



EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

25 May 2016
EMA/CVMP/758734/2015
Committee for Medicinal Products for Veterinary Use

European public MRL assessment report (EPMAR)

Copper carbonate (all food producing species)

On 12 May 2016 the European Commission adopted a Regulation¹ establishing maximum residue limits (no MRL required classification) for copper carbonate in all food producing species, valid throughout the European Union. These maximum residue limits were based on the favourable opinion and the assessment report adopted by the Committee for Medicinal Products for Veterinary Use.

Copper carbonate is intended for use in injectable products containing other minerals to treat mineral deficiency which can arise during critical phases of production or breeding life cycle in food-producing animals.

Warburton Technology submitted to the European Medicines Agency an application for the establishment of maximum residue limits, on 23 June 2015.

Based on the original data in the dossier, the Committee for Medicinal Products for Veterinary Use recommended on 10 December 2015 the establishment of maximum residue limits for copper carbonate in all food producing species.

Subsequently the Commission recommended on 15 March 2016 that maximum residue limits in all food producing species are established (no MRL required classification). This recommendation was confirmed on 5 April 2016 by the Standing Committee on Veterinary Medicinal Products and adopted by the European Commission on 12 May 2016.

¹ Commission Implementing Regulation (EU) No 2016/710, O.J. L 125, of 12 May 2016



Summary of the scientific discussion for the establishment of MRLs

Substance name:	Copper carbonate
Therapeutic class:	Alimentary tract and metabolism/mineral supplements
Procedure number:	EMEA/V/MRL/004268/FULL/0001
Applicant:	Warburton Technology
Target species:	All food producing species
Intended therapeutic indication:	Copper deficiency
Route(s) of administration:	Subcutaneous

1. Introduction

Copper carbonate is a copper salt. Copper is an essential nutrient and a normal constituent of the diet in humans and animals.

Copper carbonate is intended for use in injectable products containing other minerals to treat mineral deficiency which can arise during critical phases of production or breeding life cycle in food-producing animals. The intended dose of copper for cattle is 0.30 mg/kg bw for animals less than 1 year old, 0.20 mg/kg bw from 1 to 2 years, and 0.15 mg/kg bw over 2 years, as a single subcutaneous injection.

Several copper salts are used orally in feed or in injectable solution to treat copper deficiency in food-producing animals. The recommended doses are 50 to 100 mg copper per animal given as a single injection.

Copper is authorised as a feed additive.

Copper also has antimicrobial properties and is used in plants and animals as a fungicide and as a foot-bath for the control of foot-rot in cattle and sheep.

2. Scientific risk assessment

2.1. Safety assessment

The CVMP has previously evaluated several copper salts (copper chloride, copper gluconate, copper heptanoate, copper oxide, copper methionate, copper sulphate and dicopper oxide) which have been included in the list of substances approved for use in all food-producing animal species with a 'No MRL required' classification (Summary Report EMEA/MRL/431/98-Final²).

In view of the similarity between copper carbonate and other copper salts, the safety evaluation is based on the pharmacology and toxicology information in the EMA Summary Report on the other copper salts (EMEA/MRL/431/98-Final, 1998). It was noted that the recent European Food Safety Authority (EFSA) report³ on copper salts, including copper carbonate and a number of the other salts previously evaluated by the CVMP, does not distinguish between the different salts for the safety assessment.

² Copper chloride, copper gluconate, copper heptanoate, copper oxide, copper methionate, copper sulphate and dicopper oxide: Summary Report EMEA/MRL/431/98-Final (1998) – CVMP/EMA, London

³ Scientific Opinion on the safety and efficacy of copper compounds (E4) as feed additives for all animal species (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), based on a dossier submitted by FEFANA - EFSA Panel on Additives and Products or Substances used in Animal Feed (FEEDAP) - European

Since the CVMP assessment of the other salts of copper in 1998, additional information relevant to consumer safety evaluation of the substance has been generated. In particular, the Scientific Committee on Food (SCF) recommended a tolerable upper intake level for copper.

