Committee for Risk Assessment

RAC

Opinion

proposing harmonised classification and labelling
at EU level of

Bordeaux mixture; Reaction products of copper sulphate with calcium dihydroxide

EC number: -
CAS number: 8011-63-0

CLH-O-0000001412-86-36/F

Adopted

4 December 2014

04 December 2014

CLH-O-0000001412-86-36/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 Regulation (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: Bordeaux mixture; Reaction products of copper sulphate with calcium dihydroxide

EC number: -

CAS number: 8011-63-0

The proposal was submitted by **France** and received by the RAC on **13 December 2014.**

In this opinion, all classifications are given firstly 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 **Bordeaux mixture; Reaction products of copper sulphate with calcium dihydroxide** 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	n/a										
Dossier submitters proposal	029-RST-00-Y	Bordeaux mixture; Reaction products of copper sulphate with calcium dihydroxide	-	8011-63-0	Acute Tox. 4 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 2	H332 H318 H400 H411	GHS07 GHS05 GHS09 Dgr	H332 H318 H410		M=10	
RAC opinion	029-022-00-9	Bordeaux mixture; Reaction products of copper sulphate with calcium dihydroxide	-	8011-63-0	Acute Tox. 4 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H332 H318 H400 H410	GHS07 GHS05 GHS09 Dgr	H332 H318 H410		M=10 M=10	
Resulting Annex VI entry if agreed by COM	029-022-00-9	Bordeaux mixture; Reaction products of copper sulphate with calcium dihydroxide	-	8011-63-0	Acute Tox. 4 Eye Dam. 1 Aquatic Acute 1 Aquatic Chronic 1	H332 H318 H400 H410	GHS07 GHS05 GHS09 Dgr	H332 H318 H410		M=10 M=10	

SCIENTIFIC GROUNDS FOR THE OPINION

RAC general comment

In addition to Bordeaux mixture, ECHA received CLH proposals for nine other copper compounds or forms of copper from the same dossier submitter (France). 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. 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, sometimes also other copper compounds. The sections on acute toxicity, skin irritation/corrosion, eye damage/irritation and sensitisation in the CLH reports are specific for each substance/form.

RAC considered the dossier submitter's proposal to group the substances together for consideration of STOT RE and the CMR endpoints. RAC noted 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, whereas RAC concluded that these would need a more detailed analysis. As none of the studies with bordeaux mixture or the other tested copper substances yielded positive evidence for the classification for these endpoints, RAC did not pursue the aspect of grouping the nine substances any further.

RAC evaluation of physical hazards

Summary of the Dossier submitter's proposal
Bordeaux mixture is a reaction product of stable inorganic salts and minerals with copper in a high oxidation state. Tests indicate it is not flammable, pyrophoric, explosive or oxidising (Van Beijnen, 2000). The dossier submitter proposes 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 Bordeaux mixture does not have explosive or oxidising properties and is 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
Two rat oral acute toxicity studies are included in the report, both conducted according to OECD TG 401 (Griffon, 2000a and Jackson, 1994b). Combined (male and female) LD_{50} values were 2437 mg/kg bw and 2302 mg/kg bw, respectively, with very little sex difference. Two rat acute inhalation studies, conducted according to OECD TG 403, showed lowest LC_{50} values in males of 1.97 mg/L (whole-body) and 3.98 mg/L (nose only), respectively. No mortality was seen in females tested (Merkel, 2001; Anderson and Stewart, 2002) although in Merkel (2001), only one dose was tested for female animals. Animals were exposed to a dust atmosphere for four hours.

The two rat acute dermal toxicity studies (Griffon, 2000b and Jackson, 1994d) reported both determined LD_{50} values at above 2000 mg/kg bw. As the LC_{50} inhalation values for males lie within the range of 1.0 - 5.0 mg/l (dusts/mists), the dossier submitter proposed classification as Acute Tox. 4 – H332 for acute inhalation toxicity. Since all LD_{50} values for the oral and dermal route lie above the guidance values for classification, no classification was proposed by the dossier submitter.

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

Two MSCAs and one trade organisation expressed their general agreement with the classification proposed.

