Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Copper sulphate pentahydrate

EC number: 231-847-6
CAS number: 7758-99-8

CLH-O-0000001412-86-33/F

Adopted

04 December 2014

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CLH-O-0000001412-86-33/F

OPINION OF THE COMMITTEE FOR RISK ASSESSMENT ON A DOSSIER PROPOSING HARMONISED CLASSIFICATION AND LABELLING AT EU LEVEL

In accordance with Article 37 (4) of (EC) No 1272/2008, the Classification, Labelling and Packaging (CLP) Regulation, the Committee for Risk Assessment (RAC) has adopted an opinion on the proposal for harmonised classification and labelling (CLH) of:

Chemicals name: Copper sulphate pentahydrate

EC number: 231-847-6

CAS number: 7758-99-8

The proposal was submitted by **France** and received by the RAC on **19 July 2013**.

In this opinion, all classifications are given in the form of CLP hazard classes and/or categories, the majority of which are consistent with the Globally Harmonised System (GHS).

PROCESS FOR ADOPTION OF THE OPINION

France has submitted a CLH dossier containing a proposal together with the justification and background information documented in a CLH report. The CLH report was made publicly available in accordance with the requirements of the CLP Regulation at ***http://echa.europa.eu/harmonised-classification-and-labelling-consultation*** on **18 December 2013**. Concerned parties and Member State Competent Authorities (MSCA) were invited to submit comments and contributions by **3 February 2014**.

ADOPTION OF THE OPINION OF THE RAC

Rapporteur, appointed by RAC: **Stephen Dungey**

Co- rapporteur, appointed by RAC: **Betty Hakkert**

The opinion takes into account the comments provided by MSCAs and concerned parties in accordance with Article 37(4) of the CLP Regulation and the comments received are compiled in Annex 2.

The RAC opinion on the proposed harmonised classification and labelling was reached on **4 December 2014**.

The RAC opinion was adopted by **consensus**.

OPINION OF THE RAC

RAC adopted the opinion that **copper sulphate pentahydrate** should be classified and labelled as follows:

Classification and labelling in accordance with the CLP Regulation (Regulation (EC) 1272/2008)

	Index No	International Chemical Identification	EC No	CAS No	Classification		Labelling			Specific Conc. Limits, M-factors	Notes
					Hazard Class and Category Code(s)	Hazard statement Code(s)	Pictogram, Signal Word Code(s)	Hazard state-ment Code(s)	Suppl. Hazard statement Code(s)		
Current Annex VI entry	029-004-00-0	Copper sulphate	231-847-6	7758-98-7	Acute Tox. 4 * Eye Irrit. 2 Skin Irrit. 2 Aquatic Acute 1 Aquatic Chronic 1	H302 H319 H315 H400 H410	GHS07 GHS09 Wng	H302 H319 H315 H410			
Dossier submitters proposal		Copper sulphate pentahydrate	231-847-6	7758-99-8	Acute Tox. 4 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 2	H302 H318 H400 H411	GHS07 GHS05 GHS09 Dgr	H302 H318 H411		M=10	
RAC opinion		Copper sulphate pentahydrate	231-847-6	7758-99-8	Acute Tox. 4 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H318 H400 H411	GHS07 GHS05 GHS09 Dgr	H302 H318 H410		M = 10 M = 10	
Resulting Annex VI entry if agreed by COM		Copper sulphate pentahydrate	231-847-6	7758-99-8	Acute Tox. 4 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H302 H318 H400 H411	GHS07 GHS05 GHS09 Dgr	H302 H318 H410		M = 10 M = 10	

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

In addition to copper sulphate pentahydrate, ECHA has received CLH proposals for nine other copper compounds or forms of copper from the same dossier submitter (France). The dossier submitter stated 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. For the systemic endpoints the dossier submitter therefore proposed to read-across between the different copper compounds, and introduced identical sections on specific target organ toxicity, mutagenicity, carcinogenicity and reproductive toxicity in the CLH reports for all compounds. The studies reported in these common sections mostly concern copper sulphate pentahydrate, but sometimes also other copper compounds. The sections on acute toxicity, skin irritation/corrosion, eye damage/irritation and sensitisation in the CLH reports were specific for each substance/form.

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal

Copper sulphate pentahydrate is a stable inorganic salt with copper in a high oxidation state. Its physicochemical properties indicate that it is neither explosive, nor flammable or oxidising. The dossier submitter proposed no classification for physical hazards.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Since copper sulphate pentahydrate does not have explosive or oxidising properties and are not (auto-)flammable, RAC supports the non-classification for physical hazards, as proposed by the dossier submitter.

HUMAN HEALTH HAZARD ASSESSMENT

RAC evaluation of acute toxicity

Summary of the Dossier submitter's proposal

The CLH report includes three acute toxicity studies (two via the oral route, one via the dermal route), all conducted with copper sulphate pentahydrate. The first oral study (Lheritier, 1994), conducted according to OECD TG 401, determined the LD_{50} to be 481-482 mg/kg bw (male and female rats combined). The second oral study (Manciaux, 1998), also conducted according to OECD TG 401, determined an LD_{50} for females at 666 mg/kg bw. In the rat dermal study (Lheritier, 1993), conducted according to OECD TG 402, no animals (5/sex) died at the dose level of 2000 mg/kg bw tested.

The dossier submitter proposed classification as Acute Tox. 4 – H302 (harmful if swallowed). No classification was proposed for the dermal route, nor for the inhalation route for which no data were available.

The CLH report also contains a review of seven studies reporting on a possible association between copper exposure and Metal Fume Fever (MFF) in humans (Borak *et al.,* 2000). MFF presents as an influenza-like illness with cough and dyspnoea followed by fever, sweating and shivering, accompanied by nausea, headache, weakness, a sweet metallic taste and muscle and joint pain. The dossier submitter concluded (in agreement with the authors of the review) that none of the reports contain enough conclusive evidence to associate copper fumes or particles with MFF. Another review (Chuttani *et al.,* 1965) reports on several cases of self-poisoning by oral ingestion of copper sulphate. Intoxication is associated with nausea, epigastric burning, vomiting,

diarrhoea, ulcerations of the gastric and intestinal mucosa, and liver and kidney histopathology. Rapid chelation therapy increases survival.

Comments received during public consultation
One MSCA agreed with the proposed classification for acute toxicity during the public consultation.

Assessment and comparison with the classification criteria
Following a comparison of the available LD_{50} and LC_{50} values in rats with the criteria, RAC agrees with the conclusion of the dossier submitter that copper sulphate pentahydrate should be classified for acute oral toxicity with **Acute Tox. 4 – H302**. RAC also concludes that the available dermal LD_{50} values do not warrant classification for acute dermal toxicity.
For the inhalation route, no animal data are available and the available human data are insufficient for classification. No conclusion can be drawn for classification for acute inhalation toxicity.

RAC evaluation of specific target organ toxicity – single exposure (STOT SE)
Summary of the Dossier submitter's proposal
No clear evidence of specific toxic effects on organs was reported in the acute toxicity studies. Clinical signs of toxicity were transient in nature and considered to be unspecific signs of general acute toxicity. Liver and kidney damage in human case studies were seen as secondary to massive or poorly reported doses. The dossier submitter concluded that no classification is warranted for STOT SE.

