Section 6.1.4.2 Eye Irritation

Annex Point IIA6.1

		19 RESULTS AND DISCUSSION
19.1	**Clinical signs**	Iris lesion Conjunctival redness and chemosis Cornea opacity
19.2	**Average score**	
19.2.1	Cornea	0 (24 h); 0.5 (48 h), 0.5 (72 h)
19.2.2	Iris	1.5 (24 h); 1.5 (48 h), 1.5 (72 h)
19.2.3	Conjunctiva	
19.2.3.1	Redness	2.7 (24 h); 2.5 (48 h), 2.5 (72 h)
19.2.3.2	Chemosis	2.5 (24 h); 2.2 (48 h), 1.8 (72 h)
19.3	**Reversibility**	not stated
19.4	**Other**	The clinical signs occurred also in the rinsed eyes(50 %) but the effects were less severe.
19.5	**Overall result**	Average scores calculated over all time points: cornea: 0.25 iris: 1.29 conjunctival redness: 2.63 conjunctival chemosis: 2.25 overall irritation score: 1.33
		20 APPLICANT'S SUMMARY AND CONCLUSION
20.1	**Materials and methods**	6 WNZ rabbits were used for the eye irritation test of copper hydroxide. After insertion into the conjunctival sac of the rabbit all eye reactions were observed at 1 h, 24 h, 48 h and 72 h p.a. (Draize method). This test was conducted in accordance with OECD guideline no 405 and according to directive 84/449/EEC, B.5 "Acute toxicity (eye irritation)"
20.2	**Results and discussion**	Copper hydroxide caused iris lesion, conjunctival redness and chemosis(score 1 to 3) in all animals 1, 24, 48 and 72 h after instillation Corneal opacity was also observed in two cases (score 1 to 2). The effects of iris lesion, conjunctival redness and chemosis occurred slightly less severe when the eyes were rinsed immediately after treatment.
20.3	**Conclusion**	According to the Directives 67/548/EEC (as amended, adapting to technical progress, by Directives 93/21/EEC; 1999/45/EC and 2001/59/EC in relation to classification, packaging and labelling of dangerous substances and preparations), copper hydroxide is classified as 'Irritant' (Xi) and requires the Risk Phrase R36 'Irritating to eyes'.
20.3.1	Reliability	1
20.3.2	Deficiencies	No

Section 6.1.4.2 Eye Irritation

Annex Point IIA6.1

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	Give date of action
Materials and Methods	3.3.2 - In this test, eyes of three animals were rinced 4 seconds after administration. In the current guidelines, eyes can be rinced but only after one hour of exposure. In the case of Copper Hydroxyde, this difference of method can be very important for classification purposes.
Results and discussion	If rinced and non-rinced eye are taken separately, results are quite different. (see table below). Moreover in the table of results, some modification should be made according to general principles of scientific rounding (i.e. 1.29 is rounded to 1.3 not 1.2) or 2 decimals should be given.
Conclusion	According results seen with non-rinced eyes, a classification R41 "severely irritant to the eyes" is warranted. Results obtained with eyes rinced only 4 second after application cannot be taken into account. Moreover, the test observation period was only 72 hours, as non reversibility of the effects were seen after the observation period of three days, the lesions were not reversible at the end of the study. As no other data is available for Copper hydroxyde, this result is also in favour of a classification as a severe irritant to the eyes: Xi; R41.
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section 6.1.4.2 Eye Irritation

Annex Point IIA6.1

Appendix

Table A6_1_4E-1. Results of eye irritation study

Use this table, if relevant effects occur.

	Cornea	Iris	Conjunctiva	
			redness	chemosis
score (average of 6 animals investigated)	0 to 4	0 to 2	0 to 3	0 to4
60 min	0	1.7	2.8	2.5
24 h	0	1.5	2.7	2.5
48 h	0.5	1.5	2.5	2.2
72 h	0.5	0.5	2.5	1.8
Average 24h, 48h, 72h	0.3	1.2	2.6	2.2
Area effected	n.a.	n.a.	n.a.	n.a.
Maximum average score (including area affected, max 110)	4	2	3	4
Reversibility*	n.a.	n.a.	n.a.	n.a.
average time for reversion	n.a.	n.a.	n.a.	n.a.
Give method of calculation maximum average score. * *c :* *completely reversible* *n c :* *not completely reversible* *n :* *not reversible*				

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1

Guinea pig maximisation test (GPMT)

			Official use only
		21 REFERENCE	
21.1	**Reference**	[redacted] (1992): Guinea Pig Maximisation Test of Skin Sensitisation with "URA-08740-F-0-WP". Project no.: 10-05-0714/00-92. - Study performed by [redacted] Unpublished study. URA-97-08740-050	
21.2	**Data protection**	Yes	
21.2.1	Data owner	Spiess-Urania Chemicals GmbH, Hamburg, Germany	
21.2.2	Companies with letter of access		
21.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing active substance for the purpose of its entry into Annex I	
		22 GUIDELINES AND QUALITY ASSURANCE	
22.1	**Guideline study**	Yes OECD guideline 406	
22.2	**GLP**	Yes	
22.3	**Deviations**	No	
		23 MATERIALS AND METHODS	
23.1	**Test material**	Copper hydroxide (URA-08740-F-0-WP)	
23.1.1	Lot/Batch number	250592	
23.1.2	Specification	The product conforms to the FAO-Specifications. 50 % copper as copper hydroxide.	
23.1.2.1	Description	Fine blue-green powder	
23.1.2.2	Purity	77 % copper hydroxide (pure active ingredient) corresponding to 50 % copper	
23.1.2.3	Stability	3 years	
23.1.2.4	Preparation of test substance for application	according to the test requirements	
23.1.2.5	Pretest performed on irritant effects	Yes Pilot study for range finding	X

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1 Guinea pig maximisation test (GPMT)

23.2	**Test Animals**		
23.2.1	Species	Guinea pig	
23.2.2	Strain	Pirbright white, Bor. DHPW (SPF)	
23.2.3	Source	[redacted]	
23.2.4	Sex	male and female	
23.2.5	Age/weight at study initiation	male: 377 - 500 g female: 337 - 487 g	
23.2.6	Number of animals per group	20 test animal	
23.2.7	Control animals	20 control animal	
23.3	**Administration/ Exposure**	Adjuvant	
23.3.1	Induction schedule	day 0 intradermal day 7 dermal see also table A6.1.5-1	
23.3.2	Way of Induction	Intradermal and dermal Occlusive	
23.3.3	Concentrations used for induction	0.1 mL FCA 50 % (w/w) diluted in aqua ad iniect. 0.1 mL test substance in aqua ad iniect. (final concentration: 0.1 %) 0.1 mL test article diluted in FCA/aqua ad iniect. (final concentration: 0.1 %)	
23.3.4	Concentration Freunds Complete Adjuvant (FCA)	5 % or (diluted with aqua ad iniect. and FCA)	
23.3.5	Challenge schedule	day 21 challenge procedure	
23.3.6	Concentrations used for challenge	maximum non-irritating concentration of the test article (50 % in petrolatum)	
23.3.7	Rechallenge	Not applicable	
23.3.8	Scoring schedule	24h and 48h after challenge (patch removal)	
23.3.9	Removal of the test substance	Removal after 24 h	
23.3.10	Positive control substance	2,4 dinitrochlorobenzene and benzocaine	
23.4	**Examinations**		
23.4.1	Pilot study	yes	
23.5	**Further remarks**	not stated	X

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1

Guinea pig maximisation test (GPMT)

		RESULTS AND DISCUSSION
23.6	**Results of pilot studies**	**Intradermal (48 h after injection):** 5 %, 1 % and 0.5 %: Immoderate irritating with test article-dependent discoloration of the partly indurated injection sites were observed 0.25 %: Immoderate irritating were observed 0.10 %: No specific findings were observed **Dermal (48 h after application):** No skin reactions were observed
23.7	**Results of test**	
23.7.1	24h after challenge	0 animals with signs of allergic reactions
23.7.2	48h after challenge	0 animals with signs of allergic reactions
23.7.3	Other findings	--
23.8	**Overall result**	The sensitisation rate of at 24 and at 48 was 0 %
23.9		
		24 APPLICANT'S SUMMARY AND CONCLUSION
24.1	**Materials and methods**	Guinea Pig maximisation Test, OECD 406
24.2	**Results and discussion**	No animal showed an allergic response on the exposure of the test substance.
24.3	**Conclusion**	According to OECD guideline for testing chemicals the test substance copper hydroxide may be classified as a "non-sensitizer".
24.3.1	Reliability	1
24.3.2	Deficiencies	No

Section A6.1.5 Skin sensitisation

Annex Point IIA6.1 **Guinea pig maximisation test (GPMT)**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	06 January 2005
Materials and Methods	3.1.2.5 Pilot Study: Intradermal injection tested: 0.1, 0.25, 0.5 and 1%. Dermal application: the test article was incorporated in petroleum to provide a final concentration of 50% (w/w). A closed patch exposure was effected by means of an occlusive bandage. 3.5 Further remarks: Because the test article was non-irritating at the highest permissible concentration in the pilot study, the area was reclipped and pre-treated with 10% sodium lauryl sulphate (SLS) in petroleum 24 hours before application of the test article at a concentration of 50% in petroleum.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.1.5 **Skin sensitisation**

