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Safety and efficacy of dicopper oxide as feed additive for all animal species

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)

Abstract

The compound under assessment, dicopper oxide, contains ≥ 86% copper (Cu). Dicopper oxide is a safe source of copper for chickens for fattening. This conclusion is extended to all animal species/categories provided the maximum authorised copper content in feed is respected. There is no indication that the toxicity of dicopper oxide is essentially different from that described for divalent copper. As dicopper oxide is used as a substitute for other copper-containing additives and its bioavailability is in the range of copper sulphate, the standard copper-containing additive, no influence of the use of dicopper oxide in animal nutrition on the copper content of food of animal origin is expected. The EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel) concludes that the use of dicopper oxide in animal nutrition is of no concern for consumer safety. Dicopper oxide is not an irritant to skin but a moderate irritant to the eye. Owing to the nickel content in the additive, it should be considered as a dermal and respiratory sensitiser. Dicopper oxide poses a risk to users upon inhalation exposure. There was no available evidence suggesting that dicopper oxide would pose additional risks to the environment than the other sources of copper already authorised. The substitutive use of dicopper oxide for other copper compounds would therefore not change the previous conclusion of the Panel concerning safety from the environment. Based on the results of a tolerance study in chickens for fattening and a short-term bioavailability study, the FEEDAP Panel concluded that dicopper oxide is an effective source of copper for all animal species.

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Summary

Following a request from the European Commission, the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) was asked to deliver a scientific opinion on the safety and efficacy of dicopper oxide (Cu_2O or copper(I) oxide) for all animal species.

The compound under assessment, dicopper oxide, contains $\geq$ 86% Copper (Cu).

Dicopper oxide is a safe source of copper for chickens for fattening. This conclusion is extended to all animal species/categories provided the maximum authorised copper content in feed is respected.

There is no indication that the toxicity of dicopper oxide is essentially different from that described for divalent copper. As dicopper oxide is used as a substitute for other copper-containing additives and its bioavailability is in the range of copper sulphate, the standard copper-containing additive, no influence of the use of dicopper oxide in animal nutrition on the copper content of food of animal origin is expected. The FEEDAP Panel concludes that the use of dicopper oxide in animal nutrition is of no concern for consumer safety.

Dicopper oxide is not an irritant to skin but a moderate irritant to the eye. Owing to the nickel content in the additive, it should be considered as a dermal and respiratory sensitiser. Dicopper oxide poses a risk to users upon inhalation exposure.

There was no available evidence suggesting that the dicopper oxide would pose additional risks to the environment than the other sources of copper already authorised. The substitutive use of dicopper oxide for other copper compounds would therefore not change the previous conclusion of the Panel concerning safety from the environment.[1]

Based on the results of a tolerance study in chickens for fattening and a short-term bioavailability study, the FEEDAP Panel concluded that dicopper oxide is an effective source of copper for all animal species.

The FEEDAP Panel noted a discrepancy between the measured lead content in the additive, exceeding legal threshold, but considered safe.

[1] Potential risks to soil organisms have been identified after the application of piglet manure; there might be a potential concern related to sediment contamination with copper. Drawing final conclusions would need further model validation and refinement to the assessment of copper-based additives in livestock. The use of copper compounds in aquaculture is not expected to pose a risk. The extent to which copper-resistant bacteria contribute to the overall antibiotic resistance situation cannot be quantified at present.

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1. Introduction

1.1. Background and Terms of Reference

Regulation (EC) No 1831/2003[2] establishes the rules governing the Community authorisation of additives for use in animal nutrition. In particular, Article 4(1) of that Regulation lays down that any person seeking authorisation for a feed additive or for a new use of a feed additive shall submit an application in accordance with Article 7.

The European Commission received a request from the company Animine[3] for authorisation of the product dicopper oxide, when used as a feed additive for all animal species (category: nutritional additives; functional group: compounds of trace elements).

According to Article 7(1) of Regulation (EC) No 1831/2003, the Commission forwarded the application to the European Food Safety Authority (EFSA) as an application under Article 4(1) (authorisation of a feed additive or new use of a feed additive). EFSA received directly from the applicant the technical dossier in support of this application. The particulars and documents in support of the application were considered valid by EFSA as of 15 December 2014.

According to Article 8 of Regulation (EC) No 1831/2003, EFSA, after verifying the particulars and documents submitted by the applicant, shall undertake an assessment in order to determine whether the feed additive complies with the conditions laid down in Article 5. EFSA shall deliver an opinion on the safety for the target animals, consumer, user and the environment and on the efficacy of the product dicopper oxide, when used under the proposed conditions of use (see Section 3.1.6).

1.2. Additional information

Copper is an essential trace element for humans and animals and it is present in all organs and cells. It is involved in numerous biological processes, primarily as an integral part of enzymes (see Prohaska, 2006; Suttle, 2010; Ellingsen et al., 2015).

The feed additive *Dicopper oxide* has not been previously authorised in the European Union (EU). Several other copper compounds are authorised in the EU to be used as nutritional feed additives (trace elements): cupric acetate, monohydrate; basic cupric carbonate, monohydrate; cupric chloride, dihydrate; cupric methionate; cupric oxide; cupric sulphate, pentahydrate; cupric chelate of amino acids hydrate; copperlysine sulphate[4]; cupric chelate of glycine, hydrate[5]; copper chelate of hydroxy analogue of methionine[6]; dicopper chloride trihydroxide[7]; copper bilysinate.[8]

The Scientific Committee on Animal Nutrition (SCAN) delivered reports on the use of copper methionate for pigs (European Commission, 1981), copper compounds in feedingstuffs (European Commission, 1982) and in feedingstuffs for pigs (European Commission, 1983) and the use of copper in feedingstuffs (European Commission, 2003a). EFSA issued opinions on the safety of the chelated forms of iron, copper, manganese and zinc with synthetic feed grade glycine (EFSA, 2005), on the safety and efficacy of a copper chelate of hydroxy analogue of methionine (Mintrex®Cu) as feed additive for all animal species (EFSA, 2008a; EFSA FEEDAP Panel, 2009), on the safety and efficacy of di copper chloride tri hydroxide (tribasic copper chloride, TBCC) as feed additive for all animal species (EFSA FEEDAP Panel, 2011a) and on the safety and efficacy of copper chelate of L-lysinate-HCl as feed additive for all animal species (EFSA FEEDAP Panel, 2014). In the frame of re-evaluation EFSA has delivered three opinions on copper-based additives including: cupric acetate, monohydrate; basic cupric carbonate, monohydrate; cupric chloride, dihydrate; cupric oxide; cupric sulphate, pentahydrate; cupric chelate of amino acids, hydrate; cupric chelate of glycine, hydrate (EFSA FEEDAP Panel, 2012a, 2013, 2015).