Comments received during public consultation
No comments were received during the public consultation.

Assessment and comparison with the classification criteria
In the acute dermal toxicity study, no behavioural and clinical signs were observed.

For the inhalation route, no acute toxicity studies are available.

In the acute oral toxicity studies, various effects were observed. In Lheritier (1994), only minor effects were observed and these included initial body weight loss followed by body weight gain, stomach distention and slightly congested intestines. In the study of Manciaux (1998), effects such as sedation, hypoactivity, piloerection, dyspnoea, tonic-clonic convulsions and lateral incumbency were observed. Symptoms appeared 30 minutes post-exposure and persisted in some animals until day 2 post-exposure.
Due to the general and transient nature of the observed effects, and the fact that they occur at lethal dose levels whereas copper sulphate pentahydrate is already proposed to be classified for lethality, no classification is warranted.

In human self-poisoning cases the most frequently observed symptoms (nausea, epigastric burning, vomiting, diarrhoea) are also indicative of non-specific, general acute toxicity.

RAC concludes that based on th weight of evidence, copper sulphate pentahydrate should not be classified for specific target organ toxicity – single exposure (STOT SE).

RAC evaluation of skin irritation/corrosion
Summary of the Dossier submitter's proposal
One rabbit skin irritation study is included in the CLH report, conducted according to OECD TG 404 with copper sulphate pentahydrate (Mercier, 1994a). Mean scores for each animal for erythema were 0, 0.33 and 0.33, respectively (mean of evaluation over 24, 48 and 72 h) while no oedema was observed. All effects were reversible within 7 days. The dossier submitter concluded that no classification for corrosion/skin irritation is warranted.

Comments received during public consultation
Two MSCAs agreed with the proposal for no classification during the public consultation.

Assessment and comparison with the classification criteria
With a mean score for erythema of 0.33 over 24/48/72h in two animals and 0 in the remaining animal, and scores for edema of 0 in all test-animals, the results of the skin irritation study do not fulfil the criteria for classification (a mean score of ≥2.3 - ≤4 for erythema or for edema in at least 2 out of 3 animals).

RAC agrees with the conclusion of the dossier submitter that copper sulphate pentahydrate should not be classified for skin irritation.

RAC evaluation of eye damage/irritation
Summary of the Dossier submitter's proposal
One rabbit eye irritation study was included in the CLH report, conducted according to OECD 405 with 100 mg of copper sulphate pentahydrate instilled in the left eye and no rinsing of the eye after application (Mercier, 1994b). Values were not reported individually but instead as a mean of three animals. Mean scores (of evaluations at 24, 48 and 72h) were 2.56 for corneal opacity and 1 for iritis and mean scores for conjunctive redness and chemosis were 2 and 3.78, respectively. Ocular lesions were present in all three animals on day 21. The dossier submitter concluded that classification as Eye Dam. 1 – H318 (causes serious eye damage) is warranted.

Comments received during public consultation
Two MSCAs agreed with the proposed classification for eye damage during the public consultation.

Assessment and comparison with the classification criteria
Copper sulphate pentahydrate caused eye irritation in the one available eye irritation study. Observed effects included cornea opacity, iritis, conjunctival redness and chemosis. Ocular lesions which were observed at 72 h were still present at day 21 in all three test animals. These effects are considered as irreversible.

RAC concludes that copper sulphate pentahydrate should be classified for eye damage/irritation as **Eye Dam. 1 – H318**.

RAC evaluation of skin sensitisation
Summary of the Dossier submitter's proposal
One guinea pig maximisation test (GPMT), performed according to OECD TG 406 with copper sulphate pentahydrate, was included in the CLH report (Mercier, 1994c). For induction, an intradermal injection of 0.1% (w/w) at day 1 and topical application of 10% (w/w) at day 8 were used. Animals were challenged with 10% (w/w) on day 22. No reactions were seen in the tested (n=20) and control (n=10) animals at 24 h and 48 h after challenge.

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. The dossier submitter concluded, based on negative GPMT and extremely rare cases of allergic reactions to copper compounds in humans, that no classification for skin sensitisation for copper sulphate pentahydrate is warranted.

Comments received during public consultation
No comments were received during the public consultation.

Assessment and comparison with the classification criteria
Given the absence of skin reactions in the available skin sensitisation study, and the few individual cases of allergic reactions in humans, RAC agrees with the conclusion of the dossier submitter that copper sulphate pentahydrate should not be classified for skin sensitisation.

RAC evaluation of specific target organ toxicity – repeated exposure (STOT RE)

Summary of the Dossier submitter's proposal

The CLH report includes several repeated dose toxicity studies. 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 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 conducted with dicopper oxide (Kirkpatrick, 2010) is included as well as 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). A human case study of chronic oral self-administration of copper resulting in liver failure (O'Donohue *et al.*, 1993) and human volunteer studies demonstrating nausea associated with copper sulphate in drinking water (Araya *et al.*, 2001, 2003) are also reported, as are human case studies of chronic inhalation exposure to Bordeaux Mixture causing pulmonary lesions (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 is 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

One MSCA identified some errors in doses and units.

Assessment and comparison with the classification criteria

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

<u>Oral</u>

For the oral route, the following animal studies were available:

- 15-day drinking water studies in rats and mice
- 15-day feeding studies in rats and mice
- 90-day feeding studies in rats and mice
- 15-week feeding studies in rats

Longer-term studies (44- and 104-week feeding studies in rats by Harrison *et al.*, 1954 and a 52-week study by Haywood & Loughran, 1985; described in the carcinogenicity section of the CLH report) were also available.

In the drinking water studies, mortalities were observed at copper sulphate pentahydrate concentrations of 3000 (1/10 rats and 4/10 mice) and 10000 ppm (all rats and mice) within 2 weeks (no further details on time or cause of death reported). Clinical signs of both rats and mice in these highest two groups included emaciation, abnormal posturing, hypoactivity, dyspnoea, tremors and prostration. Surviving rats and mice in the 3000 ppm groups (with calculated intakes of 175/121 mg/kg bw/day copper sulphate pentahydrate for male/female rats and 226/245 mg/kg bw/day copper sulphate pentahydrate for male/female mice) had significantly reduced body weights and showed a decreased water consumption, which was attributed to poor

palatability of the copper solution. Microscopic lesions 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 male rats in the 300 and 1000 ppm groups. These lesions were reported to be slight. Female rats and mice of either sex were not affected.

In feed, copper sulphate pentahydrate concentrations of up to 16000 ppm (corresponding to calculated intakes of 1275/1121 mg/kg bw/day copper sulphate pentahydrate for male/female rats and 2817/3068 mg/kg bw/day copper sulphate pentahydrate for male/female mice) for 15 days did not result in mortality in either rats or mice. Final body weights of male and female rats of the 8000 and 16000 ppm groups and of female mice receiving 16000 ppm were reduced due to a decrease in food consumption as a consequence of poor palatability of the feed mixture. Microscopic findings were more severe in rats than in mice and at levels of 2000 ppm and above included hyperplasia and hyperkeratosis of the squamous mucosa of the limiting ridge between the forestomach and glandular stomach. This finding was of minimal severity only in mice, and may have been associated with the irritant effects of the sulphate rather than the copper ion. In rats, but not in mice, copper sulphate pentahydrate administration was further associated with chronic inflammation of the liver (minimal to mild, at 8000 and 16000 ppm), changes in the kidney similar to those seen in the drinking water study (at ≥4000 ppm, corresponding to calculated intakes of ≥363/637 mg/kg bw/day copper sulphate pentahydrate for male/female rats; no level of severity reported), depletion of haematopoietic cells in bone marrow (at 8000 and 16000 ppm) and a minimal to mild decrease in erythroid haematopoiesis in spleen (at 16000 ppm).