Annex Point IIA6.1 **Guinea pig maximisation test (GPMT)**

Table A6.1.5-1 **Detailed information including induction/challenge/scoring schedule for skin sensitisation test**

Inductions	GPMT		Observations/Remarks
	day of treatment	application	
Induction 1	0	Intradermal 0.1 %	3 pairs of intradermal injections No skin reaction were observed
Induction 2	7	dermal at a concentration of 50 % in petrolatum	Area was pretreated with 10 % sodium lauryl sulfate
challenge	21	topical on the shaved area on each flank left side: test article (50 % in petrolatum) right side: vehicle using patch technique	

Table A6.1.5-2 **Result of skin sensitisation test**

	Number of animals with signs of allergic reactions / number of animals in group	
	Control group	**Test group**
scored after 24h	0 / 20	0 / 20
scored after 48h	0 / 20	0 / 20

Section A6.2

Annex Point IIA6.2

Absorption, distribution, metabolism and excretion of copper in humans and in domestic and laboratory animals

			Official use only
		25 REFERENCE	
25.1	**Reference**	**A6.2/01: Doc.No.:00620B-IIA-62a** [redacted] (2003): Absorption, distribution, metabolism and excretion of copper in humans and in domestic and laboratory animals; [redacted], November 05, 2003 (unpublished).	
25.2	**Data protection**	Yes	
25.2.1	Data owner	Spiess-Urania Chemicals GmbH, Hamburg, Germany	
25.2.2	Companies with letter of access	--	
25.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		26 GUIDELINES AND QUALITY ASSURANCE	
26.1	**Guideline study**	No Review of the comprehensive literature on copper in order to satisfy the requirements of EC method B.36 (88/303/EEC).	
26.2	**GLP**	No Not applicable – literature review	
26.3	**Deviations**	Not applicable	
		27 MATERIALS AND METHODS	
27.1	**Test material**	Copper, not specified	X
27.2	**Literature**	Review article based on information taken from the following four major sources: • International Programme on Chemical Safety (IPCS) Environmental Health Criteria 200 Copper, (1998, World Health Organisation, Geneva); • LINDER (1991, Biochemistry of Copper, Plenum Press New York); • RALPH and McARDLE (2001, Copper metabolism and copper requirements in the pregnant mother, her fetus, and children. International Copper Association New York, N.Y.USA); • LINDER (2002, Handbook of Copper Pharmacology and Toxicology Ch 1 ed. Massaro E.J., Humana Press NJ). Other published papers are also referred to.	

Section A6.2

Annex Point IIA6.2

Absorption, distribution, metabolism and excretion of copper in humans and in domestic and laboratory animals

		28 RESULTS	
28.1	**Absorption**	The principal route of absorption is oral, via copper in food and in drinking water. Most absorption from dietary sources takes place in the intestine, where absorption of copper at the intestinal wall appears to have at least two mechanisms (passive diffusion and active uptake). The uptake and transport of copper in the intestinal cell is presented in Figure A6.2- 2. Cellular transporter proteins have been identified in intestinal epithelial cell membranes (the brush border). These include high-affinity copper transporter CRT1 and DMT1, a divalent metal ion transporter. Once inside the intestinal epithelial cell, copper is rapidly transferred to either a copper 'chaperone' protein, or the tripeptide glutathione (GSH) or the protein metallothionein (MT). Copper is used within the cell, and is also transported to the trans-Golgi network (TGN) where the MNK transporter protein pumps it to vesicles for exocytic release to albumin and transcuprein for transport to the liver via the hepatic portal system. Copper may also be pumped out of the cell by CTR1. There is evidence from yeast cells that there is effectively no free copper in cells, that all copper is bound to various proteins and is handed from one carrier to another. The amount of copper absorbed varies with the nutritional status of the individual. In humans, absorption has been shown to vary between 56 % for subjects on a low-copper diet, and 12 % for subjects on a high copper diet, with subjects on adequate diet absorbing 36 %. Rats have been shown to absorb 30 to 50 %, although studies in rats have also shown that absorption can be reduced to 10 % at high dietary intakes, as in humans.	X
28.2	**Distribution**	Most of the absorbed copper is rapidly deposited in the hepatocytes of the liver, although a small amount enters the kidney. The liver is the main storage organ for copper, and the principal organ of regulation of secretion of ceruloplasmin to the blood and of excess copper in the bile. Ceruloplasmin appears to be the main source of copper for other tissues. Up to 70 % of the copper in the blood is in the form of ceruloplasmin, with the majority of the rest bound to albumin and transcuprein. The copper uptake and transport in hepatocytes is presented in Figure A6.2- 3. The copper uptake and transport in non-hepatic cells is presented in Figure A6.2- 4.	X
28.3	**Metabolism**	In terms of the ADME regulatory requirement, copper is not 'metabolised', as it is a monatomic metal. However, it is incorporated in a large number of proteins. Thus, copper is used in every cell in the body and every cell is capable of regulating its copper content.	

Section A6.2 Annex Point IIA6.2		Absorption, distribution, metabolism and excretion of copper in humans and in domestic and laboratory animals	
28.4	Excretion	Any surplus copper is excreted from non-hepatic cells either via the transporter protein CTR1 and via the TGN with following exocytosis, and is transported to the liver by albumin and transcuprein where it is taken up by hepatocytes. Most cells express the MNK protein at the TGN, in the same way as the intestinal cells, although cells such as the placenta and mammary gland express both the MNK and WND proteins at the TGN to eliminate excess copper. Excess ceruloplasmin is denatured by liver endothelial cells and taken up by hepatocytes. The hepatocytes excrete any surplus copper to the bile, bound to a trypsin-independent protein fragment and the surplus copper is then lost in the faeces. Enterohepatic circulation of biliary excreted copper does not take place. Copper is also lost in faeces through sloughing of intestinal cells. Minor amounts are lost via urine, sweat and loss of hair and dead skin cells.	
28.5	Homeostasis	The copper transport mechanisms at the level of the organism form part of the system of homeostasis, the process by which the levels of copper in the body (and ultimately the cell) are regulated. Copper can be considered to show a flattened 'U'-shaped dose-response curve, as presented in Figure A6.2- 1. The left side of the 'U' curve represents deficiency, where intake is less than the requirement. Copper deficiency is associated with growth retardation, anaemia, skin lesions, impaired immunity, intestinal atrophy, impaired cardiac function, reproductive disturbance, neurological defects and skeletal lesions. Copper is essential for normal physiological function such as cellular respiration, free radical defence, synthesis of melanin, connective tissue, iron metabolism, regulation of gene expression and normal function of the heart, brain and immune system. The central near-horizontal part of the 'U' curve represents homeostasis, where intake and excretion are balanced. The right-hand part of the 'U' represents toxicity or excess copper disease. The natural homeostatic regulation of copper means that an individual on a low copper diet will retain more of an artificial dose of copper than an individual on a high copper diet. No adverse effects were observed following administration of 10 mg Cu/person/day for 12 weeks to a group of 14 adults. A case history was reported, describing a male who self-administered 30 mg Cu/day as a dietary supplement for two years without showing ill-effects. However, when he increased the dose to 60 mg Cu/day in the third year he suffered liver failure, indicating that a long-term repeat daily dose of 60 mg Cu/day is toxic, but that 30 mg Cu/day was not toxic following administration over two years.	X

Section A6.2

Annex Point IIA6.2

Absorption, distribution, metabolism and excretion of copper in humans and in domestic and laboratory animals

28.6	**Differences between humans and other mammalian species**	It has been established that the genetic codes for proteins that transport copper across cell membranes, copper chaperones and copper-containing enzymes are similar in yeast and vertebrates, indicating that they have changed little during evolution.	
		Humans show two genetic disorders involving copper metabolism: Menkes' disease and Wilson's disease. Menkes' disease is a X-linked copper deficiency disease that is usually fatal in early childhood. The disease is caused by a defect in the MNK protein, which is producing an inability to export copper from cells particularly from the basal membrane of the small intestine, where copper is absorbed. Wilson's disease is a defect in the ATPase for copper transport, resulting in faulty copper transport, impaired incorporation of copper into ceruloplasmin and impaired biliary excretion and consequently causing accumulation of copper in liver and brain.	X
		Given the stability of copper transporter proteins across species, there are however differences in some proteins, and differences in the expression of the genes (i.e. the gene may be the same in two species, but the degree to which the gene is expressed, or put into effect, may vary considerably, because of the effect of other genes known as modifier genes). Notable differences, relevant to species used in risk assessments were discussed by the authors of the literature review and are summarised below. Generally, copper regulation in rats was reported to be similar to humans. The rabbit as an obligate herbivore contains bacteria in the caecum that can digest cellulose. The rabbit exploits the additional resource of commensal bacteria by eating its faeces, which might increase the dose by passage of unabsorbed material through the alimentary canal 'twice' or which might alter the nutritional status of the animal by adverse effects on the commensal bacteria. Thus, no observed effect levels obtained in rabbit studies should be treated with caution, while observed effects may be relevant to human risk assessment. Albumin, one of the major copper transporter proteins of the blood, contains histidine in position 3 which is essential for tight binding of copper. In dogs and pigs, this histidine is replaced by a tyrosine, and consequently the albumin does not have the same affinity for copper. In contrast, dog and pig albumins only have low-affinity sites for copper. Dogs show unusually high levels of copper in the liver, ten times the levels in other species. While dog liver rapidly took up copper injected intravenously, dogs do not appear to be able to excrete copper via the bile as readily as other species. It is possible that dogs express the WND protein less than other species resulting in accumulation of copper in the liver. Based on these differences in albumin structure and the liver of the dog, it was concluded that the dog is not a good animal model for human risk assessment of copper. Despite the alteration in albumin, the biliary excretion and copper homeostasis in the pig is similar to other mammals. Cattle are tolerant of very high copper levels, while sheep are highly sensitive to copper, due to apparent inability to excrete copper.	

