.3	No
pH:>7.5-8.5	40	1.2	No

The acute ERV used for the comparison of criteria for classification correspond to the ERV calculated using the geomean only when at least 4 data are available. It should be noted that there is no impact for acute classification of granulated copper if the acute ERV calculated using the geomean even when less than 4 data are available.

For chronic classification, regarding the richness of the data-base at each pH-class, the surrogate approach is not relevant. As indicated in the table below, the releases of copper ions after 28 days (1 mg/L loading) are above the chronic ERVs of the dissolved form of copper at pH 6 and 7 which lead to classify granulated copper Aquatic chronic 2. The releases of copper ions after 28 days at 1 mg/L loading are below the chronic ERV at pH 8.

The releases of copper ions after 28 days at 0.1 mg/L loading are below the chronic ERVs at all pH-classes (6, 7 and 8).

Therefore, granulated copper is classified aquatic chronic 2.

Table 96: Comparison of chronic ERV and release of copper ions after 7 days

Chronic	**Chronic ERV (µg/L)**	**Release of copper after 28 days at 0.1 mg/L loading (µg/L)**	**Release of copper after 28 days at 1 mg/L loading (µg/L)**	**Classification**
pH: 5.51-6.5	8.7	1.3	13	Aquatic chronic 2
pH: >6.5-7.5	5.9	0.86	8.6	Aquatic chronic 2
pH:>7.5-8.5	12.6	0.49	4.9	No

The chronic ERV used for the comparison of criteria for classification correspond to the ERV calculated using the geomean only when at least 4 data are available. It should be noted that there is no impact for chronic classification of granulated copper if the acute ERV calculated using the geomean even when less than 4 data are available.

5.6 Conclusions on classification and labelling for environmental hazards (sections 5.1 – 5.4)

Considering aquatic acute classification, taking into account the recommendations of the Annex IV of the Guidance to Regulation (EC) No 1272/2008 Classification, Labelling and Packaging of substances and mixtures,

- the dissolved metal ion after a period of 7 days at a loading rate of 1 mg/L are below the acute ERVs of the dissolved form of copper, whatever the pH range. Therefore, **granulated copper does not have acute aquatic classification**.

Considering aquatic chronic classification, taking into account the recommendations of the Annex IV of the Guidance to Regulation (EC) No 1272/2008 Classification, Labelling and Packaging of substances and mixtures,

- the estimated dissolved metal ion after a period of 28 days at a loading rate of 0.1 mg/L are below the chronic ERVs at all pH-classes (6, 7 and 8). Therefore, granulated copper does not have aquatic chronic 1 classification
- the estimated dissolved metal ion after a period of 28 days at a loading rate of 1 mg/L are above the chronic ERVs at at pH 6 and 7. Therefore, granulated copper is classified Aquatic chronic 2.

RAC evaluation of aquatic hazards (acute and chronic)

Summary of the Dossier Submitter's proposal

Copper metal (massive, powder and granulated) is not currently listed in Annex VI of the CLP Regulation (EC) 1272/2008. The DS proposed that granulated copper does not require classification for Aquatic Acute hazard because the dissolved copper ion concentrations after a period of 7 days at a loading rate of 1 mg/L are below the acute ecotoxicological reference values (ERVs) of the dissolved form of copper, regardless of pH. It is, however, proposed to be classified as Aquatic Chronic 2 (H411) based on the dissolved metal ion concentrations after a period of 28 days at a loading rate of 1 mg/L, which are above the chronic ERVs at pH 6 and 7 (the estimated dissolved metal ion concentrations at a notional loading rate of 0.1 mg/L are below the chronic ERVs at pH 6, 7 and 8).

Degradation

The substance is an element and so is not degradable by definition. It is therefore not relevant to assess degradation rate as is usually done for organic compounds. However, copper is subject to chemical transformation processes and the vast majority of copper in aquatic systems is rapidly bound to particles, precipitated as insoluble inorganic salts, or bound to organic matter. In pure water, very low levels of free copper (II) ions are present in solution, with amounts governed by the propensity of the metal cation to hydrolyse in water. For a given mass of substance, the concentration of copper (II) ions in solution are

highest at low pH (also depending on the type and concentration of ligands present in the water).

The DS summarises the previous RAC opinions for several copper compounds adopted in December 2014, which concluded that copper (II) ions are not subject to rapid environmental transformation for the purposes of classification and labelling. New evidence is available in the REACH registration dossier for copper (dated 18/01/2017), but as there is no new guidance available about the "rapid removal concept" for metal compounds, the data were not presented or discussed further.

Bioaccumulation

The DS refers to the previous RAC opinions on copper compounds adopted in December 2014, which conclude that the bioaccumulation behaviour of copper (II) ions is complicated by essentiality and homeostatic mechanisms in organisms, but does not need to be considered further because it does not influence the determination of the chronic M-factor (in view of the degradability conclusion).

Aquatic toxicity

The available database for copper (II) cations is large because several soluble (and less soluble) copper compounds have been tested under a wide range of abiotic conditions involving a variety of species. There are close to 800 acute and 200 chronic data points. Many of the studies are academic research papers rather than formal regulatory reports. The DS took account of the information included in the REACH registration dossier for copper (dated 18/01/2017), which was based on existing regulatory reviews and two further reports (Heijerick and Van Sprang, 2016a & 2016b). The general approach is as follows:

- *Species selection and test duration*: Although data for many species are available, only the "standard" species and endpoints from standardised methods have been selected. For example, 96-h LC_{50} values for fish generated according to OECD TG 203 and OECD TG 236 have been used for acute classification. However, some exceptions were made. For example, acute but not chronic data were included for *Ceriodaphnia dubia* alongside *Daphnia magna* for the invertebrate trophic group; the argument being that the 7-d NOEC endpoint is not mentioned in the CLP Guidance and was 'rejected' in a Joint Research Centre (JRC) report on criteria for environmental long-term aquatic hazard classification [no further details are provided in the documentation, but following a request from the rapporteur, a copy of this report was provided and the statement about the use of *Ceriodaphnia* data appears to be based on a simplistic reading of the CLP Guidance; RAC notes that data for this species were also used in previous RAC opinions for other copper compounds]. On the other hand, test durations as low as 7 days were included in the chronic fish data set (*e.g.* a sub-chronic test with Fathead Minnow (*Pimephales promelas*) larvae by Norberg & Mount, 1985 [referred to as Nordberg *et al*. in the CLH dossier]).

- *Quality criteria*: Reported adverse effect levels must be expressed as measured, dissolved copper concentrations. Nominal data are not acceptable.

- *Physico-chemical conditions of test media*: Three factors were considered:
  - Data were split into three pH categories: 5.5-6.5, >6.5-7.5 and >7.5-8.5 to be in line with the UN GHS transformation/dissolution protocol (T/Dp), which specifies a pH range of 6-8.5 for the 7-day test and 5.5 to 8.5 for the 28-day test.
  - The effect of dissolved organic carbon (DOC) was taken account of by deriving ERVs based on both the whole data set and data normalised to a DOC level of 2 mg/L (which is the limit value in OECD TGs) where the data allow. The actual normalisation technique was not described. In addition, the OECD TGs recommend that 2 mg/L is a maximum limit for Total Organic Carbon (TOC) (the DOC level is not explicitly mentioned), and several studies had DOC levels much higher than this.
  - Water hardness does not influence the sensitivity of algae to copper, and reduces the acute sensitivity of invertebrates and fish; it has little influence on the chronic sensitivity of invertebrates but reduces the chronic sensitivity of fish. The median hardness of the media in the selected tests is generally in the lower end of the range of the OECD recommendations (10-250 mg $CaCO_3$/L) for each pH class. Therefore, the DS concluded that the ecotoxicity data are generally conservative with regards to the hardness of the test media.

- *Data aggregation*: The CLP Guidance (version 4.1, p. 500-501, Section 4.1.3.2.4.3) states that geometric means can be used if four or more equivalent data points are available for a species. The splitting of the data set according to pH reduces the overall number of data points for a species in any particular pH band. The REACH Registrants have argued that geometric means can still be used after splitting the database by pH band as long as at least four data points are available for the species across all pH values because this otherwise leads to "double" conservatism (i.e. if averaging is not applied, the lowest value within each pH band is selected due to data scarcity; this skews the data to the most sensitive value of all regardless of the mass of other information available for a species, and it should be born in mind that the hardness considerations may already introduce a level of conservatism). To analyse the impact of the use of geomean or lowest value (if less than 4 data points are available) in a pH band, the DS presents both approaches.

The following description presents the available toxicity information for each trophic group, with the lowest values highlighted in bold.

Acute fish toxicity

Acute data are reported for five fish species, which becomes three species when the data are normalised for DOC (due to missing information for the other two species). The large majority of studies have been conducted in the highest (most alkaline) pH band, so data are only available for two fish species in the acidic pH band (5.5-6.5) at which toxicity is greatest, as outlined in the follwoing Table (see also Figure below).

Table : *Summary of acute LC_{50} data (µg/L) for fish at acidic pH normalised to a DOC level of 2 mg/L*

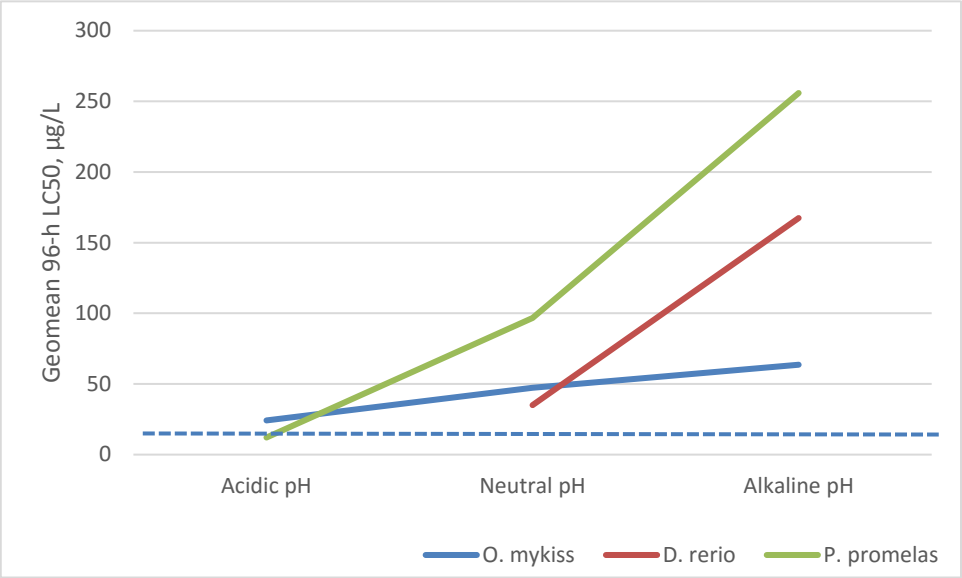
	Species	
	Oncorhynchus mykiss (Rainbow Trout)	*Pimephales promelas* (Fathead Minnow)
No. of values	8	3
Minimum	6.28	14.9
Maximum	99.3	40.1
Geometric mean	40.6	23.5[a]
Lowest value (when n<4)	-	14.9

Note: a - Less than 4 data points are available. However, two additional data points for *P. promelas* are available if DOC normalisation is not performed, so that a geometric mean that is compliant with the CLP Guidance can be derived (n>4). In the previous RAC opinions for other copper compounds, the lowest LC_{50} was 8.1 µg/L for larval *P. promelas* at pH 5.5-6.5 from a single study (this was a geomean of two values – 15.0 and 4.4 µg/L). This study did not report DOC levels, so is not included in the Table, but it is part of the non-normalised data set. The geomean LC_{50} changes to 12.1 µg/L when all data for this species are included for this pH band.

For comparison, there are 69 additional acute data points for *Oncoryhnchus mykiss* and 253 for *P. promelas* in the two other pH bands (the geometric mean LC_{50} is in the range 10-100 µg/L for *O. mykiss* at both neutral and alkaline pH, whereas it is above 100 µg/L for *P. promelas* at alkaline pH). This demonstrates an unequal spread of data across pH bands (although it should be noted that for the majority of substances, there are typically only 1-3 acute fish studies covering all pHs).

When data are normalised for DOC, the third species (Zebrafish *Danio rerio*) has only two data points at pH >7.5-8.5 (alkaline) (LC_{50}: geometric mean 117.9 µg/L, lowest 94.7 µg/L) and one data point at pH >6.5–7.5 (neutral) (LC_{50}: 26 µg/L; the non-normalised LC_{50} is 11.7 µg/L). The lowest value was obtained in a very soft water test medium (hardness 7.8 mg/L as $CaCO_3$, which is outside the range of the OECD TG recommendation). This result is therefore highly conservative. In contrast, the studies at alkaline pH were performed using relatively hard waters (141 mg/L as $CaCO_3$). The use of a very soft water at neutral pH makes it difficult to compare sensitivities between species, and RAC considers that it is not relevant to use data obtained at hardness levels outside of the recommended range for hazard classification purposes. There is a second study at neutral pH that gave an LC_{50} of 35 µg/L (Bresch, 1982; this study used a reconstituted water with a hardness of 100 mg/L as $CaCO_3$ but DOC levels were not reported). When the trends between fish species are compared, as a worst case, it is possible that the LC_{50} for *D. rerio* might be below 10 µg/L at acidic pH (see Figure 1, prepared by RAC). This could be considered a data gap despite the large amount of data for acute fish toxicity. Zebrafish can be tested in waters down to pH 6 (OECD TG 203) or 6.5 (OECD TG 236) so it would be possible to perform a test to see if they are more sensitive.

Figure: *Summary of acute LC_{50} data (µg/L) for fish (not normalised for DOC level, excluding data from very soft waters; dashed line = 10 µg/L)*



Note: The slopes of the lines change if DOC normalisation is performed, though the general trends are still apparent.

To summarise, the lowest acute LC_{50} value for fish in the data set when not normalised for DOC is **12.1 µg/L** (geomean for *P. promelas* at pH 5.5-6.5, n = 5). If geomeans are only used when there are ≥4 data points for a species in a pH band, the lowest fish LC_{50} would be **11.7 µg/L** (for *D. rerio* at pH >6.5-7.5) [though as noted above, RAC does not think this is an appropriate data point as it was obtained in very soft water]. If DOC normalisation is performed, the lowest fish LC_{50} value is **14.9 µg/L** (for *P. promelas* at pH 5.5-6.5, n = 3; the geomean is in the same concentration band, as indicated in the Table above). The hardness of the test media used to derive these values was in the range 22 - 48 mg/L as $CaCO_3$ for the *P. promelas* studies, and 100 mg/L as $CaCO_3$ for the *D. rerio* study. These reflect low hardness conditions and are therefore conservative. However, RAC notes that a lower LC_{50} (potentially below 10 µg/L) cannot be ruled out for *D. rerio* at pH 5.5-6.5.

Acute invertebrate toxicity

Over 300 individual acute data points are available for two "standard" aquatic invertebrate species (*Daphnia magna* and *Ceriodaphnia dubia*). There are more than 4 studies available for each pH band, with greatest sensitivity apparent at acidic pH. Geometric mean acute EC_{50} values at pH 5.5-6.5 are 16.3 µg/L (not normalised, n=29) and **11 µg/L** (normalised for a DOC level of 2 mg/L, n=26) for *D. magna* and **12.6 µg/L** (not normalised, n=9) and 16 µg/L (normalised for a DOC level of 2 mg/L, n=8) for *C. dubia.*

Acute algal/macrophyte toxicity

Over 50 individual acute data points are available for three "standard" algal species (*Pseudokirchneriella subcapitata* (n=36), *Chlamydomonas reinhardtii* (n=3) and *Chlorella* sp. (n=16)). Unlike fish and invertebrates, copper appears to become more acutely toxic to algae with increasing pH. When all data are considered, *P. subcapitata* is the most sensitive species, with more than 4 studies available for each pH band: the lowest geometric mean E_rC_{50} (duration not specified) is **104.9 µg/L** (n=12) at pH >7.5-8.5

(alkaline). *P. subcapitata* is still the most sensitive species when data are normalised for a DOC level of 2 mg/L, with a lowest geometric mean E_rC_{50} (duration not specified) of **31.6 µg/L** (n=11) at pH >7.5-8.5 (alkaline), which is effectively the same result as for *C. reinhardtii* (**31.4 µg/L**, n=1). For comparison, the E_rC_{50} values at pH 5.5-6.5 (acidic) are above 100 µg/L for all species regardless of DOC normalisation.

RAC notes that no acute data are presented for *Lemna* sp., whereas chronic data are available (this is briefly discussed further below).

Long-term fish toxicity

Chronic data are available for three species (*O. mykiss*, *P. promelas* and Brook Trout *Salvelinus fontinalis*). The DS has separated mortality, growth and reproduction endpoints for each species, giving 70 chronic endpoints in total. However, the number of actual studies is lower since more than one endpoint will have been reported for some studies. RAC notes that the most sensitive endpoint should be selected from a study for a particular species, so RAC is uncertain how much double counting of studies has taken place. The data set includes two specifically commissioned studies for *O. mykiss* and *P. promelas* at acidic pH, and some previously accepted data have been re-evaluated by the DS. Nevertheless, there still remains a relative scarcity of information for the acidic pH band (a single study for *O. mykiss*, three for *P. promelas* and none for *S. fontinalis*). The available information is summarised in the following Table.

Table : *Summary of long-term fish NOEC/EC10 data (µg/L) normalised to a DOC level of 2 mg/L*

Species	**End point**	**pH band**		
		Acidic	**Neutral**	**Alkaline**
Oncoryhchus mykiss	Mortality	43.8 (n=1)	16.5 or 17.5 (n=2) [a]	60.6 (n=8)
	Growth	43.3 (n=1)	40.5 (n=4)	31.3 (n=5)
	Reproduction	-	-	-
Pimephales promelas	Mortality	14.6 or 17.9 (n=3) [a]	11.6 or 24.8 (n=3) [a, b]	60.5 (n=5)
	Growth	14.6 or 17.3 (n=3) [a, b]	27 (n=4)	63.8 (n=6)
	Reproduction	-	30.6 or 32.7 (n=2) [a]	57 (n=9)
Salvelinus fontinalis	Mortality	-	28.4 (n=4)	42.3 (n=1)
	Growth	-	28.2 (n=4)	42.3 (n=1)
	Reproduction	-	**10.7** (n=3) [b, c]	-

Note: a - Lowest value presented first, followed by geometric mean.

b - All of the data are in the same classification range for each pH band and endpoint *without* DOC normalisation, with the exception of *P. promelas* mortality and growth, for which the minimum value is **5.9 µg/L** at the neutral pH band and 8.7 µg/L at the acidic pH band, respectively; and *S. fontinalis* reproduction, for which the minimum value is **6.4 µg/L** at the neutral pH band.

c - A geometric mean was not derived by the DS because there are less than four data points across the whole pH range for this endpoint.