Following 90 days of dietary administration of copper sulphate pentahydrate in concentrations of 500-8000 ppm (rats; equal to 31.4-550 and 35.4-526.5 mg/kg bw/day copper sulphate pentahydrate for males and females, respectively) or 1000-16000 ppm (mice; equal to 173-3201 and 205-4157 mg/kg bw/day copper sulphate pentahydrate for males and females, respectively), no mortality was observed in either species. Final body weights were reduced in both rats and mice from 4000 ppm. As in the 15-day study, mice were less sensitive than rats, with findings limited to the forestomach (dose-related minimal to moderate hyperplasia with hyperkeratosis of the squamous mucosa at the site of the limiting ridge at 4000 ppm and above). This effect was also seen in rats (at 2000 ppm and above), but rats in addition showed histological changes in the liver and the kidneys (mainly from 2000 ppm), accompanied by changes in clinical chemistry and urinalysis parameters indicative of hepatic and renal injury. Haematological changes were also observed (an initial increase in haemoglobin, haematocrit, platelet and red blood cell count, followed by a decrease in haemoglobin and haematocrit). Histological liver changes consisted of a dose-related increase in minimal to moderate chronic inflammation (characterised by multiple foci of a mixture of mononuclear inflammatory cells). Histological changes in the kidneys consisted of a dose-related increase in the size and number of cytoplasmic protein droplets present in the epithelium of proximal convoluted tubules of rats at doses of 2000 ppm and higher, and was less severe in females than in males. Minimal nuclear enlargement (karyomegaly) in renal tubule cells was present in the 8000 ppm group, and three females in this group also showed degeneration of renal tubule epithelium.

In three additional studies with administration of copper sulphate in the diet of rats at one or several doses for up to 15 weeks and animals sacrificed at several intervals, histological changes in the liver and kidney were demonstrated to reach a maximum after approximately six weeks of treatment, followed by regeneration and recovery to week 15, at doses up to 5000 ppm (equivalent to 982 mg/kg bw/day copper sulphate). A dose of 6000 ppm resulted in unsustainable liver damage and all animals were dead or sacrificed by week 6 of treatment. In another study with focus only on the liver, male weanling rats were administered 0 or 3000 ppm copper as copper sulphate in the diet for 15 weeks, after which part of the control and treated animals were killed and the remaining animals were given 6000 ppm for three weeks. The change of diet did not affect the condition of the 'primed' rats, but the unprimed group was lethargic with ruffled coats. Microscopically, the 'primed' animals showed almost complete recovery of the liver lesions, with no further changes in liver copper content or histopathology when receiving 6000 ppm for an additional three weeks. In the unprimed group the animals showed a marked increase in liver copper content, and moderate to severe hepatocellular necrosis with associated inflammatory response after three weeks treatment with 6000 ppm.

According to the rather limited descriptions of two 44-week dietary studies, administration of copper sulphate (at doses equivalent to 530 or 1600 ppm copper) or copper gluconate (at a dose equivalent to 1600 ppm copper) to rats resulted in a high(er) rate of mortality in the 1600 ppm groups (equivalent to 314 mg/kg bw/day copper sulphate and 571 mg/kg bw/day copper gluconate). The animals at 1600 ppm showed markedly reduced weight gain, high blood nonprotein nitrogen levels, marked accumulation of copper in liver, kidney and spleen, and icteric pigmentation with abnormal cytoplasmic staining properties in the liver. The kidneys only showed minor changes. At 530 ppm (equivalent to 106 mg/kg bw/day copper sulphate) apparently only accumulation of copper in the liver was seen.

Male weanling rats treated with 3000 ppm copper as copper sulphate (stated to be equivalent to 982 mg/kg bw/day copper sulphate) via the diet for 52 weeks (with interim kills at 15, 20 and 29 weeks) showed an adaptive response so that no long-term evidence of liver toxicity was seen.

Finally, there was a 104-week study (with interim sacrifices after 10 and 52 weeks) with dietary administration of 0, 0.1, 1 or 3% potassium sodium copper chlorophyllin (equivalent to 0, 53, 530 or 1600 ppm copper) to rats. The description of this study and its results was rather limited. Slightly higher plasma copper and liver copper levels were found at 1600 ppm (equivalent to 1500 mg/kg bw/day potassium sodium copper chlorophyllin). The kidneys, liver, stomach, small intestine and spleen of the high dose animals sacrificed after 52 weeks showed only tinctorial changes with no cell injury. At termination the histopathological examination apparently did not reveal differences between controls and treated animals.

With respect to human data, a case study is available on an individual who consumed 30 mg/day of copper as a dietary supplement for 2 years with no apparent ill effects. He then increased the copper intake to 60 mg/day and was eventually 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. Additionally, some human volunteer studies are briefly described demonstrating nausea associated with copper sulphate in drinking water.

When looking at the oral animal data, most of the available studies concern administration of copper sulphate pentahydrate via the diet. Target organs of copper toxicity in these dietary studies were the liver (inflammation), kidneys (histopathological changes) and forestomach (hyperplasia and hyperkeratosis) in rats, with some evidence of haematological changes. Mice were less sensitive, with adverse effects limited to the forestomach. The forestomach effects, that were possibly due to the irritant effect of the sulphate ion rather than of copper, may be of less relevance to humans given that humans do not have a forestomach. As to the effects on liver and kidney in rats, the lowest effective dose was around 2000 ppm in the 90-day rat study, corresponding to 126-134 mg copper sulphate pentahydrate (or 32-34 mg copper)/kg bw/day. But at this dose the severity of the effects was only minimal (see table in the section *Supplemental information* in Annex 1), whereas STOT RE is to be assigned on the basis of findings of 'significant' or 'severe' effects. Only at 8000 ppm, corresponding to 526.5-550 mg copper sulphate pentahydrate (or 134-140 mg copper)/kg bw/day, was the severity moderate, as was degeneration (of the renal tubule epithelium) seen in three females. In studies of longer duration, marked toxicity was only observed at doses equivalent to 1600 ppm copper, corresponding to 314 mg copper sulphate pentahydrate (or 80 mg copper)/kg bw/day. RAC concluded that these dose levels are above the (extrapolated) cut-off values for classification (100, 30 and 12.5 mg/kg bw/day for a 90-day, 44-week and 104-week study, respectively), on copper sulphate pentahydrate- basis as well as on copper-basis.

