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SCIENTIFIC OPINION

Scientific Opinion on the safety and efficacy of copper compounds (E4) as feed additives for all animal species: cupric sulphate pentahydrate based on a dossier submitted by Manica S.p.A.[1]

EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

ABSTRACT

Copper sulphate pentahydrate is safe for all animal species up to the maximum total copper content authorised in feed. No concerns for consumer safety are expected from the use of the feed additive. The maximum residue limits (MRLs) for copper in foods of animal origin established by European Union pesticides legislation are not consistent with legal practices in animal nutrition. As copper is an essential micronutrient, the FEEDAP Panel is not in favour of establishing MRLs for animal products, unless there is a clear consumer safety issue; if MRLs are to be maintained, the Panel has proposed amended values. The additive is an eye irritant and may induce allergic dermatitis in sensitive persons which might be exacerbated by the contamination with nickel. Users may be exposed to hazardous copper concentrations by inhalation. Potential risks to soil organisms have been identified after the application of piglet manure; there might be a potential concern related to sediment contamination. 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 limited database available on the influence of copper to the development of antibiotic resistance in gut and soil bacteria indicates that high copper concentrations in the microbial environment increase the number of copper-resistant bacteria, and copper resistance seems to be correlated with more frequent resistance to several antibiotics in certain bacterial species. A potential copper threshold concentration could not be derived. The total pool of macrolide resistance in animals probably originates from antibiotic treatment and not from the use of high dietary copper. The extent to which copper-resistant bacteria contribute to the overall antibiotic resistance can not be quantified at present. Copper sulphate pentahydrate is efficacious in meeting animal requirements.

KEY WORDS

Nutritional additive, compounds of trace elements, cupric sulphate pentahydrate, safety, MRL, antibiotic resistance, environment

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[1] On request from the European Commission, Question No EFSA-Q-2011-00743, adopted on 14 November 2012.

[2] Panel members: Gabriele Aquilina, Alex Bach, Vasileios Bampidis, Maria De Lourdes Bastos, Gerhard Flachowsky, Josep Gasa-Gasó, Mikolaj Gralak, Christer Hogstrand, Lubomir Leng, Secundino López-Puente, Giovanna Martelli, Baltasar Mayo, Derek Renshaw, Guido Rychen, Maria Saarela, Kristen Sejrsen, Patrick Van Beelen, Robert John Wallace and Johannes Westendorf. Correspondence: FEEDAP@efsa.europa.eu

[3] Acknowledgement: The Panel wishes to thank the members of the Working Group on Trace Elements, including Noël Albert Dierick, Jürgen Gropp, Joop de Knecht, Alberto Mantovani and the late Reinhard Kroker, for the preparatory work on this scientific opinion.

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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 cupric sulphate pentahydrate when used as feed additive for all animal species.

Copper sulphate pentahydrate is safe for all animal species/categories up to the maximum total copper content authorised in feed.

Consumption surveys include copper from foodstuffs of animal origin. Since the supplementation of animal feed with copper-containing compounds has not essentially changed over the last decade, no change in the contribution of foodstuffs originating from supplemented animals to the overall copper intake of consumers is expected. No concerns for consumer safety are expected from the use of copper sulphate pentahydrate in animal nutrition.

The maximum residue limits (MRLs) for copper in edible tissues and products of animal origin established by European Union pesticides legislation are found not to comply with the upper intake level set by the Scientific Committee on Food—as shown by different model calculations—and with legal feeding practices. The FEEDAP Panel is generally not in favour of establishing MRLs for essential nutrients, such as copper, in foods of animal origin, unless there is a clear consumer safety issue to do so; however, any such MRL has to consider animal health and welfare. In case MRLs for animal products are to be retained, the FEEDAP Panel proposes amended values.

Copper sulphate pentahydrate is an eye irritant but not a skin irritant or skin sensitiser; it may induce allergic dermatitis in sensitive persons, which might be exacerbated by the contamination with nickel. The dusting potential of the additive indicates that users may be exposed to hazardous copper concentrations by inhalation, which could result in a reduced immune response of the lung. The inhalation of nickel resulting from handling the additive is by itself unlikely to be of concern.

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 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 limited database available on the influence of copper on the development of antibiotic resistance in gut and soil bacteria allows to conclude that (i) high copper concentrations in the microbial environment increase the number of copper-resistant bacteria and (ii) copper resistance seems to be correlated with more frequent resistance to several antibiotics in certain bacterial species. A co-transfer of plasmid genes encoding for resistance to copper and erythromycin is plausible at least in *Enterococcus faecium*. The current database does not allow any conclusion on a potential threshold concentration of copper in feeds, below which a significant increase in copper resistance could not be expected. The total pool of macrolide resistance in animals probably originates from antibiotic treatment and not from the use of high dietary copper. The extent to which copper-resistant bacteria contribute to the overall antibiotic resistance situation can not be quantified at present. More precise (and quantitative) conclusions will require further studies.

The use of copper sulphate pentahydrate in animal nutrition is extensively documented in the scientific literature. It is recognised as an efficacious source of copper in meeting animal requirements.

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BACKGROUND

Regulation (EC) No 1831/2003[4] establishes the rules governing the Community authorisation of additives for use in animal nutrition. Article 10(2) of that Regulation also specifies that for existing products within the meaning of Article 10(1), an application shall be submitted in accordance with Article 7, at the latest one year before the expiry date of the authorisation given pursuant to Directive 70/524/EEC for additives with a limited authorisation period, and within a maximum of seven years after the entry into force of this Regulation for additives authorised without time limit or pursuant to Directive 82/471/EEC.

The European Commission received a request from the company Manica S.p.A.[5] for re-evaluation of authorisation of the copper-containing additive *Cupric sulphate pentahydrate* when used as 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) under Article 10(2) (re-evaluation of an authorised feed additive). EFSA received directly from the applicants the technical dossier in support of this application.[6] According to Article 8 of that Regulation, 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. The particulars and documents in support of the application were considered valid by EFSA as of 21 September 2011.

The additive cupric sulphate pentahydrate had been authorised in the European Union (EU) under the element Copper-Cu for all animal species "Without a time limit" (Commission Regulation (EC) No 1334/2003)[7] and amendments. Following the provisions of Article 10(1) of Regulation (EC) No 1831/2003 the compound was included in the EU Register of Feed Additives under the category "Nutritional additives" and the functional group "Compounds of trace elements".[8]

The Scientific Committee on Animal Nutrition (SCAN) delivered reports on the use of copper methionate for pigs (EC, 1981), copper compounds in feedingstuffs (EC, 1982) and in feedingstuffs for pigs (EC, 1983) and the use of copper in feedingstuffs (EC, 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 species (EFSA, 2008a, 2009a), and on the safety and efficacy of di copper chloride tri hydroxide (tribasic copper chloride, TBCC) as feed additive for all species (EFSA, 2011).

4 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.

5 Manica S.p.A., Via All'Adige, 4, I-38068 Roveredo, Italy.

6 EFSA Dossier reference: FAD-2010-0331.

7 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.

8 European Union Register of Feed Additives pursuant to Regulation (EC) No 1831/2003. http://ec.europa.eu/food/food/animalnutrition/feedadditives/comm_register_feed_additives_1831-03.pdf

TERMS OF REFERENCE

According to Article 8 of Regulation (EC) No 1831/2003, EFSA shall 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 the efficacy of the Cupric sulphate pentahydrate, when used under the conditions described in Table 1.

Additionally, the EC requested EFSA to consider, in the margins of the re-evaluation of the copper dossiers, a scientific information on the issue of copper contained in animal excreta and the development of multidrug-resistance in pathogenic bacteria.[9]

[9] Berg J, Thorsen MK, Holm PE, Jensen J, Nybroe O, Brandt KK. 2010. Cu exposure under field conditions coselects for antibiotic resistance as determined by a novel cultivation-independent bacterial community tolerance assay. Environmental Science and Technology 44, 8724-8728.

Table 1: Description and conditions of use of the additive as proposed by the applicant Manica S.p.A.

Additive	Cupric Sulphate Pentahydrate
Registration number/EC No/No (if appropriate)	E4
Category(-ies) of additive	3. Nutritional additives
Functional group(s) of additive	b. Compounds of trace elements

Description			
Composition, description	Chemical formula	Purity criteria (if appropriate)	Method of analysis (if appropriate)
Cupric Sulphate pentahydrate	**$CuSO_4x5H_2O$**	**Complies with EU law on undesirable substances.**	**ICP-AES**

Trade name (if appropriate)	-
Name of the holder of authorisation (if appropriate)	-

Conditions of use				
Species or category of animal	Maximum Age	Minimum content	Maximum content	Withdrawal period (if appropriate)
		mg or Units of activity or CFU/kg of complete feedingstuffs (select what applicable)		
All	-	-	to supplement Cu within legal limits for each species	-

Other provisions and additional requirements for the labelling	
Specific conditions or restrictions for use (if appropriate)	Can only be used through a premixture (Article 13, point 1, Council directive 96/51/EC of 23 July 1996 amending Directive 70/524/EEC concernine additives in feedingstuffs)
Specific conditions or restrictions for handling (if appropriate)	If handled uncovered, arrangements with local exhaust ventilation should be used if possible. Wear personal protective clothing. When using do not eat, drink, smoke, sniff. Avoid generation of dust. Depositing of dust. Protect drains and sewers from entry of the product. Provide for retaining containers, eg. floor pan without outflow. Keep in locked storage or only make accessible to specialists or their authorised assistants. Containers have to be labelled clearly and permanently. Keep container tightly closed in a cool, well-ventilated place.
Post-market monitoring (if appropriate)	There is no need for specific requirements of postmarket monitoring. It is recommend to conduct post marketing monitoring in compliance with EU law on feed hygiene, namely by use of HACCP and tracebility systems, and formal monitoring of customer feedback through product or service complaints.
Specific conditions for use in complementary feedingstuffs (if appropriate)	To supply Cu in final feeds within EU legal limits for each species.

Maximum Residue Limit (MRL) (if appropriate)			
Marker residue	Species or category of animal	Target tissue(s) or food products	Maximum content in tissues
-	-	-	-

ASSESSMENT

This opinion is based in part on data provided by an applicant involved in the production/ distribution of copper-containing compounds. It should be recognised that these data covers only a fraction of the existing cupric sulphate pentahydrate in the market.