2.1.1. Overview of pharmacological properties

Pharmacodynamics

Copper is an essential trace element and present in food of animal and plant origin. Copper forms an integral part of a number of metalloproteins notably caeruloplasmin, monoamine oxidase, lysyl oxidase, cytochrome C and superoxide dismutase enzymes. Deficiency of copper eventually leads to anaemia in all species studied. Bone disorders, failure of elastin and collagen biosynthesis leading to cardiovascular disorders, achromotrichia and abnormal keratinisation of hair, wool or fur are all recognised in many different animal species. Nerve disorders, especially swayback, have been described for a smaller variety of species. Copper deficiency has also been associated with reproductive failure. Copper deficiency arises either from a primary deficit of dietary copper or as a secondary deficiency caused by a dietary excess of other elements, especially zinc, molybdenum, sulphur and iron¹.

Pharmacokinetics

Copper is absorbed from the gastrointestinal tract by both active and passive transport mechanisms so that efficiency of absorption ranges from 25 to 60% and is influenced by animal requirements. Absorbed copper appears first in plasma as the cupric ion loosely bound to albumin. In this form it is available for metabolism by the liver. The liver is the major storage organ for copper where it is protein bound. Copper is transported onwards to peripheral tissues as part of the caeruloplasmin. Since liver contains the highest concentrations of copper, it is the target organ to consider in the risk assessment of copper. Excess copper is excreted mainly via the bile and faeces, though urinary losses account for 0.5 to 3 % of the daily intake¹.

A published study by Ward *et al.*⁴ (1996) showed that the oral bioavailability of copper carbonate was similar to that of copper sulphate, when given to heifers in the feed. Chapman and Bell⁵ (1963) however, showed that the rate of absorption (as determined by radioactivity in whole blood) and the rate of excretion in urine plus faeces of (⁶⁴Cu) copper carbonate ranked highest when compared to copper chloride, copper sulphate, copper nitrate, and copper oxide. Ledoux *et al.*⁶ (1995) reported a somewhat higher average absorption of copper carbonate when compared to copper chloride, copper acetate and copper oxide when given to sheep in their feed; however the absorption was somewhat lower than that of copper sulphate. The absorption appeared linear to the dose. In 208 day old male chicks, the oral absorption of copper acetate was highest, followed by copper sulphate, copper carbonate, and copper oxide (Ledoux *et al.*⁷, 1991).

2.1.2. Calculation of pharmacological ADI, if relevant

Not relevant.

Food Safety Authority (EFSA), Parma, Italy - EFSA Journal, 2015, 13(4): 4057

⁴ J. D. Ward, J. W. Spears, and E. B. Kefley - Bioavailability of Copper Proteinates and Copper Carbonate Relative to Copper Sulfate in Cattle - J. Dairy Sci., 1996, 79: 127-132

⁵ H. Chapman H. and M. Bell - Relative absorption and excretion by beef cattle of copper from various sources. - J. Anim. Sci., 1963, 22: 82-85

⁶ D.R. Ledoux, E.B. Pott, P.R. Henry, C.B. Ammerman, A.M. Merritt, and J.B. Madison- Estimation of the relative bioavailability of inorganic copper sources for sheep - Nutr. Res., 1995, 15(12): 1803-1813.

⁷ D. R. Ledoux, P. R. Henry, C. B. Ammerman, P. V. Rao and R. D. Miles - Estimation of the relative bioavailability of inorganic copper sources for chicks using tissue uptake of copper - J. Anim. Sci., 1991, 69: 215-222

2.1.3. Overview of toxicology

Single-dose toxicity

Short term studies showed that monogastric species have a high tolerance for copper¹. The EFSA report² also highlights the marked species differences in sensitivity for copper toxicity.