In addition to the dietary studies, two 15-day drinking water studies with copper sulphate pentahydrate were available. In these sub-acute studies, severe copper toxicity including mortality seemed to occur at rather low doses (≥175/121 mg/kg bw/day copper sulphate pentahydrate in male/female rats and ≥226/245 mg/kg bw/day copper sulphate pentahydrate in male/female mice). This is markedly different from e.g. the 15-day feeding studies where no mortalities were observed at doses as high as 1-3 g/kg bw/day. Unfortunately, the drinking water studies were rather limitedly described, as there were no data on time or cause of death reported,

nor on microscopic lesions in surviving animals at these doses. Furthermore, no other drinking water studies with copper sulphate pentahydrate were available. RAC noted that these dose levels are below the extrapolated cut-off value for classification (600 mg/kg bw/day for a 15-day study), but noted at the same time that it cannot be excluded that the mortality observed in these sub-acute studies reflects (in part) its acute toxicity, an effect for which copper sulphate pentahydrate is already proposed to be classified.

Inhalation

In a guideline inhalation study, rats were exposed (whole body) to 0.2, 0.4, 0.8 or 2.0 mg/m^3 cuprous oxide (= dicopper oxide = copper (I) oxide), 6 hours/day, 5 days/week for 1, 2, 3 or 4 weeks, followed by a 13-week recovery period. There was a concentration-related increase in microscopic findings in the lungs, and increased lung, bronchial lymph node, and mediastinal lymph node weights. Lung histopathology showed alveolar histiocytosis (minimal to moderate), acute inflammation, and perivascular mononuclear cell infiltrates. Further, increased blood neutrophil counts, higher LDH, protein and total cell counts, and higher proportion of neutrophils in the bronchoalveolar lavage (BAL) fluid were seen, and some minimal to mild nasal histopathology. Most effects showed a peak prior to 4 weeks of exposure, possibly indicating a plateau. Following the recovery period, the microscopic findings had disappeared, haematology and BAL fluid parameters were normal, and the effects on lung weights were greatly reduced. As the observed effects were only minimal to moderate in nature and seemed to regress already within the exposure period of 4 weeks, they seem to be of insufficient severity to warrant classification.

In the CLH report a non-guideline study in guinea pigs is also described, in which six guinea-pigs were placed in poorly ventilated glass cages and exposed via inhalation to finely pulverised Bordeaux Mixture (solution of copper sulphate neutralised with hydrated lime) for 6 months. The spray was applied three times a day so that the atmosphere of the cage was completely saturated. Two of the six guinea pigs were retained untreated for a further 3 months. Exposure to Bordeaux Mixture (prepared "in the proportion of 1.5 kg of hydrated lime for 100 litres of water") induced radiographic changes and slight histopathological changes of the lungs including 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. One animal also showed a small histiocytic granuloma in the septa with the appearance of fibro-hyaline scars. An apparent total regression of the lesions was noted on radiograph in the two recovery animals, but microscopic examination of these animals showed fibrous bands, small groups of alveoli filled with macrophages, hyaline deposits and small areas of condensation of the reticulin fibres of the septa. The absence of a clear copper dose level does not permit a comparison with the classification threshold levels.

RAC noted a mismatch between the description of the study in section 4.7.1.2 and the summary of this study in table 20. The latter seems to present the results of a different study with a different substance (copper oxychloride), which RAC was not able to evaluate further as a proper reference was lacking.

Similar interstitial pulmonary lesions to those in guinea pigs have been described for workers whose main occupations were spraying vineyards with Bordeaux Mixture. Cases were mostly from Portugal but also from the former Yugoslavia. The condition is known as Vineyard Sprayer's Lung (VSL), in which the pulmonary lesions, that may lead to respiratory insufficiency, have a well-defined histological picture characterised by three stages: intra-alveolar desquamation of macrophages, formation of predominantly histiocytic granulomas in the septa, and the healing of these lesions generally in the form of fibro-hyaline nodules very similar to those found in silicosis (Pimentel & Marques, 1969). Hepatic changes including proliferation and diffuse swelling of Kupffer's cells and the formation of well defined histiocytic or sarcoid-type granulomas near the portal tracts, all with inclusions of copper, have also been seen. Most of the published findings date from the 1970s and 1980s, and were associated with on-site preparation of the mixture and use of primitive application techniques at high application rates, without any protective respiratory measures. This is not representative for current practice 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. Although the

data seem to point to a link between Bordeaux Mixture and lung lesions, there is no detailed information on the dose levels responsible or on possible confounding factors (such as smoking and exposure to other pathogenic dusts).

Dermal
In a dermal study with rabbits, animals were treated with a 53% w/v aqueous suspension of the wettable powder formulation Kocide 101 (containing 77% copper dihydroxide) for 6-8 hours/day, 5 days/week for 3 weeks. Effects were only observed at the highest dose tested (2000 mg formulation/kg bw/day, equivalent to 1540 mg copper dihydroxide (or 1000 mg copper)/kg bw/day) and included body weight loss and skin abnormalities at histopathological examinations (epidermal thickening, focal leukocyte infiltration of dermis, keratin thickened or distorted, atrophied hair follicles). There were also single instances of dermal fibrosis, oedema, eschar formation and slight ulceration. RAC notes however that the effective dose level, on copper dihydroxide-basis as well as on copper-basis, is above the extrapolated cut-off value for classification (800 mg/kg bw/day for a 21-d study).

Overall conclusion
After considering all available human and animal data, RAC concludes that they provide insufficient evidence for classification. RAC therefore agrees with the conclusion of the dossier submitter that classification for STOT RE is not warranted for copper sulphate pentahydrate.

	Incidence and mean severity ( ) at dose level (ppm)					
	0	**1000**	**2000**	**4000**	**8000**	**16000**
Male						
Dose in mg copper sulphate pentahydrate/kg bw/day	0	172.9	381.9	735.9	1563	3201
Dose in mg Cu/kg bw/day	0	44	97.2	187.3	397.8	814.7
Forestomach, hyperplasia and hyperkeratosis	0	-	0	2 (1.0)	6 (1.0)	10 (1.6)
Female						
Dose in mg copper sulphate pentahydrate/kg bw/day	0	205.1	493.9	1048	2106	4157
Dose in mg Cu/kg bw/day	0	52.2	125.7	266.7	536	1058
Forestomach, hyperplasia and hyperkeratosis	0	-	0	5 (1.0)	8 (1.0)	10 (1.7)

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

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Several mutagenicity studies conducted with different copper compounds were included in the CLH report. 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 were all reported as negative as well as a rec-assay with copper chloride. 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, where 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 copper sulphate pentahydrate.

Comments received during public consultation

For five of the ten copper compounds under consideration, one MSCA commented that the available genotoxicity data are insufficient to evaluate, and thus to conclude on, the genotoxic potential of copper compounds. The dossier submitter responded that in their opinion the data do not meet the criteria for classification, but acknowledged that insufficient evidence exists to exclude a genotoxic potential via the IP route, referring also to the EFSA peer review of copper substances (EFSA, 2008) where it was concluded that genotoxicity is not of concern following oral administration, but that there is insufficient evidence to exclude a (local) genotoxic potential following non-oral administration.