[2] Regulation (EC) No 1831/2003 of the European Parliament and of the Council of 22 September 2003 on additives for use in animal nutrition. OJ L 268, 18.10.2003, p. 29.
[3] Animine, 335 Chemin du Noyer, 74330 Sillingy, France.
[4] Commission Regulation (EC) No 1334/2003 of 25 July 2003 amending the conditions for authorisation of a number of additives in feedingstuffs belonging to the group of trace elements. OJ L 187, 26.7.2003, p. 11.
[5] Commission Regulation (EC) No 479/2006 of 23 March 2006 as regards the authorisation of certain additives belonging to the group compounds of trace elements. OJ L 86, 24.3.2006, p. 4.
[6] Commission Regulation (EU) No 349/2010 of 23 April 2010 concerning the authorisation of copper chelate of hydroxy analogue of methionine as a feed additive for all animal species. OJ L 104, 24.4.2010, p. 31.
[7] Commission Implementing Regulation (EU) No 269/2012 of 26 March 2012 concerning the authorisation of dicopper chloride trihydroxide as feed additive for all animal species. OJ L 89, 27.3.2012, p. 3.
[8] Commission Implementing Regulation (EU) No 1230/2014 of 17 November 2014 concerning the authorisation of copper bilysinate as feed additive for all animal species. OJ L 331, 18.11.2014, p. 18.

Dicopper oxide is authorised as a pharmacologically active substance and classified regarding MRLs in foodstuffs of animal origin as 'Allowed substances, no MRL required' (table 1 of the Annex of Regulation 37/2010).[9] Dicopper oxide (Cu_2O) is authorised as a biocidal product in the EU.[10] A voluntary risk assessment report on copper was submitted Copper and Copper Compounds to the European Chemicals Agency (ECHA) on copper and copper compounds, including dicopper oxide; this report is available in the ECHA website.

2. Data and methodologies

2.1. Data

The present assessment is based on data submitted by the applicant in the form of a technical dossier[11] in support of the authorisation request for the use of dicopper oxide as a feed additive. The technical dossier was prepared following the provisions of Article 7 of Regulation (EC) No 1831/2003, Regulation (EC) No 429/2008[12] and the applicable EFSA guidance documents.

The FEEDAP Panel used the data provided by the applicant together with data from other sources, such as previous risk assessments by EFSA or other expert bodies, peer-reviewed scientific papers, other scientific reports and experts' elicitation knowledge, to deliver the present output.

EFSA has verified the European Union Reference Laboratory (EURL) report as it relates to the methods used for the control of the dicopper oxide in animal feed. The Executive Summary of the EURL report can be found in Annex A.[13]

2.2. Methodologies

The approach followed by the FEEDAP Panel to assess the safety and the efficacy of dicopper oxide is in line with the principles laid down in Regulation (EC) No 429/2008 and the relevant guidance documents: Guidance on nutritional additives (EFSA FEEDAP Panel, 2012b), Technical guidance: Tolerance and efficacy studies in target animals (EFSA FEEDAP Panel, 2011b), Technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008b), Guidance for establishing the safety of additives for the consumer (EFSA FEEDAP Panel, 2012c), Guidance on studies concerning the safety of use of the additive for users/workers (EFSA FEEDAP Panel, 2012d).

3. Assessment

The applicant is seeking authorisation for the use of dicopper oxide (Cu_2O) in feed for all animal species/categories. This copper compound is not currently authorised in the EU as feed additive.

3.1. Characterisation

For the purpose of this opinion, 'dicopper oxide' is the compound of copper intended to release the element for its nutritional function in the organism. The compound is further processed to produce a formulated additive, which is referred to as the 'additive' throughout the scientific opinion.

3.1.1. Manufacturing process

The starting raw materials used for the production of the additive are recycled copper, hydrochloric acid, sulphuric acid, water, lignosulphonate and bentonite. The manufacturing process of the product is fully described in the technical dossier.[14]

[9] These copper compounds are classified as 'Allowed substances, no MRL required': copper chloride, copper gluconate, copper heptanoate, copper methionate, copper oxide, copper sulphate and dicopper oxide.

[10] Commission Regulation (EC) No 1451/2007 of 4 December 2007 on the second phase of the 10-year work programme referred to in Article 16(2) of Directive 98/9/EC of the European Parliament and of the Council concerning the placing of biocidal products on the market, and amending Regulation (EC) No 1896/2000.

[11] FEED dossier reference: FAD-2014-0034.

[12] Commission Regulation (EC) No 429/2008 of 25 April 2008 on detailed rules for the implementation of Regulation (EC) No 1831/2003 of the European Parliament and of the Council as regards the preparation and the presentation of applications and the assessment and the authorisation of feed additives. OJ L 133, 22.5.2008, p. 1.

[13] The full report is available on the EURL website: https://ec.europa.eu/jrc/sites/default/files/finrep-fad-2014-0034-Cu2O.pdf

[14] Technical Dossier/Section II.

According to the applicant, contaminants are controlled in the product at two steps during the manufacturing process, before melting and granulation of the raw material and before the bagging.[15]

3.1.2. Characterisation of dicopper oxide

The compound 'dicopper oxide' is identified with the Chemical Abstracts Service (CAS) No 1317·39·1. Its International Union of Pure and Applied Chemistry (IUPAC) name is copper(I) oxide. Its molecular formula is Cu_2O and it has a molecular weight of 143.08 Da. The theoretical content of copper is 88.8%.

The compound contains a minimum of 97% and maximum 86% for cuprous oxide and total copper, respectively.