In summary, the lowest $NOEC/EC_{10}$ value for fish is **5.9 µg/L** for *P. promelas* mortality at pH >6.5-7.5 (not normalised for DOC level). If the geomean is used irrespective of the number of available data points, the lowest $NOEC/EC_{10}$ value for fish is **6.4 µg/L** for *S. fontinalis* reproduction at pH >6.5-7.5 (not normalised for DOC level). If DOC normalisation is taken into account, the lowest $NOEC/EC_{10}$ value for fish is **10.7 µg/L** for *S. fontinalis* reproduction at pH >6.5-7.5.

RAC notes that the long-term mortality NOEC/EC_{10} for *P. promelas* (not normalised for DOC level) is in the range 10.1-13.8 µg/L at acidic pH, which is effectively the same as the acute LC_{50} value selected for this species (12.1 µg/L). The same conclusion can be drawn when DOC normalisation is performed (acute LC_{50} of 14.9 µg/L, long-term mortality NOEC/EC_{10} of 14.6 or 17.9 µg/L). Further comments on this are provided in the section on public consultation.

In addition, RAC notes that there is no information about reproduction for any fish species at acidic pH, or any data about the long-term sensitivity of *D. rerio*, which may be highly acutely sensitive at acidic pH (see discussion under acute toxicity above).

Long-term invertebrate toxicity

44 individual chronic data points are available for two "standard" aquatic invertebrate species (*D. magna* and *C. dubia*), but the DS only discusses the data for *D. magna* (for which there are about 25 data points). There are only two data points for the neutral pH band, but more than 4 studies are available for the other two pH bands, with greatest sensitivity apparent at acidic pH. The geometric mean 21-d $NOEC_{reproduction}$ values are 13.2 µg/L (not normalised for DOC) and **10.5 µg/L** (normalised for a DOC level of 2 mg/L) at pH 5.5-6.5 (n=7). It should be noted that these are effectively the same as the acute EC_{50} values for mortality, and further comments are provided in the section on public consultation.

In addition, there is only one study reporting effects on growth, in the alkaline pH band (21-d $NOEC_{growth}$ = **12.6 µg/L**, regardless of DOC normalisation). RAC notes that if the same trend in toxicity applies as for other endpoints, a lower 21-d $NOEC_{growth}$ (potentially below 10 µg/L) cannot be ruled out for *D. magna* at pH 5.5-6.5.

Long-term algal/macrophyte toxicity

Over 50 individual chronic data points are available for three "standard" algal species (*P. subcapitata* (n=34), *C. reinhardtii* (n=4) and *Chlorella vulgaris* (n=16)) and the macrophyte *Lemna minor* (n=1). Due to the limited number of data points for some species and pH ranges RAC considers that it is not possible to draw a clear conclusion about chronic toxicity trends with pH. When data are not normalised for DOC, *C. reinhardtii* is the most sensitive species, with a lowest NOE_rC (duration not specified) of **22 µg/L** at pH 5.5-6.5 (n=2; the geometric mean is 62.6 µg/L). This is similar to the NOE_rC (duration not specified) of 30 µg/L for *L. minor* (n=1) at pH 5.5-6.5. When the data are normalised to a DOC level of 2 mg/L, the lowest geometric mean NOE_rC (duration not specified) is **13.3 µg/L** (n=15) for *P. subcapitata* at pH >6.5-7.5.

For comparison, the acute and chronic data for *P. subcapitata* and *C. reinhardtii* are presented in the following Table (with acute-to-chronic ratios (ACRs) calculated by RAC).

Table: *Summary of algal toxicity data (µg/L) normalised to a DOC level of 2 mg/L*

Species	**End point**	**pH band**		
		Acidic	**Neutral**	**Alkaline**
Pseudokirchneriella subcapitata	Acute E_rC_{50} [a]	132.6	33	31.6
	Chronic NOE_rC [a]	34.9	13.3	14.1
	ACR	*3.8*	*2.5*	*2.2*
Chlamydomonas reinhardtii	Acute E_rC_{50} [b]	143.2	80.4	31.4
	Chronic NOE_rC [b]	61.7	27.4	24.7
	ACR	*2.3*	*2.9*	*1.3*

Note: a - Geometric mean.

b - Lowest value as there are insufficient data for a geometric mean to be derived in accordance with the CLP Guidance.

RAC notes that if the same ACRs are applied to the *L. minor* NOE_rC (75.8 µg/L when normalised to a DOC level of 2 mg/L), the putative *Lemna* E_rC_{50} would be in the range 136 – 290 µg/L, providing some reassurance that it is unlikely to be an especially acutely sensitive species.

ERV derivation

The lowest acute and chronic toxicity values selected by the DS are summarised in the following Table. This is based on geomeans only if there are four or more data points for a species in a particular pH band (otherwise the lowest value is selected).

Table: *ERVs derived by the Dossier Submitter (µg/L)*

		pH band		
		5.51-6.5 (acidic)	**>6.5-7.5 (neutral)**	**>7.5-8.5 (alkaline)**
Values not normalised for DOC level				
Acute ERV	**$L(E)C_{50}$**	12.1 (*Pimephales promelas*)	11.7 (*Danio rerio*) [a]	40 (*Ceriodaphnia dubia*)
Chronic ERV	**EC_{10}/NOEC**	8.7 (*Pimephales promelas*)	5.9 (*Pimephales promelas*)	12.6 (*Daphnia magna*)
Values normalised to a DOC level of 2 mg/L				
Acute ERV	**$L(E)C_{50}$**	11 (*Daphnia magna*)	24.1 (*Ceriodaphnia dubia*)	31.4 (*Chlamydomonas reinhardtii*)
Chronic ERV	**EC_{10}/NOEC**	10.5 (*Daphnia magna*)	10.7 (*Salvelinus fontinalis*) [b]	12.6 (*Daphnia magna*)

Note: a - As noted above, RAC does not think this value should be used as it represents very low hardness conditions. The next lowest value is 14 µg/L based on data for *C. dubia*.

b - There is a mistake in Table 92 in the CLH report, where the lowest chronic value with DOC normalisation at the neutral pH band is incorrectly stated to be 5.6 µg/L for *O. mykiss*.

When geomeans are applied, even if there are less than four data points for a species in a particular pH band, effectively the same ERV values are obtained, with the exception of the chronic ERV at acidic pH without DOC normalisation (for which a value of 11.4 µg/L is obtained for *P. promelas*, instead of 8.7 µg/L).

Since similar ERV values are obtained when DOC normalisation is performed, the non-normalised ERVs were carried forward for comparison with the environmental classification criteria.

T/Dp data

Based on the Guidance on the Application of the CLP criteria (2015) the classification of metals is based on a comparison of acute and chronic ERVs (derived from soluble metal species) with the concentration of metal ions in solution after a period of 7 days (short-term test) and 28 days (long-term test), respectively, at different loadings following the T/Dp protocol. Two studies are available for granulated copper. The first (ECTX, 2016a) used one particle of granulated copper in each vessel to attain the desired mass loading

of 1 mg/L at pH 6. A copper release of 1.4 µg/L was obtained after 7 days and 6.0 µg/L after 28 days (coefficients of variation were 23 and 27 %). To overcome the high variability, a second study (ECTX, 2016b) was performed with granulated copper particles embedded in epoxy resin. This reportedly allows the exposed surface area to be set more accurately, avoids abrasion and the surfaces were polished before exposure. These results had much lower coefficients of variation (7—11 %) and showed higher copper releases than the first experiment as indicated in the Table below, so the DS prefers the results of this second study for classification purposes.

Table: *T/Dp releases from granulated copper at a loading of 1 mg/L*

Time	Copper concentration (µg/L) [a]		
	pH 6	pH 7	pH 8
7 days	3.4	2.3	1.2
28 days [b]	13	8.6	4.9

Note: a - Considering the specific surface area of granulated copper of 2.56 mm^2/mg.
b - Values were also extrapolated to a loading rate of 0.1 mg/L, yielding copper concentrations of 1.3, 0.86 and 0.49 µg/L at pH 6, 7 and 8, respectively. Extrapolation is in principle allowed according to the CLP Guidance (footnote 106 in Annex IV.2.2.3), but no further details are provided about this extrapolation in the CLH report (but see supplemental analysis below).

A comparison with the ERVs (Table above) demonstrates that dissolution of granulated copper over acute time periods at a loading of 1 mg/L does not lead to concentrations of dissolved metal ions that exceed the acute ERVs, *i.e.* no acute classification is proposed. Dissolution over chronic time periods at a loading of 1 mg/L leads to concentrations of dissolved metal ions that exceed the chronic ERVs at acidic and neutral pH. The extrapolated concentrations for a notional loading rate of 0.1 mg/L are below the chronic ERVs at all pHs. Therefore the DS proposes to classify this substance as Aquatic Chronic 2.

Comments received during public consultation

One Member State Competent Authority (MSCA) made specific comments on the environmental classification (supporting the proposal to classify as Aquatic Chronic 2), with all other comments submitted by Industry and individuals or organisations with connections to them. An additional MSCA made comments about the definition of the substance to be covered by the proposal. The comments cover a range of issues, which can be summarised as follows:

a) *A request to specify the term "granulated" including particle size and specific surface area*: This is provided at the beginning of this opinion.

b) *Further consideration of "rapid removal"*: Additional arguments are available in the copper REACH registration dossier, but have not been submitted separately during public consultation. RAC therefore cannot incorporate them into the current opinion.

c) *Preference to use data for "standard test species"*: RAC believes that, in principle, it is preferable to base classification decisions on data from standard test guideline studies, since these methods have been ring-tested and approved for use for regulatory purposes. However, the data selection in the CLH dossier is not consistent – some "non-standard" fish and algal species were included for some

endpoints (*Salvelinus fontinalis* and *Chlamydomonas reinhardtii*), and other standard species that have been used to make classification decisions for other substances (and referred to in ECHA guidance) have been omitted entirely without adequate explanation (*e.g.* crustaceans (including *Ceriodaphnia* sp., *Daphnia pulex*, *Gammarus* sp.), insects and molluscs). RAC considers that these should be included in the data set and subject to grouping and data normalisation for appropriate abiotic conditions (see supplemental analysis below).

d) *Provision of further "chronic" studies for P. promelas which are claimed to support the view that there is no significant trend in toxicity between pH 6 and 7*: This includes a new standard 7-d US EPA "short-term chronic" study at pH 6 available in draft (OSU, 2017). The Industry points out that including these data in the acidic pH band for *P. promelas* increases the number of chronic data points to four and so a geometric mean can be derived (13.3 and 13.9 µg/L for mortality and growth, respectively, without DOC normalisation), replacing the minimum value of 8.7 µg/L for this species and pH band (non-normalised). The non-normalised chronic ERV at pH 5.5-6.5 then becomes 13.2 µg/L (*D. magna* $NOEC_{reproduction}$; geometric mean of 7 values).

RAC notes that 7 days is rather short for a chronic fish endpoint. The acute OECD TG 203 duration is 4 days; the OECD TG 212 (Fish, Short-term Toxicity Test on Embryo and Sac-Fry Stages), which is used as a chronic test, specifies 8-9 days for *P. promelas*, and is considered less sensitive than the Early Life Stage test. The main difference between the Norberg & Mount (1985) study and OECD TG 212 seems to be that the larvae were fed from the outset (the OECD test begins with sac-fry feeding on the yolk sac). It is possible that feeding might reduce bioavailability of the copper due to the addition of organic carbon in the water. On the other hand, in this case, the 7-d growth results of Norberg & Mount (1985) were very similar to those derived from separate 32-d Early Life Stage and 327-d full life cycle tests (reported in 1978 and 1969, respectively). RAC has not attempted to assess whether this relationship holds if a larger data set including more modern studies were examined.

RAC also notes that using this information creates some inconsistency with the acute data set. For example, when the new data are taken into account the "chronic" mortality LC_{10} for *P. promelas* (not normalised for DOC level) is around 13 µg/L at acidic pH, which is slightly higher than the acute LC_{50} value selected for this species (12.1 µg/L). The same conclusion can be drawn when DOC normalisation is performed, and is also apparent for the invertebrate *Daphnia magna*. This might simply reflect the differences in the amount of data available (and also potentially hardness), but raises some doubts over the comparability of the acute and chronic data set. This is discussed further in the section that presents RAC's assessment of the classification below. In addition, RAC notes that there is no information about reproduction for any fish species at acidic pH, or any data about the long-term sensitivity of *Danio rerio*, which may be highly acutely sensitive at acidic pH (see discussion under *Acute Fish Toxicity* above).

The implications of excluding the 7-d data are provided in the supplemental analysis below. On balance, RAC prefers not to include them.

e) *Appropriateness of data aggregation and summarisation for data-rich substances*: In principle, RAC considers that splitting the data to reflect the pH bands defined for T/Dp testing is appropriate. In addition, it seems logical to attempt to minimise variability in the data set based on known factors such as the influence of hardness and DOC. However, in this context, RAC notes that the justification to normalise the data to a DOC level of 2 mg/L, is weak and not necessarily appropriate (as it is a maximum recommended in the OECD TGs, which is likely to protect against the toxic effect to some extent). It also introduces some uncertainties due to the omission of studies that lack sufficient background information (which also reduces the size of the data set). In addition, this may not be entirely consistent with the T/Dp data, which are produced in the absence of DOC. Therefore RAC is not in a position to recommend an appropriate DOC value. Both normalised and non-normalised ERVs are presented in the RAC assessment below, and the most stringent classification derived. RAC notes that a BLM approach might have been attempted, although this is based on an assumption that relationships established for a small number of species are generally applicable to others. RAC also considers that the resulting limitations in the data set must be recognised even if the overall number of data points is very large. An example is the lack of acute toxicity data for Zebrafish at acidic pHs, which might prove sensitive (see discussion under *Acute Fish Toxicity* above).

In terms of choice of values, RAC recognises that there needs to be a way of avoiding the default selection of a very low value from a large data set due to the method of splitting the information, provided that factors that can affect the variability in toxicity are fully considered. In particular, sensitive conditions should not be overlooked or 'diluted' by the choice of averaging method. Consequently, RAC considers that it is only appropriate to use the geometric mean when there are 4 or more data available in a pH band for specific endpoints (*e.g.* reproduction) for a species. Preferably the same effect concentrations should be used, but if insufficient data points are available EC_{10} and NOEC values can be mixed.

RAC agrees that it would be worthwhile to perform a "reality check" of the relationship between pH and selected ERV values based on specific experimental studies that have investigated effects of pH on copper toxicity, notably for fish (*e.g.* Erickson et al., 1996; Ng et al., 2010; and OSU, 2016), provided that these followed typical standard approaches to measuring acute/chronic toxicity. For example, the OSU (2016) study included a 56-days Early Life Stage test with juvenile *O. mykiss* performed under similar conditions to the study of Ng *et al*. (2010), but involving formal replication, statistical assessment, acclimation of organisms to low pH, and pre-equilibrated test waters. The EC_{10} values were 28.5 (survival) and 36.0 (wet weight) µg/L at pH 6, and 49.3 (survival) and 47.3 (wet weight) µg/L at pH 7. The Industry concludes that there are little or no sensitivity differences to fish between pH 6 and 7 based on this information. RAC notes that the fish appear to have been slightly more sensitive under acidic conditions, although without information on the uncertainty in the EC_{10} values, these data neither confirm nor exclude a pH effect.

Further evidence from Species Sensitivity Distribution (SSD), bootstrapping and Biotic Ligand Model (BLM) approaches would also be useful to consider in terms of

the overall weight of evidence. However, this has not been performed by the DS or provided during public consultation, and is not essential for a conclusion. Further interpretation of such approaches would in any case also benefit from a discussion at global level.

f) *A suggestion that the lowest chronic NOEC/EC_{10} value for P. promelas mortality at neutral pH without DOC normalisation (5.9 µg/L) is an outlier*: RAC tends to agree that this value may be unusually low (see RCOM for further discussion) but notes that when DOC normalisation is applied, the value changes to 11.6 µg/L, which is preferred as a more relevant indicator of toxicity.

g) *A suggestion to use the results of an old (1974) study on reproduction in S. fontinalis which showed no effects up to the highest test concentration of 9.4 µg/L as supporting information to justify the use of a geometric mean at neutral pH for this species and endpoint*: There is (undescribed) "uncertainty" related to the pH during this test and given the age of the test RAC does not think sufficient justification has been provided to include this data point.

h) Industry agrees with the way that the transformation/dissolution protocol data have been used, and highlights that the classification proposal is specific for the test material used. RAC has added a note to the opinion about this.

i) Based on Industry's re-analysis of the data set, they propose not to classify granulated copper as hazardous to the aquatic environment. This depends on the selection of geometric mean or the lowest value, data normalisation and also the inclusion of chronic data for relevant additional species, as well as the way inconsistencies between the acute and chronic data sets are handled.

j) It should be clarified that the proposal does not cover nanoforms of copper. RAC agrees and has added a note to this effect.

Assessment and comparison with the classification criteria

Degradation

Based on the data provided in the CLH dossier and submitted during public consultation, granulated copper is not considered to be rapidly transformed by normal environmental processes. RAC recommends that future CLH dossiers for other copper compounds could take account of all relevant information once an internationally agreed approach to this issue has been reached. This may in turn affect classification decisions drawn for this substance and previous copper compound cases.

Bioaccumulation

The bioaccumulation behaviour of copper (II) ions is complicated by essentiality and homeostatic mechanisms in organisms, but does not need to be considered further because it does not influence the determination of the chronic M-factor (in view of the conclusion about removal).

Aquatic toxicity

RAC has not independently verified all of the ecotoxicity information in the CLH dossier given the quantity of data and previous evaluations. Based on the information provided in the CLH report, public comments and supplemented by the DS during RAC discussions (see supplemental analysis), RAC considers that the following ERVs are most appropriate:

		pH band		
		5.51-6.5 (acidic)	**>6.5-7.5 (neutral)**	**>7.5-8.5 (alkaline)**
Values not normalised for DOC level				
Acute ERV	**$L(E)C_{50}$**	12.1 (*Pimephales promelas*)	11.7 (*Danio rerio*)	40 (*Ceriodaphnia dubia*)
Chronic ERV	**EC_{10}/NOEC**	13.2 (*Daphnia magna*) [a]	4 (*Ceriodaphnia dubia*) [b]	12.6 (*Daphnia magna*)
Values normalised to a DOC level of 2 mg/L				
Acute ERV	**$L(E)C_{50}$**	11 (*Daphnia magna*)	24.1 (*Ceriodaphnia dubia*)	31.4 (*Chlamydomonas reinhardtii*)
Chronic ERV	**EC_{10}/NOEC**	10.5 (*Daphnia magna*)	6.2 (*Ceriodaphnia dubia*) [b]	11.8 (*Ceriodaphnia dubia*)

Note: a – If 7-d data for *P. promelas* were used, the ERV would be 8.7 µg/L (n=3), or 13.3 µg/L if the OSU (2017) study is taken into account.

b – This is the main difference from the DS's proposal. The lowest reported long-term NOEC at neutral pH for *C. dubia* in the previous CLH reports for the copper compounds was 7.4 µg/L, which was a geomean of the 4 available (non-normalised) NOEC values without distinguishing between mortality and reproductive effects. As the CLH dossier now splits this information, the lowest NOEC becomes 4 µg/L without DOC normalisation, corresponding to 6.2 µg/L with DOC normalisation.