1. Introduction

The biological role of copper, its requirements/recommendations, deficiency and toxicity symptoms in farm animals have been already described in a former opinion of the Scientific Committee on Animal Nutrition (SCAN) (EC, 2003a); the maximum levels authorised for total copper in feedingstuffs are derived from that opinion. To the knowledge of the FEEDAP Panel, there is no additional relevant information that might lead it to modify the SCAN opinion.

Cupric sulphate pentahydrate is currently authorised as a nutritional additive under the functional group "Compounds of trace elements" to be used in feed for all animal species/categories. The present application is for the re-evaluation of that compound.

The FEEDAP Panel has been requested to evaluate the potential relation between the copper supply to 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.[10]

A compilation of risk assessments carried out on copper, including opinions from EFSA Panels other than the FEEDAP Panel, can be found in Appendix C. A list of authorisations for copper in the EU, other than as feed additive, is reported in Appendix D.

EFSA commissioned two studies, from which technical reports have been delivered; information from these reports has been used in this opinion. One of the studies was done on selected trace and ultratrace elements in animal nutrition by the University of Gent (Belgium) (Van Paemel et al., 2010); copper was included in that study. The other study concerned the pre-assessment of the environmental impact of zinc and copper used in animal nutrition (Monteiro et al., 2010).

2. Cupric sulphate pentahydrate

2.1. Characterisation and identity

Cupric sulphate pentahydrate (CAS No 7758-99-8) has the chemical formula $CuSO_4 \cdot 5H_2O$ (molecular weight 249.68 g/mol, 25 % copper).

Based on the analysis of 46 batches, the copper content in the additive ranged from 25.1 % to 25.5 %.[11] Heavy metals (lead and cadmium) and arsenic content complied with the limits set by legislation.[12] The content of nickel measured on the same batches varied between 27 and 60 mg/kg. The highest values for dioxins and the sum of dioxins plus dioxin-like polychlorinated biphenyls (PCBs) measured in five batches were 0.09 ng WHO-PCDD/F-TEQ/kg and 0.19 ng WHO-PCDD/F-PCB-TEQ/kg, respectively.[13] Control methods are in place.

The additive consists of blue and odourless crystals. Its solubility in water is 220 g/L (temperature and pH not stated). It has a bulk density of 1.2-1.33 kg/L.

[10] Regulation (EC) No 149/2008.
[11] Technical Dossier/Section II/Annex II_08.
[12] 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.
[13] Supplementary Information/Annex_II_12.

The fraction of particles with a diameter below 50–µm is about 8–10 % (v/v). Dusting potential, measured by the Stauber–Heubach test in one batch, is about 0.25 g/m^3.[14]

Cupric sulphate pentahydrate is produced from pure copper wires or recycled copper materials. Copper reacts in the presence of oxygen/air with a hot (approximately 70°C) mixture of copper sulphate solution and sulphuric acid. Subsequently, the hot solution is cooled and an anticaking agent is added to the resulting cupric sulphate pentahydrate crystals, which are further sieved and dried to produce the final additive.

2.2. Stability and homogeneity

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

The homogeneous distribution of cupric sulphate pentahydrate was measured in two different premixture formulations with nominal copper contents of 4.5 and 28 g/kg. Ten samples were taken from each premix and analysed in triplicate for copper. The measured concentrations of copper in both premixtures confirmed the intended levels. The coefficients of variation were < 5 %.[15]

2.3. Physico-chemical incompatibilities in feed

According to current knowledge, no incompatibilities resulting from the use of copper in compound feed are expected, other than those widely known and considered by feed manufacturers when formulating diets.

2.4. Conditions of use

The copper compound under application, cupric sulphate pentahydrate, is intended to supply copper in final feed for all animal species/categories up to a maximum total content of 170 mg Cu/kg complete feeddingstuffs for piglets (up to 12 weeks) and 25 mg Cu/kg for other pigs; 15 mg Cu/kg complete feeddingstuffs 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 feeddingstuffs for ovine; 50 mg Cu/kg complete feeddingstuffs for crustaceans; 25 mg Cu/kg complete feeddingstuffs for fish; and 25 mg Cu/kg complete feeddingstuffs for other species.

2.5. Evaluation of the analytical methods by the European Union Reference Laboratory (EURL)

EFSA has verified the EURL report as it relates to the methods used for the control of copper (seven compounds, including cupric sulphate pentahydrate) in animal feed. The Executive Summary of the EURL report can be found in Appendix A.

3. Safety

3.1. Safety for the target species

Huge differences in the maximum tolerable copper concentrations between animal species exist (e.g. 500 mg/kg for rodents; 250 mg/kg for poultry, pigs and horses; 100 mg/kg for fish; 40 mg/kg for cattle, 15 mg/kg for sheep) (NRC, 2005). Therefore, it cannot be expected that all animal species would share the same margin of safety of a copper-containing additive considering the maximum copper content in feed set by Regulation (EC) No 1334/2003. Consequently a safe copper-containing additive could theoretically have a margin of safety (maximum tolerable concentration/maximum content authorised) of 10 in poultry and pigs (250/25), of 4 for fish (100/25), of 1.1 for cattle (40/35) and of 1 for ovines (15/15). Most of the relevant studies on copper tolerance in animals were performed with copper sulphate pentahydrate.

[14] Technical Dossier/Section II. Supplementary information/Annexes II_9, II_10, II_13 and II_14.
[15] Supplementary Information/Annex_II_11.

The FEEDAP Panel concludes that copper sulphate pentahydrate is safe for all animal species/categories up to the respective maximum total copper content authorised in feed.

Considering the nickel background levels in animal feed being 500−4000 µg/kg dry matter (DM) feed (Nicholson et al., 1999; Van Paemel et al., 2010), nickel would be incorporated by supplementing feeds with copper sulphate at extremely low quantity: 16−36 µg Ni/kg piglet feed, when adding 150 mg Cu from copper sulphate pentahydrate and 2−6 µg Ni/kg feed for other animal species/categories, when adding 25 mg Cu from copper sulphate pentahydrate. Therefore, no concerns for the safety of the target animals would arise from this particular aspect.

3.2. Safety for the consumer

3.2.1. Metabolic and residue studies

Copper is absorbed from the diet in the upper jejunum by active and passive processes, stored in the liver and kidney, secreted in the bile and excreted in faeces. Copper excretion via the kidneys is quantitatively insignificant if complex-forming substances are not administered (thiomolybdate from oral molybdenum in ruminants, dimethyl cysteine). Copper status is not easy to determine, and the homeostatic mechanisms that control copper distribution and metabolism are not completely understood. Copper interacts with other divalent cations, such as calcium, iron and zinc, for gastrointestinal absorption and metabolism. Absorption and availability may be influenced by the carbohydrate content of the diet, with reduced availability by phytate containing diets or those containing fructose (EC, 2003b).

The SCAN delivered an opinion on copper (EC, 2003a) in which the metabolism and tissue deposition of copper was reviewed. Other reviews are available from McDowell (2003) and Suttle (2010). The distribution of total copper in the body varies with species, age and copper status of the animal. In general, levels in newborn and suckling animals are higher, followed by a steady fall during growth to the time when adult values are reached. The main target organ for copper deposition is the liver. Other edible tissues containing high concentrations of copper are the heart, brain and kidney. Lower levels are found in muscle. Liver and kidney copper concentrations are related to dietary intake, whereas muscle is less affected. Its presence in milk is generally very low and not influenced by dietary supplementation levels. Clearance is higher in poultry than in mammals; the copper concentration in eggs is generally low. In fish, copper is primarily stored in the liver.

3.2.2. Toxicological studies

The Scientific Committee on Food (SCF) (EC, 2003b) summarised data on the toxic properties of copper.

Tolerance to high intakes of copper varies greatly from one species to another, in relation to the vulnerability of the species and the levels of zinc, iron and molybdenum in the diet. Copper excess causes impaired growth and extensive necrosis of hepatocytes. Susceptibility to copper excess is also influenced by the chemical form. Manifestations of copper toxicity include weakness, tremors, anorexia and jaundice. As tissue copper levels increase, haemolytic crisis may ensue, resulting liver, kidney and brain damage.

3.2.2.1. Genotoxicity/Mutagenicity/Carcinogenicity

Copper has been reported to be genotoxic *in vitro* and also in some *in vivo* bone marrow micronucleus assays in mice, after intraperitoneal injection. As other essential trace elements (zinc, iron), copper is known to have a genotoxic potential when present at high local concentrations. In particular, copper is a redox active transition element, potentially able to catalyse the Fenton/Haber−Weiss chemistry, with consequent production of reactive oxygen species. As copper is physiologically present in the intracellular environment at very low levels, a genotoxic concern for the human population is not foreseen, except under conditions of overload, which are not relevant to the use of the additive under evaluation.

The International Agency for Research on Cancer (IARC) allocated copper (II) 8-hydroxyquinoline in Group 3 “Not classifiable as to their carcinogenicity to humans” (IARC, 1987). The SCF (EC, 2003b) concluded that studies on the carcinogenicity of copper compounds in rats and mice have given no indication of carcinogenic potential; however, some degree of uncertainty exists owing to limitations in available studies.

3.2.2.2. Reproduction toxicity

With regard to influence on reproduction, studies in rodents demonstrated that exposure to copper compounds during gestation induced embryo-/fetotoxic effects at doses of 12 mg Cu per kg bw and day, and above (IPCS, 1998).

3.2.3. Assessment of consumer safety

A tolerable upper intake level (UL) of 5 mg/day for adults and 1 mg/day for toddlers (1-3 years) was defined by the SCF (EC, 2003b). This figure was derived from an overall no observed adverse effect level (NOAEL) of 10 mg Cu/day identified in the study by Pratt et al. (1985) (daily single dose levels only administered to seven male adult volunteers for 12 weeks, and serum liver markers as endpoints), applying an uncertainty factor of 2 for potential variability in the normal population. This UL value has been consistently used in the assessments of copper in different forms by the following EFSA Scientific Panels: NDA Panel (EFSA, 2006), AFC Panel (EFSA, 2008b), FEEDAP Panel (EFSA, 2008a, 2009a), ANS Panel (EFSA, 2009b) and CEF Panel (EFSA, 2010).