The identity of dicopper oxide has been confirmed by X-ray crystallography.[16]

The analysis of five batches for copper(I) oxide, copper(II) oxide[17] and total copper[18] content were submitted; average values were 98.24 ± 0.15%, 0.01 ± 0.15% and 86.8 ± 0.15%, respectively.

Heavy metals (cadmium (Cd), lead (Pb)), arsenic (As) and nickel (Ni) were analysed in five batches.[19] Mercury was analysed in three batches.[20] The average values were 3.7 (2.5–5.1) mg Cd/kg, < 0.09 (< 0.05 to < 0.09) mg Hg/kg, 138 (112–170) mg Pb/kg, 4.4 (2.9–6.2) mg As/kg and 82 (75–97) mg Ni/kg compound. With the exception of lead, all the reported values are within limits set in the Directive 2002/32/EC on Undesirable Substances in animal feed[21] for feed additives belonging to the functional group compounds of trace elements or, where no specific limit is mentioned, do not represent a safety concern. The lead content in the compound is above the currently authorised levels for compounds of trace elements in the European Directive on undesirable substances.[21] Calculating the potential lead addition to a complete feed, assuming 160 mg supplemented Cu/kg piglet feed (background 10 mg Cu), the use of dicopper oxide (87% Cu) would provide about 40 μg Pb/kg feed (from 184 mg dicopper oxide/kg) assuming that dicopper oxide contains 200 mg Pb/kg, the level authorised for cupric carbonate. This is less than the same amount of supplemental copper from cupric carbonate would provide at the highest permitted lead level (about 60 μg/kg feed) or from copper sulphate also containing the highest lead level permitted (100 mg Pb/kg) resulting in 70 μg Pb/kg feed. The FEEDAP Panel has therefore no concerns on the measured lead concentration in dicopper oxide, provided it would not exceed 200 mg/kg.

Dioxins and the sum of dioxins and dioxin-like polychlorinated biphenyls (PCBs) were measured in three batches,[22] and levels found were 0.17–0.56 ng WHO-PCDD/F-TEQ (World Health Organization (WHO) polychlorinated dibenzodioxin/dibenzofuran (PCDD/F)-toxic equivalent (TEQ))/kg additive and 0.21–0.63 ng WHO-PCDD/F-PCB-TEQ/kg additive, respectively. These concentrations comply with those set in Directive 2002/32/EC.

3.1.3. Characterisation of the additive

The additive is composed of 86.2% copper source (dicopper oxide),[23] 12.8% sodium lignosulphonate and around 1% bentonite. The copper content ranges from 73% to 77%.[24]

Heavy metals (Cd, Pb and Hg), arsenic and nickel were analysed in five batches.[25] The average values were 7.9 (5.5–10.3) mg Cd/kg, < 0.05 mg Hg/kg, 118 (85–157) mg Pb/kg, 5.4 (2.9–9.0) mg As/kg and 69.4 (43–105) mg Ni/kg additive.

The lead content of the additive also exceeded the maximum allowed content in compounds of trace elements in three out of five batches is not considered of concern (see Section 3.1.2). It is noted that the cadmium content of the additive exceeded (in one out of five batches) numerically the maximum allowed content set in the Directive of Undesirable substances −10.3 mg compared to 10 mg/kg additive; this difference is, considering its magnitude, of no concern. It should be mentioned

[15] Technical Dossier/Supplementary Information September 2015.
[16] Technical Dossier/Section II/Annex II.11.
[17] Technical Dossier/Section II/Annex II.2.
[18] Technical Dossier/Section II/Annex II.1.
[19] Technical Dossier/Section II/Annex II.8_Supplementary information September 2015.
[20] Technical Dossier/Supplementary Information September 2015/Annex II.8.
[21] Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002, p. 10.
[22] Technical Dossier/Section II/Annex II.9.
[23] Estimated by calculations, as in the final product the applicant stated it is technically not possible to measure dicopper oxide.
[24] Technical Dossier/Section II/Annex.
[25] Technical Dossier/Section II/Annex II.8.

that the cadmium content of dicopper oxide (see Section 3.1.2.) is in line with the thresholds set in the above-mentioned Directive.

Dioxins and the sum of dioxins and dioxin-like PCBs were measured in three batches,[22] and levels found were 0.05–0.10 ng WHO-PCDD/F-TEQ/kg additive and 0.08–0.14 ng WHO-PCDD/F-PCB-TEQ/kg additive, respectively. These concentrations comply with those set in Directive 2002/32/EC.

3.1.3.1. Physical state of the product

The additive is presented in the form of fine, red-brown granules. The density of the product (average of five batches) is 1.63 g/cm^3.[26] According to the material safety data sheet, the finished product is insoluble in water.

Particle size distribution was determined by laser diffraction in three batches of the product.[27] Average values were 0.24% (0.09–0.43%) for particles < 10 μm; 3.6% (1.7–7.2%) for particles < 50 μm and 23.1% (13.8–32.7%) for particles < 100 μm.

The dusting potential as measured by the Stauber–Heubach method in five batches ranged from 3.8 to 11.3 mg/50 g additive, corresponding to 0.190–0.565 g/m^3.[26]

3.1.4. Stability and homogeneity

Stability data are not required for inorganic compounds of trace elements.

The capacity to homogeneously distribute in feed was studied in a complete feed for piglets (154 mg Cu/kg) with 10 subsamples (five samples from the top of the mix; five from the bottom). The copper recovery was 92.3% and the coefficient of variation of copper concentration was 3.0%.

3.1.5. Physico-chemical incompatibilities in feed

According to current knowledge, no incompatibilities or adverse interactions with feed components, carriers, other approved additives or medicinal products are to be expected other than those widely recognised for copper in animal nutrition.

3.1.6. Conditions of use

The copper compound under application, dicopper oxide, is intended to supply copper in final feed for all animal species/categories up to a maximum total content of 170 mg Cu/kg in complete feedingstuffs for piglets (up to 12 weeks) and 25 mg Cu/kg for other pigs; 15 mg Cu/kg complete feedingstuffs for bovine before the start of rumination (milk replacers and other complete feedingstuffs) and 35 mg Cu/kg for other bovine; 15 mg Cu/kg complete feedingstuffs for ovine; 50 mg Cu/kg complete feedingstuffs for crustaceans; 25 mg Cu/kg complete feedingstuffs for fish; and 25 mg Cu/kg complete feedingstuffs for all other species and categories.