The data aggregation exercise results in an unusual conclusion for acidic pH, *i.e.* the concentration that causes 50 % mortality in acute tests is effectively the same as that which causes no adverse effects in long-term tests (with the same species in the case of the DOC-normalised values). In a reply to a question from the RAC rapporteur, the DS considers that the acute-to-chronic ratios (ACRs) are generally low, and tend to decrease with decreasing pH (approaching unity at around pH 6). RAC has some concerns about this general conclusion, because although there may be reasons for similar acute and chronic sensitivities (*e.g.* acclimation, provision of food that could affect bioavailability, etc.), there is far more acute than chronic data especially at lower pH, which might produce misleading ACRs (since the result is highly dependent on the representive nature of a very small number of chronic values). As an example, an ACR below 1 is obtained for *O. mykiss* mortality at acidic pH, implying that the organisms are less sensitive over long-term exposure and/or at sensitive life stages. As a possible "worst case", applying the apparent ACR for *C. dubia* from the DOC-normalised ERVs at neutral pH (3.9) to the acute ERV for *D. magna* at acidic pH would result in a theoretical DOC-normalised chronic ERV for *D. magna* of 2.8 µg/L at acidic pH.

The change in species sensitivity across the pH bands could also be an artefact of the varying amounts of data available. RAC concludes that the amalgamation of such a diverse data set is not ideal for classification purposes, and that it might have been better to focus more on standard studies that have been specifically designed to investigate pH variation

under specific DOC and hardness conditions in a single laboratory. In the absence of such an analysis, the derived ERVs have to be used.

As pointed out in the discussion above, even though the data set is relatively large, there are still potential information gaps, including for Zebrafish *D. rerio* and Brook Trout *S. fontinalis* at acidic pH (e.g. an acute LC_{50} below 10 µg/L (normalised for DOC) cannot be ruled out). RAC considers that if such data became available, the acute and chronic ERVs at acidic pH could be lower than 10 µg/L.

Before presenting the classifications, it is appropriate to recall the T/Dp data at a loading of 1 mg/L:

Time	**Copper concentration (µg/L)**		
	pH 6	**pH 7**	**pH 8**
7 days	3.4	2.3	1.2
28 days	13	8.6	4.9

Acute aquatic hazard

Dissolved copper concentrations arising from granulated copper do not exceed 3.4 µg/L at any pH over 7 days at a loading rate of 1 mg/L. This is below the acute ERVs (≥ 11-12 µg /L) by at least a factor of three, so the substance will not achieve acutely toxic dissolved concentrations over a relevant time period at 1 mg/L. **It therefore does not require classification for Aquatic Acute hazard.**

Chronic aquatic hazard

Dissolved copper concentrations arising from granulated copper are 13 and 8.6 µg/L over 28 days at a loading rate of 1 mg/L at acidic and neutral pH, respectively. These exceed the chronic ERVs for these pH bands (13.2 or 10.5 and 4 or 6.2 µg/L, respectively). The extrapolated copper concentrations at a notional loading rate of 0.1 mg/L (i.e. 0.49 – 1.3 µg/L) do not exceed the chronic ERVs at any pH. Chronic toxicity may therefore be expressed at a loading rate of >0.1 to ≤1 mg/L, which results in classification as Aquatic Chronic 2 for a substance that is not rapidly transformed. This conclusion only applies to the substance that was tested in the T/Dp test, since the metal release per unit surface is an intrinsic property of the material.The chronic ERV would have to be less than 1.3 µg/L at acidic pH to affect this conclusion, which seems unlikely even though there is some uncertainty in the data set.

Conclusion on the classification

RAC agrees with the DS that granulated copper requires **classification as Aquatic Chronic 2**.

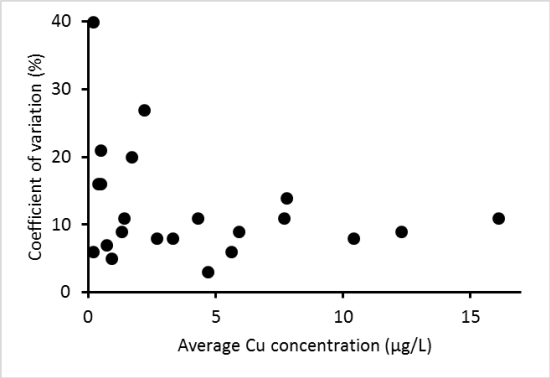
This opinion does not address either copper massive or powder (the CAS and EC number given in the proposal is in fact for metallic copper, and so to avoid misunderstandings a clear message may need to be provided if a future entry for granulated copper). Nano-forms should also be considered separately.

The ERVs selected for this substance may also affect the classification of other copper compounds already considered by RAC, but this has not been assessed.

Supplemental information - In depth analyses by RAC

1. The DS considers that a T/Dp test at 0.1 mg/L is not technically feasible, since it would likely result in copper concentrations within the range of ambient background (possibly below or close to the detection limit of 0.2 µg/L) and/or highly variable copper release that would fail the validity criteria (see Figure below).

Figure: *The uncertainty of copper concentrations measured in transformation-dissolution tests*



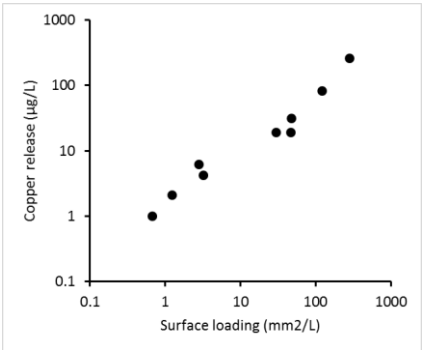
Note: Data are from annexes 3b, 4b, and 5b of the transformation-dissolution tests on granulated copper (ECTX, 2016). Note the high variability below 2 µg/L.

The DS assumes that copper release increases linearly with the surface loading (below approximately 3 mm^2/L, *i.e.* below 1 mg/L mass loading for granulated copper), based on the following information:

- For copper powders, Skeaff and Hardy (2005) noted a non-linear relationship between copper release (µg/L) and surface area at loadings between 48 and 10 700 mm^2/L (with lower release at higher loadings).

- For massive copper, the relationship between copper release and surface loading is approximately linear between 1 and 1 000 mm^2/L, with some scatter (Figure below).

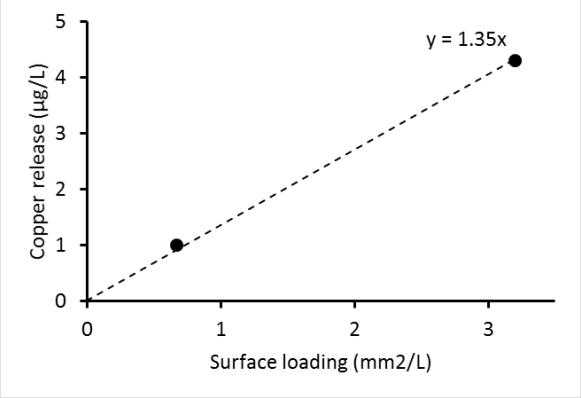
Figure*: Log-log plot of copper release at various surface loadings, tested for 7 days at pH 6*

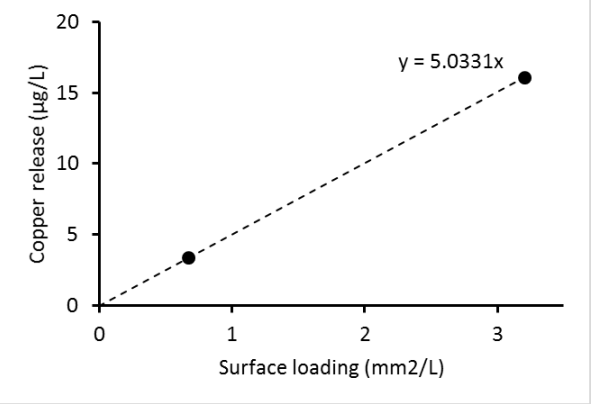


Note: Data compiled from Rodriguez *et al.* (2007, 2011, and 2012) and ECTX (2016).

- At low loadings involving polished, epoxy embedded surfaces, the relationship is linear, both after 7 days and after 28 days (Figure below).

Figure: *Copper release from polished surfaces embedded in epoxy resin, when tested at pH 6, at low loadings, and for 7 days (left) or 28 days (right)*





Note: Data compiled from Rodriguez *et al.* (2012) on copper massive, and ECTX (2016) on granulated copper. The dotted line is a linear regression forced through the origin.

RAC notes that a 0.1 mg/L loading of granulated copper is equivalent to a surface loading of 0.26 mm^2/L. In the absence of measured data in the sub-1 mm^2/L range, RAC considers that a linear relationship between release and surface loading is an appropriate assumption. Based on the regression equation derived in the above Figure, the copper release at a loading rate of 0.1 mg/L at pH 6 would be 1.31 µg/L over 28 days. This is consistent with the value derived by the DS.

2. At the request of the RAC rapporteur, the DS presented an additional table to investigate the influence of the 7-d chronic NOECs reported for *Pimephales promelas* (see Table below).

Table*: Summary of chronic toxicity data for P. promelas considering DOC normalisation at 2 mg/L*

Endpoint	**NOEC/EC_{10} (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Mortality			
n	3	3	2	5
Min	14.6	11.6	11.6	51
Max	23.5	40.9	40.9	78.2
Geometric mean	17.9	-	-	60.5
Lowest value (when n<4)	14.6	11.6	11.6	-
Growth			
n	3	4	3	6
Min	14.6	7.5	7.5	31.4
Max	23.5	49.3	49.3	115.9
Geometric mean	17.3	27	-	63.8
Lowest value (when n<4)	14.6	-	7.5	-
Reproduction			
n	-	2	9
Min	-	30.6	36.4
Max	-	34.9	68.5
Geometric mean	-	-30.6	57
Lowest value (when n<4)	-	30.6	-

Note: In green: results including the 7-d NOEC for *Pimephales promelas*

It can be seen that if these results are removed from the data set, there appear to be no chronic data for this species at acidic pH (although in fact there are additional data, but these cannot be normalised due to lack of information about DOC levels), and the most sensitive value at neutral pH drops from 11.6 to 7.5 µg/L.

The "chronic" mortality LC_{10} for *P. promelas* is ≥15 µg/L at acidic pH with DOC normalisation, which is greater than the acute LC_{50} value selected for this species (12.1 µg/L). In principle the chronic endpoint value (representing "no effects") should be lower than the acute one. This might simply reflect the differences in the amount of data available (and also potentially hardness), but raises some doubts over the comparability of the acute and chronic data sets using the 7-d end point. RAC therefore prefers to exclude these data from further consideration.

3. Following a request by the RAC rapporteur, the DS provided additional chronic data for *Ceriodaphnia dubia*, and these are summarised in Table below.

Table: *Summary of chronic toxicity data for Ceriodaphnia dubia considering DOC normalisation at 2 mg/L*

Endpoint	**NOEC (µg Cu/L)**		
	pH: 5.5-6.5	**pH: >6.5-7.5**	**pH: >7.5-8.5**
Mortality			
n	1	2	7
Min	11.5	11.8	11.3
Max	-	15.4	55
Geometric mean	-	-	29.5
Lowest value (when n<4)	11.5	11.8	-
Reproduction			
n	-	2	11
Min	-	6.2	3.3
Max	-	15.4	33.1
Geometric mean	-	-	11.8
Lowest value (when n<4)	-	6.2	-

Only a single data point is available for this species at acidic pH. The DS did not provide any further summary for other species, but referred to the previous RAC opinion on copper flakes (2014) which indicated that *"the omission of data for other invertebrates groups does not appear to make a significant difference as the most sensitive data all lie in the same range 1-10 µg/L".*

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7 ANNEXES

Table I: Fish acute ecotoxicity database used for classification purposes

Species	E(L)C50 Value µg/L	E(L)C50 Normalised value µg/L	pH	DOC mg/L	Hardness mg/L as CaCO3	Temperature °C	Ca mg/L	Mg mg/L	Na mg/L	K mg/L	SO4 mg/L	Cl mg/L	Alk mg CaCO3/L	Type of water	Reference	Lifestage
Fish acute database																
Danio rerio	11,7	26	7,34	0,9	7,8	28	2,4	0,4	1,6	0,1	7,5	3,7	4,9	Soft water	Alsop and Wood, 2011	larvae
Danio rerio	148,4	94,7	7,8	3,5	141	28	54,1	8,2	16,1	1,5	111,6	54,9	14,1	Hard water from Lake Ontario	Alsop and Wood, 2011	larvae
Danio rerio	212,1	146,9	7,8	3,5	141	26,5	54,1	8,2	16,1	1,5	111,6	54,9	14,1	Hard water from Lake Ontario	Alsop and Wood, 2011	Adult
Oncorhynchus mykiss	4,2	6,28	5,7	1,3	9,2	15.8	2,8	0,6	0,54	0,14	1,63	0,81	0,11	well water	Cusimano et al, 1986	Fry
Oncorhynchus mykiss	31,9	43,9	6	1,3	99	15	29,80	6,00	5,81	1,47	17,53	8,70	0,22	well water	Howarth and Sprague, 1978	juveniles
Oncorhynchus mykiss	22,4	63,8	6	0,36	32	15	9,60	1,90	1,88	0,48	5,67	2,81	0,22	well water	Howarth and Sprague, 1978	juveniles
Oncorhynchus mykiss	40	76,2	6	0,56	101	15	30,40	6,10	5,93	1,50	17,88	8,88	0,22	well water	Howarth and Sprague, 1978	juveniles
Oncorhynchus mykiss	28,9	76,4	6	0,36	31	15	9,30	1,90	1,82	0,46	5,49	2,73	0,22	well water	Howarth and Sprague, 1978	juveniles
Oncorhynchus mykiss	70	85,9	6	1,3	366	15	110,00	22,20	21,48	5,45	64,80	32,18	0,22	well water	Howarth and Sprague, 1978	juveniles
Oncorhynchus mykiss	82,2	99,3	6	1,3	371	15	111,50	22,50	21,77	5,52	65,69	32,62	0,22	well water	Howarth and Sprague, 1978	juveniles
Oncorhynchus mykiss	5,9	8,4	6,2	1,4	14-22	n.r.	3,3	1,0	2,3	0,20	11,3	7,2	12,7	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
Oncorhynchus mykiss	47,4	101,7	7	0,56	101	15	30,40	6,10	5,93	1,50	17,88	8,88	2,22	well water	Howarth and Sprague, 1978	n.r.
Oncorhynchus mykiss	81,1	146,7	7	0,56	100	15	30,1	6,05	5,87	1,485	17,705	8,79	2,22	well water	Howarth and Sprague, 1978	n.r.
Oncorhynchus mykiss	298	336,3	7	1,3	361	15	108,50	21,90	21,19	5,38	63,91	31,74	2,22	well water	Howarth and Sprague, 1978	n.r.

Oncorhynchus mykiss	9,2	13,1	7,1	1,4	14-22	n.r.	6,7	1,3	3,1	0,33	4,6	8,0	34,0	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
Oncorhynchus mykiss	21	19,1	7,5	2,2	57	13	17,1	3,5	3,3	0,85	39,7	19,5	7,1	Carbon & biofiltered city water	Vardy et al., 2013	swim-up larvae
Oncorhynchus mykiss	22	20	7,5	2,2	57	13	17,1	3,5	3,3	0,85	39,7	19,5	7,1	Carbon & biofiltered city water	Vardy et al., 2013	juvenile
Oncorhynchus mykiss	24	21,8	7,5	2,2	57	13	17,1	3,5	3,3	0,85	39,7	19,5	7,1	Carbon & biofiltered city water	Vardy et al., 2013	juvenile
Oncorhynchus mykiss	40	36,4	7,5	2,2	57	13	17,1	3,5	3,3	0,85	39,7	19,5	7,1	Carbon & biofiltered city water	Vardy et al., 2013	yolc sac age
Oncorhynchus mykiss	60	127,2	7,84	0,4	103	12	31,0	6,3	6,0	1,53	71,8	35,3	15,5	well water + deionized water	Ingersoll & Mebane, 2014	1d post-hatch
Oncorhynchus mykiss	56,6	120,5	7,97	0,4	104	12	31,3	6,3	6,1	1,55	72,5	35,7	20,9	well water + deionized water	Ingersoll & Mebane, 2014	18d post-hatch
Oncorhynchus mykiss	30	69,8	8	0,36	31	15	9,30	1,90	1,82	0,46	5,49	2,73	22,4	well water	Howarth and Sprague, 1978	n.r.
Oncorhynchus mykiss	40,8	99,0	8	0,4	103	12	31,0	6,3	6,0	1,53	71,8	35,3	22,4	well water + deionized water	Ingersoll & Mebane, 2014	60d post-hatch
Oncorhynchus mykiss	42,4	101,2	8	0,4	103	12	31,0	6,3	6,0	1,53	71,8	35,3	22,4	well water + deionized water	Ingersoll & Mebane, 2014	60d post-hatch
Oncorhynchus mykiss	309	347,6	8	1,3	360	15	108,20	21,90	21,13	5,36	63,73	31,66	22,4	well water	Howarth and Sprague, 1978	n.r.
Oncorhynchus mykiss	516	561,9	8	1,3	371	15	111,50	22,50	21,77	5,52	65,69	32,62	0,22	well water	Howarth and Sprague, 1978	n.r.
Oncorhynchus mykiss	50,1	110,9	8,04	0,4	103	12	31,0	6,3	6,0	1,53	71,8	35,3	24,6	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
Oncorhynchus mykiss	59	122,4	8,04	0,4	103	12	31,0	6,3	6,0	1,53	71,8	35,3	24,6	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
Oncorhynchus mykiss	19,1	55,7	8,05	0,4	105	12	30,5	6,2	5,9	1,51	70,7	34,8	94,0	well water + deionized water	Ingersoll & Mebane, 2014	95d post-hatch