Section A6.2

Annex Point IIA6.2

Absorption, distribution, metabolism and excretion of copper in humans and in domestic and laboratory animals

29 APPLICANT'S SUMMARY AND CONCLUSION

29.1 Materials and methods

Review of the extensive available literature on copper, which satisfies the requirements of EC method B.36 (88/303/EEC).

29.2 Results and discussion

Absorption of copper takes place in the small intestine, where the cells of the brush border take up copper and bind it. Bound copper diffuses through the cell and is pumped out of the cell by an active process. Once outside the cell, the copper is bound mainly to albumin and transcuprein and is transported to the liver via the hepatic portal vein in the bound form. The liver is the main storage organ for copper, and the principal organ of regulation of secretion of ceruloplasmin to the blood and of excess copper in the bile. Ceruloplasmin appears to be the main source of copper for other tissues. Copper is distributed throughout the body, and it is used in all cells. Under normal circumstances, it does not accumulate in cells. Any surplus copper is excreted from non-hepatic cells, and is transported to the liver by albumin and transcuprein where it is taken up by hepatocytes. Excess ceruloplasmin is denatured by liver endothelial cells and taken up by hepatocytes. The hepatocytes excrete any surplus copper to the bile, bound to a trypsin-independent protein fragment and the surplus copper is then lost in the faeces. Enterohepatic circulation of biliary excreted copper does not take place.

In mammals, internal levels of copper are regulated to maintain normal physiological processes. In humans, the extent of copper absorption varies between 12 and 56 % depending on diet content and status of the individual. In studies with humans, no adverse effects were observed at dietary supplements of 10 mg Cu/person/day for 12 weeks and no ill-effects were recorded in a case of self-administration of 30 mg Cu/person/day for two years.

Section A6.2 Annex Point IIA6.2	Absorption, distribution, metabolism and excretion of copper in humans and in domestic and laboratory animals	
29.3 Conclusion	The requirements of any toxicokinetic study that would be conducted according to EEC B.36/OECD 417 on an active substance are to investigate the following: • how much of an oral dose is absorbed; • where it is distributed within the body; • the extent to which the active substance is metabolised; • the identity of any metabolites; • the extent to which it and/or its metabolites accumulate in various organs and tissues; • the route by which it and/or its metabolites are excreted; • the rate at which it and/or its metabolites are excreted. • Addressing these for copper: • Absorption in both rats and humans varies according to diet: for humans on a copper-adequate diet, absorption is 36%; on a low copper diet 56%, and on a high copper diet 12%. Similar figures have been obtained for rats • Distribution is directly from the intestine to the liver, which is the main organ of regulation. The liver controls the distribution of copper to the rest of the body via the bloodstream, bound to ceruloplasmin. • Metabolism does not occur: copper is a monatomic ion and cannot be metabolised. It is however used in every cell in the body, and every cell can regulate its copper content. Many enzymes and other proteins containing copper have been described. • Accumulation does not occur except in cases of genetic disease or chronic administration of exceptionally high doses (60 mg/person/day), where copper accumulates in the liver. • Excretion in most species is via the bile, in a trypsin-independent protein fragment such that entero-hepatic circulation does not occur. A significant amount of copper is excreted bound to metallothioneins contained in intestinal brush border cells sloughed off and lost in faeces. Minor amounts are also lost in urine and from skin and hair. • Excretion is rapid. An oral dose of 20 mg Cu/kg to rats was completely eliminated from the liver by 48 hours. Blood plasma levels did not increase during this period. This review shows that whereas a full guideline ADME study has not been performed on copper, the knowledge of copper in the human body at the level of the organism, organ, cell and gene far exceeds the knowledge of any traditional biocide, and the information available is therefore sufficient to meet the requirements of Directive 98/8/EC.	X
29.3.1 Reliability	2	
29.3.2 Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	23/11/04
Materials and Methods	Agree with applicant's version But, no justification is given to allow a read across between all Copper salts (3.1). Some data concerning solubility and tests results could have been given to justify the read across.
Results and discussion	Agree with applicant's version Comments: - <u>for absorption (4.1)</u>: no data are given for dermal or inhalation absorption. Regarding the title of the study, some data were expected. For dermal absorption, data can be taken from the following studies. For inhalation absorption, no data at all are presented in the kinetic summary. Copper carbonate can be inhaled and is harmful to the rats (see A6.1.3) showing that a large amount of substance can be absorbed by this route. As no data is available for this end point, a worst case absorption factor of 100 % will be taken into account if necessary (if some exposure scenarios shows that inhalation exposure is not negligible). Data of the last paragraph comes from the following publications: Turnlund, 1989 and linder, 1991. - <u>For distribution (4.2)</u>: blood concentration is almost constant whichever the dose administered. Almost all blood copper is bound to transport protein and only a very small faction is free. - <u>Homeostasis (4.5)</u>: this section can be completed as following: This homeostasis was demonstrated in the Turnlund study (Turnlund *et al.*, 1989) in which 11 human volunteers received a diet containing total copper in the range 0.8 to 7.5 mg/day during 90 days. Under these conditions, no significant changes were observed in subjects. Turnlund also noted that plasma copper levels were not altered at 7.5 mg Cu/day, and conclude that this level was well within the homeostatic range. Copper plasma levels were not altered at the low copper diet. Pratt (1985), in a study intended to assess the effects of oral administration of copper in the treatment of pain management, gave doses of 10 mg/person/day for 12 weeks to a group of 14 adults (in addition to the normal diet). There were no adverse effects of copper administration in any of

	the subjects. The upper limit of homeostasis has never strictly defined in humans. It has been shown to be more than 7.5 mg/person/day (Turnlund, J.R. et al., 1989), with no ill effects at 10 mg/person/day (Pratt et al. 1985) and probably lies in the region of 10 to 30 mg/person/day. - In the homeostasis section, it can be noted that milk copper concentration is constant whichever the dose of copper administered. This can be useful for developmental assessment. - Differences between humans and other mammalian species: Add in paragraph of Menkes' disease (4.6): This leads to very high concentrations of copper in sloughed intestinal cells, but the failure to export to 'absorbed' copper to the bloodstream results in an effective copper deficiency for the rest of the body. The disease shows progressive mental retardation, hypothermia, seizures, poor muscle tone, feeding difficulties, jaundice, diarrhoea and a general failure to thrive. Add in paragraph of Wilson's disease: Hepatic copper levels range from 200 to 800 µg/d dry weight (normal range 20 to 50 µg/g), and patients present with hepatic cirrhosis and fatty infiltration of the liver. Urinary copper is much higher than normal. In the second or third decade of the disease, neurological symptoms can occur. Copper accumulation in the brain causes degeneration of the basal ganglia, resulting in defective movement, slurred speech, difficulty in swallowing , facial and other muscular spasms, dystonia and poor motor control (reference A6.2/01).
Conclusion	Agree with applicant's version See previous comments.
Reliability	2
Acceptability	Acceptable
Remarks	

	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Figure A6.2- 1: Dose response curve for copper (after WHO)

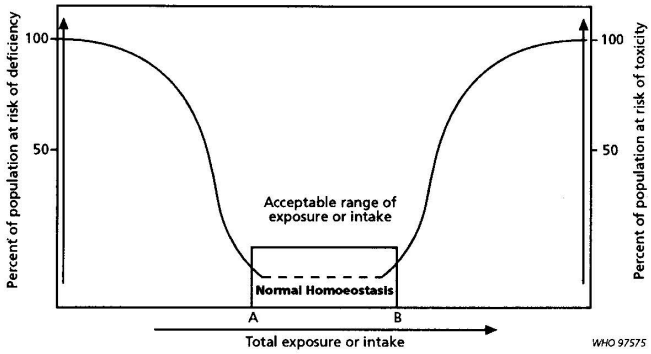


Figure A6.2- 2: Uptake and transport of copper in the intestinal cell (after Linder 2002); Copper (released by digestion) enters at the brush border through transport proteins CTR1 and DMT1. Copper is picked up from the transporter proteins by several chaperones HAH1/ATOX1, CCS, COX17 and GSH and methallothoinein MT. Copper is used within the cell, and is also transported to the trans-Golgi network (TGN) where the MNK transporter protein pumps it to vesicles for exocytic release to albumin (Alb) and transcuprein (Tc) for transport to the liver via the hepatic portal system. Copper may also be pumped out of the cell by CTR1.