3.2. Safety

Absorption, distribution, metabolism and excretion of copper in humans and animals are described in detail by the FEEDAP Panel (EFSA FEEDAP Panel, 2015) and Ellingsen et al. (2015). The majority of data come from studies with divalent copper. Copper(I) can easily be oxidised. It is considered likely that Cu(I) is converted to Cu(II) under acidic conditions of the stomach. No data could be found indicating the percentage of this conversion. However, the Committee for Veterinary Medicinal Products of the European Medicines Agency stated in its conclusion on several copper compounds, including dicopper oxide, that absorbed copper appears first in plasma as a cupric ion, loosely bound to albumin (EMEA, 1998).

3.2.1. Safety for the target species

The maximum tolerable levels and requirements for copper have been reviewed by the FEEDAP Panel in previous opinions (e.g. EFSA FEEDAP Panel, 2015).

3.2.1.1. Tolerance studies for the target species

The applicant provided a study in chicken for fattening (day old, male, Ross 308) in the first data set, comparing dicopper oxide with copper sulphate, each at three supplemental levels (20, 95 and

[26] Technical Dossier/Section II/Annex II.10.
[27] Technical Dossier/Supplementary Information September 2015/Annex II.14.

195 mg Cu/kg complete feedingstuff) for 37 days.[28] Due to the weaknesses of the experimental design (only one replicate/treatment; no statistical analysis), this study was not further considered.

The applicant submitted a second study to support tolerance of dicopper oxide.[29] A total of 576 day-old male broiler chickens (Ross 308) was randomly distributed to six experimental groups with eight replicates each (12 birds/replicate). The treatments were 15, 150 and 300 mg Cu/kg complete feed from $CuSO_4$ or Cu_2O. The diets (maize, wheat, soya bean meal, amino acid supplemented, background Cu 8 mg/kg) were given as starter (days 1–14), grower (days 15–28) and finisher (days 29–35). The diets were analytically controlled. The lowest dose (15 + 8 mg Cu/kg feed) should represent the copper concentration near to the requirement of chickens for fattening (8 mg Cu/kg; NRC, 1994); the maximum tolerable level (MTL) for chickens (and turkey) was set by the NRC (2005) as 250 mg Cu/kg feed. Body weight and feed intake were measured for the respective phases. At the end of the experiment, one bird per replicate was taken for haematology[30] and clinical chemistry[31]; after euthanasia, the weight of liver, kidneys and breast muscle was determined. The results were statistically analysed with ANOVA using the GLM procedure; group differences were examined by the Tukey–Kramer test. Table 1 summarises the main results at 35 days.

Table 1: Summary results of the 35-day tolerance study in chicken for fattening

Cu source	$CuSO_4$			Cu_2O		
mg Cu intended/kg	15	150	300	15	150	300
mg Cu analysed/kg						
Starter	12	176	382	15	149	307
Grower	25	121	399	16	120	344
Finisher	20	188	388	26	136	395
Final body weight (g)	$2,444^{ab}$	$2,448^{ab}$	$2,221^{c}$	$2,361^{b}$	$2,512^{a}$	$2,463^{ab}$
Feed intake (g/day)(1)	111	111	119	113	111	113
Feed/gain ratio	1.61^{b}	1.62^{b}	1.92^{a}	1.70^{b}	1.58^{b}	1.64^{b}
Cu concentration (mg/kg fresh matter)						
Liver	2.69^{b}	3.41^{b}	11.42^{a}	2.73^{b}	2.89^{b}	4.63^{b}
Kidney	1.96^{ab}	2.07^{ab}	2.18^{a}	1.95^{ab}	1.90^{b}	2.16^{a}
Breast	0.30^{b}	0.40^{ab}	0.51^{a}	0.35^{b}	0.34^{b}	0.48^{a}
Fat	0.31	0.48	0.73	0.31	0.20	0.50
Serum (mg Cu/L)	0.153	0.145	0.162	0.145	0.144	0.158

^{a,b,c}: Different superscripts within a row indicate statistical differences ($p < 0.05$).
(1): The only significant difference was observed in the copper sulphate group between the low and the high doses ($p < 0.05$).

Mortality was low (0.69%) without differences between treatments. No significant differences in zootechnical parameters were observed between the groups with 15 and 150 mg Cu/kg feed from either source; however, the final body weight of the 300 mg Cu group from copper sulphate was significantly lower and feed per gain ratio higher than in all other groups including the 300 mg Cu from dicopper oxide. The latter findings are difficult to interpret as (i) the analysed dietary copper concentrations were considerably above the intended levels and (ii) the copper intake per bird in the first 4 weeks amounted to 824 mg in the high copper group from dicopper oxide and amounted to 1007 mg (+ 22%) in the corresponding copper sulphate group. The observed growth depression in the high copper group from copper sulphate was related to an increased feed intake which is normally not expected when the growth depression results from a toxic effect. Conclusions from the tolerance study will be based on the comparison on the two copper sources on the low and intermediate doses in feed.

Copper deposition in liver, kidney, breast muscle, fat and serum at dietary levels of 15 and 150 mg Cu/kg was not different between the copper sources. No significant differences were found concerning haematological and biochemical endpoints.

[28] Technical Dossier/Section III/Annex III.1.
[29] Technical Dossier/Supplementary Information September 2015/Annex III.2.
[30] Red blood cell count, haemoglobin concentration, packed cell volume or haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, white blood cell count, mean corpuscular haemoglobin concentration, lymphocytes, monocytes, azurophils, heterophils, eosinopohils, basophils, thrombocyte count.
[31] Alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, gamma-glutamyltransferase; albumin; total protein; glucose and ceruloplasmin.

3.2.1.2. Studies in literature

The applicant identified two previous publications (short-term studies) in chickens for fattening with dicopper oxide in which liver deposition (Baker et al., 1991) or bile concentration (Aoyagi and Baker, 1993) were measured in comparison with copper sulphate.