Oncorhynchus mykiss	60,6	112,7	8,05	0,4	105	12	30,5	6,2	5,9	1,51	70,7	34,8	94,0	well water + deionized water	Ingersoll & Mebane, 2014	74d post-hatch
Oncorhynchus mykiss	63	127,2	8,05	0,4	105	12	31,6	6,4	6,1	1,56	73,2	36,0	25,2	well water + deionized water	Ingersoll & Mebane, 2014	46d post-hatch
Oncorhynchus mykiss	8,5	13,1	8,1	1,3	14-22	n.r.	3,8	1,1	5,6	0,23	3,7	10,2	37,9	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
Oncorhynchus mykiss	30,9	70,1	8,1	0,5	108	12	32,5	6,6	6,3	1,61	75,3	37,0	101,0	Reconstituted water	Little et al., 2012	160d post-hatch
Oncorhynchus mykiss	36,5	78,9	8,1	0,5	107,7	12	32,4	6,5	6,3	1,60	75,1	36,9	88,8	Reconstituted water	Little et al., 2012	30d post-hatch
Oncorhynchus mykiss	59,9	122,4	8,1	0,4	105	12	31,6	6,4	6,1	1,56	73,2	36,0	28,3	well water + deionized water	Ingersoll & Mebane, 2014	32d post-hatch
Oncorhynchus mykiss	62,9	126,3	8,11	0,4	95	12	28,6	5,8	5,6	1,42	66,2	32,6	29,0	well water + deionized water	Ingersoll & Mebane, 2014	1d post-hatch
Oncorhynchus mykiss	6,7	8,9	8,5	1,5	14-22	n.r.	4,0	1,1	8,7	0,23	5,6	6,0	70,1	Tap water with reverse osmosis water	Ng et al., 2010	juvenile
Oncorhynchus mykiss	56,6	107,8	8,0-8,1	0,4	95-108	12	30,5	6,2	5,9	1,51	70,7	34,8	94,0	Well water + deionized water	Calfee et al., 2014	18d post-hatch
Oncorhynchus mykiss	62,9	115,5	8,0-8,1	0,4	95-108	12	30,5	6,2	5,9	1,51	70,7	34,8	94,0	Well water + deionized water	Calfee et al., 2014	1d post-hatch
Oncorhynchus mykiss	59,9	111,8	8,0-8,1	0,4	95-108	12	30,5	6,2	5,9	1,51	70,7	34,8	94,0	Well water + deionized water	Calfee et al., 2014	32d post-hatch
Oncorhynchus mykiss	59	110,8	8,0-8,1	0,4	95-108	12	30,5	6,2	5,9	1,51	70,7	34,8	94,0	Well water + deionized water	Calfee et al., 2014	46d post-hatch
Oncorhynchus mykiss	42,4	90,1	8,0-8,1	0,4	95-108	12	30,5	6,2	5,9	1,51	70,7	34,8	94,0	Well water + deionized water	Calfee et al., 2014	60d post-hatch
Oncorhynchus mykiss	60,6	124,2	8,0-8,1	0,4	95-108	12	31,6	6,4	6,1	1,56	73,2	36,0	25,2	Well water + deionized water	Calfee et al., 2014	74d post-hatch
Oncorhynchus mykiss	19,1	64,6	8,0-8,1	0,4	95-108	12	31,6	6,4	6,1	1,56	73,2	36,0	25,2	Well water + deionized water	Calfee et al., 2014	95d post-hatch

Pimephales promelas	12,2	14,9	6	1,58	48	25	10,5	4,9	15,6	0,4	0,6	54,0	48,5	reconstituted water	OSU, 2016	larvae
Pimephales promelas	13	21,8	6	1,29	44	25	10,0	4,4	15,1	0,4	0,53	46,0	12,0	reconstituted water	OSU, 2016	larvae
Pimephales promelas	24,4	40,1	6,5	0,98	48	25	10,3	4,5	16,3	0,4	0,6	47,5	15,0	reconstituted water	OSU, 2016	larvae
Pimephales promelas	5,9	22,6	7,01	0,5	17,8	25	5,35	1,08	13,50	0,27	34,7	17,1	12,0	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	12,8	44,0	7,01	0,5	23,8	25	7,15	1,45	27,70	0,35	63,7	31,3	10,0	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	7,8	29,7	7,13	0,5	17,8	25	5,35	1,08	3,90	0,27	17,5	8,6	10,0	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	180	232,6	7,2	0,7	1213	n.r.	403	50,4	80	4,6	70,0	1090,0	16,0	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	13,2	45,2	7,22	0,5	28,4	25	8,54	1,73	6,70	0,42	28,8	14,2	19,3	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	30,2	75,4	7,28	0,5	108,5	25	32,61	6,59	6,70	1,62	76,2	37,5	19,6	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	7,5	29,1	7,29	0,5	4,2	25	1,26	0,26	7,10	0,06	15,2	7,5	18,8	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	10,2	37,4	7,29	0,5	19,3	25	5,80	1,17	6,70	0,29	23,4	11,5	17,7	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	7,2	27,6	7,3	0,5	10,9	25	3,28	0,66	6,90	0,16	18,8	9,3	19,0	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	21,4	62,6	7,31	0,5	52,3	25	15,72	3,18	6,90	0,78	43,3	21,3	19,6	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	102	94,4	7,5	2,2	57	22	17,13	3,46	3,3	0,85	39,7	19,5	7,1	Carbon & biofiltered city water	Vardy et al., 2013	yolk sack stage
Pimephales promelas	15,3	50,7	7,58	0,5	19,8	25	5,95	1,20	12,30	0,29	33,7	16,6	30,0	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	17,4	52,8	7,6	<0,5	1245	n.r.	316	111	85,48	21,72	259,8	128,7	8,89	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	31,7	82,2	7,8	0,5	97	25	29,2	5,9	5,7	1,45	67,6	33,3	14,1	Reconstituted water	Nimmo et al., 2006	larvae
Pimephales promelas	337,6	97,5	7,9	8,5	107	25	32,2	6,5	6,3	1,59	74,6	36,7	17,8	Natural water	Nimmo et al., 2006	larvae
Pimephales promelas	24,4	65,9	7,94	0,5	23,8	25	7,15	1,45	27,70	0,35	63,7	31,3	58,0	Reconstituted water	Van Genderen et al., 2008	<24h old
Pimephales promelas	769	552,6	8	6,9	438	n.r.	104	43,3	370	16,6	530,0	185,0	180,0	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	684	658,3	8,1	2,5	187	n.r.	56,1	11,4	45,6	6,6	46,5	48,2	164,0	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	1870	1669,4	8,1	4,4	66	n.r.	18,3	4,9	88,8	8,5	64,7	50,3	124,0	Natural water	Van Genderen et al., 2007	larvae

Pimephales promelas	300	384,9	8,2	<0,5	287	n.r.	72	26,1	19,89	5,05	60,5	29,9	35,80	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	906	550,2	8,2	9,8	288	n.r.	71,9	26,3	150	14,1	200,0	161,0	204,0	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	544	580,2	8,2	1,2	294	n.r.	66,7	30,9	98	2,9	172,0	1,2	228,0	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	1390	1185,1	8,2	5,4	794	n.r.	174	87,4	274	29,4	325,0	718,0	128,0	Natural water	Van Genderen et al., 2007	larvae
Pimephales promelas	250	330,8	8,3	<0,5	156	n.r.	37	15,4	11,22	2,85	34,2	16,9	45,20	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	262	341,7	8,3	<0,5	284	n.r.	72	25,4	19,53	4,96	59,4	29,4	45,20	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	315	395,8	8,3	<0,5	767	n.r.	202	64	51,13	12,99	155,2	76,9	45,20	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	197	273,8	8,4	<0,5	70	n.r.	18,1	6	4,72	1,2	14,3	7,1	57,20	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	473	562,5	8,5	<0,5	445	n.r.	115	38,3	30,05	7,64	91,3	45,2	72,40	Reconstituted water	Van Genderen et al., 2007	larvae
Pimephales promelas	137	104,3	7,85-8,17	2,96	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	96,6	159,1	7,85-8,17	0,42	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	106,1	170,6	7,85-8,17	0,41	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	259,5	178,8	7,85-8,17	3,94	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	129	192,0	7,85-8,17	0,51	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	597,7	245,5	7,85-8,17	9,53	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	283,6	263,7	7,85-8,17	2,42	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	494,8	332,5	7,85-8,17	5,14	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	363,6	339,8	7,85-8,17	2,46	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae

Pimephales promelas	499,5	344,2	7,85-8,17	4,97	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	468,1	349,4	7,85-8,17	4,26	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	395,5	356,9	7,85-8,17	2,73	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	402,4	378,1	7,85-8,17	2,45	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	383,7	379,9	7,85-8,17	2,07	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	429	380,9	7,85-8,17	2,89	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	422,1	388,9	7,85-8,17	2,61	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	596,3	394,0	7,85-8,17	5,68	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	613,3	418,3	7,85-8,17	5,47	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	774,3	531,4	7,85-8,17	5,95	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	726,5	542,1	7,85-8,17	4,98	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	671,2	610,5	7,85-8,17	2,94	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	2034	825,5	7,85-8,17	18,23	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1405	843,9	7,85-8,17	9,56	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1526	903,0	7,85-8,17	10,16	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae

Pimephales promelas	1564	957,0	7,85-8,17	9,77	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1681	1039,0	7,85-8,17	9,94	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1959	1192,1	7,85-8,17	10,94	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1514	1251,9	7,85-8,17	5,01	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	2013	1260,1	7,85-8,17	10,58	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Pimephales promelas	1949	1327,5	7,85-8,17	8,93	84,5-91,1	25	26,4	5,3	5,1	1,31	61,2	30,1	60,0	Reconstituted hard water (US EPA)	Ryan et al., 2004	larvae
Brachydanio rerio	35		7,2		100	24								reconstituted water	Bresch, 1982	juveniles (6 mo)
Brachydanio rerio	149		8,2		362	25								well water	Fogels and Sprague, 1977	n.r.
Cyprinus carpio	820		7,6		220	24								dechlorinated well water	Dehghani et al, 2012	Adult
Cyprinus carpio	810		7,8		53	17								field water	Rehwoldt, 1971	n.r.
Cyprinus carpio	800		8		55	28								field water	Rehwoldt, 1972	n.r.
Lepomis macrochirus	1000		7,2		40,2	22								tap water	Thompson et al, 1980	n.r.
Lepomis macrochirus	1100		7,5		45	20								field water	Benoit, 1975	juveniles
Lepomis macrochirus	9150		8		200	24								lab water	Geckler et al, 1976	n.r.
Lepomis macrochirus	4250		8,2		318	15								field water	Geckler et al, 1976	n.r.
Lepomis macrochirus	4300		8,2		316	19								field water	Geckler et al, 1976	n.r.
Oncorhynchus mykiss	94		6,8		18,3	6.0								field water	Mudge et al, 1993	parr
Oncorhynchus mykiss	93		6,9		23,7	4.4								field water	Mudge et al, 1993	parr
Oncorhynchus mykiss	2,8		7		9,2	15.8								well water	Cusimano et al, 1986	fry

Oncorhynchus mykiss	17		7,1		22	12.2								field water	Chapman, 1978	fry
Oncorhynchus mykiss	18		7,1		22	12.2								field water	Chapman, 1978	parr
Oncorhynchus mykiss	28		7,1		22	12.2								field water	Chapman, 1978	alevins
Oncorhynchus mykiss	29		7,1		22	12.2								field water	Chapman, 1978	smolt
Oncorhynchus mykiss	68		7,2		28,6	15.3								field water	Mudge et al, 1993	parr
Oncorhynchus mykiss	164		7,3		33	13.5								field water	Buckley, 1983	juveniles
Oncorhynchus mykiss	890		7,4		300									lab water	Calamari and Marchetti, 1973	n.r.
Oncorhynchus mykiss	253		7,4											field water	Hale, 1977	juveniles (2 mo)
Oncorhynchus mykiss	286		7,5		41	13.5								field water	Buckley, 1983	juveniles
Oncorhynchus mykiss	18		7,5		25,1	9.8								well water	Marr et al, 1998	fry
Oncorhynchus mykiss	20		7,5		54	15								tap water	Skidmore and Firth, 1983	n.r.
Oncorhynchus mykiss	57		7,6		42	9								well water	Chapman and Stevens, 1978	alevins
Oncorhynchus mykiss	9,5		7,6		24,2	10								well water	Marr et al, 1999	fingerlings
Oncorhynchus mykiss	10,5		7,6		24,2	10								well water	Marr et al, 1999	fingerlings
Oncorhynchus mykiss	12,5		7,6		24,2	10								well water	Marr et al, 1999	fingerlings
Oncorhynchus mykiss	16,5		7,6		24,2	10								well water	Marr et al, 1999	fingerlings
Oncorhynchus mykiss	21,5		7,6		24,2	10								well water	Marr et al, 1999	fingerlings
Oncorhynchus mykiss	80		7,7		120	12								well water	Seim et al, 1984	alevins
Oncorhynchus mykiss	83,3		7,8		194	12.8								well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	85,3		7,8		194	12.8								well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	103		7,8		194	12.8								well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	128		7,8		194	12.8								well water	Chakoumakos et al, 1979	n.r.

Oncorhynchus mykiss	165		7,8		194	12.8							well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	169		7,8		194	12.8							well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	197		7,8		194	12.8							well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	221		7,8		194	12.8							well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	243		7,8		194	12.8							well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	274		7,8		194	12.8							well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	514		7,8		194	12.8							well water	Chakoumakos et al, 1979	n.r.
Oncorhynchus mykiss	190		7,8		125	10							tap water	Spear, 1977	juveniles
Oncorhynchus mykiss	200		7,8		125	10							tap water	Spear, 1977	juveniles
Oncorhynchus mykiss	210		7,8		125	10							tap water	Spear, 1977	alevins
Oncorhynchus mykiss	102		8,2		362	15							well water	Fogels and Sprague, 1977	n.r.
Pimephales promelas	4,4		6		22,5	22							field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	15		6,3		290	25							reconstituted water	Schubauer-Berigan et al, 1993	larvae
Pimephales promelas	5,9		6,53		49	22							field water	Erickson et al, 1996	larvae
Pimephales promelas	12,4		7		24	22							field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	75		7,1		31,4	22							field water	Mount and Stephan, 1969	fingerlings
Pimephales promelas	84		7,1		31,4	22							field water	Mount and Stephan, 1969	fingerlings
Pimephales promelas	71,2		7,1		87,1	22							field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	970		7,2		308	26							field water	Geckler et al, 1977	n.r.
Pimephales promelas	865		7,2		316	19							field water	Geckler et al, 1977	n.r.
Pimephales promelas	650		7,2		318	15							field water	Geckler et al, 1977	n.r.
Pimephales promelas	21		7,2		45	22							field water	Erickson et al, 1996	larvae

Pimephales promelas	18,2		7,2		46	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	663		7,2		251,2	22								lab water	Erickson et al, 1996	larvae
Pimephales promelas	44		7,3		290	25								reconstituted water	Schubauer-Berigan et al, 1993	larvae
Pimephales promelas	810		7,3		310	18.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	79,7		7,3		85,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	427		7,3		90	25								field water	Nelson et al, 1985	larvae
Pimephales promelas	96		7,4		43,9	25								field water	Spehart and Fiandt, 1986	larvae (30 d)
Pimephales promelas	1090		7,4		260	11								field water	Geckler et al, 1977	n.r.
Pimephales promelas	1400		7,4		298	25								field water	Geckler et al, 1977	n.r.
Pimephales promelas	19,7		7,4		45,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	171		7,4		36	25								field water	Nelson et al, 1985	larvae
Pimephales promelas	210		7,4		103	22								reconstituted water	Birge et al, 1983	alevins
Pimephales promelas	360		7,4		103	22								reconstituted water	Birge et al, 1983	alevins
Pimephales promelas	390		7,4		262,5	22								reconstituted water	Birge et al, 1983	alevins
Pimephales promelas	410		7,4		262,5	22								reconstituted water	Birge et al, 1983	alevins
Pimephales promelas	920		7,5		240	15								field water	Geckler et al, 1977	n.r.
Pimephales promelas	<640		7,5		280	25.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	>780		7,5		280	21.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	960		7,5		282	24.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	780		7,5		324	21								field water	Geckler et al, 1977	n.r.
Pimephales promelas	20,5		7,5		19	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	23,2		7,5		34	22								field/lab water	Erickson et al, 1996	larvae

Pimephales promelas	26,7		7,5		27	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	35		7,5		51	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	232		7,5		55	25								field water	Nelson et al, 1985	larvae
Pimephales promelas	363		7,5		68	25								field water	Nelson et al, 1985	larvae
Pimephales promelas	820		7,6		284	21								field water	Geckler et al, 1977	n.r.
Pimephales promelas	760		7,6		312	14.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	136,5		7,6		44	22								reconstituted water	Curtis et al, 1979	alevins
Pimephales promelas	12,4		7,6		25	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	20,3		7,6		27	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	26,7		7,6		30	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	28		7,6		29	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	99,6		7,6		88,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	905,4		7,6		252,2	22								lab water	Erickson et al, 1996	larvae
Pimephales promelas	230		7,7		80-120	23								Reconstituted water	Johnson et al., 2008	< 24h old
Pimephales promelas	830		7,7		284	24								field water	Geckler et al, 1977	n.r.
Pimephales promelas	42,1		7,7		27	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	132,8		7,7		87;1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	52		7,7		52	25								field water	Nelson et al, 1985	larvae
Pimephales promelas	460		7,8		202	22								field water	Pickering et al, 1977	sub-adult
Pimephales promelas	490		7,8		202	22								field water	Pickering et al, 1977	fingerlings
Pimephales promelas	580		7,8		252	6.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	750		7,8		308	7								field water	Geckler et al, 1977	n.r.