A GLP-compliant study in cattle examined what can be regarded as a worst case in terms of administration of copper by parenteral injection of calcium copper edetate. The data obtained indicated that administration of 200 mg of copper to cattle over 100 kg was adequately tolerated and produced only mild and probably transient symptoms of copper toxicity in the overtly normal animal. The more obvious effect of treatment was the swelling, moderate inflammation and sensitivity to pain seen at the treatment site, which persisted at least until day 3. Administration of a second dose 35 days after the first to animals that were probably tending towards hypercupraemia was tolerated, but the animals showed signs of acute (haematuria) and chronic (gross pathological changes of the liver and spleen) copper poisoning. Animals treated with a single 600 mg dose of copper suffered from acute copper toxicity, which proved fatal in 75% of cases. The data indicate the relatively low safety margin between effective and toxic dose, but do not show any unexpected effects of administration of high dose copper¹.

Sheep are particularly sensitive to the adverse effects of excess copper intake and there are several published reports of acute and chronic copper toxicity in this species after both oral and parenteral administration of copper reviewed by Joint the FAO/WHO Expert Committee on Food Additives (JECFA)⁸.

Repeated dose toxicity

The long term ingestion of copper at levels higher than those required for maintenance of normal copper status may lead to chronic hypercuprosis. In this condition copper is accumulated in the liver until a limit is reached at which time the copper is released into the bloodstream resulting in acute vascular haemolysis (haemolytic crisis) and finally in death. Overdose of cattle or sheep over a short period of time results in acute copper toxicity.

Shanaman *et al.*, 1972, cited in WHO Food Additives Series 17, reported a 1-year chronic study conducted with male and female beagle dogs to evaluate the potential oral toxicity of copper gluconate administered at levels of 0.012, 0.06 and 0.24% of the diet. These levels were equivalent to 3, 15 and 60 mg/kg bw/day. Weight gains and food consumption values were similar for the control and treated groups. Overall health, haematology and urinalysis were comparable to controls. After 1 year, minimal liver function changes were observed in 1 of 12 dogs receiving the 0.24% copper gluconate diet, a change that was reversed following a 12-week withdrawal period. Accumulation of copper in liver, kidneys and spleen was seen at the high dose. No compound-related effects were seen at the lowest dose and there were no compound-related deaths or gross or microscopic pathological lesions in any dog. It is concluded that the NOEL of copper in a 1-year feeding study in the dog was approximately 3 mg/kg bw per day.

Reproductive toxicity, including developmental toxicity

Study of the effects on reproduction

No data were available but it should be remembered that copper is a natural element present in almost all food ingredients and in drinking water. No effects on reproduction are thus likely to be induced by the ingestion of copper quantities not exceeding the normal levels in food and drinking water.

⁸ Copper - Summary of Evaluations Performed by the Joint FAO/WHO Expert Committee on Food Additives, Geneva 1982

Study of developmental toxicity

Neither copper gluconate nor iodide were embryotoxic in the mouse and rat. Studies on embryotoxicity and teratology in mice, rats and hamsters have been conducted with copper chloride, metallic copper, cupric citrate, copper sulphate and copper gluconate. The effect of orally administered copper gluconate on fertility in male and female rats has also been published. All these studies are reviewed and included in the final evaluation of the 26th JECFA report⁸.

By contrast, other studies in rodents demonstrated that oral exposure to copper during gestation induced embryo/fetotoxic and developmental effects. Copper(II) sulphate induced embryo lethality in mink and mice but only when administered at the very high dose-levels of 12 and 80 mg Cu/kg bw/day, respectively³. Nevertheless, in a controlled study the teratogenic potential of copper releasing intrauterine devices (IUD) on the developing human embryo was investigated⁹. No malformations or copper deposits were observed in the organs/placentae of copper IUD-exposed embryos (n=11) examined between 7 and 12 weeks gestation. The results from the small study suggest that copper releasing IUDs have no observed negative effects on the developing embryo, in agreement with the results described in the JECFA assessment.