Baker et al. (1991) fed graded levels of supplemental copper 0 (control), 75 and 150 mg/kg from copper sulphate or dicopper oxide to groups of 4 replicates with 7-day-old chickens for fattening for 2 weeks. The control diet contained 290 mg Cu/kg by addition of 275 mg Cu from copper sulphate. The liver of the control animals contained 40 mg Cu/kg; the liver of animals fed additional 175 mg Cu from sulphate and oxide contained 186 and 174 mg Cu/kg, respectively. The relative bioavailability of copper from dicopper oxide was calculated to be 92.5%.

The study of Aoyagi and Baker (1993) followed a similar design. Groups of 15 chickens (7-day old, three replicates with five birds each) were fed copper-deficient diets (0.6 mg Cu/kg) supplemented with 0, 0.5, 1.0 and 2.0 mg Cu/kg from copper sulphate or dicopper oxide for 2 weeks. The addition of 2 mg Cu/kg feed markedly increased copper in bile (3,373 and 3,053 μg Cu/L for copper sulphate and dicopper oxide, respectively) compared to the control group (400 μg Cu/L). The relative copper availability in dicopper oxide compared to copper sulphate was calculated to be 97.9%.

The data are considered supportive to the tolerance study. Similar values for the relative bioavailability are at least indicative of a similar internal exposure of the target species with the element.

3.2.1.3. Conclusions on the safety for the target species

No relevant differences between dicopper oxide and copper sulphate were observed in the tolerance study on chickens for fattening at a use level of 15 mg Cu/kg feed and its 10-fold level. Published studies indicate approximately the same bioavailability relative to copper sulphate at levels below and above the requirements. It is concluded that dicopper oxide is a safe copper source as copper sulphate in chickens for fattening. This conclusion can be extended to all animal species/categories provided the maximum authorised copper content in feed is respected.

3.2.2. Safety for the consumer

The concentration of copper in the organism is efficiently regulated by homeostatic mechanisms that include gastrointestinal absorption, the uptake and metabolism by the liver and biliary excretion into faeces. Copper toxicity ensues when the capacity of the homeostatic mechanisms is exceeded. Among edible tissues of animal origin, the highest concentration of copper is found in the liver (the main deposition organ), followed by the kidney and muscle. Among products of animal origin, milk shows the lowest values (Souci et al., 2008; see also Appendix A).

In its previous opinions in the context of the re-evaluation of copper compounds as nutritional feed additives, the FEEDAP Panel has reviewed the metabolic and toxicological profile of copper, consumer exposure and its safety (see e.g. EFSA FEEDAP Panel, 2015). The Panel concluded that the use of the copper compounds assessed in animal nutrition is of no concern for the safety of consumers, provided that the current maximum total contents of copper authorised in feed are respected. Consequently, only results and conclusions concerning the additive under assessment are reported below.

3.2.2.1. Deposition studies

The applicant submitted a study on tolerance of chickens for fattening to dicopper oxide (see Section 3.2.1). This study provided data on copper deposition in tissues and organs (see Table 1).

Copper content in tissues and organs increased with the concentration in diets. No differences between copper sources were observed (both groups with intended 300 mg Cu/kg feed were not considered; see Section 3.2.1). In breast muscle, the copper concentrations were approximately 10 times lower than in liver, similar between the sources of copper. The contents of copper in animal fat were not significantly different. Serum copper levels were similar for both copper sources and tested doses.

In addition, there is no evidence that the bioavailability of copper from dicopper oxide is higher than the bioavailability from copper sulphate (see Section 3.2.1.2). Consequently, there is no reason to expect a significant difference between the tissue/product depositions of copper from the dicopper oxide and the tissue/product depositions of copper from copper sulphate. Based on the available evidence, there is no indication that feed supplementation with dicopper oxide up to EU maximum authorised copper levels in feedingstuffs would lead to a greater copper deposition in edible tissues or products, compared to copper sulphate.

3.2.2.2. Toxicological studies

The toxicology of copper has been recently reviewed by Ellingsen et al. (2015). Toxicology of divalent copper has been summarised by EFSA FEEDAP Panel (2015).

The primary target of copper toxicity is the hepatocyte; copper excess impairs the turnover of liver tissue and causes extensive necrosis of hepatocytes. As tissue copper levels increase, haemolytic outbursts, kidney and brain damage are observed (European Commission, 2003b, 2008).

Both the Scientific Committee on Food (SCF) and Scientific Committee on Health and Environmental Risks (SCHER) identified the effects of the liver as the critical ones for hazard characterisation (European Commission, 2003b, 2008). A tolerable upper intake level (UL) of 5 mg Cu/day for adults and 1 mg/day for toddlers (1–3 years of age) was defined by the SCF (European Commission, 2003b). This figure was derived from an overall NOAEL of 10 mg Cu/day identified in the study by Pratt et al. (1985) where 10 mg/kg was daily administered to seven male adult volunteers for 12 weeks, with serum liver markers as endpoints. The SCF applied an uncertainty factor of 2 for potential variability in the normal population and a factor of 10 for toddlers. The FEEDAP Panel notes that using the 90-day rat NOAEL of 16.3 mg/kg body weight (bw), as proposed by the SCHER (European Commission, 2008), and an uncertainty factor of 200 (accounting for the duration of the study), would lead to figures essentially equal to the UL derived by SCF, i.e., 4.9 mg/day and 0.8 mg/day for a 60-kg adult and a 10-kg toddler, respectively.

3.2.2.3. Assessment of consumer safety

The available data on copper intake by the European population (Van Dokkum, 1995; European Commission, 2003b; Sadhra et al., 2007; Rubio et al., 2009; Turconi et al., 2009; Klevay, 2011, 2012) indicate an average intake below 50% of the UL. Consequently, there are no concerns about the copper content of edible tissues of animal origin.

The copper intake of the European population reflects therefore also the current use of copper supplementation of feed. Dicopper oxide will be used as a substitute for other copper-containing additives. Considering its similar copper availability to that of copper sulphate, no increases in the copper content in animal tissues and products would result from the use of dicopper oxide in animal nutrition.