Pimephales promelas	44,7		7,8		44	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	62,5		7,8		41	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	78,9		7,8		37	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	103,8		7,8		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	111,8		7,8		47	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	113,4		7,8		47	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	122,9		7,8		138,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	125,4		7,8		60,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	129,9		7,8		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	131,1		7,8		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	151		7,8		46	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	155,7		7,8		76,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	158,1		7,8		107,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	160,3		7,8		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	166,7		7,8		103,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	167,3		7,8		103,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	167,4		7,8		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	172,4		7,8		151,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	176,9		7,8		46	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	181		7,8		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	183		7,8		44	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	190,6		7,8		45,5	22								field water	Erickson et al, 1996	larvae

Pimephales promelas	191,7		7,8		189,2	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	199,8		7,8		134,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	226,8		7,8		134,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	242,6		7,8		139,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	289,1		7,8		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	430		7,9		198	20.8								field water	Mount, 1968	fingerlings
Pimephales promelas	470		7,9		198	20.8								field water	Mount, 1968	fingerlings
Pimephales promelas	820		7,9		228	17.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	940		7,9		150	21.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	820		7,9		260	4.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	490		7,9		290	15								field water	Geckler et al, 1977	n.r.
Pimephales promelas	17,2		7,9		45,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	21,6		7,9		45,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	62,3		7,9		45,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	70,1		7,9		50	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	101,5		7,9		59,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	114,6		7,9		46	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	117,4		7,9		75,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	125,7		7,9		46	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	126,6		7,9		74,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	167,4		7,9		76,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	172,6		7,9		43	22								field water	Erickson et al, 1996	larvae

Pimephales promelas	172,8		7,9		133,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	175,4		7,9		52	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	996,3		7,9		252,2	22								lab water	Erickson et al, 1996	larvae
Pimephales promelas	470		8		160-180	22								Reconstituted hard water (US EPA)	Dwyer et al., 2005	larvae
Pimephales promelas	980		8		224	18								field water	Brungs et al, 1976	n.r.
Pimephales promelas	750		8		280	3								field water	Brungs et al, 1976	n.r.
Pimephales promelas	730		8		294	23.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	690		8		210	3								field water	Geckler et al, 1977	n.r.
Pimephales promelas	750		8		244	6.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	1060		8		224	18								field water	Geckler et al, 1977	n.r.
Pimephales promelas	950		8		260	12.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	650		8		276	19.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	750		8		280	3								field water	Geckler et al, 1977	n.r.
Pimephales promelas	1000		8		303	24								field water	Geckler et al, 1977	n.r.
Pimephales promelas	540		8		314	24								field water	Geckler et al, 1977	n.r.
Pimephales promelas	920		8		322	8								field water	Geckler et al, 1977	n.r.
Pimephales promelas	26,9		8		23	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	41,1		8		44	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	42,6		8		44,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	46,3		8		44,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	68,6		8		44,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	70,8		8		45	22								field water	Erickson et al, 1996	larvae

Pimephales promelas	77		8		44	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	77,5		8		51	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	81,6		8		51	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	83,9		8		45,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	84,3		8		52	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	94		8		44,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	97,9		8		51	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	110,9		8		53	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	123,3		8		46,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	143		8		45,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	152		8		53	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	168,4		8		44,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	183		8		90,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	262,3		8		90,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	292,3		8		88,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	370,3		8		140,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	405,1		8		140,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	830		8,1		228	17.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	930		8,1		150	21.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	820		8,1		260	12.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	640		8,1		242	8.5								field water	Geckler et al, 1977	n.r.
Pimephales promelas	680		8,1		262	2.5								field water	Geckler et al, 1977	n.r.

Pimephales promelas	52,7		8,1		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	70,5		8,1		47	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	75,8		8,1		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	96,7		8,1		60,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	112		8,1		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	122,9		8,1		243,2	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	127,1		8,1		91,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	137,2		8,1		87,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	183		8,1		120,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	189,1		8,1		87,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	190,8		8,1		180,2	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	193		8,1		45,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	223,7		8,1		93,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	230		8,1		45	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	253,5		8,1		92,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	256,2		8,1		45,5	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	268,4		8,1		138,1	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	271,4		8,1		107,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	283,3		8,1		92,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	289,1		8,1		43	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	496		8,1		194,2	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	521		8,1		235,2	22								lab water	Erickson et al, 1996	larvae

Pimephales promelas	758,1		8,1		200,2	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	38,4		8,1		255,7	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	750		8,2		244	6.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	630		8,2		280	25.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	870		8,2		310	18.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	600		8,2		206	3								field water	Geckler et al, 1977	n.r.
Pimephales promelas	36,9		8,2		47	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	92,9		8,2		43	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	148,7		8,2		90,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	150,3		8,2		91,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	752,9		8,2		440,4	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	297		8,2		164	12								reconstituted water	Richards and Beitinger, 1995	n.r.
Pimephales promelas	311		8,2		164	22								reconstituted water	Richards and Beitinger, 1995	n.r.
Pimephales promelas	450		8,2		164	5								reconstituted water	Richards and Beitinger, 1995	n.r.
Pimephales promelas	513		8,2		164	32								reconstituted water	Richards and Beitinger, 1995	n.r.
Pimephales promelas	690		8,3		120	21.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	860		8,3		298	22.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	840		8,3		308	26								field water	Brungs et al, 1976	n.r.
Pimephales promelas	645		8,3		274	17								field water	Geckler et al, 1977	n.r.
Pimephales promelas	229,9		8,3		47	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	646,8		8,3		218,2	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	653,8		8,3		217,2	22								field/lab water	Erickson et al, 1996	larvae

Pimephales promelas	940,4		8,3		212,2	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	892,1		8,3		251,2	22								lab water	Erickson et al, 1996	larvae
Pimephales promelas	698		8,3		292,3	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	770		8,4		280	21.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	58,5		8,4		48	22								field water	Erickson et al, 1996	larvae
Pimephales promelas	182,4		8,4		87,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	644,9		8,4		144,1	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	953		8,4		226,2	22								field/lab water	Erickson et al, 1996	larvae
Pimephales promelas	600		8,5		212	9.5								field water	Brungs et al, 1976	n.r.
Pimephales promelas	660		8,5		212	9.5								field water	Geckler et al, 1977	n.r.

Table II: Algae acute ecotoxicity database used for classification purposes

	ErC50 Value	ErC50 Normalised value		DOC	Hardness	Temperature	Ca	Mg	Na	K	SO4	Cl	Alk		
Species	**µg/L**	**µg/L**	**pH**	**mg/L**	**mg/L as CaCO3**	**°C**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg/L**	**mg CaCO3/L**	**Type of water**	**Reference**
Algae acute database															
Chlamydomonas reinhardtii	380	143,2	6,02	9,84	250	n.r.	80,2	12,0	106,0	0,4	104,7	358,0	0,55	field water	De Schamphelaere & Janssen, 2006
Chlamydomonas reinhardtii	315	80,4	7,03	9,84	250	n.r.	80,2	12,0	106,0	0,4	104,7	303,8	9,88	field water	De Schamphelaere & Janssen, 2006
Chlamydomonas reinhardtii	146	31,4	8,11	9,84	250	n.r.	80,2	12,0	106,0	0,4	104,7	221,9	92,50	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	602	305,2	5,5	10,27	250	n.r.	80,2	12,4	83,0	0,4	73,4	266,2	0,08	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	440	319,3	6,01	5,03	400	n.r.	128,2	19,6	46,7	0,4	86,4	292,1	0,53	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	333	199,9	6,03	5,17	100	n.r.	32,1	4,9	47,8	0,4	29,2	122,3	0,58	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	773	236,8	6,04	15,49	100	n.r.	32,1	5,3	115,6	0,4	604,2	211,6	0,59	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	987	404,7	6,05	15,24	400	n.r.	128,2	19,9	114,0	0,4	117,2	379,3	0,60	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	254	59,4	7,01	10,03	500	n.r.	160,3	24,5	111,7	0,4	121,0	386,4	9,43	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	296	63,7	7,03	10,81	250	n.r.	80,2	12,4	117,9	0,4	38,6	250,3	9,84	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	364	87,5	7,03	10,81	250	n.r.	80,2	12,4	117,9	0,4	75,0	250,3	9,92	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	60	74,3	7,04	1,5	250	n.r.	80,2	12,2	59,3	0,4	46,9	169,1	10,00	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	208	43,6	7,04	10,23	250	n.r.	80,2	12,4	115,4	0,4	73,3	245,3	10,00	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	446	49,3	7,05	19,9	250	n.r.	80,2	12,7	173,8	0,4	99,9	322,6	10,40	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	238	48,1	7,07	10,26	25	n.r.	8,1	1,5	117,0	0,4	36,9	112,4	10,80	field water	De Schamphelaere & Janssen, 2006

Chlorella vulgaris	111	44,2	7,88	5,31	100	n.r.	32,1	5,0	119,1	0,4	29,6	117,3	58,50	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	380	54,1	7,88	15,66	100	n.r.	32,1	5,3	186,2	0,4	60,9	207,7	58,50	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	99	42,1	7,92	5,04	400	n.r.	128,2	19,5	120,5	0,4	85,5	285,4	64,00	field water	De Schamphelaere & Janssen, 2006
Chlorella vulgaris	506	89,1	7,97	15,82	400	n.r.	128,2	19,9	191,3	0,4	119,1	382,9	69,60	field water	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	756	411,2	5,68	9,84	250	25	80,2	12,2	70,6	0,4	68,4	221,9	0,20	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	205	113,1	5,99	5,64	400	25	128,2	19,4	53,1	0,4	86,1	274,7	0,50	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	368	65,2	6,17	14,9	100	25	32,1	4,9	99,8	0,4	53,0	175,5	10,00	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	122	56,5	6,18	5,07	100	25	32,1	4,9	43,0	0,4	26,9	100,3	10,00	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	230	122,0	6,19	5,23	100	25	32,1	4,9	128,5	0,4	13,6	266,2	1,10	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	199	97,5	6,2	5,31	100	25	32,1	4,9	44,1	0,4	13,4	109,9	1,31	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	811	205,9	6,2	15,6	100	25	32,1	4,9	101,4	0,4	13,5	204,9	1,10	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	824	227,7	6,22	15,8	100	25	32,1	4,9	358,6	0,4	13,9	680,6	1,10	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	174	18,9	6,95	18,2	250	25	80,2	12,2	141,6	0,4	90,6	285,7	8,79	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	100	19,7	7,01	10,2	250	25	80,2	12,2	107,4	0,4	69,5	224,8	9,60	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	30	30,7	7,02	1,95	250	25	80,2	12,2	59,3	0,4	47,6	161,3	9,72	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	105	20,6	7,02	10,4	500	25	160,3	24,3	108,0	0,4	118,2	368,7	9,68	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	102	20,5	7,03	9,98	250	25	80,2	12,2	105,7	0,4	68,8	223,0	10,10	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	99	19,6	7,04	10,1	250	25	80,2	12,2	106,7	0,4	69,4	224,0	10,30	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	190	42,0	7,04	9,89	500	25	160,3	24,3	264,4	0,4	90,6	677,1	10,20	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	35	31,8	7,05	2,21	250	25	80,2	12,2	96,3	0,4	42,4	233,3	10,50	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	35	34,0	7,08	2,06	250	25	80,2	12,2	59,5	0,4	42,3	165,2	11,20	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	156	32,5	7,09	9,99	250	25	80,2	12,2	264,4	0,4	42,7	538,8	11,40	Synthetic-Bihain	De Schamphelaere & Janssen, 2006

Pseudokirchneriella subcapitata	178	33,1	7,09	11,1	250	25	80,2	12,2	109,4	0,4	42,3	247,8	11,40	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	685	94,2	7,11	19,9	250	25	80,2	12,2	158,2	0,4	42,3	328,6	12,00	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	281	63,2	7,12	10,5	500	25	160,3	24,3	106,4	0,4	90,3	386,4	12,30	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	462	57,7	7,17	18,5	250	25	80,2	12,2	455,2	0,4	42,7	868,5	13,80	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	193	37,1	7,19	10,4	25	25	4,8	0,3	127,8	0,4	33,4	92,9	14,40	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	151	20,0	7,78	15,2	400	25	128,2	19,4	177,7	0,4	111,4	348,5	48,00	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	37	37	7,8	2										Reconstitued medium	De Schamphelaere et al, 2005
Pseudokirchneriella subcapitata	51	19,2	7,92	5,46	400	25	128,2	19,4	114,3	0,4	85,6	273,3	63,10	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	93	32,7	7,92	5,99	400	25	128,2	19,4	217,2	0,4	71,3	467,9	63,50	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	268	38,8	8,01	15,1	400	25	128,2	19,4	416,1	0,4	71,5	822,4	76,20	Synthetic-Bihain	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	59	22,2	8,02	5,42	100	25	32,1	4,9	114,0	0,4	27,9	102,8	77,70	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	209	27,9	8,05	15,3	100	25	32,1	4,9	186,2	0,4	54,2	179,0	82,30	Synthetic-Ankeveen	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	346	48,7	8,05	16,1	400	25	128,2	19,4	178,6	0,4	71,2	379,3	81,80	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	92	34,0	8,07	5,75	400	25	128,2	19,4	121,4	0,4	71,1	284,3	84,90	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	161	33,8	8,25	10,3	250	25	80,2	12,2	319,5	0,4	42,3	241,1	120,50	Synthetic-Ossenkolk	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	219	49,8	8,37	10,3	250	25	80,2	12,2	485,1	0,4	42,7	549,5	152,70	Synthetic-Bihain	De Schamphelaere, 2006

Table III: Inverterbrates acute ecotoxicity database used for classification purposes

Species	E(L)C50 Value µg/L	E(L)C50 Normalised value µg/L	pH	DOC mg/L	Hardness mg/L as CaCO3	Temperature °C	Ca mg/L	Mg mg/L	Na mg/L	K mg/L	SO4 mg/L	Cl mg/L	Alk mg CaCO3/L	Type of water
Invertebrates acute database														
Ceriodaphnia dubia	52	52	6	2	113,6	25	34,1	6,9	6,7	1,7	79,2	39,0	121,9	field water
Ceriodaphnia dubia	14	10,1	6	2,9	97,6	25	29,3	5,9	5,7	1,5	68,0	33,5	74,2	field water
Ceriodaphnia dubia	56	42,3	6	3	182	25	54,7	11,1	10,7	2,7	126,8	62,4	144,3	field water
Ceriodaphnia dubia	9,0	7,4	6,1	2,5	375	25	61,9	53,6	16,0	1,6	247,6	121,9	5,0	Natural water
Ceriodaphnia dubia	12,0	9,8	6,1	2,5	140	25	23,1	20,0	16,0	1,6	112,2	55,2	5,0	Natural water
Ceriodaphnia dubia	1,6	16,4	6,5	0,1	44	25	7,0	6,3	14,0	1,1	53,0	4,5	30,0	Reconstituted soft water
Ceriodaphnia dubia	1,6	11,6	6,5	0,1	374	25	61,7	53,5	32,6	8,3	282,8	139,2	30,0	Reconstituted soft water
Ceriodaphnia dubia	73	14,3	6,5	10	44	25	7,0	6,3	14,0	1,1	53,0	4,5	30,0	Reconstituted soft water
Ceriodaphnia dubia	23	18,4	7	2,5	25	25	4,0	3,6	16,0	1,6	45,9	22,6	13,0	Natural water
Ceriodaphnia dubia	30	24,2	7	2,5	374	25	61,7	53,5	32,6	8,3	282,8	139,2	2,22	Natural water
Ceriodaphnia dubia	32	25,7	7	2,5	140	25	23,1	20,0	12,2	3,1	105,9	52,1	2,22	Natural water
Ceriodaphnia dubia	76	76,0	7	2	113,6	25	34,1	6,9	6,7	1,7	79,2	39,0	121,9	field water
Ceriodaphnia dubia	28	19,6	7	2,9	97,6	25	29,3	5,9	5,7	1,5	68,0	33,5	74,2	field water
Ceriodaphnia dubia	84	62,6	7	3	182	25	54,7	11,1	10,7	2,7	126,8	62,4	144,3	field water
Ceriodaphnia dubia	5,38	14,1	7,2	0,7	1213	n.r.	403	50,4	80	4,6	1090,0	70,0	16,0	Natural water
Ceriodaphnia dubia	50	4,9	7,2	15,5	6,3	25	1,9	0,4	0,4	0,1	4,4	2,2	3,53	Natural water
Ceriodaphnia dubia	2,2	27,7	7,5	0,1	44	25	7,0	6,3	14,0	1,1	53,0	4,5	30,0	Reconstituted soft water
Ceriodaphnia dubia	2,8	32,3	7,5	0,1	44	25	7,0	6,3	14,0	1,1	53,0	4,5	30,0	Reconstituted soft water
Ceriodaphnia dubia	5,02	17,9	7,6	0,5	1245	n.r.	316	111	9,1	5,8	1040,0	9,7	20,0	Reconstituted water
Ceriodaphnia dubia	23,6	7,0	7,7	6,5	114	25	34,3	6,9	6,7	1,7	79,4	39,1	3,53	Natural water
Ceriodaphnia dubia	39,0	31,3	7,8	2,5	140	25	23,1	20,0	16,0	1,6	112,2	55,2	19,0	Natural water
Ceriodaphnia dubia	42	33,7	7,8	2,5	25	25	4,0	3,6	16,0	1,6	14,4	7,1	19,0	Natural water

Ceriodaphnia dubia	44,0	35,5	7,8	2,5	375	25	61,9	53,6	16,0	1,6	247,6	121,9	19,0	Natural water
Ceriodaphnia dubia	14	3,1	7,9	8,5	110	25	33,1	6,7	6,4	1,6	76,7	37,7	3,53	Natural water
Ceriodaphnia dubia	42,2	9,5	7,9	8,5	107	25	32,2	6,5	6,3	1,6	74,6	36,7	3,53	Natural water
Ceriodaphnia dubia	77,4	28,0	8	5,8	159	n.r.	44,6	11,6	46,1	6,3	76,9	49,9	108,0	Natural water
Ceriodaphnia dubia	148	55,2	8	6,4	268	n.r.	77,4	18,3	92,1	7,9	115,0	49,9	156,0	Natural water
Ceriodaphnia dubia	279	131,5	8	6,9	438	n.r.	104	43,3	370	16,6	185,0	530,0	180,0	Natural water
Ceriodaphnia dubia	257	100,5	8	7,7	349	n.r.	84,1	33,7	292	18,6	321,0	436,0	184,0	Natural water
Ceriodaphnia dubia	10,4	31,2	8	0,5	158	n.r.	41,1	13,5	60	8,7	142,0	10,2	124,0	Reconstituted water
Ceriodaphnia dubia	91	91,0	8	2	113,6	25	34,1	6,9	6,7	1,7	79,2	39,0	121,9	field water
Ceriodaphnia dubia	31	21,6	8	2,9	97,6	25	29,3	5,9	5,7	1,5	68,0	33,5	74,2	field water
Ceriodaphnia dubia	93	68,5	8	3	182	25	54,7	11,1	10,7	2,7	126,8	62,4	144,3	field water
Ceriodaphnia dubia	6,5	48,0	8,1	0,1	44	25	7,0	6,3	14,0	1,1	53,0	4,5	60,0	Reconstituted soft water
Ceriodaphnia dubia	44,1	36,4	8,1	2,5	187	n.r.	56,1	11,4	45,6	6,6	48,2	46,5	164,0	Natural water
Ceriodaphnia dubia	131	68,8	8,1	4,4	66	n.r.	18,3	4,9	88,8	8,5	50,3	64,7	124,0	Natural water
Ceriodaphnia dubia	79,2	26,4	8,1	6,3	149	n.r.	35,4	14,8	68,7	5,5	19,8	124,0	120,0	Natural water
Ceriodaphnia dubia	17,7	44,5	8,1	0,5	305	n.r.	79,5	25,9	84	6,2	214,0	8,6	160,0	Reconstituted water
Ceriodaphnia dubia	36,3	51,4	8,2	1,2	294	n.r.	66,7	30,9	98	2,9	1,2	172,0	228,0	Natural water
Ceriodaphnia dubia	78,5	49,4	8,2	3,5	223	n.r.	61,8	16,6	60,6	7,9	57,7	58,6	184,0	Natural water
Ceriodaphnia dubia	207	105,0	8,2	5,4	509	n.r.	174	87,4	274	29,4	718,0	325,0	128,0	Natural water
Ceriodaphnia dubia	302	91,5	8,2	9,8	288	n.r.	71,9	26,3	150	14,1	161,0	200,0	204,0	Natural water
Ceriodaphnia dubia	23,1	53,0	8,2	0,5	287	n.r.	72	26,1	98,9	7,1	256,0	6,5	192,0	Reconstituted water
Ceriodaphnia dubia	26,2	58,6	8,2	0,5	220	n.r.	50,8	22,6	104	7,4	233,0	8,3	184,0	Reconstituted water
Ceriodaphnia dubia	147	55,8	8,3	5,8	235	25	26,0	8,5	9,2	1,0	20,0	11,0	93,0	natural well water
Ceriodaphnia dubia	157	60,6	8,3	5,8	238	25	26,0	8,5	9,2	1,0	20,0	11,0	94,0	natural well water
Ceriodaphnia dubia	115,0	23,4	8,3	9,8	198	20	71,1	5,1	23,6	2,2	160,5	40,4	45,1	Natural water
Ceriodaphnia dubia	267	61,3	8,3	10	249	25	26,0	8,6	9,4	1,1	29,0	16,0	96,0	natural well water
Ceriodaphnia dubia	14,5	42,2	8,3	0,5	164	n.r.	43,3	13,7	52,2	10,3	142,0	10,6	112,0	Reconstituted water
Ceriodaphnia dubia	20,3	51,3	8,3	0,5	156	n.r.	37	15,4	76,5	6,8	167,0	7,3	156,0	Reconstituted water
Ceriodaphnia dubia	29,3	63,1	8,3	0,5	260	n.r.	67	22,6	99,9	7,1	184,0	9,4	196,0	Reconstituted water