Studies on copper dietary intakes in industrialised countries did provide comparable results. Mean dietary copper intakes by adults in different European countries have been estimated to be within a range of about 1.0-2.0 mg/day (Van Dokkum, 1995; EC, 2003b; Sadhra et al., 2007; Rubio et al., 2009; Turconi et al., 2009). Based on 11 independent, peer-reviewed surveys considering only analytically confirmed copper (n = 849) in Belgium, Canada, the UK and the USA, the mean copper intakes for men and women were estimated to be 1.48 (2.87 for P95) and 0.92 (2.18 for P95) mg/day, respectively (Klevay, 2011). A recent analytical study of Catalonian diets showed a copper intake of 1.2 mg/day (Domingo et al., 2012). It has been suggested that calculated copper intakes are overestimated when only food composition tables are used in nutrition surveys (Klevay, 2012).

Among edible tissues of animal origin, the highest concentration of copper is found in liver (the main deposition organ), followed by kidney and muscle. Among products of animal origin, milk shows the lowest values (for quantitative figures, see section 3.2.4).

Since the supplementation of animal feed with copper-containing compounds has not essentially changed over the last decade, it is reasonable to assume that food of animal origin recorded in the above-mentioned consumption surveys originated from animals fed copper supplemented diets and showing copper concentrations of tissues and products in the range mentioned above. As copper sulphate pentahydrate is considered as a kind of standard for other copper-containing compounds, the continued use of cupric sulphate pentahydrate in animal nutrition would not modify consumer exposure to copper.

3.2.4. Maximum residue limits for copper in animal tissues and products

Copper fulfils vital functions in living organisms and it is an essential micronutrient. The FEEDAP Panel is generally not in favour of establishing MRLs for essential nutrients in foods of animal origin, unless there is a clear consumer safety issue to do so; however, any such MRL has to consider animal health and welfare.

As copper sulphate (and other copper compounds) is also used for pesticides purposes, MRLs have been established under Regulation (EC) No 396/2005[16] (Annex IIIA, temporary MRLs). The following MRLs are set for copper in products of animal origin from swine, bovine, sheep, goat, horses, poultry and other farm animals (rabbits, kangaroos) (Regulation (EC) No 149/2008):[17]

- liver: 30 mg/kg; kidney: 30 mg/kg; edible offal: 30 mg/kg; meat: 5 mg/kg; fat (free of lean meat): 5 mg/kg; other: 5 mg/kg
- milk (including cream, butter, cheese and curd): 2 mg/kg; eggs (fresh or cooked): 2 mg/kg.

It is worth noting that for the setting of the MRLs the acceptable daily intake (ADI) of 0. 15 mg/kg bw per day,[18] was considered. This ADI was derived from a NOAEL of 15 mg/kg bw per day observed in a 1-year study in dogs, applying an uncertainty factor of 100, resulting in an ADI of 10.5 mg Cu in a 70-kg adult. This value is about double the UL set by the SCF in 2003 (5 mg Cu per adult person and day, 1 mg Cu per toddler and day). An exposure calculation following the default consumption figures set in Regulation (EC) No 429/2008, results in 9.45 mg Cu per person and day, corresponding to 90 % of the proposed ADI,[19] but to 190 % of the UL set by the SCF (EC, 2003b).

However, Regulation (EC) No 429/2008 foresees that in certain situations (e.g. some nutritional additives) it may be appropriate to subsequently refine the human exposure assessment using more realistic consumption figures, but still keeping the most conservative approach. Where this is possible, this shall be based on Community data.

To calculate exposure to copper from animal tissues and products, the default values for high-consuming adults and toddlers were used, as derived from the EFSA Comprehensive European Food Consumption Database and laid down in the EFSA guidance for consumer safety (EFSA, 2012). Milk containing copper at the MRL level of 2 mg/kg would result, following consumption of 1050 mL milk, in an exposure of 2.1 mg Cu per day for toddlers, this figure alone being twice the UL. A comparable calculation for adults consuming 1500 mL milk and 60 g liver (from poultry, pigs and ruminants in equal amounts) results in an intake of 4.8 mg Cu per day, which is already close to the UL; adding a background intake from non-animal-derived food (up to 60 %[20] of the average copper intake) of 0.9 mg/day would result in a total intake of 5.7 mg Cu per day, higher than the UL. These two examples indicate that the MRLs for copper in animal tissues and products do not comply with the UL established by the SCF.

In the conclusion of the pesticides peer review regarding copper compounds (EFSA, 2008c) it was stated that "... it was noted by the experts that not all possible sources of copper (e.g. use as feed additives resulting in residues higher than background levels in animal products; copper levels in seafood) have been taken into account in the presented estimates yet."

The FEEDAP Panel compared the current MRLs for copper with tissue concentrations that would be expected from the use of copper-supplemented diets not exceeding the maximum copper content

[16] Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. OJ L 70, 16.3.2005, p. 1.

[17] Commission Regulation (EC) No 149/2008 of 29 January 2008 amending Regulation (EC) No 396/2005 of the European Parliament and of the Council by establishing Annexes II, III and IV setting maximum residue levels for products covered by Annex I thereto. OJ L 58, 1.3.2008, p. 1.

[18] Draft assessment report prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC COPPER VOLUME 3, ANNEX B.6 Rapporteur Member State's Summary, Evaluation And Assessment Of The Data And Information. 2007. The UL of 0.15 mg Cu per bw and day proposed in the EFSA's Conclusion (EFSA, 2008c) was based on an evaluation of all information submitted with the application for the inclusion of copper compounds as an active substance in Annex I to Directive 91/414/EEC.

[19] For essential nutrients a UL is commonly used to characterise the upper tolerated safe level. For clarification, the FEEDAP Panel continues to use the expression "ADI" for the value proposed in the "Draft assessment report prepared in the context of the possible inclusion of the following active substance in Annex I of Council Directive 91/414/EEC copper Volume 3 Annex B.6" and the expression "UL" for the value proposed by the SCF (EC, 2003).

[20] EFSA, 2008a.

authorised in complete feed (EC, 2003a; Van Paemel et al., 2010; Danish Food Consumption Databank[21]). Copper concentrations in liver are highest in ruminants, with figures ranging from 40 to 140 mg/kg. In steers fed unsupplemented feed, the copper content of liver was 63 mg/kg DM (corresponding to *ca.* 25 mg/kg wet weight) but feed supplemented with 20 mg Cu/kg DM resulted within six months in a copper liver level of 290-380 mg/kg DM (corresponding to *ca.* 116-152 mg/kg wet weight) (Engle et al., 2000). Levels in pigs' liver of ranged from 22.5 to 121.9 mg/kg DM (*ca.* 10 and 48 mg/kg wet weight, respectively) corresponding to supplementation levels of 7.5–120 mg Cu/kg (Bradley et al., 1983). In other studies the level of copper in pigs' liver was shown to be 9 mg Cu/kg (average of 126 samples; Jorhem & Sundström, 1993) and 14.9 mg/kg (average of 62 samples; López-Alonso et al., 2007). Levels in the range 60–80 mg Cu/kg are found in the liver of ducks and geese, whereas considerably lower copper levels (5–15 mg/kg) are reported in chicken liver. Kidney shows a copper content ranging from 3 to 12 mg/kg, muscle tissue from 0.3 to 7 mg/kg, milk up to 0.1 mg/kg and egg up to 1 mg/kg.

Herland et al. (2011) reported figures of up to 0.6 mg Cu/kg in the muscle of salmon and trout and up to 2.7 mg Cu/kg fillet in cod fed diets supplemented with up to 10 mg Cu/kg complete feed; in commercial samples of farmed seabass up to 1.1 mg Cu/kg fillet (Trocino et al., 2012). In Appendix E presents relevant information on the copper concentrations in edible tissues and products from various sources relating to this section.

From the data above, it is evident that MRLs considerably underestimate the natural occurrence of copper in liver from ruminants (and water fowl), and simultaneously reflect unrealistically high concentrations in all other tissues and products. Copper concentration in the liver of ruminants and water fowl is related to the high affinity of copper to hepatocytes.

The 2010 annual monitoring of the EU Member States for residues of veterinary drugs in live animals and animal products[22] was examined and non-compliances on copper were identified. The German data are available on the website of the Federal Office of Consumer Protection and Food Safety (BVL), including copper residues in liver.[23] Of a total of 563 pig liver samples, 6.9 % exceeded the current MRL (range: 31−239 mg/kg; median: 59 mg/kg). The corresponding figure for bovines is 187 liver samples, of which 15 % exceeded the MRL (range: 34−374 mg/kg; median: 138 mg/kg). Since the use of copper as a feed additive is authorised, the BVL stated that the MRLs derived from the use of copper as a pesticide should be modified, if appropriate. The results of the 2011 annual monitoring show comparable non-compliances.[24]

Based on the copper concentrations found in tissues and products of animal origin, and considering the species differences in copper metabolism, the FEEDAP Panel proposes a modification of the current MRLs for copper in animal tissues and products:

- swine, ruminants, horses, poultry and other farm animals (rabbits, kangaroos): liver (except ruminants, swine and water fowl) 20 mg/kg; kidney 12 mg/kg; edible offal 12 mg/kg; meat 3 mg/kg; fat (free of lean meat) 3 mg/kg; other 3 mg/kg
- ruminants liver: 140 mg/kg; swine liver: 30 mg/kg; water fowl liver: 100 mg/kg
- fish flesh (salmonids): 1 mg/kg
- milk: 0.2 mg/kg; eggs (fresh or cooked): 1.5 mg/kg.

[21] Danish Food Consumption Databank—Ed. 7.01. National Food Institute—Technical University of Denmark. http://www.foodcomp.dk/v7/fcdb_foodnutrlist.asp?CompId=0064

[22] Report for 2010 on the results from the monitoring of veterinary medicinal product residues and other substances in live animals and animal products. http://www.efsa.europa.eu/en/supporting/pub/212e.htm

[23] http://www.bvl.bund.de/DE/01_Lebensmittel/01_Aufgaben/02_AmtlicheLebensmittelueberwachung/07_NRKP/01_berichte_nrkp/07_NRKP_ErgaenzendeDokumente_2010/lm_nrkp_bericht_2010_node.html

[24] Data communicated to the European Commission under Council Directive 96/23; retrieved from the EU residue database in September 2012. EFSA's report not published at the time of publishing this opinion.