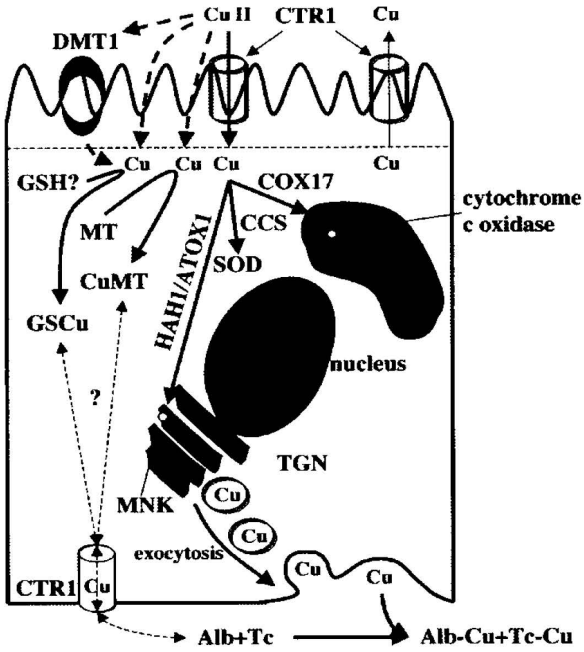


Figure A6.2- 3: Copper uptake and transport in hepatocytes (after Linder 2002); The central cells depicted are parenchymal hepatocytes, separated from the blood circulation by the space of Disse. Copper bound to transcuprein (Tc) or albumin (Alb) is passed to the transporter protein CTR1 or DMT1, crosses the cell membrane to copper chaperones COX17, CCS, HAH1/ATOX1 and Wilson's disease protein WND for incorporation in ceruloplasmin Cp-Cu, which is released to the blood. Excess copper is transported to bile as a trypsin-independent protein fragment incapable of resorption in the intestine, and is lost in the faeces.

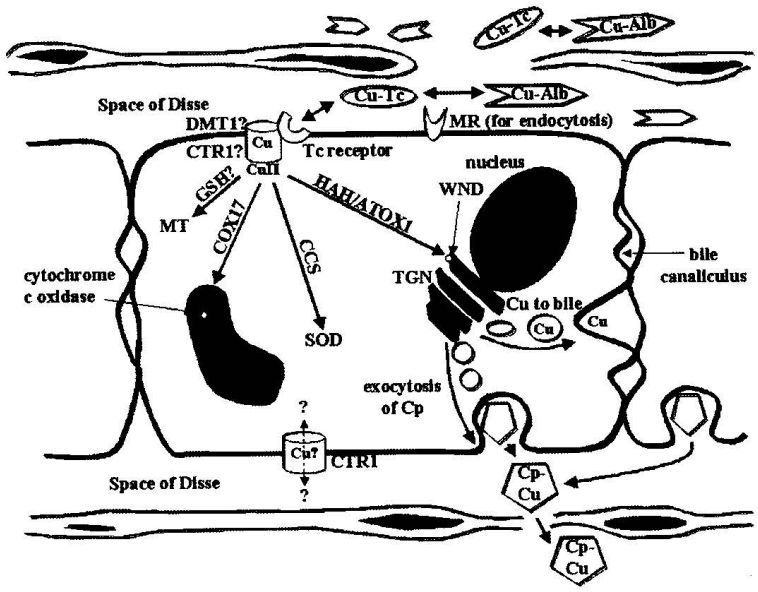
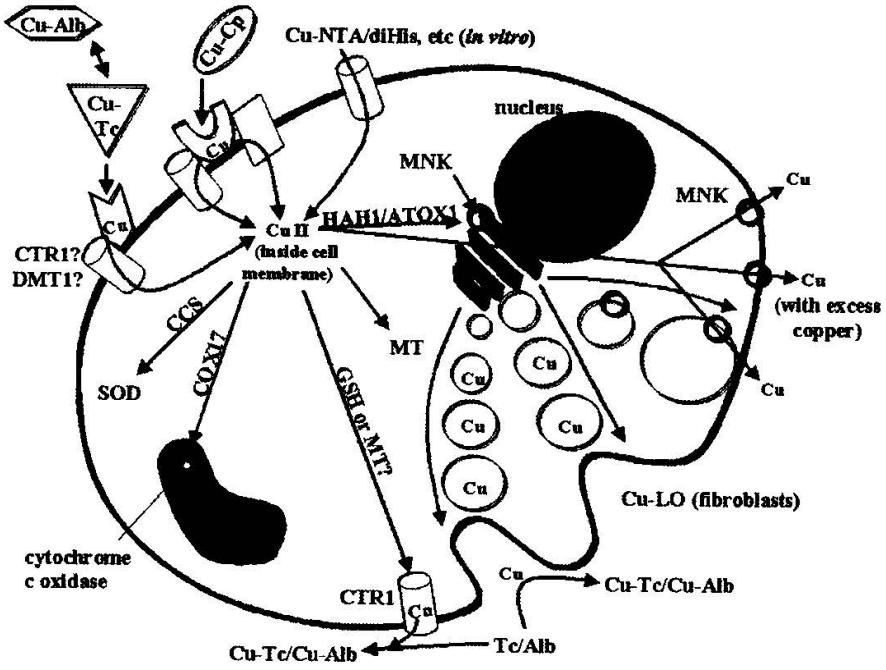


Figure A6.2- 4: Copper uptake and transport in non-hepatic cells (after Linder 2002); Most cells probably take up copper from ceruloplasmin (and probably transcuprein) via CTR1 and DMT1. Some cells (heart, aorta) have specific ceruloplasmin receptors that pass copper to the transporter proteins. As in other cells, copper moves in the cell bund to various chaperones, and is used in many enzymes. It leaves the cell possibly via CTR1 and two types of exocytosis, attached to specific secreted proteins such as lysyl oxidases or for attachment to albumin or transcurprein in the plasma. In cases of excess copper, the MNK protein may also be involved.



Section A6.2 Absorption, distribution and excretion in male rats

Annex Point IIA6.2

			Official use only
		30 REFERENCE	
30.1	**Reference**	**A6.2/02: Doc.No. 00620B-IIA-62b** [redacted] (2003): Five copper substances – absorption, distribution, and excretion in male rats; [redacted]; Report no.: 11784, 18 November 2003 (unpublished draft).	
30.2	**Data protection**	Yes	
30.2.1	Data owner	Spiess-Urania Chemicals GmbH, Hamburg, Germany	
30.2.2	Companies with letter of access	--	
30.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		31 GUIDELINES AND QUALITY ASSURANCE	
31.1	**Guideline study**	Yes	
		EC method B.36 (87/302/EEC); OECD 417 (1984)	
31.2	**GLP**	Yes	
		Self-certified laboratory	
31.3	**Deviations**	No	
		32 MATERIALS AND METHODS	
32.1	**Test material**	(i) Copper hydroxide	X
		(ii) Copper oxide	
		(iii) Copper oxychloride	
		(iv) Tribasic copper sulphate	
		(v) Bordeaux mixture	
		(vi) Copper sulfate pentahydrate	
32.1.1	Lot/Batch number	(i) 380-71-05	
		(ii) 280802	
		(iii) 27003B	
		(iv) 471/2002	
		(v) 1/170	
		(vi) 03415PU	
32.1.2	Specification	Not specified	

Section A6.2 Absorption, distribution and excretion in male rats

Annex Point IIA6.2

32.1.3	Purity	(i) 60.1 % copper (ii) 87 % copper (iii) 57.39 % copper (iv) 31.12 % copper (v) 27.38 % copper (vi) 25.45 % copper
32.1.4	Description	(i) light blue powder (ii) red-brown powder (iii) light green, fine homogenous powder (iv) greenish-blue solid (v) green powder (vi) blue crystals
32.1.5	Stability	No evidence of instability was observed for all test substances.
32.1.6	Radiolabelling	None
32.2	**Test animals**	
32.2.1	Species	Rat
32.2.2	Strain	Crl:CD® (SD) IGS BR
32.2.3	Source	[redacted]
32.2.4	Sex	Male
32.2.5	Age/weight at study initiation	Age: approx. 7 weeks Body weight: variation did not exceed ± 20 % of the mean weight by dose group
32.2.6	Number of animals per group	Exp. 1A: 4 Exp. 1B: 4 Exp. 2: 1 Exp. 3: 5 Exp. 4: 5
32.2.7	Control animals	Yes, concurrent vehicle for all experiments
32.3	**Administration/ Exposure**	Oral
32.3.1	Type	Gavage
32.3.2	Concentration of test substance	Dose levels are specified below for each experiment
32.3.3	Volume applied	approx. 4 mL/kg bw
32.3.4	Vehicle	ultra-pure water/diet slurry