Genotoxicity

Copper compounds have been recorded to be mutagenic in mammalian cells in culture but in bacteria copper sulphate did not induce DNA-repair using the bacterial colorimetric assay SOS chromotest. Similarly copper gluconate and cuprous iodide were not mutagenic in the Ames test with and without metabolic activation. In vivo copper sulphate administered intraperitoneally to mice was found to induce a significant increase in the frequency of chromosomal aberrations in bone marrow cells at all concentrations used (1.1 to 6.6 mg/kg bw). However, cuprous and cupric ions are not generally considered to be genotoxic when taken orally¹.

So, copper(II) has been reported to be genotoxic *in vitro* and also in some *in vivo* bone marrow micronucleus assays in mice after intraperitoneal injection. Similar to iron, copper is a redox-active transition element, potentially able to catalyse the Fenton/Haber–Weiss reaction, with the consequent production of reactive oxygen species. Therefore, copper is known to have genotoxic potential when present at high local concentrations. A genotoxic concern for the human population is not foreseen, except under conditions of overload, which are not relevant to the use as a veterinary medicine.

Carcinogenicity

The International Agency for Research on Cancer (IARC) allocated copper(II) 8-hydroxyquinoline to Group 3 “Not classifiable as to their carcinogenicity to humans” (IARC, 1987, cited in (3)). The Scientific Committee for Food (see (3)) 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. Nevertheless, CVMP considered that cuprous and cupric irons are not genotoxic when taken orally at relevant doses and stated, based on a number of studies that examined the effect of administration of copper on tumour induction or antitumour activity of other compounds, that dietary copper sulphate at levels of 0.05% and 0.1% did potentiate the antitumour activity of pyrovate bis (thiosemicarbazone) in mice implanted with a number of tumour systems.

Studies of other effects including immunotoxicity and neurotoxicity

There is no evidence from the literature that copper compounds have a specific effect on the immune system.

⁹ S.T. Beer, S.M. Bradberry, W.N. Harrison, J.A. Vale - UKPID MONOGRAPH on COPPER CARBONATE – National Poisons Information Service, Birmingham B18 7QH, UK, (1998)

The SCF (EC, 2003, in EFSA, 2015³) summarised data on the toxic properties of copper. Manifestations of copper toxicity include weakness, anorexia and jaundice. As tissue copper levels increase, a haemolytic crisis may ensue producing liver, kidney and brain damage, which may explain the tremors observed at high doses, but copper is not a direct neurotoxic agent.

2.1.4. Calculation of the toxicological ADI or alternative limit

In the previous evaluation of copper salts (EMA's Summary on Copper salts residues²), the CVMP considered the JECFA review of copper (26th Report, 1982⁸) which established a maximum tolerated intake for copper in food of 0.5 mg/kg bw for humans. This maximum tolerated dose (corresponding to an ADI) has not been revised meanwhile but an upper intake level has been established by the Scientific Committee on Food as indicated below. In addition, the EFSA recently concluded that the use of copper salts (including copper carbonate) as feed additives remains safe for the consumer.

Since the EMA summary report (EMEA/MRL/431/9–FINAL) of 1998, the Scientific Committee on Food (2003) established an upper intake level of 5 mg/person/day (adults) and 1 mg per day for toddlers. EFSA (2008) established for pesticides an ADI of 0.15 mg/kg bw corresponding to 9 mg/person per day based on a NOAEL of 15 mg/kg bw per day from the 1-year dog study and a standard safety factor of 100. Since copper is an essential trace element and a normal constituent of nutrition it is proposed to retain the tolerable upper intake level of 5 mg/person per day in line with the SCF (2003). This value was derived from the NOEL of 10 mg/day identified in the study by Pratt *et al.* (1985)¹⁰ (daily single dose levels administered only to seven male adult volunteers for 12 weeks, and serum liver markers as endpoints) and an uncertainty factor of 2 for potential variability in the human population. The results from the 1-year study in dogs support this upper limit.