Assessment and comparison with the classification criteria

Ten *in vitro* and five *in vivo* mutagenicity studies are presented in the CLH report. All *in vivo* studies tested copper sulphate pentahydrate, whereas in the *in vitro* studies several copper compounds were tested (copper sulphate (pentahydrate), oxine copper, Bordeaux Mixture, dicopper chloride trihydroxide, copper Nordox Technical and copper chloride). The *in vitro* studies were only briefly summarised in tabular form, without further details given. Of these *in vitro* studies, all Ames tests (7x) were negative (with and without metabolic activation), as well as a rec-assay with *Bacillus subtilis* (in the absence of metabolic activation). UDS tests in primary rat hepatocytes and Chinese hamster V79 cells were positive (in the absence of metabolic activation), and a weak positive results was further observed in an SCE test in Chinese hamster V79 cells (in the absence of metabolic activation).

Two *in vivo* studies using the oral route (a rat liver UDS assay and a mouse bone marrow micronucleus assay) showed clear negative results for mutagenicity (Ward, 1994; Riley, 1994). These studies seem well-performed with respect to quality and validity. In another oral study a positive response for chromosal aberrations was found (Bhunya & Pati, 1987). This study was however of lower quality, deviating from test guidelines as to the number of animals tested (only 3 per group per time point), the pooling of data from these three animals, and the absence of a positive control. The chromosomal aberrations were predominantly chromatid gaps, and when these were excluded similar results were obtained as for negative controls.

Following non-oral exposure (i.e. via the IP route), two studies showed positive results. Chromosomal aberrations (predominantly chromatid gaps), micronuclei induction and sperm abnormalities were observed in the study by Bhunya & Pati (1987), but as remarked above, this study was of lower quality. Further, the increased incidences of micronuclei coincided with cytotoxic effects in all doses investigated. Agarwal *et al.* (1990) also found chromosomal aberrations, but this study also deviated somewhat from OECD TG 475 (low number of animals tested, no cytotoxicity observed and reported, and scoring of only 50 metaphases (instead of at least 100)). The positive results seem to be in conflict with a third study using the IP exposure route (Tinwell & Ashby, 1990), which showed clearly negative results for micronuclei induction. Deviations which could influence the validity and quality of the results were not observed for this study.

RAC considers the positive *in vitro* UDS tests less relevant given the negative result in the oral *in vivo* UDS test. Without further details available, the weak positive response in the SCE assay cannot be adequately evaluated. RAC nevertheless considers the result of this indicator test less

relevant, given the presence of a negative oral *in vivo* micronucleus test of good quality. Following IP exposure, which bypasses the normal process of copper absorption and distribution, negative and positive results have been observed, but the positive studies included deficiencies which may have affected the reliability of the results.

Although there is insufficient evidence to exclude a (local) genotoxic potential upon non-oral administration, RAC concludes, in line with the dossier submitter, that overall the available data do not support the classification of copper sulphate pentahydrate for germ cell mutagenicity.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

The CLH report refers to 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). 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 are 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 is 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 in humans and animals is that copper is not carcinogenic and that therefore no classification for carcinogenicity is warranted for copper compounds, including copper sulphate pentahydrate.

Comments received during public consultation

No comments were received during public consultation.

Assessment and comparison with the classification criteria

The CLH report includes three longer-term feeding studies in rats (Harrison *et al.*, 1954) for which it was stated that there were no observations of increased tumour incidences. The studies concerned were two 44-week studies with copper sulphate (at doses equivalent to 530 or 1600 ppm copper) and copper gluconate (at a dose equivalent to 1600 ppm copper) and a 104-week study with 0.1, 1 or 3% potassium sodium copper chlorophyllin (equivalent to 53, 530 or 1600 ppm copper). However, only a small number of animals were included in these studies (20-25/sex/group), a number of organs/tissues were not examined, and the duration of treatment in the studies with copper sulphate and copper gluconate was too short for proper evaluation of the carcinogenic potential. For a fourth longer-term study, with administration of 3000 ppm copper as copper sulphate via the diet for 52 weeks to male weanling rats (Haywood & Loughran, 1985), no information on tumour development was given.

The dossier submitter further referred to the repeated dose toxicity studies in rats and mice, but these studies were considered not relevant for carcinogenicity evaluation due to the limited exposure and observation duration and the limited number of animals tested. Nevertheless, no microscopic evidence of (pre)neoplastic lesions was observed in these studies.

Some additional studies where copper was administered together with known carcinogens (*p*-dimethylaminobenzene, acetylaminofluorene, dimethylnitrosamine, dimethyl-benz(α)anthracene) were referred to by the dossier submitter for illustrative purposes only. RAC considers that these studies did not provide data relevant for classification and therefore did not evaluate them.

The available human epidemiological data or information from cohort studies (mining, smelting, refining or alloy industries) do not provide clear evidence for an increased risk of (lung) cancer upon exposure to copper compounds due to e.g. confounders (such as co-exposures to other carcinogenic substances, smoking status), lack of individual exposure data, etc. Also for the occupational disease VSL, with several cases of lung cancer among the patients, the data available do not permit a direct link between lung cancer and exposure to Bordeaux Mixture to be established, among others because smoking habits were not adequately addressed.

For two genetic abnormalities which lead to accumulation of copper (Wilson's disease - accumulation in liver, kidney and brain; Menkes' disease – accumulation in intestinal epithelium, kidney and fibroblasts) there is no evidence for increased incidence of cancer in victims of these diseases, despite the chronic high tissue copper levels.

When looking at all available data, it can be concluded that in the animal studies no increased incidences of tumours were observed and that the human data do not provide conclusive evidence for a direct link between copper exposure and cancer. Whereas the shortcomings in the animal and human data were noted, RAC concludes that there is insufficient evidence to warrant classification of copper sulphate pentahydrate for carcinogenicity.

RAC evaluation of reproductive toxicity

Summary of the Dossier submitter's proposal

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 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, the dossier submitter concluded that no classification for reproductive and developmental effects is warranted for copper compounds, including copper sulphate pentahydrate.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

The reproductive toxicity of copper compounds was studied in several studies in the rat, mouse, rabbit and hamster.

Fertility

No adverse effects on sexual function and fertility were observed in a well-performed 2-generation rat study in which the animals were given diets containing 0, 100, 500, 1000, or 1500 ppm copper sulphate pentahydrate. The only potentially adverse effect seen in this study was a decrease in spleen weight in P1 females and F1 and F2 weanlings of the high dose group in periods that coincided with high food (and thus copper) intake. Two other non-guideline studies, one with copper gluconate in rats (at gavage doses of 0, 3 or 30 mg/kg bw/day) and one with copper sulphate in mice (at 0, 500, 1000, 1500, 2000, 3000 or 4000 ppm in the diet) also did not show adverse effects on fertility parameters. Further, no adverse effects on the reproductive organs were observed in the oral 90-day studies with rats and mice, and from human data there is no evidence for adverse effects of oral exposure to copper through normal diets on pregnancy, parturition, lactation or growth and development. Based on the available data, RAC therefore agrees with the conclusion of the dossier submitter that classification for effects on sexual function and fertility is not required for copper sulphate pentahydrate.