Section A6.2 Absorption, distribution and excretion in male rats

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32.4	**Pilot experiments**	Experiment 1A: Plasma and liver pilot experiment with copper sulphate at concentrations of 0, 5, 20, or 65 mg Cu/kg bw to determine a single appropriate dose level for subsequent experiments. Serial blood samples were removed from the jugular vein cannula (pre-dose, 30 min and at 1, 2, 4, 6, 9, 13, 18, 24, 32, 40 and 48 hours after dose administration). Liver samples from one rat per dose group were also submitted to Cu analysis. Experiment 1B: Plasma copper and surgical status pilot experiment to assess the potential impact of jugular vein and bile duct cannulation on plasma Cu concentrations of control animals. A water/diet slurry (0 mg Cu/kg bw) was administered to four rats unaltered by surgery, four rats with jugular vein cannulae and four rats with bile duct cannulae. Bile was collected approx. 17 to 0 hours before dosing and 0 - 24 and 24 – 48 hours after dosing and tail vain blood was collected approx. 17 and 0 hours before dosing and 24 and 48 hours after dosing. Experiment 2: Absorption and disposition pilot experiment to test the feasibility of using excreta and tissue Cu burdens to compare bioequivalence from the different test substances. 65 mg Cu/kg bw as Copper hydroxide, Copper oxide, Copper oxychloride, Tribasic copper sulphate, Bordeaux mixture, or Copper sulfate pentahydrate was administered to bile duct cannulated rats. Bile, liver, plasma, post-biliary intestinal content, post-biliary intestine wall, pancreas, urine, faeces and carcass were sampled at 24 hours after administration.
32.5	**Main experiments**	Experiment 3: Distribution and excretion experiment with biliary cannulation, to estimate bioavailability by summation of Cu recovery from tissues, carcass, excreted dose in bile and urine. Experiment 4: Quantification of the time course of Cu in liver and plasma after oral administration of one representative copper substance, copper hydroxide.
32.5.1	Concentration of test substance	Exp. 3: 20 mg Cu/kg bw for each of the six copper substances Exp. 4: 20 mg Cu/kg bw as copper hydroxide
32.5.2	Post-exposure period	Exp. 3: 24 hours Exp. 4: 48 hours
32.5.3	Samples (sampling time)	Exp. 3: blood, plasma, liver, combined stomach and contents, combined post-biliary intestines and contents, carcass, bile, urine, faeces, and cage wash (collection period: 24 hours) Exp. 4: whole blood, plasma, liver (0, 1.5, 3, 6, 9, 12, 18, 24, 30, 36, 48 hours)
32.5.4	Examinations	Clinical observations (daily), mortality (daily), body weights (at study initiation and upon termination), Cu analysis of dose suspensions, representative food samples, whole blood, plasma, tissues, excreta and cage wash as appropriate
32.5.5	Copper analysis	Microwave digestion was used to prepare non-aqueous samples. Copper was analysed by ICP-AES. The method detection limit (MDL) and limit of quantitation (LOQ) were 0.0054 and 0.021 ppm, respectively.
32.6	**Further remarks**	Analysis of dose samples confirmed that the dose had been prepared accurately and that the dose formulations were homogenous.

Section A6.2 Absorption, distribution and excretion in male rats

Annex Point IIA6.2

		33 RESULTS	
33.1	**Pilot experiments**	Experiment 1A: No consistent dose response in plasma Cu concentration was observed over the 48 hour collection period, which was attributed to the bioregulation of Cu uptake. Elevated copper concentrations were determined in livers of high dose animals. Generally all dose groups including the vehicle control exhibited a drop in plasma concentrations after dose administration which was attributed to fasting prior and after administration. For this reason fasting was discontinued in subsequent experiments. Experiment 1B: All animals appeared healthy. Plasma copper concentrations were higher in rats with cannulation surgery compared to control rats. This finding was attributed to a generalised inflammatory response which is known to raise levels of ceruloplasmin and thus the carrying capacity of Cu in plasma. Rats without surgery showed the greatest body weight gain during the study followed by jugular vein cannulated rats and then by bile cannulated rats. Experiment 2: Absorption for copper sulphate and the five other copper substances ranged from 4.67 to 6.86 % of the administered dose, based on the amount of copper measured in bile, liver, pancreas, plasma and urine. Some distention and fluid retention in the intestinal tract was noted during sample collection. Thus the dose was adjusted to 20 mg/kg bw for the main experiments. The results of the pilot experiments were used to adjust the experimental designs of the main experiments.	X
33.2	**Experiment 3**	All rats appeared healthy with no signs of infection. The actual dose levels ranged from 21.8 to 24.3 mg Cu/kg bw for the six copper test substances. Control animals received 0.052 mg Cu/kg bw from copper present in the diet. The absorbed dose ranged from 10.7 to 12.9 % based on percent of the dose measured in whole blood, liver, carcass, bile and urine. No statistically significant difference in absorption was observed between the six copper test substances. The ranking of copper concentrations in excreta and tissue samples was generally faeces > GI tissue and contents (post-biliary) > stomach and contents > liver > bile > plasma ~ whole blood ~ carcass > urine > cage wash. The total recovery ranged between 109 and 141 %, the majority of which was measured in the faeces and in the GI tract tissue and contents. Recovery values in excess of 100 % of the administered dose were explained with continued uptake of Cu from normal dietary intake. Based on the results of this experiment, it was concluded that the six copper test substances have essentially the same relative bioavailability under the conditions of this experiment. The results are summarised in Table A6.2- 1.	

33.3	Experiment 4	Animals of the control and test group received mean measured doses of 0.025 or 24.9 mg Cu/kg bw, respectively. Similar Copper concentrations were determined in plasma of the vehicle control and the treatment group. Mean values ranged from 0.7 to 1.1 µg Cu/g plasma and were consistent to values obtained for control animals without surgery in the pilot experiment 1B. Administration with 20 mg Cu/kg bw as copper hydroxide caused clearly elevated copper concentrations in liver. While the time course in the control was essentially unchanged during the 48 hours after dosing, a peak concentration of 10.2 µg Cu/g tissue was reached at 12 hours after administration of the test substance. Background copper concentrations measured in livers of the control rats were approximately one-half of the peak concentration found in rats of the treatment group. Overall, 0.96 to 1.85 % of the administered dose of 20 mg Cu/kg bw was recovered in the liver. Non-compartmental kinetic analysis of the liver concentration data gave an apparent elimination half-life of 31 hours, estimated from the mean data 12 to 48 hours after administration of the test substance. However, the estimation of the linear elimination half-life ignores the fact that continuing exposure to copper occurs from dietary intake and that systemic uptake is subject to bio-regulatory control. It also ignores the natural background level of copper already present, and is therefore not an accurate representation. By subtracting the initial T_0 value, the apparent half-life of the dosed copper is 10.134 hours. The AUC for the 20 mg Cu/kg bw group (343 hr* µg/g) was 1.4-fold greater than the AUC for the control group (239 hr* µg/g). Upon study termination, copper concentrations in the liver were equivalent to the control, indicating complete clearance of the administered test substance. The results are presented in Table A6.2- 2 and Figure A6.2- 5.

34 APPLICANT'S SUMMARY AND CONCLUSION

34.1	Materials and methods	Based on the results of three pilot experiments two main experiments were conducted, a distribution and excretion experiment with biliary cannulation to estimate the bioavailability of copper from six different test substances, and an experiment investigating the time course of copper in liver and plasma after oral administration of one representative copper substance, i.e. copper hydroxide. The study was conducted according to EC method B.36 (87/302/EEC) and OECD 417 (1984).
34.2	Results and discussion	The results of the main experiments showed that the five forms of copper were similarly absorbed to copper sulphate, following oral administration to bile cannulated rats. The absorbed dose ranged from 10.7 to 12.9 % based on percent of the dose measured in whole blood, liver, carcass, bile and urine. The ranking of copper concentrations in excreta and tissue samples was generally faeces > GI tissue and contents (post-biliary) > stomach and contents > liver > bile > plasma ~ whole blood ~ carcass > urine > cage wash. Administration of 20 mg Cu/kg bw as copper hydroxide had no effect on Copper plasma levels. In contrast, increased concentrations of copper in liver were observed with a peak of 10.2 µg Cu/g tissue at 12 hours after administration. Thereafter copper concentrations decreased to control levels upon study termination. A relative terminal half-life of 10.134 hours was calculated.