The value of 5 mg/person/day is therefore used as the reference value for consumer safety evaluation.

It is concluded that there is no need to change the conclusions on the previous safety evaluation for other copper derivatives within the scope of this application and that those conclusions are also applicable to copper carbonate.

2.1.5. Overview of microbiological properties of residues

Although copper has antimicrobial properties, there is no evidence from the literature that, after treatment of animals for copper deficiency, residues would have any microbiological effect on gut flora, or on the micro-organisms used for industrial food processing.

2.1.6. Calculation of microbiological ADI

The establishment of a microbiological ADI is not considered necessary.

2.1.7. Observations in humans

In 1974 WHO concluded that the fatal oral human dose of various copper salts is about 200 mg/kg bw. Chronic copper poisoning in man is very rare, suggesting that the human body can adapt to a wide range of copper intake without detrimental effects. There are a few reports of chronic copper toxicity in human infants but the only report in adults concerns chronic effects such as pulmonary deposition and fibrosis, granulomas and malignant liver tumours in vineyard workers.

¹⁰ W. B. Pratt WB, J. L. Omdahl JL, J. R. Sorenson JR. Lack of effects of copper gluconate supplementation. *Am. J. Clin. Nutr.*, 1985, 42: 681-682

The most sensitive parameter of copper toxicity is diarrhoea frequently observed in infants after drinking contaminated water. Copper has also been implicated in the aetiology of Indian Childhood Cirrhosis in children exposed to high levels of copper in the drinking water. The direct relationship of this potentially lethal liver damage to copper intoxication awaits final confirmation.

In the JECFA review of copper (26th Report, 1982⁸) a maximum tolerated intake for copper in food of 0.5 mg/kg bw was set for humans. Assuming a 60 kg man and a 100 g meal of liver and no other source of copper in the diet an approximate limit for liver can be estimated to be 300 mg copper/kg fresh liver (average copper content in normal bovine liver is 25 to 315 mg/kg). However, liver from apparently normal ruminants can contain more than 350 mg copper/kg fresh liver. In this case it is likely that the animals will also develop symptoms of copper poisoning. Consequently, copper poisoning on the farm is a marker for excessive copper accumulation in ruminant liver. On the other hand, copper bound to tissue proteins is likely to be less toxic than soluble copper salts which in an aqueous environment liberate copper ions. The JECFA concluded that the copper present in liver is unlikely to be as toxic to man as the worst case which the putative maximum tolerated intake must accommodate. In addition the maximum tolerated intake includes a significant safety margin over the no effect level for the critical group¹.

More recently, the Scientific Committee on Food (EC, 2003, in EFSA, 2015³) summarised data on the toxic properties of copper. Tolerance to high intakes of copper varies greatly from one species to another, in relation to species vulnerability and the levels of zinc, iron and molybdenum in the diet.

A tolerable upper intake level for copper of 5 mg/day for adults and 1 mg/day for toddlers based on human data was defined by the Scientific Committee on Food as mentioned in the previous section. Copper excess causes impaired growth and extensive necrosis of hepatocytes. The 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, a haemolytic crisis may ensue producing liver, kidney and brain damage.

2.1.8. Findings of other EU or international scientific bodies

A tolerable upper intake level for copper of 5 mg/day for adults and 1 mg/day for toddlers was defined by the Scientific Committee on Food as mentioned in previous sections. This tolerable upper intake level value has been consistently used in the assessments of copper in different forms by the following EFSA scientific panels: the Scientific Panel on Dietetic Products, Nutrition and Allergies (NDA Panel, EFSA, 2006), the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC Panel, EFSA, 2008), the Panel on Additives and Products or Substances used in Animal Feed (FEEDAP Panel, EFSA, 2008, 2009, 2012), the Panel on Food Additives and Nutrient Sources added to Food (ANS Panel, EFSA, 2009) and the Panel on Food Contact Materials, Enzymes, Flavourings and Processing Aids (CEF Panel, EFSA, 2010).