Developmental toxicity

In the key rabbit developmental toxicity study, pregnant rabbits were dosed via gavage with 0, 6, 9 or 18 mg copper/kg bw/day (as copper dihydroxide) on days 7-28 of gestation. The two highest doses resulted in significant maternal toxicity, characterised by marked initial body weight loss (resulting in an overall 31% and 72% lower body weight gain in mid and high dose animals compared to controls) and inappetance (17 and 30% lower food consumption), abortion (2 high dose dams) and death (3 high dose dams). Pups in the high dose group had slightly lower foetal bodyweight (9%) and slightly increased incidences of retarded ossification of skull and pelvic bones. Incidences of retarded sternebral ossification were decreased in the high dose group but were slightly increased in the mid dose group where there was no effect on foetal body weight. Rib alterations occurred at a very high incidence across all groups, including controls. There were no treatment-related foetal malformations. The biological significance of some slightly increased incidences in common skeletal variants is not clear, but it is to be noted that they were observed in the presence of maternal toxicity.

Litter parameters (such as number of pups, viability and lactation index, pup weight) were not affected in the rat 2-generation study with copper sulphate pentahydrate. Two oral (gavage) developmental toxicity studies with 0, 0.1, 3 or 30 mg copper gluconate/kg bw/day in rats (on days 5-15 of gestation) and mice (on days 6-14 of gestation) did not show embryotoxicity or teratogenicity. No differences in external and visceral examination were observed in offspring of rats that received drinking water with copper acetate (increased stepwise to a concentration of 0.185% over a period of 7 weeks) before mating. Skeletal examination showed some delayed ossification in 21.5 day old foetuses but not in new-born pups. Further, the studies using copper exposure via intrauterine devices in rats, rabbits and hamsters did not reveal significant developmental effects.

From human data there is no evidence for adverse effects of oral exposure to copper through normal diets on foetal development and on growth and development of neonates and infants. Further, there is no clear association between copper IUDs and foetal abnormalities in humans.

Overall, RAC concludes that there is insufficient evidence for copper fulfilling the classification criteria for developmental toxicity. RAC therefore supports the conclusion of the dossier submitter that copper sulphate pentahydrate should not be classified for developmental toxicity.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier Submitter's proposal

Copper sulphate pentahydrate has a current harmonised classification under the entry "copper sulphate" (Index No 029-004-00-0). The dossier submitter's (DS) proposal specified an acute M-factor and changed the chronic classification from Aquatic Chronic 1 to Aquatic Chronic 2, based on the following arguments:
The water solubility of 220,000 mg/L exceeds the acute Ecotoxicity Reference Value (ERV) of the dissolved metal ion, so the substance is considered to be a readily soluble metal compound.

For acute aquatic classification, the lowest acute $ERV_{CuSO45H2O}$ of 0.073 mg/L was considered to be below the trigger value of 1 mg/L, the DS concluded the classification as Aquatic Acute 1 is appropriate.

As the lowest acute $ERV_{CuSO45H2O}$ (0.073 mg/L) is above 0.01 mg/L but ≤0.1 mg/L, the DS proposed an acute M-factor of 10.

In order to demonstrate removal from the water column (> 70% removal within 28 days) to assess the "persistence" or lack of degradation of metal ions the DS considered information provided by the copper task force (Rader, 2013). Evidence of rapid removal from the water column was based on the TICKET-Unit World Model (UWM), which describes partitioning to dissolved organic carbon, particulates, etc., deposition and transformation to sulfides in sediment. Together with evidence from field studies, the dossier submitter considered that this provides a satisfactory description of copper ion dynamics, and was therefore of the opinion that more than 70% of dissolved copper (II) ions are removed from the water column within 28 days, i.e. that dissolved copper compounds are rapidly removed. The potential for copper remobilisation from sediment was expected to be limited in oxic and anoxic conditions.

For aquatic chronic classification, the lowest chronic $ERV_{CuSO45H2O}$ is 0.019 mg/L. As the substance is considered to be a readily soluble metal compound and subjected to rapid removal, chronic classification is on the basis of the chronic ERV. Since this is above 0.01 mg/L but ≤0.1 mg/L, the DS proposed a classification as Aquatic Chronic 2. A chronic M-factor is not applicable.

Comments received during public consultation

Six comments were submitted on the environmental part of the DS's proposal, of which two agree but with some observations, and the remaining four provide extensive comments challenging the DS's proposal.

An industry associationpointed to disagreements in the selection and interpretation of ecotoxicity data between the CLH report and the REACH dossier, but agreed with the proposal. Four MSCAs objected to the use of the TICKET-UWM, for several reasons. Among them the fact that the model is designed for shallow lakes (so is not representative of turbulent or flowing systems or circumstances where sediment is not present), it includes significant assumptions about transformation to sulfides, and uses default assumptions for factors (like concentration of the particulate matter) that may vary spatially and temporally. One MSCA pointed out that dissolution data for copper (II) oxide (CuO) show an increase in dissolved copper ion concentrations by a factor of four between day 7 and day 28 at a loading rate of 1 mg/L, which does not suggest rapid transformation to less soluble forms. The lack of an existing international agreement about how to apply the rapid removal concept was also highlighted (including by one other CA, although they did not object to the approach taken). These four CAs therefore indicate that dissolved copper (II) ions should not be considered to be rapidly removed from the aquatic environment, and that the chronic classification should therefore be Aquatic Chronic 1 (M-factor of 1) rather than Aquatic Chronic 2. In response, the dossier submitter agreed that copper (II) ions cannot currently be considered to be rapidly removed from the water column, and proposed changes to the proposed classification accordingly.

In addition, in several comments, MSs requested changes to, or better justification of, the selection of the lowest ecotoxicity data values, since there appeared to be discrepancies between some of the source documents and the way the information was summarised in the CLH report. Some of the differences were related to the use of geometric means rather than the lowest value

for a species, and in other cases it was due to uncertainties about whether the cited data referred to the compound itself or to the metal ion. Furthermore one CA pointed out that it may be appropriate to apply the surrogate approach, since there is no chronic test result available for the most sensitive species (*Pimephales promelas*) in the acute tests. In addition, the same CA noted that there are data on other invertebrate species and it was not clear why these were not included in the CLH report. Moreover, considering the amount of ecotoxicological data available for copper, it was proposed to use the species sensitivity distribution (SSD) curve for each trophic level for both short and long-term effects.

Another MSCA suggested that an explicit statement should be included that nano-forms should be considered separately.

Assessment and comparison with the classification criteria

Substance identity: The DS proposed to have a separate entry for copper sulphate and its pentahydrate form for human health endpoints, consequently the environmental classification should also be based on the molecular weight (MW) of the pentahydrate. However, if the foreseen Annex VI entry is meant to cover both hydrated and anhydrous forms of the substance, the more precautionary approach of basing the calculations on the MW of the anhydrous form should be adopted. For the purposes of this opinion, the MW of $CuSO_4 5H_2O$ will be used.

Water solubility: The CLH report does not present transformation/dissolution data for $CuSO_4 5H_2O$ over different timescales, pH values or loading rates. RAC notes that such data do not exist according to the industry comments submitted during public consultation, so in its absence the available water solubility data were used. Section 1 of the CLH report indicates that the water solubility value is 220,000 mg/L at 25 °C (no pH specified).