Section A6.2 Absorption, distribution and excretion in male rats

Annex Point IIA6.2

34.3	**Conclusion**	
34.3.1	Reliability	1
34.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	23/11/2004
Materials and Methods	Agree with applicant's version (i) Copper hydroxide CAS 20427-59-2 (ii) Copper oxide CAS 1317-39-1 (iii) Copper oxychloride CAS 1332-40-7 (iv) Tribasic copper sulphate CAS 1333-22-8 (v) Bordeaux mixture CAS 8011-63-0 (vi) Copper sulfate pentahydrate CAS 7758-99-8
Results and discussion	Agree with applicant's version Remarks: 4.1 – Experiment 1A: for the 20 and 65 mg/kg groups, blood copper at the beginning of the study is twice higher than low dose and control groups (1.6 µg/g vs 2.9 µg/g). The only difference except the dose was that the former were tested one week after. It was concluded that inflammation due to bile duct canulation was responsible of the high blood copper by increasing the amount of copper transport proteins. 4.1 – Experiment 1B: Due to the results obtained, it was decided to limit the durations that bile-duct canulated rats were used to no longer than one week.
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	

Reliability	
Acceptability	
Remarks	

Table A6.2- 1: Mean disposition of Cu in bile-duct cannulated rats (Mean values, n = 5, Experiment 3)

Sample matrix	$CuSO_4$	$Cu(OH)_2$	Cu_2O	CuOxyCl	$TbCuSO_4$	Bordeaux	Vehicle
Actual mean dose (mg Cu/kg bw)							
	23.7	24.3	22.4	21.8	24.1	23.2	0.052
Tissue concentration (µg Cu/g sample)							
Plasma	2.81	2.60	2.40	3.18	2.96	2.67	2.76
Whole blood	1.97	2.10	1.85	1.88	2.19	2.23	2.21
Liver	15.8	20.6	15.9	17.7	16.7	17.4	5.23
Carcass	1.87	1.70	1.44	1.43	2.07	1.98	1.88
Bile	6.73	9.74	7.31	6.44	6.82	7.93	1.29
Urine	0.890	0.894	0.882	0.890	1.34	1.17	0.534
Stomach	16.4	6.20	7.20	20.4	49.9	99.2	38.5
GI tract	49.9	115	117	133	85.7	102	13.8
Faeces	694	889	1099	914	845	1034	31.5
Cage wash	0.274	0.145	0.249	0.215	0.253	0.480	0.333
Mean percent of administered dose							
Blood	0.26	0.29	0.27	0.26	0.32	0.24	NA
Liver	3.02	3.90	3.01	3.66	3.15	3.74	NA
Carcass	6.43	5.63	5.29	5.20	6.88	6.80	NA
Bile	1.94	2.48	1.84	1.83	1.54	1.78	NA
Urine	0.39	0.20	0.27	0.23	0.28	0.35	NA
Stomach	2.45	0.53	1.06	2.66	4.75	9.72	NA
GI tract	19.75	39.86	42.04	44.27	30.96	40.43	NA
Faeces	73.82	67.71	74.05	63.97	72.14	76.34	NA
Cage wash	0.90	0.59	0.89	0.79	0.85	1.70	NA
Absorbed	12.03	12.50[a]	10.69[a]	11.18[a]	12.17[a]	12.91[a]	NA
Unabsorbed	96.92	108.69	118.04	111.69	108.71	128.20	NA
Recovered	108.96	121.19	128.73	122.87	120.87	141.11	NA

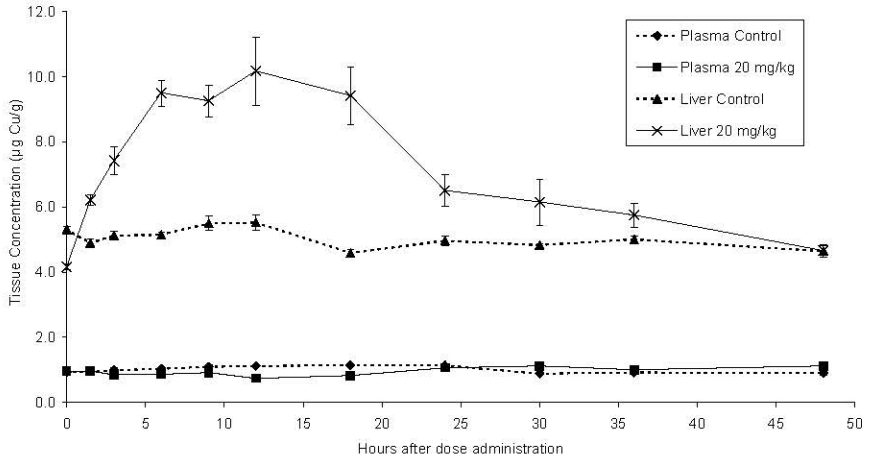
[a] Not significantly different from $CuSO_4$ group by Dunnett's multiple comparisons test at $p < 0.05$.
NA = not applicable

Table A6.2- 2: Mean liver and plasma copper time course concentration data after administration of 0 or 20 mg Cu/kg bw by gavage (Experiment 4)

Sampling time [hours]	Tissue concentration (µg/g)[a]			
	Liver		Plasma	
	Vehicle control	20 mg Cu/kg bw	Vehicle control	20 mg Cu/kg bw
0	5.30 ± 0.10	4.16 ± 0.08	0.931 ± 0.032	0.963 ± 0.017
1.5	4.89 ± 0.13	6.21 ± 0.17	0.950 ± 0.012	0.961 ± 0.056
3	5.12 ± 0.12	7.35 ± 0.44	0.988 ± 0.067	0.841 ± 0.024
6	5.14 ± 0.09	9.49 ± 0.40	1.03 ± 0.03	0.863 ± 0.055
9	5.49 ± 0.22	9.25 ± 0.48	1.10 ± 0.05	0.908 ± NA
12	5.52 ± 0.23	10.2 ± 1.0	1.12 ± 0.01	0.737 ± 0.035
18	4.58 ± 0.11	9.41 ± 0.88	1.14 ± 0.04	0.818 ± 0.126
24	4.96 ± 0.16	6.50 ± 0.49	1.13 ± 0.06	1.06 ± 0.03
30	4.82 ± 0.06	6.14 ± 0.72	0.883 ± 0.062	1.11 ± 0.05
36	5.00 ± 0.10	5.75 ± 0.37	0.917 ± 0.045	0.995 ± 0.085
48	4.64 ± 0.17	4.65 ± 0.18	0.902 ± 0.064	1.11 ± 0.09

[a] Mean and SE for up to n = 5 rats per timepoint

Figure A6.2- 5: Copper time course in liver and plasma (mean and SE) following administration of 0 or 20 mg Cu/kg bw as copper hydroxide by gavage (Experiment 4)



Section A6.2
Annex Point IIA6.2

Percutaneous absorption through human skin (*in vitro* test)

Official use only

35 REFERENCE

35.1	**References**	**A6.2/03:** [redacted] (2003): The in vitro percutaneous absorption of copper from various formulations through human skin. [redacted] Report no.: 22829; August 27, 2003 (unpublished). **A6.2/12:** [redacted] (2004): The in vitro percutaneous absorption of copper from various formulations through human skin – dermal delivery. [redacted], Report no.: 23873; June 18, 2004 (unpublished).
35.2	**Data protection**	Yes
35.2.1	Data owner	EU Copper Task Force
35.2.2	Companies with letter of access	Spiess-Urania
35.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

36 GUIDELINES AND QUALITY ASSURANCE

36.1	**Guideline study**	Yes OECD 428 (Draft, 2002); OECD Draft guidance document No. 28 (2002)
36.2	**GLP**	Yes
36.3	**Deviations**	No

37 MATERIALS AND METHODS

37.1	**Test material**	(i) Kocide PM-E (containing: cupric hydroxide) (ii) MACC 80 (Bordeaux Mixture 20 % WP) (iii) Nordox Super 75 WP (containing: copper oxide) (iv) Copper Oxychloride 50 WP (containing: Dicopper chloride trihydroxide) (v) Cuproxat Flowable (containing: Tribasic copper sulphate)

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Percutaneous absorption through human skin (*in vitro* test)

37.1.1	Lot/Batch number	(i)	1020105705
		(ii)	1/155
		(iii)	200601
		(iv)	11313B
		(v)	141/2001
37.1.2	Specification	Not specified.	
37.1.3	Purity	(i)	76.1 % cupric hydroxide (metallic copper equivalent 49.6 % w/w)
		(ii)	19.55 % copper w/w
		(iii)	75 % copper w/w
		(iv)	49.64 % copper w/w
		(v)	18.8 % copper w/v
37.1.4	Description	(i)	not specified
		(ii)	not specified
		(iii)	not specified
		(iv)	not specified
		(v)	not specified
37.1.5	Stability	(i)	stable for at least 2 years from date of analysis
		(ii)	not specified
		(iii)	not specified
		(iv)	not specified
		(v)	not specified
37.1.6	Radiolabelling	None	
37.2	**Skin preparations**		
37.2.1	Skin samples	Full thickness human skin samples	
37.2.2	Source	Patients (22 to 51 years old), who gave informed consent for their skin to be taken for scientific research purposes, prior to undergoing routine surgery at the Plastic Surgery Unit, St. Johns Hospital NHS Trust, Livingston, UK.	
37.2.3	Preparation	Split-thickness skin membranes were prepared.	
37.3	**Administration/ Exposure**	Dermal	
37.3.1	Application	The 5 test preparations were topically applied to human split-thickness skin membranes mounted in flow through diffusion cells *in vitro*. (Samples were generated from Inveresk study no. 203885).	