It should be noted that the proposed MRLs are derived from the highest values found in the literature, and therefore the average copper content of tissues and products of animal origin would be lower.

Applying the default consumption figures of Regulation (EC) No 429/2008 to these proposed MRLs, the maximum adult consumer intake would be 8.2 mg Cu/day, corresponding to 78 % of the proposed ADI, but to 164 % of the UL set by the SCF.

When applying the values in EFSA's guidance for Consumer safety (EFSA, 2012), lower values are obtained. Milk containing copper at the MRL level of 0.2 mg/kg would result, following the consumption of 1050 mL milk, in an exposure of 0.21 mg Cu/day for toddlers; when adding 0.27 mg Cu from the consumption of 90 g meat, the result would be 0.48 mg Cu/day. Both values are clearly below the UL of 1 mg Cu/day. A comparable calculation for adults consuming 1500 mL milk and 60 g liver (from poultry, pig and ruminant at equal amounts) results in an intake of 4.3 mg Cu/day. Adding a background intake from non-animal-derived food of 0.9 mg/day would result in a total intake of 5.2 mg Cu/day, close to the UL. The same calculations with the proposed MRLs for meat, instead of milk, would lead to higher exposure of the consumer: 290 g meat and 60 g liver would result in an intake of 4.9 mg Cu/day, and 5.8 mg Cu/day, including background levels. This calculated excess (15 %) of the UL by adult high consumers of meat and liver (scenario II, Appendix F) is overly conservative as it assumes liver consumption on a daily basis with liver copper levels equalling the proposed MRLs. Therefore, it is highly unlikely that the consumption of animal products at the proposed MRLs will lead to exceedance of the UL in individual consumers. For details of MRLs and the calculations of consumer exposure, see Appendix F.

3.2.5. Conclusions on safety for consumers

Consumption surveys indicated that the mean dietary copper intake by adults in Europe is between 1.0 and 2.0 mg/day. These data include copper from foodstuffs of animal origin. Since the supplementation of animal feed with copper-containing compounds has not essentially changed over the last decade, no change in the contribution of foodstuffs originating from supplemented animals to the overall copper intake of consumers is expected. Since copper sulphate pentahydrate is considered as a kind of standard for other copper-containing compounds, the continued use of copper sulphate pentahydrate in animal nutrition would not modify consumer exposure to copper. No concerns for consumer safety are expected from the use of copper sulphate pentahydrate in animal nutrition.

Copper is an essential micronutrient. The FEEDAP Panel is generally not in favour of establishing MRLs for essential nutrients in foods of animal origin, unless there is a clear consumer safety issue to do so; however, any such MRL has to consider animal health and welfare. The MRLs for edible tissues and products of animal origin established by EU pesticides legislation are found not to comply with the UL set by the SCF and legal feeding practices. If MRLs are to be retained, the FEEDAP Panel proposes the following amended values:

- swine, ruminants, horses, poultry and other farm animals (rabbits, kangaroos): liver (except ruminants, swine and water fowl) 20 mg/kg; kidney 12 mg/kg; edible offal 12 mg/kg; meat 3 mg/kg; fat (free of lean meat) 3 mg/kg; other 3 mg/kg
- ruminants liver: 140 mg/kg; swine liver: 30 mg/kg; water fowl liver: 100 mg/kg
- fish flesh (salmonids): 1 mg/kg
- milk: 0.2 mg/kg; eggs (fresh or cooked): 1.5 mg/kg.

3.3. Safety for the users

No specific studies have been provided by the applicant.

3.3.1. Skin and eye irritancy and sensitisation

The voluntary risk assessment report (VRAR) of 2008, available on the European Chemicals Agency (ECHA) website[25] concluded for copper sulphate pentahydrate that the compound (i) is an irritant to the eye, (ii) is not a skin irritant, and (iii) is not considered as a skin sensitiser (based on a study on guinea pigs). However, copper or copper salts may also induce allergic contact dermatitis in susceptible individuals producing cutaneous itching and eczema (ACGIH, 1991; IPCS, 1998).

Although the concentrations of nickel in the additive are low, the presence of nickel might contribute to the for potential contact dermatitis in workers.

3.3.2. Inhalation toxicity

The VRAR (2008) derived for copper a systemic NOAEL for inhalation repeated dose effects of about 4 mg/kg bw per day, by extrapolating a no observed effect level (NOEL) of 16 mg/kg bw per day observed in an oral 90-day repeated dose study on cupric sulphate (Hébert et al., 1993).

Data on inhalation toxicity for copper reported by the IPCS (1998) suggest an inhibition of the immune response of the lung. In mice, single or repeated three-hour exposures to copper(II) sulphate aerosol resulted in significant immunosuppressive effects, as indicated by reduced resistance to pulmonary infection by *Klebsiella pneumoniae* and *Streptococcus zooepidemicus*. These effects were evident after a single exposure to 0.28 mg Cu/m^3 and above and after 5 or 10 daily exposures to 0.06–0.07 mg Cu/m^3. In hamsters, a single four-hour exposure to copper(II) sulphate pentahydrate aerosol resulted in reduced pulmonary macrophage activity from 3.2 mg Cu/m^3 and above within 1 h after exposure; no effect was observed at 0.3 mg Cu/m^3. The relevant aerosol fractions in copper sulphate were estimated from a graph (data provided by the applicant) to be about: 20 % inhalable (below 100 µm diameter), 10 % thoracic (below 50 µm) and 4 % respirable (below 10 µm). Inhalation exposure to copper from copper sulphate can therefore be calculated to be 12.5 mg inhalable Cu and 2.5 mg respirable Cu/m^3 air. These concentrations in air are higher than the presumed lowest observed adverse effect concentration (LOAEC) in mice (0.28 and 0.06–0.07 mg Cu/m^3 after acute and repeated exposure, respectively) and higher than or similar to the presumed acute LOAEC in hamsters (3.2 mg Cu/m^3).

Inhalation exposure to soluble nickel nickel—an impurity of the additive (up to 60 mg/kg)—is of concern for occupational safety as nickel is recognised to be a respiratory sensitiser, toxic (inducing bronchitis) and a carcinogen (lung cancer)in humans. However the inhalation of nickel as result of this contamination is negligible (below the occupational exposure limit set by Scientific Committee on Occupational Exposure Limits (SCOEL; EC, 2011) and not expected to pose an additional risk to users.

3.3.3. Conclusions on safety for the users

Copper sulphate pentahydrate is an eye irritant but not a skin irritant or skin sensitiser; it may induce allergic dermatitis in sensitive persons, which might be exacerbated by the contamination with nickel. The volume of dust released by the additive in a Stauber–Heubach test indicates that users may be exposed to hazardous copper concentrations by inhalation which could result in a reduced immune response of the lung. The inhalation of nickel resulting from handling the additive is by itself unlikely to be of concern.

3.4. Safety for the environment

During the use of copper-containing feed additives, copper is unavoidably released into the environment. When used in livestock feed, copper excreted in the faeces will enter the soil environment when the faeces are applied as fertiliser to the land in the form of manure, slurry or litter. This may present two main potential risks:

[25] http://echa.europa.eu/copper-voluntary-risk-assessment-reports/-/substance/464/search/+/term

- copper accumulation within the topsoil to concentrations posing potential toxic risk to soil organisms
- leaching of copper from the soil to surface waters in concentrations posing potential toxic risk to organisms resident in the water column and bottom sediments.

When used in aquaculture, trace elements such as copper may be released directly to the broader aquatic environment around an aquaculture facility or be taken up by fish and then excreted into the environment. As stated in the EFSA technical guidance for assessing the safety of feed additives for the environment (EFSA, 2008d), the compartment of concern for fish farmed in cages is assumed to be the sediment, whereas for fish farmed in land-based systems the effluent flowing to surface water is considered to pose the main environmental risk.

EFSA commissioned a study on the environmental impact of zinc and copper used in animal nutrition (Monteiro et al., 2010). The results of this study were used as the basis for the present opinion.

To assess the potential risks from copper used as additive in feed for terrestrial animals a model was used, which integrates the physicochemical and hydrological processes that determine the accumulation and leaching of metals in soil. Input rates of metals resulting from the use of feed additives and the spreading of animal manure on the land were based on the maximum allowable metal contents of feed additives for different livestock types and the maximum allowable rates of nitrogen input of 170 kg/ha per annum. The assessment is based on the worst case assumption that the total amount of additive consumed will be excreted.

Calculation of concentrations in surface water (as dissolved metal) and sediment (as total sediment metal) was done based upon the Forum for the Coordination of Pesticide Fate Models and their Use (FOCUS) scenario methodology and taking into consideration the speciation in the environment. More specific information on the parameterisation and assumptions made is given in the report.

The predicted no-effect concentrations (PNECs) for the different compartments were calculated following the same methodologies as those presented in the EU risk assessment report for copper by correcting for bioavailability based on the assumed soil and water chemistry of the different scenarios. Likewise, it was decided to use the added PNEC approach for copper.

The environmental risks of copper arising from aquaculture were assessed using the exposure models recommended in the technical guidance (EFSA, 2008d). The estimated concentrations in surface water resulting from the use of copper as feed additive for different fish species farmed in raceways, ponds, tanks and recirculation systems and the estimated concentration in sediment arising from the use of feed additives in sea cages were all below the PNEC and therefore do not give rise to concern.

Concerning the terrestrial environment, potential risks to soil organisms have been identified as a result of application of piglet manure. However, of the nine scenarios in which a potential risk was identified, only two have local significance for pig production. Levels of copper in other types of manure are too low to create a potential risk within the timescale considered.