Degradability:

Rapid removal: RAC considers that the TICKET-UWM 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 default parameters has not (yet) been resolved, especially as some properties are likely to vary spatially and temporally. For example, comparison with monitoring data in the CLH report suggested 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 DS's proposal also relied 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 DS's proposal did 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 was demonstrated to be irreversible under all relevant circumstances. At a general level, RAC considers that decisions about rapid removal could be based on observations from a standardised OECD Transformation/ Dissolution test. In this case, T/D studies showed increasing concentrations of copper ions over 28 days (not a decline), indicating that copper (II) ions remained in solution under these test conditions.

In conclusion, RAC considers that copper (II) ions are not subject to rapid environmental transformation for the purposes of classification and labelling.

Bioaccumulation

The bioaccumulation behaviour of copper (II) ions in organisms should consider both essentiality and homeostatic mechanisms. The DS's proposal did not present a clear description of the available data for comparison with the CLP criteria. However, in view of the degradability conclusion, this end-point does not influence the determination of the chronic M-factor and so was not considered further.

Ecotoxicity

<u>*Choice of ecotoxicity data*</u>: The ecotoxicity database for copper (II) ions is extensive, with many studies of acute and chronic toxicity in fish, invertebrates and algae/higher plants using a variety of copper compounds at different pH values as well as hardness and dissolved organic carbon (DOC) levels. The two principal sources of information cited in the DS's proposal are the pesticide DAR and the vRAR (2008). RAC considers that the chronic ecotoxicity information in the vRAR is generally reliable for hazard assessment as it was evaluated in depth by the relevant industry experts and reviewed by the pre-REACH CAs[1]. However, Tables 1-3 in Annex 1 (section "Additional key elements") show that the presentation of ecotoxicity information in these sources is inconsistent (presumably due to differences in data aggregation as pointed out in the public comments). This is considered further below:

a) Given the large number of studies for individual species, the data in the CLH report were aggregated to present single values for each species in three different pH bands. The CLP Guidance for metals recommends transformation/dissolution testing at different pHs, so RAC agrees that grouping into pH bands is appropriate as there is a clear trend in toxicity that would be overlooked if all the data for a species were combined. However, the reasons for the choice of the actual pH bands were not explained, and the effects of hardness and DOC were not discussed.

b) The dossier submitter's proposal used geometric means even if there are only two data points for a species in a particular pH band. This is not consistent with the CLP Guidance (which indicates that at least four data points are preferred) or the REACH CSRs, and led to discrepancies between the data sets, which were noted during public consultation.

c) For invertebrates, data were presented for only two species of crustacean (*Daphnia magna* and *Ceriodaphnia dubia*). RAC notes that it is standard practice to consider all relevant data from reliable standard test guideline studies, and so the dossier submitter's proposal was not necessarily based on a comprehensive data set. The dossier submitter did not provide any additional information in response to the public consultation comments on this issue. However, RAC notes that the vRAR (2008) contains long-term toxicity data for several other invertebrate taxonomic groups (including molluscs and insects) as well as higher plants (*Lemna minor*). Further details are provided in Annex 1 under "Additional key elements".

    i) In the vRAR (2008), all the reliable chronic NOEC data were compiled in a species sensitivity distribution, deriving a hazardous concentration for 5% of the species (HC_5) (with the 50^{th} percentile confidence interval) of 7.3 µg/L (6.1-7.9 µg/L) based on the best fitting approach, or 6.1 µg/L (3.7-8.6 µg/L) using the log normal curve fitting. These values are very similar to the lowest NOEC in the dataset (6.0 µg/L for the mollusc *Juga plicifera*).

    ii) Due to the variation in physico-chemical conditions used in the tests, in the vRAR (2008) the data were also 'normalised' using a biotic ligand model. The

[1] 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.

lowest normalised NOEC is 5.3 µg/L for the rotifer *Brachionus calyciflorus* (at pH 8.1, hardness of 165 mg/L $CaCO_3$ and DOC of 3.2 mg/L). The lowest HC_5-50 derived for an ecoregion is 7.8 µg/L (4.4-11.7 µg/L).

iii) RAC notes that the CLH report also mentioned a NOEC of 3.12 µg/L (as copper) from an indoor microcosm study using copper hydroxide, without specifying the measured end-point or study duration; it was also pointed out, in comments during the public consultation, that in the final EFSA conclusion a NOEC of 4.8 µg/L is cited which was used for the overall risk assessment for aquatic organisms. As it was not clear how this information would be used in hazard classification, it was not considered further.

In summary, the lowest long-term NOEC reported in the CLH report is 7.4 µg/L for *Ceriodaphnia dubia* at pH 6.5-7.5. The omission of data for other invertebrate groups from the DS's proposal does not appear to make a significant difference as the most sensitive data all lie in the range 1-10 µg/L.

Discrepancies in the ecotoxicity data as presented: The lowest acute toxicity value selected in the CLH report was 0.029 mg/L (29 µg/L) at pH 5.5-6.5, giving the source as the vRAR. The origin of this data point is unclear, but RAC assumes that it relates to data for *O. mykiss* (a similar value was obtained with *Ceriodaphnia dubia* at pH >7.5-8.5). 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 (cited as coming from the vRAR – an actual study reference was not provided). This is based on two values, both for larval fish, 15.0 µg/L and 4.4 µg/L. During PC, 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 TG 236) 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 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 a 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), 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 therefore 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 were used the consequence for classification would be the same for the pentahydrate (though not the anhydrous form)). 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.

As noted above, the lowest reported long-term NOEC in the CLH report is 7.4 µg/L for *Ceriodaphnia dubia* at pH 6.5-7.5, and this value is consistent with the large amount of chronic data presented in the vRAR (2008), including the HC_5. However, this is almost identical to the acute LC_{50} for *P. promelas* at pH 5.5-6.5, and there are no measured chronic toxicity data for any fish species in the pH range of 5.5-6.5. Consequently, the adequancy of the long-term study results was questioned. At first sight it might seem disproportionate to consider the whole long-term fish toxicity data set (n=29) as 'non-adequate'. However, the acute fish test data clearly show that for the three species for which data across the total pH range of 5.5-8.5 are available, the toxicity is the highest in the lowest pH range, i.e., 5.5-6.5. Therefore, despite the large number of fish studies used in the dossier submitter's proposal, RAC believes that it is appropriate to consider the surrogate method for the fish trophic group (as was suggested in one of the public consultation comments). [N.B. The CLP criteria and guidance do not address this specific issue, but Example D in Section 4.1.3.4.4 of the CLP guidance is comparable to some extent. It describes a substance with a large data set, for which acute as well as chronic toxicity data are available for all three trophic levels. For crustacea, chronic data are available for *Daphnia magna*, which is clearly the least sensitive of the invertebrate species for which acute data are available. Hence, according to the guidance, the chronic aquatic toxicity data for *D. magna* in this case should be considered not in conformity with the definition of 'adequate chronic data'.]