Copper carbonate (basic cupric carbonate; monohydrate), as well as cupric acetate, monohydrate; cupric chloride, dihydrate; cupric oxide; cupric sulphate, pentahydrate; cupric chelate of amino acids, hydrate; and cupric chelate of glycine, hydrate were recently (EFSA 2015²) considered as safe sources of copper for all animal species/categories when used up to maximum European Union-authorized copper levels in complete feed. EFSA considered that no concerns for consumer safety are expected from the use of the copper compounds under application in animal nutrition when used up to the maximum EU-authorized levels in feed.

2.1.9. Overall conclusions on the ADI

The CVMP retains the SCF tolerable upper intake level of 5 mg per person and day based on the NOEL of 10 mg copper/person/day and an uncertainty factor of 2 from the human study for the consumer safety evaluation of copper carbonate.

2.2. Residues assessment

2.2.1. Pharmacokinetics in target species

CVMP² considered that ruminants have a high capacity for hepatic storage of copper and residues in liver can be as high as 79% of total body copper and up to 12% in muscle. In cattle and sheep, the actual concentration is dependent on the diet and health of the animal. Liver is a good indicator of the copper burden of ruminants and most surveys of copper residues have concentrated on this tissue where, following adequate diet supplement, copper concentration in the range of 25 to 315 mg/kg wet weight have been found. Copper concentration in kidney ranges from 1.2 to 1.5 mg/kg wet weight whereas concentrations tend to be lower in muscle (0.01 to 0.6 mg/kg wet weight) and fat. The concentration in tissues other than liver, kidney, blood, spleen, lungs and brain are not significantly affected by administration of copper products. The concentration of copper in milk is affected by the concentration of copper in the diet and varies considerably depending on the geographic location.

Caeruloplasmin renders free copper innocuous with subsequent excretion via a lysosome-to-bile pathway. This process is essential to normal copper homeostasis and provides a protective mechanism in acute copper poisoning. An impaired or overloaded biliary copper excretion system results in hepatic copper accumulation, as occurs in patients with Wilson's disease and in copper poisoning.

In a published study from Bicalho *et al.*¹¹ (2014), pregnant cows received 75 mg copper by injection (as part of a multimineral formulation), equivalent to approximately 0.12 mg Cu/kg bw, on gestation days 230 and 260 of gestation. Blood samples were collected in cows of each group at 230 and 260 days of gestation, then 3, 7, 14 and 25 days after calving for copper analyses. Injectable trace mineral-supplemented cows had moderately (+4%) increased serum concentration of copper ($p = 0.04$) in comparison with control cows.

2.2.2. Residue depletion studies

Cattle

Ward *et al.*⁴ (1996) showed that the oral supplementation of feed with cupric carbonate failed to increase copper plasma concentrations at the same extent as copper sulphate, except after 21 days where all groups supplemented with copper had similar plasma copper concentrations, which were greater ($p < 0.01$) than for control heifers. Nevertheless, copper liver concentrations were only moderately increased with copper carbonate (27.7 mg/kg vs. 14.1 mg/kg in controls) when compared with copper sulphate (47.5 mg/kg), probably as a result of the lower rate of absorption through the gastro-intestinal tract.