For the water compartment, none of the scenarios resulted in exceeding the PNEC when corrected for bioavailability. However, predicted concentrations of metals in the sediments of receiving waters, derived from the erosion of metal-enriched particles and transport in drainage and runoff, responded significantly to increases in metal inputs due to manure application. Potential risks were predicted within 50 years for several scenarios (i.e. R3, R1, D6, D5, D2 and D1). In two scenarios (D2 and R3) exceedances were predicted due to all manure types. The predictions of the D2 scenario are of particular note as it is a cracking clay soil of the type vulnerable to bypass flow during events and thus potentially to extensive transport of particles to drainage as is simulated in that scenario.

In the view of the FEEDAP Panel, these findings should be treated with caution as further model validation is needed and refinements are feasible, e.g. by taking into account the surface water chemistry of the locations of the FOCUS scenarios, more updated bioavailability models, re-suspension and washout of deposited sediment and chemical transformation of trace elements in the sediment following deposition (i.e. formation of acid-volatile sulphide and metal sulphides).

3.4.1. Conclusions on safety for the environment

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.

3.5. Influence of copper in animal nutrition on the development of antibiotic resistance in bacteria

The influence of copper in animal nutrition on the development of antibiotic resistance in gut and soil bacteria was considered by the FEEDAP Panel. The full report is presented in Appendix B.

The limited database available on the influence of copper to the development of antibiotic resistance in gut and soil bacteria allows concluding that (i) high copper concentrations in the microbial environment increase the number of copper-resistant bacteria and (ii) copper resistance seems to be correlated with a more frequent resistance to several antibiotics in certain bacterial species. For *Enterococcus faecium* it could be shown that the *ermB* gene coding for erythromycin (and generally macrolide antibiotic) resistance is located on the same plasmid as the *tcrB* (coding for copper resistance), making co-transfer plausible. The current database does not allow any conclusion on a potential threshold concentration of copper in feeds, below which a significant increase in copper resistance could not be expected. The total pool of macrolide resistance in animals probably originates from antibiotic treatment and not from the use of high dietary copper. The extent to which copper-resistant bacteria contribute to the overall antibiotic resistance situation can not be quantified at present. More precise (and quantitative) conclusions need further studies.

4. Efficacy

The use of copper sulphate pentahydrate in animal nutrition is extensively documented in the scientific literature. It is recognised as an efficacious source of copper in meeting animal requirements.

5. Post-market 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[26] and Good Manufacturing Practice.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

Copper sulphate pentahydrate is safe for all animal species/categories up to the maximum total copper content authorised in feed.

Consumption surveys include copper from foodstuffs of animal origin. Since the supplementation of animal feed with copper-containing compounds has not essentially changed over the last decade, no change in the contribution from foodstuffs originating from supplemented animals of the overall

[26] 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.

copper intake of consumers is expected. No concerns for consumer safety are expected from the use of copper sulphate pentahydrate in animal nutrition.

Copper is an essential micronutrient. The FEEDAP Panel is generally not in favour of establishing MRLs for essential nutrients in foods of animal origin, unless there is a clear consumer safety issue to do so; however, any such MRL has to consider animal health and welfare.

The MRLs for copper in edible tissues and products of animal origin established by EU pesticides legislation are found not to comply with the UL set by the SCF and legal feeding practices. After an assessment of the current MRLs values set for the use of copper as pesticide, and if MRLs for tissues and products of animal origin are to be retained, the FEEDAP Panel proposes the following amended values:

- swine, ruminants, horses, poultry and other farm animals (rabbits, kangaroos): liver (except ruminants, swine and water fowl) 20 mg/kg; kidney 12 mg/kg; edible offal 12 mg/kg; meat 3 mg/kg; fat (free of lean meat) 3 mg/kg; other 3 mg/kg
- ruminants liver: 140 mg/kg; swine liver: 30 mg/kg; water fowl liver: 100 mg/kg
- fish flesh (salmonids): 1 mg/kg
- milk: 0.2 mg/kg; eggs (fresh or cooked): 1.5 mg/kg.

Copper sulphate pentahydrate is an eye irritant, but not a skin irritant or skin sensitiser; it may induce allergic dermatitis in sensitive persons, which might be exacerbated by the contamination with nickel. The dusting potential of the additive indicates that users may be exposed to hazardous copper concentrations by inhalation which could result in a reduced immune response of the lung. The inhalation of nickel resulting from handling the additive is by itself unlikely to be of concern.

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 limited database available on the influence of copper to the development of antibiotic resistance in gut and soil bacteria allows to conclude that (i) high copper concentrations in the microbial environment increase the number of copper-resistant bacteria and (ii) copper resistance seems to be correlated with a more frequent resistance to several antibiotics in certain bacterial species. A co-transfer of plasmid genes encoding for resistance to copper and erythromycin is plausible, at least in *Enterococcus faecium*. The current database does not allow any conclusion on a potential threshold concentration of copper in feeds, below which a significant increase in copper resistance could not be expected. The total pool of macrolide resistance in animals probably originates from antibiotic treatment and not from the use of high dietary copper. The extent to which copper-resistant bacteria contribute to the overall antibiotic resistance situation can not be quantified at present. More precise (and quantitative) conclusions need further studies.

Copper sulphate pentahydrate is recognised as an efficacious source of copper in meeting animal requirements.

RECOMMENDATIONS

The specification of copper sulphate pentahydrate should include a minimum copper content. Based on the analytical values this could be 25 %.

The labelling requirements set under "Other provisions" for Copper in Regulation (EC) No 1334/2003 should be considered.

REMARKS

The MRLs for copper in edible tissues and products of animal origin established by EU pesticides legislation are found not to comply with the UL set by the SCF—as shown by different model calculations—as well as with legal feeding practices. Copper fulfils vital functions in living organisms and it is an essential micronutrient. The FEEDAP Panel is generally not in favour of establishing MRLs for essential nutrients in foods of animal origin, unless there is a clear consumer safety issue to do so; however, any such MRL has to consider animal health and welfare. In case MRLs for animal products shall be maintained, the FEEDAP Panel proposes amended values to those set in the pesticide legislation.

Controversial data are available for the safety assessment of consumer exposure to copper since two different values characterising the upper safe intake exist, an UL of 5.0 mg per person and day set by the SCF in 2003 and an ADI corresponding to 10.5 mg per person and day proposed by the EFSA PRAPeR Unit. Therefore the FEEDAP Panel proposes a reconsideration of all available data resulting in a harmonised safe maximum daily intake value.

A reduction in the maximum copper content in feed for ruminants to bring it close to the minimum requirement would reduce copper concentration in the liver. However, this measure can hardly be realised in practice because of the varying occurrence of copper antagonists in feedingstuffs (mainly molybdenum and sulphur), particularly in roughages, requiring additional copper to prevent the risk of copper deficiency and its consequences on animal health.

DOCUMENTATION PROVIDED TO EFSA

1. Dossier Cupric sulphate pentahydrate, for all animal species. November 2010. Submitted by Manica S.p.A.
2. Dossier Cupric sulphate pentahydrate, for all animal species. Supplementary information. February 2012. Submitted by Manica S.p.A.
3. Letter. Multidrug-resistant pathogenic bacteria - Copper in soil. May 2011. Sent by the European Commission.
4. Evaluation report of the European Union Reference Laboratory for Feed Additives on the methods(s) of analysis for Copper (E4). January 2012.
5. EFSA Internal Scientific Report. Protocol of the systematic review of Influence of Copper on antibiotic resistance of gut microbiota in pigs (including piglets). May 2012.
6. The Report on Copper and antibiotic resistance from University of Ghent. May 2012.
7. EFSA Internal Report. Technical assistance in retrieving and collecting data on non compliances on Copper from the EU residue database. September 2012.
8. Comments from Member States received through the ScienceNet.

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APPENDICES

APPENDIX A

Executive Summary of the Evaluation Report of the European Union Reference Laboratory for Feed Additives on the Method(s) of Analysis for Copper[27]

In the current application authorisation is sought under articles 4(1) and 10(2) for *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 (solid & liquid)* under the category/functional group 3(b) "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 additives* for all categories and species.

Applicants stated minimum *total copper* content of: 31% in *Cupric acetate monohydrate;* 55% in *Basic cupric carbonate monohydrate;* 37% in *Cupric chloride dihydrate;* 77% in *Cupric oxide;* 24% in *Cupric sulphate pentahydrate;* 10% in *Cupric chelate of amino-acids hydrate;* and 23% and 6% in solid and liquid *Cupric chelate of glycine hydrate*, respectively.

The *feed additives* are intended to be incorporated into *premixtures*, *feedingstuffs* and *water*. All Applicants suggested maximum levels of total copper in the *feedingstuffs* complying to the limits set in Regulations (EC) No 1334/2003 and 479/2006 and ranging from 15 to 170 mg/kg, depending of the animal species/category.

The EURL recommends three European Pharmocopoeia methods: - Ph. Eur. monograph 01/2008:2146 for the identification of *Copper acetate monohydrate;* - Ph. Eur. monograph 01/2008:0894, for the identification of *Copper sulphate pentahydrate*; and - the generic Ph. Eur. monograph 01/2008:20301 for the "identification reactions of ions and functional groups", such as acetates, carbonates, chlorides and sulphates. Additionally crystallographic techniques such as X-Ray diffraction could be used for the characterisation of crystalline structure of *Cupric acetate monohydrate*, *Cupric chloride dehydrate, Copper oxide* and *Cupric sulphate pentahydrate*.

For the quantification of "amino" content in the amino copper chelates (i.e. *Copper chelate of glycine hydrate* and *Copper chelate amino acids hydrate*), the Applicant proposed - upon request from the EURL - the Community method based on High Performance Liquid Chromatography (HPLC) combined with post-column derivatisation using ninhydrin as derivatisation agent and photometric detection at 570 nm. The EURL considers the Community method suitable for the characterisation of the amino compounds in the frame of official control.

For the *determination* of total copper in the *feed additive*, *premixtures* and *feedingstuffs* the Applicants submitted internationally recognised ring trial validated methods EN 15510 and CEN/TS 15621. Both methods are based on inductively coupled plasma atomic emission spectroscopy, with or without pressure digestion. The following performance characteristics were reported for EN 15510:

- a relative standard deviation of *repeatability* (RSD_r) ranging from 2.9 to 12 %;

- a relative standard deviation for *reproducibility* (RSD_R) ranging from 8 to 22 %; and

- a limit of quantification (LOQ) of 3 mg/kg.