In addition, it was noted in comments received during the public consultation that in the biocide Assessment Report for copper (II) hydroxide (Product type 8, RMS France, September 2011) the lowest reported NOEC is 2.2 µg Cu/L for growth in the fish *Oncorhynchus mykiss*. This appears to be aggregated in the CLH report with three other studies for this species in the pH 6.5-7.5 band, so that the geometric mean is 16.1 µg/L. RAC considers that this is acceptable, although as noted above, it does appear that some fish studies provide acute LC_{50}s in the range 1-10 µg/L. Similarly, it was indicated in comments received during public consultation that in the DAR for copper hydroxide, a 92-d NOEC of 1.7 µg/L was obtained in a fish early life stage test for *O. mykiss* at pH 8.0 (cited as Schäfers, 2000). This result does not appear to have been taken into account in the data aggregation used in the dossier submitter's proposal. A third reliable chronic result for this species in the pH range >7.5-8.5 was included in the CLH report (NOEC 16 µg Cu/L). Comments by industry following the public consultation raised some issues about the reliability of the lower value of 1.7 µg/L (e.g. the reported copper concentrations were highly variable in this study and the test substance was a formulation containing 10% w/w dispersant and also an adhesive). Whilst toxicity is still likely to have been driven by copper ions, the composition might have had some influence. It is also sparingly soluble, rather than a soluble salt. This result is therefore not used directly but is considered by RAC as supporting information for chronic classification purposes.

ERV derivation: The lowest acute $L(E)C_{50}$ (as dissolved copper) presented in the CLH report is 8.1 µg/L for *P. promelas* at pH 5.5-6.5. The acute $ERV_{CuSO45H2O}$ is therefore equal to 0.032 mg/L [{acute ERV of metal ion x molecular weight of the metal compound/(atomic weight of the metal x number of metal ions)}, so 0.0081 x 249.6/(63.5 x 1)]. This is lower than the acute $ERV_{compound}$ proposed in the CLH report (0.073 mg/L), which is based on a different acute toxicity value, although also uses the molecular weight for the anhydrous salt, which is incorrect (the $ERV_{compound}$ should be 0.11 mg/L based on an LC_{50} of 0.029 mg/L and the molecular weight of the pentahydrate).

The lowest long-term NOEC (as dissolved copper) presented in the CLH report is 7.4 µg/L for *Ceriodaphnia dubia* at pH 6.5-7.5. The chronic ERV for $CuSO_45H_2O$ is equal to 0.029 mg/L [{chronic ERV of metal ion x molecular weight of the metal compound/(atomic weight of the metal x number of metal ions)}, so 0.0074 x 249.6/(63.5 x 1)]. This is higher than the chronic $ERV_{compound}$ proposed in the CLH report (0.019 mg/L) since the latter value uses an incorrect molecular weight (as for the acute $ERV_{compound}$). As noted in Annex 1, other apparently reliable NOEC data exist that are lower than this value, but still in the range 1-10 µg/L (e.g. a normalised NOEC of 5.3 µg/L for the rotifer *Brachionus calyciflorus* at pH 8.1, hardness of 165 mg/L $CaCO_3$ and DOC of 3.2 mg/L). They will therefore make only a very small difference to the ERV. However, there are no chronic toxicity data for the fish species that is acutely most sensitive at pH 5.5-6.5, so the surrogate method for the fish trophic group is therefore considered.

Comments received during public consultation on the related substances Bordeaux mixture, copper dihydroxide and dicopper oxide specifically suggested that the 92-d NOEC of 1.7 µg Cu/L for *O. mykiss* (obtained with copper hydroxide) should be used as the basis for the chronic classification. As already noted, this value is the same order of magnitude as the other sensitive chronic data, but it would lead to a lower chronic ERV of 0.007 mg/L for $CuSO_45H_2O$. RAC notes that this result was obtained at pH 8, for which only one other value is available for this species in that pH range. Since aquatic toxicity appears to generally increase as the pH is lowered, the implication is that the selected chronic data set might not be sufficiently sensitive. This value is therefore considered alongside the surrogate method.

Acute aquatic hazard:

The water solubility (220,000 mg/L, apparently at all pHs) exceeds the acute ERV of the dissolved metal ion (0.0081 mg Cu/L based on the *P. promelas* data), so the substance is considered to be a readily soluble metal compound. Classification is therefore on the basis of the acute $ERV_{CuSO45H2O}$ (0.032 mg/L), and as this is below 1 mg/L, RAC agrees to classify copper sulfate pentahydrate as **Aquatic Acute 1** (H400) . As the lowest acute $ERV_{CuSO45H2O}$ is above 0.01 mg/L but ≤0.1 mg/L, the **acute M-factor is 10**.

Chronic aquatic hazard:

As the substance is considered to be a readily soluble metal compound, classification may be based on the lowest chronic ERV (0.029 mg/L based on data for *Ceriodaphnia dubia*). Since this is below 0.1 mg/L and the substance not subject to rapid environmental transformation, RAC agrees to classify copper sulfate pentahydrate as **Aquatic Chronic 1** (H410). As the chronic $ERV_{CuSO45H2O}$ is above 0.01 mg/L but ≤ 0.1 mg/L, the chronic M-factor would be 1 for a substance not subject to rapid environmental transformation. However, using the surrogate method for the fish trophic group, the chronic M-factor should be consistent with the **acute M-factor, i.e. 10**.

In summary, RAC agrees with the DS's proposal to classify copper sulphate pentahydrate as **Aquatic Acute 1 (H400)** but considers that a more stringent classification as **Aquatic Chronic 1 (H410)** is required than originally proposed (Aquatic Chronic 2 (H411)), because of the conclusions on rapid environmental transformation as well as on the most sensitive fish toxicity data. The resulting classification for copper sulphate pentahydrate is the same as the existing harmonised classification for copper sulphate, but clarifies that the M-factor applies to both acute and chronic effects. The classification is based on a MW of 249.6 g/mol and the presence of 1 copper atom per molecule.

RAC suggests that the harmonised classification for anhydrous copper sulphate ($CuSO_4$) (CAS no. 7758-98-7) could also be clarified accordingly (the difference in molecular weight does not affect the acute or chronic ERVs significantly).

Nano-forms should be considered separately.

Additional references

Additional references not included in the CLH report

European Copper Institute 2008. Appendix K1 in Voluntary Risk Assessment of copper, copper II sulphate pentahydrate, copper(I)oxide, copper(II)oxide, dicopper chloride trihydroxide. European Copper Institute (ECI). Available at (19/09/2014): http://echa.europa.eu/fi/copper-voluntary-risk-assessment-reports/-/substance/474/search/+/term

Mount, D. (1973). Chronic Effect of Low pH on Fathead Minnow Survival, Growth and Reproduction. Water Research, 7, 987-993.

Zischke, J.A., Arthur J.W., Nordlie K.J., Hermanutz R.O., Standen D.A., and Henry T.P. (1983). Acidification effects on macroinvertebrates and fathead minnows (*Pimephales promelas*) in outdoor experimental channels. Water Research, 17, 47- 63.

ANNEXES:

Annex 1 Background Document (BD) gives the detailed scientific grounds for the opinion. The BD is based on the CLH report prepared by the Dossier Submitter; the evaluation performed by RAC is contained in RAC boxes.

Annex 2 Comments received on the CLH report, response to comments provided by the Dossier Submitter and by RAC (excl. confidential information).