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 Bordeaux mixture should be classified for acute inhalation toxicity with **Acute Tox. 4 – H332**. RAC also concludes that the available oral and dermal LD_{50} values do not warrant classification for acute oral and dermal 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 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 with copper sulphate 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 oral studies, treated animals showed a variety of clinical signs such as hypoactivity, dyspnoea, piloerection, stiff gait, sedation, soft faeces, lateral recumbence and swollen abdomen, red nasal and ocular discharge, pale and hunched appearance, ataxia, diarrhoea, laboured breathing, prostration, hypothermia, alopecia and emaciation. These effects are considered to be indicative of general, non-specific toxicity, just like the most frequently observed symptoms in human self-poisoning cases (nausea, epigastric burning, vomiting, diarrhoea).
In the acute dermal toxicity studies, no specific clinical signs or abnormalities were observed except for red nasal discharge in all females two and four hours after dosing.
Based on the oral and dermal acute toxicity studies, there is no clear evidence of specific toxic effects on a target organ or tissue at dose levels fulfilling the classification criteria and no signs of narcotic effects.
In the acute inhalation studies, some effects on the respiratory tract were observed. These effects included irregular breathing, dyspnoea, laboured and slow respiration. Surviving animals recovered in both studies by day 5. In animals that died during the study, lung abnormalities were observed (edema of the lungs, discoloration of the lungs and mucus filled trachea, spongy, dark or mottled lungs). Further, lung weights and lung:body weight ratios were increased. These

respiratory effects occurred mostly at lethal dose levels, and for lethality the substance is already proposed to be classified. Further, the transient signs at the non-lethal levels are indicative of non-specific, general acute toxicity. RAC supports the conclusion of the dossier submitter that Bordeaux mixture should not be classified for specific target organ toxicity – single exposure (STOT SE).

RAC evaluation of skin corrosion/irritation

Summary of the Dossier submitter's proposal

One OECD TG 406 compliant rabbit skin irritation study is included in the CLH report (Jackson, 1994f). Bordeaux mixture caused no oedema or erythema and average scores were all 0. The dossier submitter concluded no classification for skin irritation or corrosion is warranted.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Given that all three test-animals scored zero for both erythema and oedema over 24-72h in the available skin irritation study, RAC agrees with the conclusion of the dossier submitter that Bordeaux mixture should not be classified for skin irritation.

RAC evaluation of eye damage/irritation

Summary of the Dossier submitter's proposal

Two OECD TG 405 compliant rabbit eye irritation studies are included in the CLH report. Average Draize scores from 24, 48 and 72h are reported in the table below.

	Corneal opacity	**Iritis**	**Conjuctival redness**	**Conjuctival chemosis**
Griffon (2000c)	1.67, 4, 2	1, -, 1 Assessment obscured in one animal at all time points	2.33, 3, 3	3, 4, 2.67
Jackson (1994g)	1, 2, (4)*	1, 1, (1)#	1.33, 1, (1.5)*	0.67, 1, (2)*

assessment obscured (by corneal opacity) in one animal at time point 24h; animal sacrificed prior to 72h assessment
* animal sacrificed prior to 72h assessment

In at least one study, 2 out of 3 animals had conjuctival redness and chemosis scores ≥ 3, and 3 out of 3 animals had corneal opacity score ≥ 1. In the Griffon (2000c) study, the conjunctival effects (redness and chemosis) persisted until the end of the observation period of 21 days in one animal. In the Jackson (1994g) study, corneal opacity was still present in one animal at the end of the observation period (day 21). The dossier submitter therefore proposed classification as Eye Dam. 1 – H318.

Comments received during public consultation

Two MSCAs and one trade organisation expressed their general agreement with the classification proposed.

Assessment and comparison with the classification criteria

Bordeaux mixture caused eye irritation in the available eye irritation studies. Some effects persisted until day 21 in both studies.

As some effects were shown to be irreversible (corneal opacity in one animal, and conjunctival effects (chemosis and redness) in a second animal), RAC concludes that Bordeaux mixture should be classified for irreversible eye irritation (**Eye Dam. 1 - H318**).