3.2.2.4. Conclusions on the safety for the consumer

There is no indication that the toxicity of dicopper oxide is essentially different from that described for divalent copper. As dicopper oxide is used as a substitute for other copper-containing additives and its availability is in the range of copper sulphate, the standard copper-containing additive, no influence of the use of dicopper oxide in animal nutrition on the copper content of food of animal origin is expected.

The FEEDAP Panel concludes that the use of dicopper oxide in animal nutrition is of no concern for consumer safety.

3.2.3. Safety for the user

The applicant submitted studies with the additive under assessment concerning skin and eye irritation, skin sensitisation, acute dermal toxicity and acute inhalation toxicity.

3.2.3.1. Effects on skin and eyes

An acute dermal toxicity study was performed on rats, according to the OECD TG 402.[32] A single dose of 2,000 mg dicopper oxide/kg bw was applied to intact skin (10% of body surface) of ten animals (five per sex) for 24 h. Treatment caused neither signs of systemic toxicity nor of skin irritation, such as erythema or oedema.

An acute skin irritation test was performed in rabbits (three animals) by applying dicopper oxide to intact skin (OECD TG 404).[33] The treatment area was the shaved dorsal flank area: 500 mg of the test material and 0.5 mL of distilled water were introduced under a 2.5 cm × 2.5 cm cotton gauze patch, which was removed after 4 h; skins were observed at 1, 24, 48 and 72 h after the beginning of the experiment. No erythema, oedema or other signs of irritation were observed. The study indicated that the additive has no skin irritation potential.

[32] Technical Dossier/Section III/Annex III.6.
[33] Technical Dossier/Section III/Annex III.7.

An ocular irritation study was carried out on rabbits according to the established Draize method (Draize et al., 1944).[34] One hundred milligrams of dicopper oxide in the form of fine powder was instilled into the conjunctival sac of the right eye of each rabbit; the left eye remained untreated and served as concurrent control. In three rabbits, the treated eye was rinsed with approximately 30 mL of physiological saline solution (0.9% NaCl) 20–30 s after instillation of the test substance. Ocular irritation was evaluated at 1, 24, 48, 72 h and 4 days after instillation. Conjunctivitis was observed in all unrinsed eyes after 1 h; at 24 h, corneal opacity and iritis were evident in five rabbits. After 48 and 72 h, severity of irritation decreased, and 4 days after instillation, all animals were free of ocular irritation. For rinsed eyes, conjunctivitis was observed after 1 h; no corneal opacity or iritis was observed. After 48 h, all animals were free of ocular irritation. In conclusion, the additive is classified as a moderate ocular irritant; the essential lack of irritation for rinsed eyes supports the reversibility of ocular alterations.

The skin sensitisation potential was investigated on 30 albino guinea pigs (10 for the control group and 20 for the test group), according to OECD TG 406.[35] No sensitisation reactions were observed at 24 or 48 h after challenge. The study results indicate that dicopper oxide is not a skin sensitiser.

The nickel content of the additive is up to 105 mg/kg; given its well-known sensitisation potential (European Commission, 2011), it would be prudent to consider the additive as a dermal and respiratory sensitiser (Nemery, 1990; Schnabel et al., 2010; Klein and Costa, 2015).

3.2.3.2. Effects on the respiratory system

Acute inhalation toxicity

An acute inhalation toxicity was conducted on 10 rats (five males and five females) exposed (nose only) to 5.0 mg/L dicopper oxide (nominal concentration) for a period of 4 h (OECD TG 403).[36] Animals were observed for 14 days after treatment: after the observation period, the animals were necropsied and examined for gross pathological lesions. No deaths were observed during the study period. During the exposure phase an increased respiratory rate with sporadic instances of decreased respiratory rate, wet fur and staining by test material were observed. Once removed from the exposure chamber, all animals showed increased respiratory rate, noisy respiration, hunched posture, pilo-erection and wet, stained fur. Additionally, lethargy and ataxia were observed in males. Clinical signs gradually decreased and after 6 days, all the animals appeared normal. At necropsy, cases of lung abnormalities (pale patches and dark foci) and of pale/or enlarged kidney were observed. As no histopathological examination was carried out, these abnormalities could not be further characterised. As a single dose level was tested, and residual lung and kidney abnormalities were detected in animals at the end of the 14-day post-treatment period, the study cannot provide any indication about a safe inhalation exposure to dicopper oxide. The FEEDAP Panel concludes that dicopper oxide is hazardous by inhalation.

Assessment of inhalation exposure

Copper is considered hazardous by inhalation; in laboratory animals, exposure does also impair the ability of lung tissue to respond to infections (IPCS, 1998). The Scientific Committee on Occupational Exposure Limits (SCOEL) has delivered a recommendation on the occupational exposure limit (OEL) for copper and its inorganic compounds, which sets an 8-h time-weighted average of 0.01 mg/m^3 respirable fraction (European Commission, 2014).

Considering the dusting potential, the respirable fraction of particles and the copper content of the additive, the estimated inhalation exposure is at least (the respirable fraction in the additive would be lower than in the dust where it was not measured) between 0.4 and 1.1 mg Cu/m^3, which is above the SCOEL value.

The nickel content of the additive under assessment was provided and is up to 105 mg/kg. The proposed OEL for the inhalable fraction of water soluble nickel is 0.01 mg Ni/m^3 (European Commission, 2011). The dusting potential of the product amounted up to 0.565 g/m^3, corresponding to about 0.06 mg Ni/m^3. Considering this and the low amount of respirable particles, the margin between the OEL and the nickel in dust does not indicate a risk upon inhalation.

The FEEDAP Panel recognises that the use of the OEL as a guidance value for user safety of feed additives may result in overly conservative assessments, as the exposure is unlikely to be that continuous and intense as in an industrial scenario, for which OELs have been envisaged.

[34] Technical Dossier/Section III/Annex III.5.
[35] Technical Dossier/Section III/Annex III.9.
[36] Technical Dossier/Section III/Annex III.8.

Nevertheless, even with the mentioned caveat, a copper concentration in the inhalable dust exceeding the OEL by at least one order of magnitude would point out a risk by inhalation. Therefore, dicopper oxide is considered to pose a risk by inhalation to users.