Ceriodaphnia dubia	37	72,7	8,3	0,5	284	n.r.	72	25,4	105	14,7	296,0	12,5	224,0	Reconstituted water
Ceriodaphnia dubia	39,5	80,3	8,3	0,5	767	n.r.	202	64	64,1	10,7	704,0	6,5	124,0	Reconstituted water
Ceriodaphnia dubia	16	65,5	8,4	0,1	44	25	7,0	6,3	14,0	1,1	53,0	4,5	125,0	Reconstituted soft water
Ceriodaphnia dubia	25	68,2	8,4	0,4	105	25	26,0	8,5	8,7	1,0	18,0	9,1	100,0	natural well water
Ceriodaphnia dubia	58,5	48,8	8,4	2,5	249	n.r.	73,7	15,8	60,6	5,9	50,7	54,5	192,0	Natural water
Ceriodaphnia dubia	10,4	32,6	8,4	0,5	70	n.r.	18,1	6	67,6	4	50,2	3,9	136,0	Reconstituted water
Ceriodaphnia dubia	29,8	65,6	8,4	0,5	349	n.r.	90,4	29,9	81,9	7,4	327,0	8,4	168,0	Reconstituted water
Ceriodaphnia dubia	30	77,8	8,5	0,3	174	25	31,0	21,0	51,0	4,5	188,0	6,2	128,0	Reconstitued water (ASTM hard w
Ceriodaphnia dubia	24,8	55,6	8,5	0,5	445	n.r.	115	38,3	110	5,6	318,0	8,4	220,0	Reconstituted water
Daphnia magna	35,2	6,5	5,52	10,3	8	20	2,4	0,5	7,9	6,2	2,8	2,4	0,080	field water
Daphnia magna	1	2,3	5,96	0,8	19,8	25	3,4	2,9	2,8	0,5	20,1	1,0	5,3	Reconstituted water
Daphnia magna	3,2	2,9	6,02	2,2	9,2	25	1,4	1,3	10,6	0,2	10,9	4,2	6,7	Reconstituted water with natural
Daphnia magna	33,8	29,0	6,1	2,34	12,4	20	3,1	1,1	4,4	0,7	4,6	7,8	0,740	field water
Daphnia magna	4,9	4,5	6,11	2,2	22,4	25	3,7	3,1	10,5	0,5	23,9	4,1	6,7	Reconstituted water with natural
Daphnia magna	292	133,5	6,11	6,9	179,9	20	40,1	19,4	92	3,4	76,8	5,45	0,78	reconstituted water
Daphnia magna	0,5	1,6	6,16	0,6	10,6	25	1,7	1,6	2,9	0,2	10,5	1,0	5,3	Reconstituted water
Daphnia magna	7	6,1	6,17	2,3	39,6	25	6,6	5,7	10,6	0,9	40,8	4,2	6,7	Reconstituted water with natural
Daphnia magna	4	3,1	6,19	2,6	10,6	25	1,6	1,5	11,7	0,2	12,9	4,6	5,3	Reconstituted water with natural
Daphnia magna	8,6	6,6	6,23	2,6	19,8	25	3,4	2,8	11,9	0,4	22,2	4,7	5,3	Reconstituted water with natural
Daphnia magna	7,4	7,4	6,28	2	21	20	6,3	1,3	1,2	0,3	14,6	7,2	0,42	
Daphnia magna	29,1	29,1	6,28	2	394	20	118,4	23,9	23,1	5,9	274,6	135,1	0,42	
Daphnia magna	25,2	5,3	6,28	9	21,1	25	3,5	3,1	34,1	0,5	31,1	13,5	5,3	Reconstituted water with natural
Daphnia magna	16,5	15,8	6,29	2,1	169	20	50,8	10,3	9,9	2,5	117,8	58,0	0,43	
Daphnia magna	8,3	6,4	6,29	2,6	42,2	25	7,1	5,9	11,7	0,9	43,0	4,6	5,3	Reconstituted water with natural
Daphnia magna	421	167,1	6,29	9	280	20	80,2	19,4	92	4,4	76,8	7,31	1,13	reconstituted water
Daphnia magna	25,9	5,6	6,3	8,7	9,2	25	1,5	1,4	33,0	0,2	18,9	13,4	6,7	Reconstituted water with natural
Daphnia magna	37,9	27,9	6,31	2,72	10,1	20	2,2	1,1	4,1	0,5	4,8	7,0	1,450	field water
Daphnia magna	465	204,2	6,31	8,3	220	20	80,2	4,86	114,9	2,5	19,2	8,23	1,66	reconstituted water
Daphnia magna	22,8	5,3	6,33	8,4	44,9	25	7,3	6,5	32,1	1,0	51,9	12,9	5,3	Reconstituted water with natural

Daphnia magna	18,8	4,0	6,33	8,7	10,6	25	1,7	1,6	33,1	0,2	19,9	13,2	6,7	Reconstituted water with natural
Daphnia magna	26,6	5,8	6,33	8,7	22,4	25	3,8	3,3	33,1	0,5	30,7	13,3	5,3	Reconstituted water with natural
Daphnia magna	1,6	5,9	6,34	0,5	42,2	25	6,9	6,1	2,8	0,9	40,4	0,9	5,3	Reconstituted water
Daphnia magna	40,9	16,6	6,35	4,87	18,1	20	5,7	0,9	4,4	1,0	4,0	14,2	1,770	field water
Daphnia magna	106	100,4	6,4	2,2	260	20	80,2	14,6	92	3,8	57,6	8	2,11	reconstituted water
Daphnia magna	47,3	10,6	6,42	8,7	42,2	25	7,0	6,1	33,2	1,0	50,5	13,5	5,3	Reconstituted water with natural
Daphnia magna	92,6	113,3	6,77	1,4	200,0	20	40,1	24,3	23	4,1	96,1	2,28	4,78	reconstituted water
Daphnia magna	100	124,7	6,85	1,3	480,0	20	160,3	19,4	23	6,3	76,8	8,92	7,21	reconstituted water
Daphnia magna	81,8	56,9	6,9	3,04	250,0	20	80,2	12,2	48,7	3,05	48,03	144,6	9,45	Reconstituted water with natural DOC
Daphnia magna	128	62,8	6,92	4,53	250,0	20	80,2	12,2	49,9	3,05	48,03	146,1	8,31	Reconstituted water with natural DOC
Daphnia magna	311	98,9	6,92	8,54	250,0	20	80,2	12,2	232,2	3,05	48,3	478,6	7,64	Reconstituted water with natural DOC
Daphnia magna	53,8	54,9	6,94	1,95	250,0	20	80,2	12,2	88,3	3,05	48,3	220,9	7,99	Reconstituted water with natural DOC
Daphnia magna	136	119,1	6,95	2,5	380,0	20	120,2	19,4	23	5,3	76,8	6,92	8,91	reconstituted water
Daphnia magna	192	86,9	6,97	5,35	250,0	20	80,2	12,2	63,9	3,05	52,8	142,9	7,54	Reconstituted water with natural DOC
Daphnia magna	117	138,8	6,98	1,4	460,0	20	160,3	14,6	69	5,7	57,6	10,9	7,98	reconstituted water
Daphnia magna	86,6	87,5	6,98	1,97	250,0	20	80,2	12,2	52,6	3,05	48,2	142,2	10,60	Reconstituted water with natural DOC
Daphnia magna	607	101,7	6,98	16,9	250,0	20	80,2	12,2	139,3	3,05	48,3	299,2	8,79	Reconstituted water with natural DOC
Daphnia magna	332	74,9	6,99	10,8	250,0	20	80,2	12,2	81,8	3,05	49	143,9	8,42	Reconstituted water with natural DOC
Daphnia magna	638	198,8	6,99	11,7	250,0	20	80,2	12,2	64,8	3,05	49	147,8	9,35	Reconstituted water with natural DOC
Daphnia magna	542	101,3	6,99	15,4	250,0	20	80,2	12,2	374,7	3,05	48,5	748	8,99	Reconstituted water with natural DOC
Daphnia magna	109	102,0	7	2,2	360,0	20	120,2	14,6	46	4,7	57,6	7,92	10,6	reconstituted water
Daphnia magna	261	139,4	7	5,11	250,0	20	80,2	12,2	54,3	3,05	48,5	144,3	8,21	Reconstituted water with natural DOC
Daphnia magna	101,8	22,2	7	9,01	40	20	3,19	7,13	55,90	2,45	17,30	100,10	8,00	Natural water
Daphnia magna	129	130,8	7,01	1,95	250,0	20	80,2	12,2	49,2	3,05	48,2	142,5	9,56	Reconstituted water with natural DOC
Daphnia magna	295	63,0	7,02	10,1	52,7	20	17,0	2,5	11,3	4,2	18,5	21,5	9,6	field water
Daphnia magna	275	71,0	7,03	9,22	250,0	20	80,2	12,2	97	3,05	48,03	229	9,88	Reconstituted water with natural DOC
Daphnia magna	60,6	48,1	7,06	2,58	250,0	20	80,2	12,2	60,9	3,05	54,9	164,5	10,60	Reconstituted water with natural DOC
Daphnia magna	212	31,7	7,07	13,7	250,0	20	80,2	12,2	125,1	3,05	84,4	249,6	10,80	Reconstituted water with natural DOC
Daphnia magna	50,6	48,8	7,08	2,08	250,0	20	80,2	12,2	57,5	3,05	48,03	163,8	11,10	Reconstituted water with natural DOC

Daphnia magna	9,2	16,6	7,1	1,1	81	20	21	7	17	5	32,0	31,0	12,70	Filtered natural water
Daphnia magna	372	44,7	7,1	17,8	250,0	20	80,2	12,2	148,7	3,05	95,3	281,1	11,60	Reconstituted water with natural DOC
Daphnia magna	34,4	8,7	7,13	7,28	8	20	1,34	0,91	3,84	0,60	7,50	5,70	10,00	Natural water
Daphnia magna	21	9,9	7,21	4,1	9	20	1,39	1,08	0,66	0,61	8,00	7,60	10,00	Natural water
Daphnia magna	200	144,5	7,29	3,4	440,0	20	160,3	9,7	46	5,0	38,4	9,73	17,2	reconstituted water
Daphnia magna	686	104,4	7,3	17,8	165,4	20	52,1	8,6	11,8	0,8	109,5	20,2	15,2	field water
Daphnia magna	6,183	23,1	7,3	0,5	42	20	6,2	5,5	12,5	1,7	41,0	1,0	32,0	Reconstituted water (US EPA
Daphnia magna	229	156,6	7,35	3,7	240,0	20	80,2	9,7	23	3,1	38,4	4,73	18,5	reconstituted water
Daphnia magna	792	86,8	7,35	22,8	139,7	20	47,7	7,4	14,9	3,6	93,9	24,7	17,3	field water
Daphnia magna	4,9	12,2	7,41	0,81	9	20	2,07	0,56	2,07	0,28	8,50	1,20	12,00	Natural water
Daphnia magna	1213	418,0	7,41	14,8	140,0	20	40,1	9,7	92	2,2	38,4	5,73	25,0	reconstituted water
Daphnia magna	300	191,0	7,43	4,6	280,0	20	80,2	19,4	114,9	4,4	76,8	8,73	26,0	reconstituted water
Daphnia magna	118,5	27,0	7,43	8,47	14	20	2,30	1,43	6,38	0,81	8,40	8,00	15,00	Natural water
Daphnia magna	77,3	15,3	7,45	9,4	12	20	2,04	1,36	4,48	0,60	8,10	4,30	14,00	Natural water
Daphnia magna	3,7	21,3	7,5	0,34	12	20	2,74	0,80	2,11	0,54	6,80	0,30	14,00	Natural water
Daphnia magna	648	76,3	7,5	20,4	133,4	20	44,0	6,1	26,7	0,9	86,3	6,8	27,4	field water
Daphnia magna	50	30,5	7,55	3,27	18	20	2,14	1,39	4,20	0,70	1,40	5,30	16,00	Natural water
Daphnia magna	175	63,1	7,55	6,13	131,7	20	42,5	6,2	26,7	3,5	48,0	33,0	30,3	field water
Daphnia magna	5,5	20,6	7,57	0,53	20	20	2,42	0,63	2,23	0,35	8,30	1,00	20,00	Natural water
Daphnia magna	100	116,2	7,59	1,5	360,0	20	120,2	14,6	69	4,7	57,6	8,23	36,2	reconstituted water
Daphnia magna	281	87,1	7,59	7,81	69,7	20	23,0	3,0	15,1	4,4	24,7	21,2	32,9	field water
Daphnia magna	14	14,7	7,6	1,9	75	20	20	6	17	15	32,0	31,0	9,48	Filtered natural water
Daphnia magna	61,82	10,2	7,6	11,4	50	20	10,5	5,1	6,4	3,1	23,3	2,0	40,0	natural water
Daphnia magna	529	66,5	7,6	18,4	125,1	20	40,1	6,1	19,1	21,1	41,0	36,9	37,7	field water
Daphnia magna	47	34,2	7,62	2,75	12	20	2,50	1,33	4,00	0,65	1,90	4,90	16,00	Natural water
Daphnia magna	39	34,7	7,64	2,25	12	20	2,86	1,28	0,90	0,60	2,30	5,90	16,00	Natural water
Daphnia magna	7,3	23,9	7,65	0,6	18	20	3,79	1,32	2,47	0,80	7,10	0,30	23,00	Natural water
Daphnia magna	19	23,9	7,65	1,59	13	20	3,23	1,22	3,70	0,56	2,80	5,60	16,00	Natural water
Daphnia magna	276	202,6	7,65	3,6	120,0	20	40,1	4,86	23	1,6	19,2	2,23	34,6	reconstituted water

Daphnia magna	6,596	23,7	7,65	0,5	72	20	10,9	9,6	21,7	1,6	65,0	1,0	52,0	Reconstituted water (US EPA
Daphnia magna	152	162,7	7,67	1,7	480,0	20	160,3	19,4	46	6,3	76,8	9,23	36,5	reconstituted water
Daphnia magna	11,4	23,7	7,7	0,96	14	20	3,59	1,17	3,60	0,51	3,20	5,30	16,00	Natural water
Daphnia magna	35,23	22,8	7,7	3,1	80	20	15,9	7,0	7,4	2,8	64,4	3,0	34,0	natural water
Daphnia magna	484	151,9	7,7	10	92	20	32,0	2,9	15,4	5,8	23,9	21,0	38,2	field water
Daphnia magna	526	261,7	7,71	7,8	260,0	20	80,2	14,6	46	3,8	57,6	5,23	45,3	reconstituted water
Daphnia magna	3,976	15,4	7,71	0,5	42	20	6,5	5,8	13,0	1,1	39,0	1,0	29,0	Reconstituted water (US EPA
Daphnia magna	366	138,8	7,72	7,88	108,1	20	39,0	2,6	13,0	4,4	23,2	25,2	46,0	field water
Daphnia magna	388	279,3	7,74	4	60,0	20	8,02	9,7	23	1,4	38,4	0,63	40,1	reconstituted water
Daphnia magna	3,8	20,1	7,78	0,37	14	20	3,95	1,11	3,40	0,46	3,70	4,90	16,00	Natural water
Daphnia magna	30,0	83,4	7,8	0,38	250	20	80,1	12,2	17,2	2,9	180,1	144,4	14,1	Reconstituted water
Daphnia magna	40,6	98,6	7,8	0,38	250	20	80,1	12,2	17,2	2,9	180,1	144,4	14,1	Reconstituted water
Daphnia magna	53,2	115,3	7,8	0,38	250	20	80,1	12,2	17,2	2,9	180,1	144,4	14,1	Reconstituted water
Daphnia magna	7,8	21,5	7,8	0,72	19	20	4,30	1,26	2,68	0,67	7,10	0,50	23,00	Natural water
Daphnia magna	7,7	17,0	7,8	0,9	79	20	20	7	17	15	32,0	31,0	9,69	Filtered natural water
Daphnia magna	6,4	40,9	7,81	0,25	28	20	6,65	2,05	5,55	0,94	11,50	2,00	27,00	Natural water
Daphnia magna	289	180,8	7,81	4,8	260,0	20	80,2	14,6	46	3,8	57,6	5,23	55,3	reconstituted water
Daphnia magna	6,2	30,4	7,83	0,38	23	20	6,07	1,39	4,27	0,68	11,20	0,60	24,00	Natural water
Daphnia magna	8,4	35,3	7,83	0,44	20	20	4,17	1,60	3,21	0,85	9,40	1,20	27,00	Natural water
Daphnia magna	7,4	23,9	7,83	0,6	22	20	4,78	2,41	5,60	1,00	10,10	2,20	31,00	Natural water
Daphnia magna	8,9	24,1	7,83	0,73	18	20	3,41	1,40	3,09	0,61	8,30	0,80	21,00	Natural water
Daphnia magna	380	266,1	7,83	4,2	40,0	20	8,02	4,86	23	0,8	19,2	0,63	58,7	reconstituted water
Daphnia magna	30,5	52,4	7,84	1,1	42,7	20	7,40	5,90	13,60	0,10	38,60	0,93	28,00	Reconstituted water
Daphnia magna	30,8	28,0	7,84	2,2	9,2	25	1,5	1,2	34,5	0,2	11,0	4,1	58,7	Reconstituted water with natural
Daphnia magna	56,1	53,6	7,85	2,1	42,7	20	7,40	5,90	13,60	1,10	38,60	0,93	28,02	Reconstituted water
Daphnia magna	332	66,3	7,85	11,8	112,7	20	34,9	6,2	66,2	5,4	34,3	56,7	59,3	field water
Daphnia magna	9,46	33,0	7,85	0,5	72	20	10,9	9,6	21,7	1,6	65,0	1,0	47,0	Reconstituted water (US EPA
Daphnia magna	7,2	30,5	7,86	0,44	22	20	4,92	2,35	9,55	0,95	9,70	9,20	29,00	Natural water
Daphnia magna	7,4	30,8	7,86	0,45	22	20	5,11	1,82	4,61	0,78	10,10	1,50	29,00	Natural water

