



## COMMITTEE FOR VETERINARY MEDICINAL PRODUCTS

### COPPER CHLORIDE, COPPER GLUCONATE, COPPER HEPTANOATE, COPPER OXIDE, COPPER METHIONATE, COPPER SULPHATE and DICOPPER OXIDE

#### SUMMARY REPORT

1. Copper compounds (calcium copper edetate, copper heptanoate, copper methionate, copper chloride, copper oxide, dicopper oxide and copper gluconate) are normally used, orally in feed or in an injectable solution, to treat copper deficiency in food-producing animals. In veterinary medicine, the recommended doses are 50 to 100 mg copper per animal given as a single injection. Copper has antimicrobial properties and is used in plants and animals as a fungicide and as a foot-bath for the control of foot-rot (copper sulphate 5% to 10% solution) in cattle and sheep. Copper is authorised as a feed additive in the following doses: 35 to 175 mg/kg feed in pigs, 30 to 50 mg/kg feed in calves, 15 mg/kg feed in ovine and 35 mg/kg feed in other species.

Copper is an essential trace element and a constituent of plant and animal tissues.

Copper salts have been used parenterally or orally to prevent or treat copper deficiency in human medicine for many years.

The copper concentration in food and drinks ranges from approximately 0.1 mg/litre 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/litre 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.

2. The CVMP previously considered ethylenediaminetetraacetic acid and its salts (synonyms: EDTA; edetate) and an entry into Annex II of Council Regulation (EEC) 2377/90 was recommended.
3. 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 iron.
4. 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 intake.

5. Short and long term studies showed that monogastric species have a high tolerance for copper. The NOEL of copper in a one year feeding study in the dog was approximately 5 mg/kg feed.
6. 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 Joint FAO/WHO Expert Committee on Food Additives (JECFA) report.
7. 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 copper.

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 FAO/WHO Expert Committee on Food Additives (JECFA) (1982).

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.

8. 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 irons are not generally considered to be genotoxic when taken orally.
9. A number of studies have examined the effect of administration of copper on tumour induction or antitumour activity of other compounds. Dietary copper sulphate at levels of 0.05% and 0.1% was found to potentiate the antitumour activity of pyrovate bis (thiosemicarbazone) in mice implanted with a number of tumour systems.
10. There is no evidence from the literature that copper compounds have a specific effect on the immune system.

11. 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.
12. 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.

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 relation of this potentially lethal liver damage to copper intoxication awaits final confirmation.

13. In the Joint FAO/WHO Expert Committee on Food Additives (JECFA) review of copper (26th Report, 1982) 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. Consequently 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 group.
14. 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 dependant 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 concentrations 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.
15. The effect of an injectable preparation of 100 mg per animal of calcium copper edetate has been investigated in cattle. The concentrations of copper found at the injection site were initially high (76 mg/kg on day 1) but by 7 days after treatment were not significantly different from those found in control animals (9 and 8 mg/kg, respectively). The rapid depletion of copper from the site of administration was confirmed by the results of analysis of the serum samples. The highest mean copper concentration (1.11 µg/ml) was found in samples taken 4 hours after treatment. The mean copper concentration in liver remained above 200 mg/kg in each of the treated groups examined and the results are significantly different from those found in control animals (140 mg/kg).
16. Analytical methods for the determination of copper in biological samples based on Atomic Absorption Spectrophotometry are available.

## Conclusions and recommendation

Having considered the criteria laid down by the Committee for the inclusion of substances in Annex II of Council Regulation (EEC) No. 2377/90 and in particular that:

- copper is an essential nutrient and a normal constituent of the diet in man and animals,
- the administration of copper by dietary or parenteral formulations results in residues in liver which are within the physiological range,
- copper is authorized as a feed additive;

the Committee considers that there is no need to establish an MRL for copper chloride, copper gluconat, copper heptanoate, copper methionate, copper oxide, copper sulphate and dicopper oxide and recommends their inclusion into Annex II of Council Regulation (EEC) No 2377/90 in accordance with the following table:

<b>Pharmacologically active substance(s)</b>	<b>Animal species</b>	<b>Other provisions</b>
Copper chloride	All food producing species	
Copper gluconate	All food producing species	
Copper heptanoate	All food producing species	
Copper methionate	All food producing species	
Copper oxide	All food producing species	
Copper sulphate	All food producing species	
Dicopper oxide	All food producing species	