3.2.3.3. Conclusions on the safety for users/workers

Dicopper oxide is not an irritant to skin but a moderate irritant to the eye. Owing to the nickel content in the additive, it should be considered as a dermal and respiratory sensitiser. Dicopper oxide poses a risk to users upon inhalation exposure.

3.2.4. Safety for the environment

In its previous opinions in the context of the re-evaluation of copper compounds as nutritional feed additives, the FEEDAP Panel has reviewed the safety for the environment of various copper compounds (e.g. EFSA FEEDAP Panel, 2015). The assessment was based on the data set provided by Monteiro et al. (2010). The Panel concluded that potential risks to soil organisms have been identified as a result of the application of piglet manure. Levels of copper in other types of manure are too low to create a potential risk within the timescale considered. There might also be a potential environmental concern related to the contamination of sediment owing to drainage and the run-off of copper to surface water. In order to draw a final conclusion, further model validation is needed and some further refinement to the assessment of copper-based feed additives in livestock needs to be considered, for which additional data would be required. The use of copper-containing additives in aquaculture, up to the maximum authorised copper level in feeds, is not expected to pose an appreciable risk to the environment. The extent to which copper-resistant bacteria contribute to the overall antibiotic resistance situation cannot be quantified at present. The FEEDAP Panel notes that the environmental safety of the use of copper in animal nutrition is currently under assessment.

There was no available evidence suggesting that dicopper oxide would pose additional risks to the environment than the other sources of copper already authorised. Moreover, dicopper oxide has approximately the same bioavailability as copper sulphate. The substitutive use of dicopper oxide for other copper compounds would therefore not change the above conclusion.

3.3. Efficacy

The applicant provided in the first data set a total of nine studies, which included a tolerance study and six publications on bioavailability of different copper sources (Baker et al. (1991) and Aoyagi and Baker (1993) with poultry; Cromwell et al. (1989) with weanling pigs; Baker (2005) with sheep; Kegley and Spears (1994) and Ledoux et al. (1995) with cattle) and two publications with the single administration of a ^{64}Cu-labelled Cu-compound to sheep (Lassiter and Bell, 1960) and growing cattle (Chapman and Bell, 1963) to support the efficacy of the additive. The tolerance study could not be considered (see Section 3.2.1). None of the studies submitted corresponded to a classical efficacy trial, no control group with marginal or deficient copper supply was installed and the duration was too short. Only two of the publications described studies in which the dicopper oxide was fed (Baker et al., 1991; Aoyagi and Baker, 1993), and are reported in Section 3.2.1.2. Both studies demonstrated the availability of copper from dicopper oxide to chickens for fattening at a level near to copper sulphate (93–98%). From these two short-term studies, only one with dietary copper below the maximum authorised total copper content in feed (Aoyagi and Baker, 1993) could be considered for efficacy assessment.

The tolerance study in chickens for fattening was designed in a way that could also be used to support efficacy. The study is described in Section 3.2.1; the main results are summarised in Table 1. Only the groups with a dietary copper content of 15 mg supplemental copper per kg complete poultry feed (corresponding to 12–26 mg of analysed copper per kg complete feed, see Table 1) were considered.[37] Both copper sources, the dicopper oxide and copper sulphate, resulted in similar (not statistically different at $p < 0.05$) values for final body weight, cumulative feed intake, feed to gain ratio and copper deposition in liver, kidney, breast muscle, fat and serum.

3.3.1. Conclusions on the efficacy for the target species

The FEEDAP Panel finally concludes that dicopper oxide is effective as a source of copper for all animal species.

[37] The maximum copper content permitted by EU legislation in poultry feed is 25 mg/kg.

3.4. Post-marketing monitoring

The FEEDAP Panel considers that there is no need for specific requirements for a post-market monitoring plan other than those established in the Feed Hygiene Regulation[38] and good manufacturing practice.

4. Conclusions

Dicopper oxide is a safe source of copper for chickens for fattening. This conclusion is extended to all animal species/categories provided the maximum authorised copper content in feed is respected.

There is no indication that the toxicity of dicopper oxide is essentially different from that described for divalent copper. As dicopper oxide is used as a substitute for other copper-containing additives and its bioavailability is in the range of copper sulphate, the standard copper-containing additive, no influence of the use of dicopper oxide in animal nutrition on the copper content of food of animal origin is expected. The FEEDAP Panel concludes that the use of dicopper oxide in animal nutrition is of no concern for consumer safety.

Dicopper oxide is not an irritant to skin but a moderate irritant to the eye. Owing to the nickel content in the additive, it should be considered as a dermal and respiratory sensitiser. Dicopper oxide poses a risk to users upon inhalation exposure.

There was no available evidence suggesting that dicopper oxide would pose additional risks to the environment than the other sources of copper already authorised. The substitutive use of dicopper oxide for other copper compounds would therefore not change the previous conclusion of the Panel concerning safety for the environment.[1]

Based on the results of a tolerance study in chickens for fattening and a short-term bioavailability study, the FEEDAP Panel concluded that dicopper oxide is an effective source of copper for all animal species.

5. Recommendation

The analysed lead concentration in dicopper oxide does not present a safety concern, as animal exposure by the use of this additive would be lower than that resulting from the use of other compounds of copper with a contamination up to the maximum permitted lead level. However, the lead concentration occurring in dicopper oxide is above the thresholds set in European legislation. Therefore, the FEEDAP Panel recommends identifying a solution for the noted discrepancy.

As recycled copper is used as starting material in the manufacturing process, it is recommended that the hazard analysis and critical control points (HACCP) system accounts for the foreseeable contaminants in the additive.

6. General remark

The FEEDAP Panel reviewed the potential relation between the copper supply of animals and the development of antibiotic resistance in bacteria. The Panel also considered the maximum residue limits (MRLs) set for copper in products of animal origin resulting from the use of copper as pesticide, in the light of the use of copper in animal nutrition. The risk assessment was presented in a previous opinion of the FEEDAP Panel on copper sulphate pentahydrate (EFSA FEEDAP Panel, 2012a) and, therefore, has not been repeated in the current document. The FEEDAP Panel reiterates its previous proposal concerning the modification of the current maximum residue limits for copper in animal tissues and products.