In another study, two treatments were administered to November-born calves within 24 hours of birth in alternating birth order¹². Treatments ($n = 75$ /treatment) consisted of 1 ml subcutaneous injection of either a multimineral formulation (copper 0.22 mg/kg bw as copper carbonate) or sterile saline. All 150

¹¹ M. L. S. Bicalho, F. S. Lima, E. K. Ganda, C. Foditsch, E. B. S. Meira Jr., V. S. Machado, A. G. V. Teixeira, G. Oikonomou, R. O. Gilbert, and R. C. Bicalho - Effect of trace mineral supplementation on selected minerals, energy metabolites, oxidative stress, and immune parameters and its association with uterine diseases in dairy cattle – *J. Dairy Sci.*, 2014, 97(7)

¹² J. D. Arthington, P. Moriel, P. G. M. A. Martins, G. C. Lamb, and L. J. Havenga - Effects of trace mineral injections on measures of performance and trace mineral status of pre- and postweaned beef calves - *J. Anim. Sci.*, 2014, 92: 2630–2640

treatment administrations were delivered within a 25-day calving period. Treatments were readministered to all calves 2 additional times before weaning at approximately 100 and 200 days of age. Trace mineral status of calves was assessed in liver biopsy samples collected from 12 random heifer calves per treatment on approximately days 150, 200 and weaning (day 250). Averaged across all 3 sampling times, administration of the multiminerals resulted in greater concentrations of liver copper (+34%) at the end of the observation period but generally speaking, the mean concentrations remained within a similar range in both groups, from 100 to 200 mg/kg (dry matter) in controls and 150 to 200 mg/kg (dry matter) in treated cattle. It does not appear clearly from these results if there was any significantly increased liver copper concentration on average.

From the above study, 24 calves were used in another study. After weaning, the calves were transported to a research feedlot and re-administered treatments. Liver biopsy samples were taken on day 1 (immediately before treatment) and day 13. In controls liver copper concentration were stable at 220 mg/kg (dry weight) and reached 300 to 360 mg/kg (dry weight) in calves injected with the multiminerals formulation.

Pogge *et al.*¹³ (2012) reported copper concentrations in liver biopsies taken before, and on days 1, 8, and 15 after injection of copper carbonate (as part of a multiminerals formulation) at a dose of 0.33 mg/kg bw in 10 Simmentahl and 10 Angus steers (9 months of age). On average, the copper concentration increased from 114 mg/kg dry weight (controls) to 178 mg/kg dry weight (treated animals).

Daugherty *et al.*¹⁴ showed in a study with 67 cows, that after subcutaneous two injection of multiminerals formulation (30 days prior calving (study day 0) and at study day 96), the average liver concentrations were 45.1 mg/kg dry weight in controls and 81.3 mg/kg dry weight in treated animals.

In a study of Genther *et al.*¹⁵ (2014) forty steers were equally assigned to diets for an 84-day depletion period: control (supplemental Cu, Mn, Se, and Zn) or deficient (no supplemental Cu, Mn, Se, or Zn plus Fe and Mo as trace minerals antagonists). On day 1 of the 85-day repletion period, 10 steers within each dietary treatment were injected with sterilized saline or the multiminerals formulation, containing 15, 10, 5, and 60 mg/ml of Cu, Mn, Se, and Zn, respectively, at a dose of 1 ml/68 kg bw. All steers were fed the same repletion diet. Blood was collected on day 0 and 1, and blood and liver biopsies were collected on day 8, 15, 29, 57, and 85 post-injection. Copper concentration in liver increases from 125–139 to 151–162 mg/kg dry weight.

Sheep

Ledoux *et al.*⁶ (1995) showed that the liver uptake of copper from orally administered copper carbonate to sheep was comparable with that of other copper sources i.e. copper sulphate and copper acetate. Copper oxide appeared to have a lower uptake.

Chickens

In 208 day old male chicks, the oral absorption of copper acetate was highest, followed by copper sulphate, copper carbonate, and copper oxide (Ledoux *et al.*¹⁶, 1991).

¹³ D. J. Pogge, E. L. Richter, M. E. Drewnoski and S. L. Hansen - Mineral concentrations of plasma and liver after injection with a trace mineral complex differ among Angus and Simmental cattle. - J. Anim. Sci., 2012, 90: 2692–2698

¹⁴ S. R. Daugherty, G. E. Carstens, D. B. Herd, K. S. Barling, and R. D. Randel - Effects of Prenatal and Prebreeding Trace Mineral/ Vitamin E Injections on Calf Health and Reproductive Performance of Beef Cows - Texas A&M University, College Station.