A variety of matrices (i.e. feed for pigs and for sheep, rock phosphate, a mineral premix and a mineral mix) with a total copper content ranging from 7.3 to 470 mg/kg was used in the frame of the CEN/TS

[27] The full report is available on the EURL website. http://irmm.jrc.ec.europa.eu/SiteCollectionDocuments/FinRep-CopperGroup.pdf

15621 ring-trial. The following performance characteristics were reported: - RSD_r ranging from 2.6 to 6.8 %; - RSD_R ranging from 3.8 to 12 %; and - LOQ = 1 mg/kg *feedingstuffs*.

Furthermore, a Community method is available for the determination of total copper in *feedingstuffs*, but no performance characteristics for the method were provided. The UK Food Standards Agency recently reported results of a ring-trial based on the above mentioned Community method, and reported precisions (RSD_r and RSD_R) for *feedingstuffs* ranging from 2.4 to 9.2 %.

Based on these performance characteristics the EURL recommends for official control the CEN methods EN 15510 or CEN/TS 15621 to determine total copper content by ICP-AES in the *feed additive* and *premixtures*. As for the determination of total copper content in *feedingstuffs*, the EURL recommends for official control the Community method based on AAS and the above mentioned CEN methods (EN 15510 or CEN/TS 15621).

Similarly to the "SANCO Zinc group", the EURL recommends the ring-trial validated CEN method EN ISO 11885, based on inductively coupled plasma optical emission spectroscopy (ICP-AES) for the quantification of total copper in *water*.

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.

APPENDIX B

Influence of copper in animal nutrition on the development of antibiotic resistance in bacteria

1. Introduction

In recent scientific literature, attention has been paid to the prevalence of antibiotic and copper resistance genes and especially to the assumed linkage between them and their regulation, in bacterial communities potentially exposed to high concentrations of copper. The FEEDAP Panel has considered it appropriate to undertake the evaluation of the item from two angles: (a) the copper supplementation to the animals and its influence in the gut microbiota and (b) the copper in soil and development of antimicrobial resistance of soil bacteria. For the former, a Systematic literature review "Influence of Copper on antibiotic resistance of gut microbiota on pigs, including piglets" has been conducted.

2. Systematic Literature Review: "Influence of Copper on antibiotic resistance of gut microbiota on pigs (including piglets)"

A Systematic Literature Review (SLR) was conducted and it is summarised below.

A total of 227 references was examined to assess the influence of a copper supplemented diet on antibiotic resistance of gut microbiota in pigs (including piglets). The total number of studies selected was very low: seven "field studies", eight "environmentally controlled studies" and ten "cross-sectional studies". Only the "field studies" selected (seven) could directly reply to the review question; from those, only three were deemed to have an appropriate methodological quality.

The three relevant field studies (Hasman et al., 2006; Amachawadi et al., 2010, 2011) demonstrated that elevated copper levels (125-208 mg/kg feed) may increase tolerance or passive or active resistance of the bacterial community to copper, which in turn can be associated with the selection of antibiotic resistant strains of bacteria. This was especially the case for bacterial species such as *Enterococcus faecium*, widely diffused in the animal gut and in the environment. In this species, the antibiotics of greatest concern are macrolides, erythromycin in particular. Thus, it has been demonstrated that in this species the gene responsible for copper resistance (*tcrB*, coding for an ATPase) is usually located on the same plasmid as *ermB*, a gene conferring resistance to erythromycin. The relationship between the presence of the *tcrB* gene and phenotypic resistance to copper was confirmed. *E. faecium* isolates have a higher prevalence of *tcrB*-positive isolates compared with *Enterococcus faecalis* isolates. Co-selection between resistance in copper and resistance to the glycopeptide antibiotic vancomycin (*vanA* and *vanB* genes) was lower or absent. Additionally, in another study (Ragland et al., 2006) in pigs there was no increase reported in vancomycin-resistant isolates between the control group (11.2 mg/kg feed) and the group receiving increased copper supplementation (192.4 mg/kg feed).

The co-selection of copper and erythromycin resistance also became evident from the "environmentally controlled studies". Hasman and Aarestrup (2002) found that the same dose of 63.5 mg Cu/L growth medium resulted in a different copper resistance prevalence : the *tcrB* gene was found in *E. faecium* isolated from pigs (75 %), broilers (34 %), calves (16 %) and humans (10 %), but not in isolates from sheep. The occurrence of this resistance gene in these species reflects the frequency of antibiotic use, as a feed additive or as medicine. This study could suggest that the copper resistance in these bacterial communities is related to the already existing antibiotic resistance in bacterial populations isolated from these species.

All the "cross-sectional studies" showed rather poor methodological quality. This was mainly due to the selection of isolates that was not representative for this review question or the limited information on the copper to which animals were exposed.

3. Copper in soil and development of antimicrobial resistance of soil bacteria

Forty-six soil samples (from the year 1940) were analysed for their geochemical composition and antibiotic resistance genes (*tetM*, *tetQ*, *tetW*, *blaTEM*, *blaSHV*, *blaCTX*, *blaOXA*, *ermB*, *ermC*, *ermE*) of the soil bacteria (Knapp et al., 2011). Statistical analysis examined correlations between different trace elements and the occurrence of antibiotic resistance genes. Five out of eleven genes showed significant positive correlations with copper level; positive correlations with chromium, nickel, lead, and iron were also found but at a lower frequency. Studies of soil samples treated with copper-spiked sewage gave inconsistent results. The authors concluded that geochemical metal conditions of the soil innately influence the potential for antibiotic resistance.

Berg et al. (2005) studied whether copper-amendment of field plots affects the frequency of copper resistance, and antibiotic resistance patterns in indigenous soil bacteria. Soil bacteria were isolated from untreated (8.7 mg Cu/kg soil) and copper-amended (116.7 mg Cu/kg soil, by the application of copper sulphate) field plots. Copper load significantly increased the frequency of copper-resistant isolates. A panel of isolates were characterized by Gram stain reaction, amplified ribosomal DNA restriction analysis and resistance profiling against seven antibiotics. More than 95 % of the copper-resistant isolates were Gram negative. Copper-resistant Gram-negative isolates had significantly higher incidence of resistance to ampicillin, sulphanilamide and multiple antibiotics than copper-sensitive Gram negative isolates. Furthermore, copper-resistant Gram-negative isolates from copper-contaminated plots had a significantly higher incidence of resistance to chloramphenicol and multiple antibiotics than corresponding isolates from control plots.

The current copper concentration in European agricultural soil is about 31 mg/kg DM, with a range between 16 and 58 mg/kg DM (Heijerick et al., 2006). Therefore the copper concentration in copper-loaded soil investigated by Berg et al. (2005) was higher than the current concentrations in soil. Scenarios with continuous use of manure from animals fed under the current conditions indicated that no relevant increase in copper soil concentration was to be expected within the next 50 years with one exception: different scenarios with continuous application of manure from piglets only, fed diets supplemented up to the highest EU-authorised copper maximum content, predicted copper concentrations in soil ranging between 55 and 110 mg/kg (Monteiro et al., 2010).

Berg et al. (2010) published a second study in which considerably higher copper concentrations in soil were investigated (3172 mg/kg soil). The authors concluded that high copper exposure selected for copper-tolerant bacterial communities but also co-selected for increased community-level tolerance to tetracycline and vancomycin. Copper-resistant isolates showed significantly higher incidence of resistance to five out of seven antibiotics (tetracycline, olaquindox, nalidixic acid, chloramphenicol and ampicilin) than Cu-sensitive isolates. However, this high copper concentration in soil would never be reached by the use of manure from animals fed diets supplemented up to the highest copper content authorised (i.e. 170 mg Cu/kg complete feedingstuff vs. 3172 mg Cu/kg soil).

Comparing the low dose of copper due to the spreading of manure with the high dose of copper used in the experiments of Berg (2010) is not straightforward. The occurrence of resistance is greatly enhanced by the presence of manure (Schmitt 2005). After the spreading of manure, the copper and the growing bacteria are both present in the patches of dung and not homogenised throughout the soil. The bioavailability of copper in freshly applied manure might also be much higher than in an aged copper-polluted soil.

As copper, zinc and antibiotics are generally used in pig farming the occurrence of coupled resistance genes against all of these might not be a coincidence. In a novel study the resistance of copper and zinc was coupled with the resistance of beta-lactams (Holzel, 2012). The resistance of mercury was not coupled. This might have been caused by the use of copper, zinc and antibiotics whereas mercury is not used.

4. Conclusions

High dietary copper induces an increase of copper-resistant bacteria. In one bacterial species, *E. faecium*, this resistance is coded by *tcrB* gene. In this bacterial species the *ermB* gene coding for erythromycin (and generally for macrolide antibiotics) resistance is located on the same plasmid as the *tcrB* (coding for copper resistance), making co-transfer plausible. In *E. faecium*, the resistance to copper would therefore be associated with erythromycin (macrolide) resistance. This has been confirmed by studies in *E. faecium*, and to a markedly lesser extent in *E. faecalis*. The total pool of macrolide resistance in animals probably originates from antibiotic treatment and not from the use of high dietary copper. The co-selection of resistance to copper and resistance to the glycopeptide antibiotic vancomycin (*vanA* and *vanB* genes) was less or not evident. More precise (and quantitative) conclusions need further studies, particularly in piglets feed for which shows the highest authorised copper content in Europe (170 mg/kg feed).

Data from soil bacterial isolates confirmed a principal correlation between the development of resistance to copper and resistance to various antibiotics, particularly in Gram-negative bacteria. The available data does not allow any estimate of the practical relevance of these findings, particularly because potentially critical soil levels were estimated in a scenario simulating continuous application of manure from piglets fed the maximum copper content authorised in feed in the EU. Further research is required to characterise the prevalence of antibiotic resistance in soil bacteria due to copper load.

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APPENDIX C

List of Risk Assessment Reports on copper and copper compounds

Besides the reports cited in the Background section, risk assessments have been carried out by other EU bodies, Institutions and Industry (see list below). Stern (2010) reviewed the essentiality and toxicity in copper health risk assessment.