Documentation provided to EFSA

1) Dossier Dicopper oxide. August 2014. Submitted by Animine.
2) Dossier Dicopper oxide. Supplementary information. September 2015. Submitted by Animine.
3) Dossier Dicopper oxide. Supplementary information. December 2015. Submitted by Animine.
4) Evaluation report of the European Union Reference Laboratory for Feed Additives on the Methods(s) of Analysis for Dicopper oxide.
5) Comments from Member States.

[38] Regulation (EC) No 183/2005 of the European Parliament and of the Council of 12 January 2005 laying down requirements for feed hygiene. OJ L 35, 8.2.2005, p. 1.

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Abbreviations

bw	body weight
CAS	Chemical Abstracts Service
ECHA	European Chemicals Agency
EURL	European Union Reference Laboratory
FEEDAP Panel	EFSA Panel on Additives and Products or Substances used in Animal Feed
HACCP	Hazard Analysis and Critical Control Points
IUPAC	International Union of Pure and Applied Chemistry
MRL	maximum residue limit
MTL	maximum tolerable level
NOAEL	no observed adverse effect level
OECD	Organisation for Economic Co-operation and Development
OEL	occupational exposure limit
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-para-dioxin
PCDF	polychlorinated dibenzofuran
SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
SCHER	Scientific Committee on Health and Environmental Risks
SCOEL	Scientific Committee on Occupational Exposure Limits
TBCC	tribasic copper chloride
TEQ	toxic equivalent
UL	upper intake level
WHO	World Health Organization

Appendix A – Copper content in food of animal origin

Food of animal origin	Average (range) (mg/kg fresh matter)
Milk	
Human	0.35 (0.22–0.77)
Cow (raw milk)	0.10 (0.02–0.30)
Goat	0.11 (0.08–0.75)
Sheep (ewe's milk)	0.15 (0.09–0.88)
Eggs	
Laying hens (whole egg)	0.65
Fish	
Flounder	0.47
Halibut	0.41 (0.26–2.30)
Herring (Atlantic)	1.23 (0.75–4.40)
Cod	0.53 (0.15–4.70)
Mackerel	1.14 (0.55–2.00)
Horse mackerel	0.57 (0.44–1.90)
Sardine	1.70
Plaice	0.42 (0.10–5.50)
Alaska pollock	0.35
Tuna	0.51
Eel	0.87 (0.40–0.91)
Trout	1.47 (0.39–1.70)
Carp	0.87
Salmon	1.29 (0.58 – 2.00)
Blood	
Beef	0.90
Meat/muscle	
Beef, only muscle	0.87 (0.70–1.20)
Veal, only muscle	1.60 (0.90–2.40)
Sheep, only muscle	0.90 (0.50–1.30)
Lamb, only muscle	1.70 (1.30–2.40)
Pork, only muscle	0.88 (0.60–0.90)
Rabbit, meat	1.50
Chicken for fattening	0.42
Turkey for fattening	1.10 (0.40–1.80)
Liver	
Beef	32
Calf	55 (35–79)
Sheep	76 (45–110)
Pig	13 (9–16)
Chicken for fattening	3.20 (1.50–4.10)
Heart	
Beef	3.00
Calf	3.20 (2.90–3.40)
Sheep	4.50
Pig	4.10
Lung	
Beef	2.60
Kidney	
Beef	4.30 (3.70–4.40)
Calf	3.70
Sheep	3.50
Pig	7.80 (6.0–7.9)

Source: Souci et al. (2008).

Annex A – Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Dicopper Oxide

In the current application authorisation is sought under article 4(1) for *Dicopper oxide* under the category/ functional group (3b) "nutritional additives"/"compounds of trace elements", according to the classification system of Annex I of Regulation (EC) No 1831/2003. Specifically, authorisation is sought for the use of the *feed additive* for all categories and species. The Applicant intends to market a red-brown granulated formulation (*CoRouge*®) containing ca. 86% of *dicopper oxide (feed additive)* - equivalent to a *total copper* content ranging from 73 to 77% - together with ca. 13% of lignosulphonate and 1% of bentonite. The *feed additive* is intended to be incorporated into *feedingstuffs* through *premixtures*. The Applicant suggested maximum levels of *total copper* in the *feedingstuffs* complying with the limits set in Regulations (EC) No 1334/2003 and ranging from 15 to 170 mg/kg, depending of the animal species/category.

For characterisation of *dicopper oxide* (Cu_2O) the Applicant submitted an X-ray diffraction method (XRD), described in the generic European Pharmacopoeia monograph (Eur. Ph. 6.0, 01/2008:20933), to identify the crystalline structure of Cu_2O together with a single-laboratory validated titrimetric method for the quantification of *total copper* in Cu_2O. The validity of the titration method was demonstrated for the product (*CoRouge*®) and the titration results were further confirmed by the analyses of the product performed by ICP-AES according to the protocol described in the EN 15510 standard, mentioned below.

For the quantification of *total copper* in *premixtures* and *feedingstuffs* the Applicant submitted the internationally recognised ring-trial validated EN 15510 method based on inductively coupled plasma atomic emission spectroscopy (ICP-AES) after ashing and/or treatment with hydrochloric acid. In the meantime, the EURL already evaluated in the frame of the Copper group dossiers (FAD-2010-0031; FAD-2010-0070; FAD-2010-0331) an alternative ring-trial validated EN 15621 method, based on ICP-AES after pressure digestion. Furthermore, a Community method is available for the quantification of *total copper* in *feedingstuffs,* which was further ring-trial validated by the UK Food Standards Agency. Based on the available information the EURL recommends for official control the X-ray diffraction and the titrimetric method submitted by the Applicant for the characterisation of *dicopper oxide* (*feed additive*), together with the two CEN (EN 15510 or EN 15621) and Community (Com Reg (EC) No 152/2009 – Annex IV-C) methods for the quantification of *total copper* in the *feed additive*, *premixtures* and/or *feedingstuffs*.

Further testing or validation of the methods to be performed through the consortium of National Reference Laboratories as specified by Article 10 (Commission Regulation (EC) No 378/2005) is not considered necessary.