RAC evaluation of skin sensitisation

Summary of the Dossier submitter's proposal

One guinea pig maximisation test (GPMT), conducted with Bordeaux mixture according to OECD TG 406, is included in the CLH report (Griffon, 2000d). Intradermal and topical induction doses were 0.1% (w/w) and 30% (w/w) at days 1 and 7, respectively. Prior to topical induction, animals were treated with 10% (w/w) sodium lauryl sulphate to induce irritation. Animals were challenged with 30% (w/w) at day 21. At 24h after the challenge, no reactions were seen in any of the tested (n=20) and control (n=10) animals. After 48h, positive reactions were seen in 4/20 tested and 0/10 control 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. As positive reactions were seen in less than 30% of tested animals and cases of allergic reactions to copper compounds in humans are extremely rare, the dossier submitter concluded that no classification for skin sensitisation for Bordeaux mixture is warranted.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

Given that in the GPMT at 24h no positive response was observed and at 48h a positive response was observed in 20% of the animals (4 out of 20 animals) at a 0.1% intradermal induction dose (*i.e.* below the classification threshold), and the few individual cases cases of allergic reactions in humans, RAC agrees with the conclusion of the dossier submitter that Bordeaux mixture 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

Data on Bordeaux mixture is limited to one study 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 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 causing 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

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

RAC notes that data on copper dihydroxide do not seem to warrant classification, but remarks that data is limited to one dermal animal study of relatively short duration (3 weeks) only. The CLH report further contains data on other copper compounds (predominantly copper sulphate pentahydrate), from which the dossier submitter proposed to read-across to Bordeaux mixture. 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 Bordeaux mixture.

RAC evaluation of germ cell mutagenicity

Summary of the Dossier submitter's proposal

Apart from one (negative) Ames test, RAC notes that no data on Bordeaux mixture are available. 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 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 Bordeaux mixture.

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 upon oral administration, but that there is insufficient evidence to exclude a (local) genotoxic potential upon non-oral administration.

Assessment and comparison with the classification criteria

Apart from one (negative) Ames test, RAC notes that no data on Bordeaux mixture 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 Bordeaux mixture. 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 Bordeaux mixture.

RAC evaluation of carcinogenicity

Summary of the Dossier submitter's proposal

No data on Bordeaux mixture 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 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 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 Bordeaux mixture.

Comments received during public consultation

No comments were received during the public consultation.

Assessment and comparison with the classification criteria

RAC notes that no data are available on Bordeaux mixture. The CLH report contains some data on other copper compounds (among which copper sulphate pentahydrate), from which the dossier submitter proposed to read-across to Bordeaux mixture. 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 Bordeaux mixture.

RAC evaluation of reproductive toxicity
Summary of the Dossier submitter's proposal

No data on Bordeaux mixture 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 investigating the reproductive toxicity of other copper compounds, as well as some human data.

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

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

Assessment and comparison with the classification criteria
RAC notes that no data on copper thiocyanate 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 Bordeaux mixture. 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 Bordeaux mixture.

ENVIRONMENTAL HAZARD ASSESSMENT

RAC evaluation of environmental hazards

Summary of the Dossier Submitter's proposal

Bordeaux mixture is the undefined reaction product of copper sulphate with calcium dihydroxide. This substance does not have a current harmonised classification. The DS's proposal is based on the following arguments:

The water solubility of Bordeaux mixture (2.2 mg/L at pH 6.8 and 1.1 mg/L at pH 9.8) exceeds the acute ERV of the dissolved metal ion. Taking into account the recommendations of the CLP guidance[1], this compound is considered to be a readily soluble metal compound for classification purposes.

For aquatic acute classification, the lowest acute Ecotoxicity Reference Value (acute $ERV_{Bordeaux\ mixture}$ 0.10 mg/L) was considered to be below the trigger value of 1 mg/L, the DS concluded the classification as Aquatic Acute 1 (H400) is appropriate.

As the lowest acute $ERV_{Bordeaux\ mixture}$ (0.10 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 DS proposed that rapid removal of Bordeaux mixture from the water column can be demonstrated. The lowest chronic $ERV_{Bordeaux\ mixture}$ (0.025 mg/L) is above 0.01 mg/L but ≤0.1 mg/L, hence the DS concluded that classification as Aquatic Chronic 2 (H411) is appropriate for a substance subject to rapid removal. 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 one commenter agreed without further comment, one agreed but with some observations, and five commenters provided extensive comments challenging the DS's proposal to apply the rapid removal concept in the assessment.