1. EU Risk Assessment Reports (RARs)

The Voluntary Risk Assessment Reports (VRAR) submitted to ECHA for Cu (http://echa.europa.eu/chem_data/transit_measures/vrar_en.asp)

2. EC Health and Consumers Scientific Committees Opinions

The Scientific Committee on Health and Environmental Risk (SCHER) opinion on the VRAR on Copper, Copper II sulphate pentahydrate, Copper(I)oxide, Copper(II)oxide, Dicopper chloride trihydroxide (Human health part) (http://echa.europa.eu/doc/trd_substances/VRAR/Copper/scher_opinion/scher_opinion_hh.pdf)

The Scientific Committee on Health and Environmental Risk (SCHER) opinion on the VRAR on Cu and its compounds (Environmental part) (http://echa.europa.eu/doc/trd_substances/VRAR/Copper/scher_opinion/scher_opinion_env.pdf)

3. EU Member States. Risk Assessment Reports

Risk Assessment Copper. Expert Group on Vitamins and Minerals 2003. (http://www.food.gov.uk/multimedia/pdfs/evm_copper.pdf)

Prediction of the long term accumulation and leaching of copper in Dutch agricultural soils: a risk assessment study. Published on 20 April 2006. (report n° 1278, http://www.alterra.wur.nl/NL/publicaties+Alterra/Alterra+rapporten/)

4. EFSA-ANS Panel Opinions

Copper(II) oxide as a source of copper added for nutritional purposes to food supplements (http://www.efsa.europa.eu/en/efsajournal/pub/1089.htm)

Magnesium aspartate, potassium aspartate, magnesium potassium aspartate, calcium aspartate, zinc aspartate, and copper aspartate as sources for magnesium, potassium, calcium, zinc, and copper added for nutritional purposes to food supplements - Scientific Panel on Food Additives and Nutrient Sources added to food (http://www.efsa.europa.eu/en/efsajournal/pub/883.htm)

Orotic acid salts as sources of orotic acid and various minerals added for nutritional purposes to food supplements (http://www.efsa.europa.eu/en/efsajournal/pub/1187.htm)

5. EFSA-CEF Panel Opinions

Scientific Report of EFSA on the risk assessment of salts of authorised acids, phenols or alcohols for use in food contact materials (http://www.efsa.europa.eu/en/efsajournal/pub/1364.htm)

Scientific Opinion on the safety evaluation of the substance, copper hydroxide phosphate, CAS No. 12158-74-6, for use in food contact materials (http://www.efsa.europa.eu/en/efsajournal/pub/1838.htm)

6. EFSA-AFC Panel Opinions

Opinion on certain bisglycinates as sources of copper, zinc, calcium, magnesium and glycinate nicotinate as source of chromium in foods intended for the general population (including food supplements) and foods for particular nutritional uses[1] - Scientific Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (http://www.efsa.europa.eu/en/efsajournal/pub/718.htm)

7. EFSA-NDA Panel Opinions

Scientific Opinion on the substantiation of health claims related to copper and protection of DNA, proteins and lipids from oxidative damage (ID 263, 1726), function of the immune system (ID 264), maintenance of connective tissues (ID 265, 271, 1722), energy-yielding metabolism (ID 266), function of the nervous system (ID 267), maintenance of skin and hair pigmentation (ID 268, 1724), iron transport (ID 269, 270, 1727), cholesterol metabolism (ID 369), and glucose metabolism (ID 369) pursuant to Article 13(1) of Regulation (EC) No 1924/2006 (http://www.efsa.europa.eu/en/efsajournal/pub/1211.htm)

Scientific Opinion on the substantiation of health claims related to copper and reduction of tiredness and fatigue (ID 272), maintenance of the normal function of the nervous system (ID 1723), maintenance of the normal function of the immune system (ID 1725) and contribution to normal energy-yielding metabolism (ID 1729) pursuant to Article 13(1) of Regulation (EC) No 1924/2006. (http://www.efsa.europa.eu/en/efsajournal/pub/2079.htm)

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APPENDIX D

List of authorisations for copper other than as a feed additive

The following copper compounds are authorised for use in food (Regulation (EC) No 1170/2009):[28] copper L-aspartate, copper bisglycinate, copper lysine complex, copper (II) oxide (which may be used in the manufacture of food supplements); copper lysine complex which may be added to foods.

The following copper compounds can be used for the manufacturing of dietetic foods (Commission Regulation (EC) No 953/2009):[29] cupric carbonate, cupric citrate, cupric gluconate, cupric sulphate and copper lysine complex.

The following copper compounds can be used for the manufacturing of processed cereal-based foods and baby foods for infants and young children (Commission Directive 2006/125/EC):[30] Copper-lysine complex, Cupric carbonate, Cupric citrate, Cupric gluconate, Cupric sulphate.

Regarding pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin, the following copper compounds are listed in Table 1 of the Annex of Regulation 37/2010[31] as Allowed substances, no MRL required: Copper chloride, Copper gluconate, Copper heptanoate, Copper methionate, Copper oxide, Copper sulphate and Dicopper oxide.

The following copper compounds are listed in Annex of Commission Implementing Regulation (EU) No 540/2011[32] as "Active substances approved for use in plant protection products": Copper (II) hydroxide (Copper hydroxide), Dicopper chloride trihydroxide (Copper oxychloride) and Copper oxide.

The following type of fertilisers for copper as Fertilisers containing only one micro-nutrient are listed in Annex I of Regulation (EC) No 2003/2003[33] of the European Parliament and of the Council: (a) Copper salt (chemically obtained product containing a mineral salt of copper as its essential ingredient), (b) Copper oxide (chemically obtained product containing copper oxide as its essential ingredient), (c) Copper hydroxide (chemically obtained product containing copper hydroxide as its essential ingredient), (d) Copper chelate (water-soluble product obtained by combining copper chemically with a chelating agent), (e) Copper-based fertiliser (product obtained by mixing types "a" and/or "b" and/or "c" and/or a single one of type "d" and, if required, filler that is neither nutrient nor toxic), (f) Copper fertiliser solution (product obtained by dissolving types "a" and/or one of the type "d" in water), (g) Copper oxychloride (chemically obtained product containing copper oxychloride [$Cu_2Cl(OH)_3$] as an essential ingredient), (h) Copper oxychloride suspension (product obtained by suspension of type "g").

[28] Commission Regulation (EC) No 1170/2009 of 30 November 2009 amending Directive 2002/46/EC of the European Parliament and of Council and Regulation (EC) No 1925/2006 of the European Parliament and of the Council as regards the lists of vitamin and minerals and their forms that can be added to foods, including food supplements. OJ L 314, 1.12.2009, p. 36.

[29] Commission Regulation (EC) No 953/2009 of 13 October 2009 on substances that may be added for specific nutritional purposes in foods for particular nutritional uses. OJ L 269, 14.10.2009, p. 9.

[30] Commission Directive 2006/125/EC of 5 December 2006 on processed cereal-based foods and baby foods for infants and young children. OJ L 339, 6.12.2006, p. 16.

[31] Commission Regulation (EU) No 37/2010 of 22 December 2009 on pharmacologically active substances and their classification regarding maximum residue limits in foodstuffs of animal origin. OJ L 15, 20.1.2010, p. 1.

[32] Commission Implementing Regulation (EU) No 540/2011 of 25 May 2011 implementing Regulation (EC) No 1107/2009 of the European Parliament and of the Council as regards the list of approved active substances. OJ L 153, 11.6.2011, p. 1.

[33] Regulation (EC) No 2003/2003 of the European Parliament and of the Council of 13 October 2003 relating to fertilisers. OJ L 304, 21.11.2003, p. 1.

The following copper compounds can be used for cosmetic purposes (Regulation (EC) No 1223/2009 of the European Parliament and of the Council):[34] 74160 (29H,31H-Phthalocyaninato (2-)-N29,N30, N31, N32 copper), 74260 (Polychloro copper phthalocyanine), 77400 (Copper).

According to the Annex to Regulation (EC) No 432/2012[35] the following health claims can be made only for food which is at least a source of copper as referred to in the claim *SOURCE OF [NAME OF VITAMIN/S] AND/OR [NAME OF MINERAL/S]* as listed in the Annex to Regulation (EC) No 1924/2006[36]: “Copper contributes to maintenance of normal connective tissues”, “Copper contributes to normal energy-yielding metabolism”, “Copper contributes to normal functioning of the nervous system”, “Copper contributes to normal hair pigmentation”, “Copper contributes to normal iron transport in the body”, “Copper contributes to normal skin pigmentation”, “Copper contributes to the normal function of the immune system” and “Copper contributes to the protection of cells from oxidative stress”.

[34] Regulation (EC) No 1223/2009 of the European Parliament and of the Council of 30 November 2009 on cosmetic products. OJ L 342, 22.12.2009, p. 59.

[35] Commission Regulation (EC) No 432/2012 of 16 May 2012 establishing a list of permitted health claims made on foods, other than those referring to the reduction of disease risk and to children’s development and health. OJ L 136, 25.05.2012, p.1.

[36] Regulation (EC) No 1924/2006 of the European Parliament and of the council of 20 December 2006 on nutrition and health claims made for food. OJ L 404, 30.12.2006, p.9.

APPENDIX E

Copper content in animal tissues and products.

Table E1: Copper contents in animal tissues and products. (From Van Paemel et al., 2010,[1] unless otherwise specified).