Section A6.2
Annex Point IIA6.2

Percutaneous absorption through human skin (*in vitro* test)

37.3.2	Exposure parameters	Surface area of exposed skin: 0.64 cm² Receptor chamber volume: 0.25 mL Flow rate: ca. 1.5 mL/h Receptor fluid: phosphate buffered saline containing bovine serum albumin (ca. 5 % w/v) and streptomycin and penicillin G with the pH changed to pH 7.4 using 0.2 M sodium hydroxide.
37.3.3	Concentration of test substances	Phase 1: (10 replicates/group) 3.34 mg/cm² copper oxychloride — tested in a single moist slurry (representing the formulation prior to mixing with water, but sticking to skin) 2.26 mg/cm² copper hydroxide — tested in a single moist slurry (representing the formulation prior to mixing with water, but sticking to skin) 5.40 mg/cm² copper oxide — tested in a single moist slurry (representing the formulation prior to mixing with water, but sticking to skin) 1.25 mg/cm² Bordeaux mixture — as supplied (suspension concentrate) 1.88 mg/cm² tribasic copper sulphate — as supplied (suspension concentrate) Phase 2: (10 replicates/group) 200.6 µg/cm² copper hydroxide — concentrated suspension 12.6 µg/cm² copper hydroxide — dilute suspension
37.3.4	Vehicle	Water
37.3.5	Volume applied	10 µl/cm²
37.3.6	Exposure period	6 hours At the end of the 6 hour exposure period, the exposed skin surface was washed with ca. 10 mL soap solution (ca. 2%).
37.3.7	Sampling time	Receptor fluid was collected in a single 0–24 hour sample.
37.3.8	Examinations	Measurements of copper concentrations (including method validation) were performed by ICP-MS. The skin samples generated from Inveresk study no. 203885 were removed from freezer storage, allowed to reach room temperature and thereafter, the *stratum corneum* was removed by tape stripping with up to 25 successive tape strips.

38 RESULTS

Section A6.2
Annex Point IIA6.2

Percutaneous absorption through human skin (*in vitro* test)

38.1	**Percutaneous absorption**	Following topical application of copper oxychloride (ca. 250 g/kg), copper hydroxide (ca. 188 g/kg), copper oxide (ca. 375 g/kg), Bordeaux mixture (ca. 100 g/kg) and tribasic copper sulphate (ca. 190 g/L) to human skin, absorption of copper was < 0.01, < 0.01, < 0.01, 0.01 and 0.01 % of the applied dose, respectively. Dermal delivery (including test item in the receptor fluid and the exposed skin after tape stripping) was in a range of 0.08 to 0.41% of the applied dose. Following topical application of copper in form of copper hydroxide at two states of dilution (ca. 1.25 and 20 g/L), absorption of copper was 0.12 and < 0.01 % of the applied dose, respectively. Dermal delivery amounted to 4.06 and 0.52% of the applied dose, respectively. The results are summarised in Table A6.2- 3 and Table A6.2- 4.

39 APPLICANT'S SUMMARY AND CONCLUSION

39.1	**Materials and methods**	*In vitro* percutaneous absorption of copper from various formulations through human split-thickness skin membranes was tested in flow-through diffusion cells. The study was conducted according to OECD 428 (Draft, 2002) and OECD Draft guidance document No. 28 (2002).
39.2	**Results and discussion**	The absorption of copper through human skin was negligible, with a worst case of 0.12 % of the applied dose. Dermal delivery for the 5 undiluted formulations was in a range of 0.08 to 0.41% of the applied dose, while dermal delivery for copper hydroxide at two states of dilution (ca. 1.25 and 20 g/L) amounted to 4.06 and 0.52% of the applied dose, respectively.
39.3	**Conclusion**	
39.3.1	Reliability	1
39.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	March 2007
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.2- 3: Summary of results of phase 1.

Copper form	Applied dose	Absorbed dose		Dermal delivery	
	mg/cm²	ng/cm²	% applied dose	ng/cm²	% applied dose
Copper oxychloride	3.34	82.98	0.00	2601.38	0.08
Copper hydroxide	2.26	16.05	0.00	9499.92	0.41
Copper oxide	5.40	55.56	0.00	3074.09	0.06
Bordeaux mixture	1.25	140.92	0.01	1652.42	0.12
Tribasic copper sulphate	1.88	116.54	0.01	2206.05	0.12

Absorbed dose: The mass of test item reaching the receptor fluid or systemic circulation within a specified period of time.

Dermal delivery: sum of receptor fluid and exposed skin after tape stripping

Table A6.2- 4: Summary of results of phase 2.

Copper form	Applied dose	Absorbed dose		Dermal delivery	
	mg/cm²	ng/cm²	% applied dose	ng/cm²	% applied dose
Copper hydroxide	0.0126	14.50	0.12	511.07	4.06
Copper hydroxide	0.2006	2.93	0.00	1034.33	0.52

Absorbed dose: The mass of test item reaching the receptor fluid or systemic circulation within a specified period of time.

Dermal delivery: sum of receptor fluid and exposed skin after tape stripping

Section A6.2

Annex Point IIA6.2

Percutaneous absorption through human skin (*in vitro* test)

Official use only

40 REFERENCE

40.1	**Reference**	**A6.2/11:**
		[redacted] (2003): Copper compounds – *in vitro* dermal penetration study through human skin. [redacted], Report no.: CSV 004/023929, July 15, 2003 (unpublished).
40.2	**Data protection**	Yes
40.2.1	Data owner	EU Anti-Fouling Copper Task Force
40.2.2	Companies with letter of access	Spiess-Urania
40.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.

41 GUIDELINES AND QUALITY ASSURANCE

41.1	**Guideline study**	Yes OECD 428 (Draft, 2000); OECD Draft guidance document No. 28 (2000)
41.2	**GLP**	Yes
41.3	**Deviations**	Yes The copper content was only analysed in the receptor fluid instead of all components of the test system.

42 MATERIALS AND METHODS

42.1	**Test material**	(i) Cuprous oxide Red (Spiess Urania) (ii) Red Copp 97N Premium (American Chemet Corporation) (iii) Cuprous Oxide Paint Grade Red (Nordox Industrier AS) (iv) Cuprous thiocyanate (Bardyke Chemicals Ltd.) (v) Copper Flake Powder (Wolstenholme International Ltd.) (vi) Cupric sulphate (Fisher Scientific, reference material)
42.1.1	Lot/Batch number	(i) not stated (ii) 39140 (iii) not stated (iv) not stated (v) not stated (vi) 0248057
42.1.2	Specification	Not specified.
42.1.3	Purity	(i) > 98.1 % Cu_2O (87.5 % copper) (ii) 97.5 % Cu_2O (88 % copper) (iii) 98 % Cu_2O (87 % copper) (iv) 99.51 % CuSCN (51.99 % copper) (v) > 96 % copper (vi) not stated

Section A6.2 Percutaneous absorption through human skin (*in vitro* test)

Annex Point IIA6.2

42.1.4	Description	(i)	red powder
		(ii)	red powder
		(iii)	red powder
		(iv)	Near white powder
		(v)	Copper coloured powder
		(vi)	not stated
42.1.5	Stability	(i)	not specified
		(ii)	not specified
		(iii)	not specified
		(iv)	not specified
		(v)	not specified
		(vi)	not specified
42.1.6	Radiolabelling	None	
42.2	**Skin preparations**		
42.2.1	Skin samples	Full thickness human skin samples	
42.2.2	Source	The skin samples were supplied by University College London tissue bank. Abdominal skin or skin from the back was obtained from male or female donors (age: 49–70 years).	
42.2.3	Preparation	Split-thickness skin membranes were prepared with a dermatome (thickness: approx. 300 µm).	
42.3	**Administration/ Exposure**	Dermal	
42.3.1	Application	The test preparations were topically applied to human split-thickness skin membranes mounted in static diffusion cells *in vitro*.	
42.3.2	Exposure parameters	Surface area of exposed skin: 0.95 cm^2 Diffusion cell: static Receptor fluid: 10 mM phosphate buffered saline supplemented with 5% w/v bovine serum albumin adjusted to pH 7.4. The receptor fluid was continuously stirred and maintained at 32°C.	
42.3.3	Concentration of test substances	Product name:	Achieved dose (µg Copper):
		Cuprous oxide:	997.8
		Cuprous thiocyanate:	693.1
		Copper powder:	925.7
		Cupric sulphate:	894.9
		1) Mixture from three different sources (i.e. Cuprous oxide Red (Spiess Urania), Red Copp 97N Premium (American Chemet Corporation) and Cuprous Oxide Paint Grade Red (Nordox Industrier AS).	
42.3.4	Vehicle	Distilled water	
42.3.5	Volume applied	10 µl	

Section A6.2
Annex Point IIA6.2

Percutaneous absorption through human skin (*in vitro* test)