Daphnia magna	38,2	29,6	7,86	2,6	19,8	25	3,4	2,9	35,8	0,5	2,4	4,5	57,3	Reconstituted water with natural
Daphnia magna	45,1	35,1	7,87	2,6	43,6	25	7,2	6,3	35,8	1,0	44,9	4,6	60,0	Reconstituted water with natural
Daphnia magna	72	47,2	7,87	3,1	42,7	20	7,40	5,90	13,60	1,10	38,60	0,93	28,06	Reconstituted water
Daphnia magna	100	38,9	7,87	5,2	42,7	20	7,40	5,90	13,60	1,10	38,60	4,60	28,06	Reconstituted water
Daphnia magna	108,8	24,1	7,87	8,9	10,6	25	1,7	1,6	57,9	0,2	20,5	13,4	61,3	Reconstituted water with natural
Daphnia magna	399	65,6	7,87	14,3	116,6	20	40,9	3,5	9,7	2,0	5,9	17,6	63,3	field water
Daphnia magna	6,03	22,8	7,87	0,5	46	20	7,0	6,2	14,1	1,0	53,0	1,0	32,0	Reconstituted water (US EPA
Daphnia magna	87,39	22,4	7,88	7,8	84	20	20,0	7,5	17,6	2,5	48,0	7,0	60,0	natural water
Daphnia magna	155,7	24,2	7,88	12,5	54	20	12,3	5,7	7,2	3,7	23,0	4,0	42,0	natural water
Daphnia magna	8,4	59,5	7,9	0,1	42,7	20	7,40	5,90	13,60	1,10	38,60	0,93	28,10	Reconstituted water
Daphnia magna	5,7	33,2	7,9	0,3	26	20	5,45	2,22	4,46	1,11	9,50	0,90	31,00	Natural water
Daphnia magna	5,2	29,2	7,91	0,3	9,2	25	1,5	1,4	27,0	0,2	9,2	1,0	61,3	Reconstituted water
Daphnia magna	88,8	43,0	7,91	4,2	42,7	20	7,40	5,90	13,60	1,10	38,60	4,30	28,12	Reconstituted water
Daphnia magna	15,3	13,3	7,92	2,3	9,2	25	1,6	1,2	35,1	0,2	11,2	4,4	58,7	Reconstituted water with natural
Daphnia magna	276	116,2	7,93	6,6	135,7	20	48,1	3,8	62,3	2,0	33,6	46,8	59,7	field water
Daphnia magna	12,1	52,4	7,94	0,31	132	20	22,68	3,54	6,14	0,83	45,00	0,90	37,00	Natural water
Daphnia magna	187,6	47,5	7,94	8,3	21,1	25	3,6	3,2	55,8	0,5	30,2	12,9	60,0	Reconstituted water with natural
Daphnia magna	9,3	28,2	7,95	0,6	42,2	25	6,9	6,1	26,9	0,9	40,5	1,1	61,3	Reconstituted water
Daphnia magna	16,7	13,9	7,95	2,4	21,1	25	3,3	3,1	35,0	0,5	22,0	4,3	60,0	Reconstituted water with natural
Daphnia magna	106,4	24,7	7,95	8,5	10,6	25	1,4	1,4	56,4	0,2	18,7	2,8	66,7	Reconstituted water with natural
Daphnia magna	130,5	31,3	7,96	8,4	21,1	25	3,6	2,9	56,2	0,5	29,4	12,9	62,7	Reconstituted water with natural
Daphnia magna	2,6	15,6	7,97	0,3	18,5	25	3,1	2,6	26,9	0,4	18,2	1,0	61,3	Reconstituted water
Daphnia magna	21,1	20,1	7,98	2,1	40,9	25	6,7	5,7	35,4	0,9	41,2	4,1	60,0	Reconstituted water with natural
Daphnia magna	122,5	28,7	7,98	8,6	44,9	25	7,5	6,4	56,6	1,0	52,0	13,1	62,7	Reconstituted water with natural
Daphnia magna	159	37,1	7,98	8,8	40,9	25	6,7	6,0	57,1	0,9	49,6	13,3	61,3	Reconstituted water with natural
Daphnia magna	208,0	41,0	8	10,4	198	20	55,1	14,5	67,6	6,4	246,1	124,0	22,4	Natural water
Daphnia magna	68,45	23,4	8,02	5,8	42	20	11,6	3,7	12,1	3,3	37,0	3,0	28,0	natural water
Daphnia magna	19,28	53,8	8,02	0,5	100	20	15,6	13,2	31,0	0,1	98,0	1,0	72,0	Reconstituted water (US EPA
Daphnia magna	8,3	40,8	8,03	0,31	61	20	18,22	3,40	7,02	0,56	30,30	5,30	42,00	Natural water

Daphnia magna	68,31	12,5	8,03	10,5	60	20	14,9	4,8	17,1	2,6	31,8	2,0	64,0	natural water
Daphnia magna	119	119,7	8,06	1,98	190,2	20	60,5	9,5	25,1	3,2	38,4	41,5	83,8	field water
Daphnia magna	96,23	25,0	8,06	7,8	106	20	26,3	10,3	8,8	5,2	64,0	4,0	66,0	natural water
Daphnia magna	304	49,5	8,1	14,7	229	20	77	9	36	11	59,0	59,0	153,20	Filtered natural water
Daphnia magna	27,8	45,1	8,11	1,18	38	20	6,40	4,96	5,55	1,41	8,30	1,50	51,00	Natural water
Daphnia magna	156,1	76,9	8,11	4,65	85,2	20	14,20	12,10	27,30	2,10	80,70	3,19	58,22	Reconstituted water
Daphnia magna	9,2	57,9	8,12	0,14	32	20	7,54	2,64	5,13	1,07	9,00	1,50	41,00	Natural water
Daphnia magna	104,5	61,7	8,12	3,63	85,2	20	14,20	12,10	27,30	2,10	80,70	3,19	58,24	Reconstituted water
Daphnia magna	19,24	54,3	8,13	0,5	96	20	14,9	13,0	30,1	2,2	95,0	2,0	70,0	Reconstituted water (US EPA
Daphnia magna	12,1	46,5	8,15	0,38	88	20	27,79	3,35	4,30	0,65	42,70	5,50	54,00	Natural water
Daphnia magna	32,3	67,8	8,16	0,71	270	20	35,08	7,15	13,83	3,02	82,90	6,70	59,00	Natural water
Daphnia magna	24,3	54,4	8,17	0,76	68	20	19,43	2,83	6,87	0,79	24,70	3,50	54,00	Natural water
Daphnia magna	141,6	28,4	8,19	10	54	20	13,9	4,2	17,8	3,0	9,0	3,0	76,0	natural water
Daphnia magna	116,3	21,6	8,19	10,7	90	20	23,7	7,7	18,7	3,0	48,0	7,0	74,0	natural water
Daphnia magna	25,3	84,8	8,2	0,1	85,2	20	14,20	12,10	27,30	2,10	80,70	3,19	58,33	Reconstituted water
Daphnia magna	60,3	98,1	8,2	0,87	85,2	20	14,20	12,10	27,30	2,10	80,70	3,19	58,33	Reconstituted water
Daphnia magna	69	42,4	8,2	3,5	230	20	74	11	37	4	51,0	82,0	139,10	Filtered natural water
Daphnia magna	71	37,9	8,2	4	189	20	64	7	14	4	38,0	29,0	129,10	Filtered natural water
Daphnia magna	87	37,9	8,2	5	218	20	71	10	37	4	51,0	82,0	134,10	Filtered natural water
Daphnia magna	93	37,8	8,2	5,3	174	20	60	6	14	4	38,0	29,0	119,20	Filtered natural water
Daphnia magna	314	58,8	8,2	17,3	591	20	174	38	149	16	185,0	194,0	471,90	Filtered natural water
Daphnia magna	10,14	34,6	8,2	0,5	80	20	13,1	11,5	26,3	1,3	76,0	1,0	57,0	Reconstituted water (US EPA
Daphnia magna	78,4	85,3	8,22	1,78	85,2	20	14,20	12,10	27,30	2,10	80,70	3,19	58,34	Reconstituted water
Daphnia magna	109,7	87,6	8,23	2,69	85,2	20	14,20	12,10	27,30	2,10	80,70	3,19	58,35	Reconstituted water
Daphnia magna	32	64,7	8,24	0,79	72	20	21,95	2,39	5,52	0,75	17,90	2,30	66,00	Natural water
Daphnia magna	135,5	22,2	8,24	12,3	102	20	25,6	13,3	7,8	5,2	22,5	6,0	106,0	natural water
Daphnia magna	188	72,6	8,26	6,42	219,3	20	60,9	16,3	82,5	10,4	106,6	132,9	124,0	field water
Daphnia magna	81,06	14,5	8,27	11	104	20	25,2	8,2	20,2	2,6	40,7	8,0	96,0	natural water
Daphnia magna	826	305,9	8,27	14,2	160,0	20	40,1	14,6	114,9	2,8	57,6	3,15	135,6	reconstituted water

Daphnia magna	37,78	30,6	8,29	2,5	88	20	19,1	9,3	0,4	3,5	17,0	2,0	90,0	natural water
Daphnia magna	104	53,9	8,3	4,3	203	20	63	11	37	4	51,0	82,0	124,80	Filtered natural water
Daphnia magna	170,0	43,5	8,3	8,2	236	20	66,5	17,1	76,8	9,5	290,7	127,0	45,2	Natural water
Daphnia magna	257	83,7	8,3	8,24	236,4	20	67,7	17,1	76,8	9,5	107,6	126,9	134,2	field water
Daphnia magna	178,0	37,3	8,3	9,8	198	20	71,1	5,1	23,6	2,2	160,5	40,4	45,2	Natural water
Daphnia magna	308	49,8	8,3	18	481	20	150	26	149	16	185,0	194,0	394,30	Filtered natural water
Daphnia magna	15,58	47,8	8,31	0,5	98	20	14,9	13,0	30,1	2,2	95,0	2,0	66,0	Reconstituted water (US EPA
Daphnia magna	60	123,5	8,36	0,1	166,9	20	28,30	23,40	52,80	4,40	140,90	6,00	111,06	Reconstituted water
Daphnia magna	48,2	74,6	8,36	1,07	120	20	37,23	5,31	10,10	1,34	45,70	4,40	86,00	Natural water
Daphnia magna	28,3	89,7	8,39	0,07	88	20	18,10	8,60	10,55	2,16	18,80	1,90	76,00	Natural water
Daphnia magna	210	149,5	8,39	3,8	200,0	20	40,1	24,3	114,9	4,1	96,1	3,15	159,9	reconstituted water
Daphnia magna	239,8	167,7	8,39	3,94	166,9	20	28,30	23,40	52,80	4,40	140,90	0,93	111,05	Reconstituted water
Daphnia magna	244	161,5	8,39	4,4	300,0	20	80,2	24,3	114,9	5,0	96,1	5,15	140,3	reconstituted water
Daphnia magna	109	60,1	8,4	4,1	195	20	65	8	14	4	38,0	29,0	140,40	Filtered natural water
Daphnia magna	207	33,3	8,4	14,2	294	20	75	26	147	12	94,0	310,0	195,50	Filtered natural water
Daphnia magna	59,9	91,6	8,43	0,98	112	20	32,65	6,89	9,31	1,46	36,20	2,80	97,00	Natural water
Daphnia magna	31,65	19,7	8,44	3,2	48	20	9,9	4,8	5,2	2,3	25,0	1,0	32,0	natural water
Daphnia magna	100	141,0	8,45	0,84	166,9	20	28,30	23,40	52,80	4,40	140,90	6,00	110,99	Reconstituted water
Daphnia magna	66,2	75,4	8,45	1,67	128	20	34,91	7,55	11,66	0,91	30,80	4,00	107,00	Natural water
Daphnia magna	235,9	187,6	8,45	3,25	166,9	20	28,30	23,40	52,80	4,40	140,90	0,93	110,99	Reconstituted water
Daphnia magna	125,2	140,4	8,46	1,57	166,9	20	28,30	23,40	52,80	4,40	140,90	6,00	110,97	Reconstituted water
Daphnia magna	206	193,1	8,46	2,33	166,9	20	28,30	23,40	52,80	4,40	140,90	6,00	110,97	Reconstituted water
Daphnia magna	157	141,3	8,46	2,5	80,0	20	8,02	14,6	92	2,1	57,6	0,55	209,2	reconstituted water
Daphnia magna	798	398,4	8,46	10,9	120,0	20	40,1	4,86	114,9	1,6	19,2	3,15	183,5	reconstituted water
Daphnia magna	172,8	22,8	8,48	15,7	154	20	34,9	18,6	10,5	6,9	16,0	8,0	170,0	natural water
Daphnia magna	43,1	71,1	8,5	0,91	271	20	66,24	15,72	52,47	1,82	141,20	27,30	140,00	Natural water
Daphnia magna	99	70,3	8,5	3,1	234	20	74	12	37	4	51,0	82,0	125,90	Filtered natural water
Daphnia magna	124	56,3	8,5	5	193	20	64	8	14	4	38,0	29,0	141,10	Filtered natural water
Daphnia magna	151	24,7	8,5	13,3	298	20	78	25	147	12	94,0	310,0	181,40	Filtered natural water

Daphnia magna	354	58,1	8,5	15,1	186	20	63	7	36	11	59,0	59,0	181,40	Filtered natural water

Table IV: Fish, algae, invertebrates chronic ecotoxicity database used for classification purposes

Organism	Age/size of organisms	Exposure time	Endpoint	NOEC/EC10 (µg/L)	Normalised (µg/L)	pH	DOC mg/L	Ca mg/L	Mg mg/L	Na mg/L	K mg/L	SO4 mg/L	Cl mg/L	Alk mg/L as CaCO3	Medium	Reference
Fish chronic database																
Pimephales promelas	larvae	7 d	Mortality	10,1	17,1	6	1,10	9,99	4,40	15,13	0,38	0,53	46,0	12,0	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Mortality	11,9	14,6	6	1,59	10,50	4,87	15,60	0,43	0,62	54,0	48,5	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Mortality	13,8	23,5	6,5	1,14	10,29	4,54	16,32	0,42	0,58	47,5	15,0	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Growth - dw	9	15,3	6	1,10	9,99	4,40	15,13	0,38	0,53	46,0	12,0	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Growth - dw	11,9	14,6	6	1,59	10,50	4,87	15,60	0,43	0,62	54,0	48,5	Laboratory water	OSU, 2016
Pimephales promelas	larvae	7 d	Growth - dw	13,8	23,5	6,5	1,14	10,29	4,54	16,32	0,42	0,58	47,5	15,0	Laboratory water	OSU, 2016
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	Growth - biomass	94,7	21,9	5,52	10,3	7,6	3,6	25,6	9,3	9,5	28,6	0,6	Lake	Heijerick et al., 2005
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	Growth - biomass	61,8	54,3	6,1	2,34	8,3	4,3	22,1	3,8	11,3	34,1	2,1	Lake	Heijerick et al., 2005
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	Growth - biomass	52,9	39,9	6,31	2,72	7,4	4,3	21,8	3,6	11,5	33,2	3,2	Lake	Heijerick et al., 2005
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	161,6	50,6	5,68	9,84	80,2	12,2	70,6	0,4	68,4	221,9	0,3	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	49,4	20,5	5,99	5,64	128,2	19,4	53,1	0,4	86,1	274,7	0,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	148,4	24,5	5,99	15,3	128,2	19,4	108,0	0,4	112,4	349,2	0,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	155,9	22,8	6,17	14,9	32,1	4,9	99,8	0,4	53,0	175,5	1,0	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006

Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	52,3	21,9	6,18	5,07	32,1	4,9	43,0	0,4	26,9	100,3	1,0	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	110,3	49,1	6,19	5,23	32,1	4,9	128,5	0,4	13,6	266,2	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	97,7	41,2	6,2	5,31	32,1	4,9	44,1	0,4	13,4	109,9	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	336,9	53,7	6,2	15,6	32,1	4,9	101,4	0,4	13,5	204,9	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	337,0	56,1	6,22	15,8	32,1	4,9	358,6	0,4	13,9	680,6	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Oncorhynchus mykiss	juveniles	51 d	Mortality	28,5	43,75	6	1,15	7,9	3,69	11,99	0,336	0,52	37,1	40	Laboratory water	OSU, 2016
Oncorhynchus mykiss	juveniles	51 d	Growth - wet biomass	28,2	43,33	6	1,15	7,9	3,69	11,99	0,336	0,52	37,1	40	Laboratory water	OSU, 2016
Oncorhynchus mykiss	parr	61 d	Growth	45	31,2	7,1	2,9	7,4	1,4	3,2	0,1	5,1	0,1	21,3	River (Chehalis River)	Mudge et al., 1993
Oncorhynchus mykiss	fry (0.12 g; 2.6 cm)	60 d	Growth - length	8,1	52,7	7,5	0,2	5,2	2,4	0,9	4,4	3,5	0,8	27,7	Well + deionised water	Marr et al., 1996
Oncorhynchus mykiss	fry (0.12 g; 2.6 cm)	60 d	Growth - weight	3,3	28,5	7,5	0,2	5,2	2,4	0,9	4,4	3,5	0,8	27,7	Well + deionised water	Marr et al., 1996
Oncorhynchus mykiss	parr	61 d	Mortality	28	19,3	7	8,7	1,6	1,3	0,1	0,1	0,1	24,2	21,3	River (Chehalis River)	Mudge et al., 1993
Oncorhynchus mykiss	parr	61 d	Mortality	24	16,5	7,1	2,9	7,4	1,4	3,2	0,1	5,1	0,1	21,3	River (Chehalis River)	Mudge et al., 1993
Oncorhynchus mykiss	juveniles	51 d	Growth - biomass	38,8	57,67	7	1,26	7,4	3,44	11,12	0,314	0,45	16,8	40	Laboratory water	OSU, 2016
Pimephales promelas	fry (10 - 20 mm)	327 d	Growth - length male	14,9	45,2	6,9	0,55	9,5	1,8	0,1	0,1	0,1	0,1	30,0	Spring+ deionised tap	Mount & Stephan, 1969
Pimephales promelas	fry (10 - 20 mm)	327 d	Growth - length female	16,7	49,3	6,9	0,55	9,5	1,8	0,1	0,1	0,1	0,1	30,0	Spring+ deionised tap	Mount & Stephan, 1969