¹⁵ O. N. Genther, and S. L. Hansen, - A multi-element trace mineral injection improves liver copper and selenium concentrations and manganese superoxide dismutase activity in beef steers - J. Anim. Sci., 2014, 92: 695–704

¹⁶ D. R. Ledoux, P. R. Henry, C. B. Ammerman, P. V. Rao and R. D. Miles – Estimation of the relative bioavailability of inorganic copper sources for chicks using tissue uptake of copper - J. Anim. Sci., 1991, 69: 215-222

The behaviour of copper, administered as copper carbonate, in target food producing animals, is very similar to that when administered in other copper salts, such as those already having a 'No MRL required' classification.

Selection of marker residue and ratio of marker to total residues

Not applicable as the substance is intended for a 'No MRL required' classification.

2.2.3. Monitoring or exposure data

The copper concentration in food and drinks ranges from approximately 0.1 mg/l in milk to 44 mg/kg in calf liver. Copper concentrations in drinking water vary according to local conditions but in most countries the recommended limit is 1 mg/l for domestic water supplies. The average daily dietary requirement for copper in humans has been estimated at 0.03 mg/kg bw in adults and at 0.05 mg/kg bw in infants and children. The average daily intake of copper was estimated to be in a range of about 1-2 mg/person/day (EFSA, 2015³).

2.2.4. Analytical method for monitoring of residues

Not applicable as the substance is intended for a 'No MRL required' classification.

3. Risk management recommendations

3.1. Potential effects on the microorganisms used for industrial food processing

Not relevant.

3.2. Other relevant risk management considerations for the establishment of maximum residue limits

No relevant factors were identified for consideration of the risk management recommendations.

3.3. Elaboration of MRLs

It has been shown that the administration of copper carbonate by dietary formulations leads to a systemic absorption not significantly exceeding the other copper derivatives and is therefore unlikely to result in residues in liver that exceed physiological values. It was also shown that copper residues in liver after parenteral administration, although increased when compared with controls, remained within the physiological range.

It can therefore be concluded that the establishment of MRL values for copper carbonate is not needed for the protection of the consumer, and hence that this substance can be included in the list of substances approved for use in all food-producing species with a 'No MRL required' classification.

3.4. Considerations on possible extrapolation of MRLs

Since the Committee recommends the 'No MRL required' classification for all food producing species, considerations on possible extrapolation are not applicable.

3.5. Conclusions and recommendation for the establishment of maximum residue limits

Having considered that:

- copper is an essential nutrient and a normal constituent of the diet in man and animals,
- the average daily intake of copper was estimated at 1 - 2 mg/person/day,
- a tolerable upper intake level for copper of 5 mg/person was established,
- the administration of copper by dietary or parenteral formulations results in residues in liver which are within the physiological range,
- veterinary treatment is not expected to significantly contribute to the average copper intake compared to mineral supplementation in animal diets and other sources of copper intake;

the Committee, having considered the application, concluded that the establishment of maximum residue limits for copper carbonate in all food producing species is not necessary for the protection of human health and therefore recommends by consensus the inclusion of copper carbonate in table 1 of the Annex to Regulation (EU) No 37/2010 in accordance with the following table:

Pharmaco- logically active substance	Marker residue	Animal species	MRLs	Target tissues	Other provisions	Therapeutic classification
Copper carbonate	NOT APPLICABLE	All food producing species	No MRL required	NOT APPLICABLE	NO ENTRY	Alimentary tract and metabolism/ Mineral supplement

4. Background information on the procedure

Submission of the dossier: 23 June 2015

Steps taken for assessment of the substance

Application validated: 9 July 2015

Clock started: 10 July 2015

CVMP opinion adopted: 10 December 2015