A. Data from feed supplementation studies

Species/category	Duration	Dietary Cu (mg Cu/kg)	Liver (mg Cu/kg)	Muscle (mg Cu/kg)	Egg (mg Cu/kg)
Pig	161 days	15	7.9	0.44	
		30	13.4	0.43	
Pig[2]	Not specified	22	8.8	1.5	
	"	35	16.8	0.7	
	"	14.4/10.2*	22.4	0.98	
	"	15/17*	36.4	1.08	
Laying hens	28 days	27	13.4		1.04
	28 days	23.8			< 0.9 yolk / < 0.3 white
		30.8			< 0.9 yolk / < 0.3 white
	56 days	8.1	5.0		1.61 yolk / 0.22 white
		29.9	5.4		2.02 yolk / 0.23 white
Chicks	21 days	9.8	7.7		
Steers	177 days	0**	25.3	0.87	
		10**	113	1.1	
		20**	152	0.75	
Dairy cows	60 days	5.5	67.5		
		43	191.5		
Sheep[2]	Not specified	3.5	120	1.25	
Goat[2]	Not specified	4	40	1	
	"	19	140	2	
	"	6	126	0.45	
Rainbow trout	28 days	11.4	38.4	0.26	
		277.8	45.2	0.32	
Atlantic cod[3]	2 years	2.8		0.4	
		10.1		2.7	
Sea Bass (Farmed)[4]	Not specified	11.3		1.1	
		14.8		0.99	

(*) Copper concentration in Grower/Finisher

(**) Supplemented copper. Background in feed not reported

(1) Van Paemel M, Dierick N, Janssens G, Fievez V and De Smet S, online, 2010. Selected trace and ultratrace elements: Biological role, content in feed and requirements in animal nutrition – Elements for risk assessment. Technical Report submitted to EFSA. http://www.efsa.europa.eu/en/supporting/doc/68e.pdf

(2) EC (European Commission), 2003a. Scientific Committee on Animal Nutrition (SCAN) delivered report on the use of copper in feedingstuffs (19 February 2003). http://ec.europa.eu/food/fs/sc/scan/out115_en.pdf

(3) Herland H, Cooper M, Esaiassen M and Olsen RL, 2011. Effects of Dietary Mineral Supplementation on Quality of Fresh and Salt-Cured Fillets from Farmed Atlantic Cod, *Gadus morhua*. Journal of the World Aquaculture Society 42 (2) 261–267.

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B. Data from survey studies

Species/category	No of samples	Liver (mg Cu/kg)	Muscle (mg Cu/kg)	Egg (mg Cu/kg)	Milk (mg Cu/kg)
SWINE					
Hogs	326	11.1	1.16		
Boars/sows	280	18.3	0.93		
Pigs for fattening	126	9.0	0.36-0.92		
Pigs (6 m old)	62	14.9	6.85		
Pigs(5)		10.1			
Pigs(6)		13.0	0.9		
RUMINANTS					
Calves (6 - 12 m old)	195	89.6			
Calves	327	138	1.56		
Calves(5)		23.5			
Calves(6)			1.6		
Veal	438	64.6	0.68		
Heifers/steers	287	46.1	1.77		
Bulls/cows	95	43.7	1.41		
Cattle	56		1.70		
	97	80.1	4.97		
	100	20.4	3.89		
			0.375-0.775		
Cattle(6)		32			0.1
		55			
Dairy cattle	3				0.001-0.012
	16				0.12
					0.052
Ox(5)		64			
Lambs	165	89.8	1.47		
			1.10-1.32		
Mature sheep		131.4	2.32		
Lamb(6)		76	1.35		0.15
Goat(6)					0.1
POULTRY					
Chicken	308	4.60	3.07		
Chickens(5)		5.1			
Chickens(6)		3.2	0.5		
Laying hens	22			0.604	
	19			0.507	
	40			0.43-0.52	
Hens(5)		5.1			
Hens(6)				0.65	
Turkey	60	7.14	3.68		
Turkey(6)			1.0		
Duck	111	66.7	5.9		
Duck(5)		59.6			
Duck(6)			2.4		
Goose(5)		75.2			
Goose(6)			3.3		
RABBITS(6)			1.5		
HORSES(6)			2.1		0.3
FISH					
Cod(5)		6.6			
Cod(6)			0.5		
Herring(5)			0.08		
Herring, Atlantic(6)			1.3		
Herring, Baltic see(6)			0.1		
Mackerel(5)			0.9		
Mackerel(6)			1.1		

Eel(5)	0.03
Eel(6)	0.9
Trout(5)	0.05
Trout(6)	1.5
Carp(6)	0.9
Salmon(5)	0.17
Salmon(6)	1.3

(5) Danish Food Consumption Databank – Ed. 7.01. National Food Institute – Technical University of Denmark. http://www.foodcomp.dk/v7/fcdb_foodnutrlist.asp?CompId=0064

(6) Souci SW, Fachmann W and Kraut H, 2008. Food composition and nutrition tables. 7th Edition. MedPharm Scientific Publisher, Stuttgart, Germany; and CRC Press, Taylor and Francis Group, LLC, Boca Raton, USA;

Table E2: Copper contents in liver. Monitoring of veterinary medicinal product residues and other substances in live animals and animal products, year 2010. Data from one Member State.(1)

Species	No. of samples	Category	Concentration range (mg/kg)	No. of non-compliant samples*	Percent non-compliant samples*
Bovine	188	Calves	71.3 - 374	9	4.8
		Cows	54 - 297.6	8	4.3
		Beef cattle	37 - 195	9	4.8
		Other cattle	70.4 - 129	2	1.1
Sheep	5	Fattening lambs	265	1	20
Pigs	563	Piglets	31.2 - 142	9	1.6
		Fattening pigs	35.1 - 239.4	26	4.6
		Other pigs	42.2 - 131	4	0.7

(1) COMMISSION STAFF WORKING DOCUMENT ON THE IMPLEMENTATION OF NATIONAL RESIDUE MONITORING PLANS IN THE MEMBER STATES IN 2010 (Council Directive 96/23/EC) http://ec.europa.eu/food/food/chemicalsafety/residues/workdoc_2010_en.pdf (Data from Germany)

(*) "Non-compliant" with the currently in force MRLs as set in Regulation (EC) No 149/2008

APPENDIX F

Maximum Residue Limits for copper in food of animal origin and calculations for exposure

Table F1: Maximum Residue Limits for copper in food of animal origin: set by Regulation (EC) No 149/2008 and proposed by FEEDAP Panel in the present opinion.

Item	Animal species	MRLs (in mg/kg fresh matter)	
		Currently in force	Proposed by FEEDAP Panel
TISSUES/ORGANS			
Liver	Ruminants	30	140
	Water fowl	30	100
	Swine	30	30
	Other animal species	30	20
Kidney		30	12
Edible offal		30	12
Meat		5	3
Fat (free of lean meat)		5	3
Others(1)		5	3
Milk		2	0.2
Eggs (Fresh or cooked)		2	1.5
Fish Flesh	Salmonid	---	1

(1) Refers to "Other tissues/organs", that is, tissues other than *Meat*, *Fat*, *Liver*, *Kidney* and *Edible offal*

Table F2: Exposure to Copper of high consumers (95 percentile, of toddlers and adults) when applying different sets of MRLs

Item	Toddlers			Adults (scenario I)[(1)]			Adults (scenario II)[(1)]		
	Current MRL[(2)]		Proposed MRL[(2)]	Current MRL		Proposed MRL	Current MRL		Proposed MRL
	g food	mg Cu	mg Cu	g food	mg Cu	mg Cu	g food	mg Cu	mg Cu
Liver[(3)]				60	0.18	4.0	60	0.18	4.0
Meat	90	0.45	0.27				290	1.45	0.87
Milk	1050	2.1	0.21	1500	3.0	0.3			
Background (mg Cu/day)		(*)	(*)		0.9	0.9		0.9	0.9
TOTAL Exposure (mg Cu/day)		2.55	0.48		4.08	5.2		2.53	5.77
UL (mg Cu/day)**		1	1		5	5		5	5

(1) As detailed in text (see Section 3.2.4)
(2) See Table F1
(3) Calculated for an equal amount from poultry, swine and ruminants
(*) No data available
(**) UL proposed by the SCF (EC, 2003). An ADI of 10.5 mg Cu per adult and day was considered when setting MRLs by the PRAPeR Unit of EFSA (EFSA, 2008)

EC (European Commission), 2003, online. Opinion of the Scientific Committee on Food (SCF) on the Upper Intake Level of Copper (27 March 2003). http://ec.europa.eu/food/fs/sc/scf/out176_en.pdf

EFSA (European Food Safety Authority), 2008. Conclusion regarding the peer review of the pesticide risk assessment of the active substance Copper (I), copper (II) variants namely copper hydroxide, copper oxychloride, tribasic copper sulfate, copper (I) oxide, Bordeaux mixture. EFSA Scientific Report 187, 1101. Conclusion on the peer review of copper compounds.

ABBREVIATIONS

ACGIM	American Conference of Governmental Industrial Hygienists
ADI	acceptable daily intake
AFC	EFSA Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food
ANS	EFSA Scientific Panel on Additives and Nutrient Sources added to Food
BVL	Bundesamt für Verbraucherschutz und Lebensmittelsicherheit
bw	Body weight
Cu	Copper
CEF	EFSA Scientific Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids
CVMP	Committee for Medicinal Products for Veterinary Use
DM	Dry matter
ECHA	European Chemicals Agency
EC	European Commission
EFSA	European Food Safety Authority
EU	European Union
EURL	European Union Reference Laboratory
FEEDAP	EFSA Scientific Panel on Additives and Products or Substances used in Animal Feed
FOCUS	Forum for the Coordination of Pesticide Fate Models and their Use
HPLC	High Performance Liquid Chromatography
IARC	International Agency for Research on Cancer
ICP-AES	Inductively coupled plasma atomic remission spectroscopy
IPCS	International Programme on Chemical Safety
LOAEC	Lowest Observed Adverse Effect Concentration
LOAEL	Lowest observed adverse effect level
LOQ	Limit of quantification
MRL	Maximum residue limit
NDA	EFSA Scientific Panel on Dietetic Products, Nutrition and Allergies
Ni	nickel
NOAEL	No observed adverse effect level
NOEL	No observed effect level
NRC	National Research Council
PCB	Polychlorinated biphenyl
PRAPeR	Pesticide Risk Assessment Peer Review
PCDD	Polychlorinated dibenzo-*para*-dioxin
PCDF	Polychlorinated dibenzofuran
PNECs	Predicted no-effect concentrations
RSD_r	Relative standard deviation of *repeatability*
RSD_R	Relative standard deviation for *reproducibility*
SCAN	Scientific Committee on Animal Nutrition
SCF	Scientific Committee on Food
SCOEL	Scientific Committee on Occupational Exposure Limits
SLR	Systematic literature review
TBCC	Tribasic copper chloride
TWAs	Time weighted averages
UL	Tolerable upper intake level
VRAR	Voluntary risk assessment report