An industry association pointed 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 indicated 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 or 10) 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

[1] CLP Guidance… ECHA Guidance on the Application of the CLP criteria (version 4.0 November 2013)

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

Water solubility: Transformation/dissolution data for Bordeaux mixture over different timescales, pH values or loading rates were not included in the CLH report. RAC notes that such data do not exist according to the industry comments submitted during public consultation, so in their absence the available water solubility data have been used. In section 1 of the CLH report, several water solubility values were included; >124,000 mg/L (>33,100 mg/L as dissolved copper) at pH 2.9, 2.20 mg/L (0.587 mg/L as dissolved copper) at pH 6.8 and ≤1.1 mg/L (≤0.294 mg/L as dissolved copper) at pH 9.8 (at 20 °C).

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 (under 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 lowest normalised NOEC is 5.3 µg/L for the rotifer *Brachionus calyciflorus* (at

[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.

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.

Discrepencies in the ecotoxicity data as presented: The lowest acute toxicity value selected in the CLH report is 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). However, the lowest geometric mean $L(E)C_{50}$ 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 is 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, 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 Bordeaux mixture). 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. 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 in the lowest pH range of 5.5-6.5 is highest. Therefore, despite the large number of fish studies used in the DS's proposal, RAC believes that it is appropriate to consider the surrogate method for the fish trophic group (as was suggested by 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 indicated in comments received during public consultation that in the DAR for copper hydroxide, a 92-d NOEC of 1.7 µg Cu/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 DS's proposal. There is only one other reliable chronic result for this species in the pH range >7.5-8.5 according to the CLH report (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 was still likely to have been driven by copper ions, the composition might have had some influence. The tested substance is also sparingly soluble, rather than a soluble salt. This result was therefore not used directly but was considered 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 $ERV_{Bordeaux\ mixture}$ is therefore equal to 0.028 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 878.7/(63.5 x 4)]. This acute ERV is lower than that proposed in the CLH report (0.1 mg/L), which is based on a different acute toxicity value. In one comment received during public consultation it was suggested that the lowest acute toxicity data from the Bordeaux mixture DAR (an E_rC_{50} of 0.041 mg Cu/L for *Selenastrum capricornutum*) should be used to set an acute ERV of 0.14 mg/L. RAC notes that in general terms, algae are not the most sensitive trophic group when the whole copper database is considered, and so this approach might not be sufficiently precautionary. Despite the uncertainties in the way the acute ERV has been derived in this opinion, the value of 0.028 mg/L is preferred.

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 $ERV_{Bordeaux\ mixture}$ is equal to 0.0256 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 878.7/(63.5 x 4)]. 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). Even if considered, 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.

In comments received during public consultation on Bordeaux mixture and the related substances copper dihydroxide and dicopper oxide it was 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 in the same order of magnitude as the other sensitive chronic data, but it would lead to a lower chronic ERV of 0.006 mg/L for Bordeaux mixture. 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.

Note: In the CLH report there was no discussion about the toxicity arising from the hydroxide counter ion, which might cause effects due to pH changes. In the absence of data, it is assumed by RAC that the copper ions dominate aquatic toxicity and that the classification can therefore be based on the metal ion only.

Acute aquatic hazard:
The water solubility (>33,100 mg/L at pH 2.9, 0.587 mg/L at pH 6.8 and ≤0.294 mg/L at pH 9.8, all as dissolved copper) exceeds the acute ERV of the dissolved metal ion (0.0081 mg/L based on the *P. promelas* data or 0.029 mg/L based on the value selected in the CLH report), so the substance is considered to be a readily soluble metal compound. RAC agrees to classify Bordeaux mixture as **Aquatic Acute 1 (H400)** on the basis of the acute $ERV_{Bordeaux\ mixture}$ (0.028 mg/L). As the acute $ERV_{Bordeaux\ mixture}$ 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 $ERV_{Bordeaux\ mixture}$ (0.0256 mg/L based on data for *Ceriodaphnia dubia*). Since this is below 0.1 mg/L, classification as **Aquatic Chronic 1 (H410)** is appropriate for a substance not subject to rapid environmental transformation, based on RAC conclusion on rapid removal from the environment. As the chronic $ERV_{Bordeaux\ mixture}$ 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 Bordeaux mixture as **Aquatic Acute 1 (H400)** with an **acute M-factor** of **10** but considers that a more stringent chronic classification (**Aquatic Chronic 1 (H410); chronic M-factor 10**) is required than originally proposed (Aquatic Chronic 2 (H411)), because of the conclusion on rapid environmental transformation as well as the most sensitive fish toxicity data. The classification is based on a MW of 878.7 and the presence of 4 copper atoms per molecule.

Additional references

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

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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).