Pimephales promelas	embryo-larval	32 d	Growth	3,8	7,5	7,05	1	12,8	2,9	1,1	0,5	3,4	1,2	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
Pimephales promelas	larvae	7 d	Growth - dw	22	31,5	7	1,37	10,2	4,6	14,8	0,39	0,58	33,1	32,5	Laboratory water	OSU, 2016
Pimephales promelas	fry (10 - 20 mm)	327 d	Mortality	13,1	40,9	6,9	0,55	9,5	1,8	0,1	0,1	0,1	0,1	30,0	Spring+ deionised tap	Mount & Stephan, 1969
Pimephales promelas	embryo-larval	32 d	Mortality	5,9	11,6	7,05	1	12,8	2,9	1,1	0,5	3,4	1,2	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
Pimephales promelas	larvae	7 d	Mortality	22,5	32,2	7	1,37	10,2	4,6	14,8	0,39	0,58	33,1	32,5	Laboratory water	OSU, 2016
Pimephales promelas	embryo-larval	32 d	Reproduction (hatching)	16	30,6	7,05	1	12,8	2,9	1,1	0,5	3,4	1,2	42,4	Lake (Lake Superior)	Spehar & Fiandt, 1985
Pimephales promelas	fry (10 - 20 mm)	327 d	Reproduction	10,8	34,9	6,9	0,55	9,5	1,8	0,1	0,1	0,1	0,1	30,0	Spring+ deionised tap	Mount & Stephan, 1969
Salvelinus fontinalis	fry	60 d	Growth	11,2	17,11	6,85	1,3	11,4	2,1	8,8	1,6	12,6	12,1	27,8	Well	Sauter et al., 1976
Salvelinus fontinalis	fry	30 d	Growth	44,4	58,91	6,9	1,3	56,9	10,7	20,2	3,6	39,2	31,9	177,6	Well	Sauter et al., 1976
Salvelinus fontinalis	Alevins/juveniles	189 d	Growth	9,5	18,7	7,45	1	13,7	2,6	9,7	1,8	14,3	13,5	41,6	Tap	McKim & Benoit, 1971
Salvelinus fontinalis	yearling	244 d	Growth	17,4	33,5	7,45	1	13,7	2,6	9,7	1,8	14,3	13,5	41,6	Tap	McKim & Benoit, 1971
Salvelinus fontinalis	fry	60 d	Mortality	12,4	18,91	6,85	1,3	11,4	2,1	8,8	1,6	12,6	12,1	27,8	Well	Sauter et al., 1976
Salvelinus fontinalis	fry	30 d	Mortality	41,3	55,25	6,9	1,3	56,9	10,7	20,2	3,6	39,2	31,9	177,6	Well	Sauter et al., 1976
Salvelinus fontinalis	yearling	244 d	Mortality	17,4	33,5	7,45	1	13,7	2,6	9,7	1,8	14,3	13,5	41,6	Tap	McKim & Benoit, 1971
Salvelinus fontinalis	Alevins/juveniles	189 d	Mortality	9,5	18,7	7,5	1	13,2	3,0	1,1	0,5	3,4	1,2	41,6	Tap	McKim & Benoit, 1971
Salvelinus fontinalis	fry	60 d	Reproduction	6,4	10,7	6,85	1,3	11,4	2,1	8,8	1,6	12,6	12,1	27,8	Well	Sauter et al., 1976
Salvelinus fontinalis	fry	30 d	Reproduction	36,4	49,38	6,9	1,3	56,9	10,7	20,2	3,6	39,2	31,9	177,6	Well	Sauter et al., 1976
Salvelinus fontinalis	yearling	244 d	Reproduction	17,4	33,5	7,45	1	13,7	2,6	9,7	1,8	14,3	13,5	41,6	Tap	McKim & Benoit, 1971
Oncorhynchus mykiss	juveniles	51 d	Mortality	54,2		7	1,26	7,4	3,44	11,12	0,314	0,45	16,8	40	Laboratory water	OSU, 2016
Oncorhynchus mykiss	embryo	30 d	Mortality	11,4	22,41	7,6	1	13,2	3,0	1,1	0,5	3,4	1,2	42,4	Lake (Lake Superior)	McKim et al., 1978
Oncorhynchus mykiss	eggs	63 d	Mortality	53,3	70,92	7,65	1,3	35,5	7,3	15,8	2,8	28,0	24,0	126,0	Well	Seim et al., 1984
Oncorhynchus mykiss	larvae (26 days post hatch)	21 d	Mortality	37	83,2	7,87	0,4	26,0	9,5	8,8	1,0	18,0	11,0	91,1	well water + deionized water	Ingersoll & Mebane, 2014

Oncorhynchus mykiss	larvae (1 day post hatch)	21 d	Mortality	41	88,4	7,92	0,4	26,0	9,5	8,9	1,0	18,0	11,0	91,1	well water + deionized water	Ingersoll & Mebane, 2014
Oncorhynchus mykiss	larvae (1 day post hatch)	52 d	Mortality	34	78,9	7,92	0,4	26,0	9,5	8,9	1,0	18,0	11,0	91,1	well water + deionized water	Ingersoll & Mebane, 2014
Oncorhynchus mykiss	larvae (26 days post hatch)	28 d	Mortality	34	78,9	7,95	0,4	26,0	9,5	8,9	1,0	18,0	11,0	91,1	well water + deionized water	Ingersoll & Mebane, 2014
Oncorhynchus mykiss	eyed embryo	30 d	Mortality	17	45,6	8,3	0,5	31,0	21,0	51,0	4,5	188,0	6,2	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Oncorhynchus mykiss	swim-up fry	30 d	Mortality	22	54,8	8,3	0,5	31,0	21,0	51,0	4,5	188,0	6,2	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	fry (10 - 15 mm)	330 d	Mortality	33	66,5	8	0,55	54,0	15,0	15,0	2,0	35,0	12,0	161,0	Spring+ deionised tap	Mount, 1968
Pimephales promelas	larvae	28 d	Mortality	61	78,2	8,17	1,3	56,0	15,0	76,0	1,5	59,0	71,0	211,9	Ground water	Scudder et al., 1988
Pimephales promelas	newly hatched larvae	30 d	Mortality	19	51,3	8,3	0,5	31,0	21,0	51,0	4,5	188,0	6,2	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	newly hatched larvae	30 d	Mortality	19	51	8,3	0,5	31,0	21,0	51,0	4,5	188,0	6,2	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Pimephales promelas	newly hatched larvae	30 d	Mortality	24	59,8	8,3	0,5	31,0	21,0	51,0	4,5	188,0	6,2	120,0	Reconstituted moderately hard water (US EPA)	Besser et al., 2005
Salvelinus fontinalis	embryo	30-60 d	mortality	22,3	42,3	7,6	1	13,2	3,0	1,1	0,5	3,4	1,2	42,4	Lake (Lake Superior)	McKim et al., 1978
Algae chronic database																
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	3 d	growth	178	61,7	6,02	9,84	80,1	12,0	106,2	0,4	104,7	358,1	0,5	Reconstituted	De Schamphelaere et al., 2006
Chlamydomonas reinhardtii	Inoculum: 1,000 c/ml	10 d	growth	22	64,8	6,2	0,5	4,9	2,9	0,1	11,0	11,5	8,7	0,3	Reconstituted	Schäfer et al., 1994
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	404,1	213,0	5,5	10,3	80,2	12,4	83,0	0,5	73,4	266,6	0,1	Reconstituted	De Schamphelaere et al., 2006

Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	187,8	130,3	6,01	5	128,3	19,6	46,7	0,5	86,4	292,5	0,5	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	108,3	60,2	6,03	5,17	32,1	4,9	47,8	0,5	29,2	122,5	0,6	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	407,4	99,0	6,04	15,5	32,1	5,3	115,6	0,5	60,4	211,9	0,6	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	growth	510,2	180,0	6,05	15,2	128,3	19,9	114,0	0,5	117,2	379,9	0,6	Reconstituted	De Schamphelaere et al., 2006
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	Growth - biomass	94,7	21,9	5,52	10,3	7,6	3,6	25,6	9,3	9,5	28,6	0,6	Lake	Heijerick et al., 2005
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	Growth - biomass	61,8	54,3	6,1	2,34	8,3	4,3	22,1	3,8	11,3	34,1	2,1	Lake	Heijerick et al., 2005
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	Growth - biomass	52,9	39,9	6,31	2,72	7,4	4,3	21,8	3,6	11,5	33,2	3,2	Lake	Heijerick et al., 2005
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	161,6	50,6	5,68	9,84	80,2	12,2	70,6	0,4	68,4	221,9	0,3	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	49,4	20,5	5,99	5,64	128,2	19,4	53,1	0,4	86,1	274,7	0,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	148,4	24,5	5,99	15,3	128,2	19,4	108,0	0,4	112,4	349,2	0,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	155,9	22,8	6,17	14,9	32,1	4,9	99,8	0,4	53,0	175,5	1,0	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	52,3	21,9	6,18	5,07	32,1	4,9	43,0	0,4	26,9	100,3	1,0	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	110,3	49,1	6,19	5,23	32,1	4,9	128,5	0,4	13,6	266,2	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	97,7	41,2	6,2	5,31	32,1	4,9	44,1	0,4	13,4	109,9	1,1	Reconstituted OECD medium	De Schamphelaere

																& Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	336,9	53,7	6,2	15,6	32,1	4,9	101,4	0,4	13,5	204,9	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	337,0	56,1	6,22	15,8	32,1	4,9	358,6	0,4	13,9	680,6	1,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Lemna minor	Double fronded colonies	7 d	growth	30	75,8	6,5	0,5	4,0	4,9	1,4	114,8	31,4	8,2	0,7	artificial	Teisseire et al., 1998
Chlamydomonas reinhardtii	Inoculum: 10,000 c/ml	3 d	Growth	108	27,4	7,03	9,84	80,1	12,0	106,2	0,4	104,7	303,8	9,9	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	Growth	85,4	21,3	7,01	10	160,4	24,5	111,7	0,5	121,1	387,0	9,4	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	Growth	98,9	22,6	7,03	10,8	80,2	12,4	117,9	0,5	75,1	250,6	9,8	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	Growth	83,9	19	7,03	10,8	80,2	12,4	117,9	0,5	38,6	250,6	9,9	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	Growth	36,4	54,1	7,04	1,5	80,2	12,2	59,3	0,5	46,9	169,3	10,0	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	Growth	132,3	32,6	7,04	10,2	80,2	12,4	115,4	0,5	73,3	245,7	10,0	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	Growth	282,9	38,0	7,05	19,1	80,2	12,7	173,8	0,5	99,9	323,1	10,4	Reconstituted	De Schamphelaere et al., 2006
Chlorella vulgaris	Inoculum: 10,000 c/ml	3 d	Growth	158,7	38,1	7,07	10,3	8,1	1,5	117,0	0,5	36,9	112,5	10,7	Reconstituted	De Schamphelaere et al., 2006
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	Growth (biomass)	164	18,4	7,3	17,8	57,3	11,7	29,5	3,9	116,2	46,4	18,6	Lake	Heijerick et al., 2005
Pseudokirchneriella subcapitata	Inoculum: 10,000 c/ml	3 d	Growth (biomass)	65,5	6,1	7,5	20,4	48,5	9,3	44,4	4,0	93,0	33,0	30,9	Lake	Heijerick et al., 2005
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	86,8	9,3	6,98	18,2	80,2	12,2	141,6	0,4	90,6	285,7	8,9	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006

Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	52,2	10,2	7,01	10,2	80,2	12,2	107,4	0,4	69,5	224,8	13,9	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	16,7	17,1	7,02	1,95	80,2	12,2	59,3	0,4	47,6	161,3	9,7	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	54,8	10,6	7,02	10,4	160,3	24,3	108,0	0,4	118,2	368,7	9,7	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	51,6	10,3	7,03	9,98	80,2	12,2	105,7	0,4	68,8	223,0	10,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	93,2	19,4	7,04	9,89	160,3	24,3	264,4	0,4	90,6	677,1	10,2	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	50,6	10,0	7,04	10,1	80,2	12,2	106,7	0,4	69,4	224,0	10,3	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	18,0	16,4	7,05	2,21	80,2	12,2	96,3	0,4	42,4	233,3	10,5	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	18,1	17,6	7,08	2,06	80,2	12,2	59,5	0,4	42,3	165,2	11,2	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	67,2	13,6	7,09	9,99	80,2	12,2	264,4	0,4	42,7	538,8	11,4	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	62,1	11,1	7,09	11,1	80,2	12,2	109,4	0,4	42,3	247,8	11,4	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	202,6	20,3	7,11	19,9	80,2	12,2	158,2	0,4	42,3	328,6	12,1	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006

Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	78,4	15,1	7,12	10,5	160,3	24,3	106,4	0,4	90,3	386,4	12,3	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	105,7	11,4	7,17	18,5	80,2	12,2	455,2	0,4	42,7	868,5	13,8	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Pseudokirchneriella subcapitata	1*10^4 cells/ml	72 h	Growth rate	74,9	14,0	7,19	10,4	4,8	0,3	127,8	0,4	33,4	92,9	15,4	Reconstituted OECD medium	De Schamphelaere & Janssen, 2006
Invertebrates chronic database																
Ceriodaphnia dubia	neonates (< 24 h)	7 d	reproduction	20	11,5	6	3,7	37,0	19,0	0,1	0,1	23,2	8,0	44,0	River (Clinch River)	Belanger & Cherry, 1990
Daphnia magna	neonates	21 d	Reproduction	21,5	18,4	6,1	2,34	3,1	1,1	4,4	0,7	4,6	7,8	0,7	Lake	Heijerick et al., 2002
Daphnia magna	neonates	21 d	Reproduction	28	20,6	6,31	2,72	2,2	1,1	4,1	0,5	4,8	7,0	1,4	Lake	Heijerick et al., 2002
Daphnia magna	neonates	21 d	Reproduction	8,2	8,2	6,27	2	66,8	53,4	35,7	1,3	351,2	0,9	13,4	Reconstitued EPA water	Rodriguez & Arbildua, 2012
Daphnia magna	neonates	21 d	Reproduction	5,9	5,9	6,28	2	66,8	53,4	35,7	1,3	351,2	0,9	13,4	Reconstitued EPA water	Rodriguez & Arbildua, 2012
Daphnia magna	neonates	21 d	Reproduction	6,7	6,5	6,28	2,1	28,1	22,7	36,6	1,2	153,1	0,9	13,4	Reconstitued EPA water	Rodriguez & Arbildua, 2012
Daphnia magna	neonates	21 d	Reproduction	32,9	7,3	6,41	6,1	57,7	8,8	17,9	3,2	35,0	91,7	96,2	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
Daphnia magna	neonates	21 d	Reproduction	10,7	16,2	6,41	6,1	57,7	8,8	17,9	3,2	35,0	91,7	96,2	Reconstituted water (modified M4 medium)	Van Regenmortel et al., 2013
Ceriodaphnia dubia	neonates (< 8 h)	7 d	Mortality	4	15,4	6,95	0,5	6,1	1,1	12,4	0,1	22,1	0,1	19,0	Reconstituted	Jop et al., 1995
Ceriodaphnia dubia	neonates (< 8 h)	7 d	Mortality	19	11,8	7	3,2	6,8	1,3	5,7	0,1	18,0	0,1	13,0	River	Jop et al., 1995
Ceriodaphnia dubia	neonates (< 8 h)	7 d	Reproduction	4	15,4	6,95	0,5	6,1	1,1	12,4	0,1	22,1	0,1	19,0	Reconstituted	Jop et al., 1995
Ceriodaphnia dubia	neonates (< 8 h)	7 d	Reproduction	10	6,2	7	3,2	6,8	1,3	5,7	0,1	18,0	0,1	13,0	River	Jop et al., 1995
Daphnia magna	neonates	21 d	Reproduction	181	16,5	7,5	20,4	43,3	6,1	26,7	0,9	86,3	6,8	27,3	Lake	Heijerick et al., 2002
Daphnia magna	neonates (< 24 h old)	21 d	Reproduction	300	34,4	7,3	17,8	52,1	8,6	11,8	0,8	109,5	20,2	18,2	Natural water	De Schamphelaere & Janssen, 2004

Ceriodaphnia dubia	neonates (< 24 h)	7 d	Mortality	20	55	7,6	0,5	14,0	12,0	26,3	2,0	48,0	1,9	62,5	Reconstitued	Cerda & Olive, 1993
Ceriodaphnia dubia	neonates (< 24 h)	7 d	Mortality	122	45,4	8,25	5,7	30,4	5,7	0,1	0,1	0,1	0,1	97,0	River (Lester River)	Spehar & Fiandt, 1985
Ceriodaphnia dubia	< 24 h	7 d	Mortality	20	11,3	8,3	3	25,0	8,3	8,5	0,9	16,0	9,4	91,0	natural well water	Wang et al., 2011
Ceriodaphnia dubia	< 24 h	7 d	Mortality	65	19,6	8,3	5,8	26,0	8,5	9,2	1,0	20,0	11,0	94,0	natural well water	Wang et al., 2011
Ceriodaphnia dubia	< 24 h	7 d	Mortality	91	28,5	8,3	5,8	26,0	8,5	9,2	1,0	20,0	11,0	93,0	natural well water	Wang et al., 2011
Ceriodaphnia dubia	< 24 h	7 d	Mortality	200	37,8	8,3	10	26,0	8,6	9,4	1,1	29,0	16,0	96,0	natural well water	Wang et al., 2011
Ceriodaphnia dubia	< 24 h	7 d	Mortality	10	32,92	8,4	0,4	26,0	8,5	8,7	1,0	18,0	9,1	100,0	natural well water	Wang et al., 2011
Daphnia magna	neonates	21 d	Mortality	36,8	36,8	8,1	2	67,6	13,7	13,2	3,4	39,9	19,8	28,3	Lake (Lake Ijssel)	Van Leeuwen et al., 1988