42.3.6	Exposure period	For each of the test substances, there were three sampling regimes: (1) 6 hours (termination of the test). The skin was swabbed at 6 hours after dosing using 1% Tween 80 in distilled water on cotton wool buds. (2) 6 hours, termination of the test 24 hours post application. The skin was swabbed at 6 hours after dosing using 1% Tween 80 in distilled water on cotton wool buds. (3) 24 hours (no swab was performed).
42.3.7	Sampling time	Receptor fluid was collected at the end of the experiment (either 6 or 24 hours).
42.3.8	Examinations	The copper content was analysed in receptor fluid samples by atomic absorption spectrophotometry (including method validation).
		43 RESULTS
43.1	**Percutaneous absorption**	In conclusion, following topical application of cuprous oxide (composite from three sources), cuprous thiocyanate, copper powder, and cupric sulphate to human skin for six hours, absorption of copper determined in the receptor fluid amounted to 0.157, 0.126, 0.038 and 0.136 % of the applied dose, respectively, at 24 hours after application. The results are summarised in Table A6.2- 5.
		44 APPLICANT'S SUMMARY AND CONCLUSION
44.1	**Materials and methods**	*In vitro* percutaneous absorption of copper from various formulations through human split-thickness skin membranes was tested in static diffusion cells. The study was conducted according to OECD 428 (Draft, 2000) and OECD Draft guidance document No. 28 (2000), except that the copper content was only analysed in the receptor fluid instead of all components of the test system.
44.2	**Results and discussion**	The absorption of copper through human skin was minimal, with a worst case of 0.16 % of the applied dose.
44.3	**Conclusion**	Discounting Copper powder (not of relevance for this dossier), the results of this study demonstrate that there was no appreciable difference between soluble and less soluble Copper compounds in their potential to penetrate human skin *in vitro*. The results of this study with Copper sulphate may therefore be extrapolated without restriction to other less soluble Copper salts such as Copper hydroxide or Copper carbonate. Whereas conventional thinking requires the amount of the test item also to be investigated in the epidermal layer of the tested skin (since this may be potentially absorbed in the post-exposure period), this may be considered redundant in this case in view of the cationic, polar nature of the dissolved copper, for which no feasible mechanism of transcutaneous permeation can be postulated. It is therefore proposed to take forward the observed maximal dermal penetration rate of 0.16% for human risk assessment, despite the formally restricted validity implied by the deviation from the test guideline.
44.3.1	Reliability	2

Section A6.2 Annex Point IIA6.2	Percutaneous absorption through human skin (*in vitro* test)

44.3.2 Deficiencies	Yes The copper content was only analysed in the receptor fluid instead of all components of the test system, so that a full mass balance could not be established.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	April 2006
Materials and Methods	Agree with applicant's version.
Results and discussion	Agree with applicant's version. **Comment:** Percutaneous absorption (4.1): For each test substances, there were three sampling regimes. Results for these 3 regimes should be reported and discussed even if the chosen regime is the most occupationally relevant sampling regime.
Conclusion	
Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.2- 5: Copper content in receptor fluid samples.

Test material	Dose level [mg/cm²]	Termination at [h]	Swabbed at 6 h [yes/no]	Amount absorbed [%]	Amount absorbed [ng]	Absorption rate [ng/cm²/h]	Absorption ratio[1]
Cuprous oxide composite							
	1.050	6	y	0.098	977.2	171.4	1.15
		24	y	0.157	1564.6	68.62	
		24	n	0.193	1926.0	84.47	
Cuprous thiocyanate							
	0.730	6	y	0.128	887.8	155.8	0.92
		24	y	0.126	871.3	38.22	
		24	n	0.147	1019.6	44.72	
Copper powder							
	0.974	6	y	0.086	800.0	140.4	0.28
		24	y	0.038	352.9	15.48	
		24	n	0.046	427.9	18.77	
Cupric sulphate							
	0.942	6	y	0.097	868.4	152.4	1.00
		24	y	0.136	1216.5	53.36	
		24	n	0.038	337.6	14.81	

1) Absorption ratio given with respect to cupric sulphate, using the amount absorbed at 24 hours following a swab at 6 hours post dosing.

Section A6.2 Metabolism studies in mammals

Annex Point IIA6.2 **– Supportive data, provided as IUCLID short summaries –**

References:		**A6.2/04:** Anonymous (1996): Trace elements in human nutrition and health, World Health Organization, Chapter 7, p123-143 **A6.2/05:** Anonymous (1998): Copper, Environmental Health Criteria 200, World Health Organization, Geneva, 360p.
In Vitro/in vivo	:	In vivo
Type	:	
Species	:	
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Remark	:	Copper is an essential trace element, consumed via human nutrition at daily intake rates of 0.9-2.2 mg Cu/day. Its essentiality relates to its presence in a large number of proteins in the human body. Homeostasis is controlled via metallothionein-associated mechanisms. Copper is predominantly absorbed via the gastrointestinal tract. Upon passage to blood plasma, copper is bound rapidly to serum albumin or transcuprein as transport proteins. Subsequent transport is directed primarily to the liver, the critical organ accounting for copper homeostasis. Excess copper is excreted primarily through the bile, or incorporate into ceruloplasmin for resecretion to blood. The oral absorption rate varies between 20-75% (the balance being excreted via faeces), and appears to inversely related to dietary copper content. Absorption occurs not only in the intestine, but also to a relevant extent already in the stomach. Reliable quantitative data on the extent of pulmonary or dermal absorption have not been reported. However, in view of the ionic nature of copper ions, percutaneous absorption is not considered to be a relevant route of entry into the body (WHO, 1996 and 1998).
Test substance	:	Copper, not specified
Reliability	:	(4) not assignable Summary of toxicokinetic data, secondary literature

References:	**A6.2/06:** Mason K.E. (1979): A conspectus of research on copper - Metabolism and requirements of man. J. Nutrition 109, p. 1979-2066 **A6.2/07:** Österberg, R. (1980): Physiology and pharmacology of copper, Pharmac. Ther. 9, p. 121-146.
In Vitro/in vivo	: In vivo
Type	: Toxicokinetics
Species	:
Number of animals	
Males	:
Females	:
Doses	
Males	:
Females	:
Vehicle	:

Result : The normal daily diet contains 2 to 5 mg copper. Absorption of copper is regulated at the level of the intestinal mucosa and excretion is predominantly through the intestinal tract, either via the bile or as nonabsorbed copper. Urinary excretion is negligible in normal healthy man amounting to about 1 to 2 per cent of the intake. Although the site of maximal absorption of copper varies among different mammalian species, in man absorption occurs primarily in the stomach and duodenum. This conclusion is based upon observations that after oral administration of radioactive copper the isotope appears rapidly in the blood, reaching maximum levels within 1 to 2 hours, at which time it is found bound to serum albumin. A secondary peak appears after 1 to 2 days, at which time the copper has been incorporated into the ceruloplasmin. After oral administration up to 90 percent of absorbed copper can be detected in the liver. Within the liver cells, one copper fraction is required for the synthesis of ceruloplasmin, superoxy dismutase and other copper proteins, but the largest copper fraction is directly excreted via the bile.

Animal studies indicate that at least two mechanisms are concerned in copper absorption, an energy- dependent one (involving the absorption of complexes of copper and amino acids) and an enzymatic one (involving the binding of copper to, and successive release from, macromolecular proteins). Studies of experimental animals indicate that mechanisms of copper storage and transfer involve not only metallothionein as first identified in the chick intestinal mucosa, but that there also may exist a variety of metallothioneins, differing slightly in amino-acid content and metal binding characteristics.

In animals, many effects are known to interfere with copper absorption through various mechanisms (competition for binding sites by zinc, perhaps to a much lesser extent by cadmium; interactions between molybdenum, sulphates and copper; the effects of dietary phytates and the influence of ascorbic acid intake).

On the basis of reviewed studies in humans, the authors considered that it seems reasonable to assume an absorption of 40 to 60 per cent of the oral intake of copper, accepting the fact that there is wide individual variation.

In rats dosed with [67Cu]-ceruloplasmin intravenously, the radio-labelled copper was taken up by all tissues, but primarily by the liver where it appeared in a specific protein fraction of the hepatocyte cytosol. In addition, to serving as the major pathway of copper excretion via the biliary tract, the liver releases copper to maintain the labile pool of copper in the serum and blood cells. Ceruloplasmin synthesised by the liver amounts to approximately 93 per cent of plasma copper, which is remarkably constant in healthy adult humans.

Test substance : Copper, not specified

Reliability : (4) not assignable
Summary of toxicokinetic data, secondary literature

Reference:		**A6.2/08:** Winge, D.; Mehra, R. (1990): Host defense against copper toxicosis; International Review of Experimental Pathology 31, p47-83.
In Vitro/in vivo	:	In vivo
Type	:	Toxicokinetics
Species	:	
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Result	:	Copper absorption in rats occurs predominantly from the stomach and jejunum. Copper balance is maintained in part by homeostatic mechanisms operating at the level of intestinal copper absorption. Under conditions of low copper intake, a compensatory enhancement is observed in intestinal copper absorption (from 36 per cent in normal diets to 55 per cent in low copper diets). Homeostatic mechanisms operate at high dietary intake of copper to minimise the accumulation of copper in cells and tissues. Dietary components (e.g. ascorbate, phytates, dietary protein, zinc, iron, molybdenum, sulfide) can affect the bioavailability of copper. The liver readily accumulates copper ions and transiently stores the metal. In cultured mammalian cells, radiocopper has been shown to initially reside in the cytosol associated with metallothionein. Nearly 80 per cent of the copper leaving the liver is excreted via the bile. Biliary excretion of copper correlates positively with the absorbed dose of copper, but negatively with the hepatic concentration of metallothionein. The steady state concentration of metallothionein can be markedly increased in animals by metal ions and a variety of metabolic effectors and cytokines. Overall, detoxification mechanisms to minimise the potential deleterious effects of excessive tissue concentrations of copper in animals, include the level of gastrointestinal absorption (partial regulation of copper absorption occurs at the level of the transport system as well as mucosal copper sequestration), the preferential clearance of absorbed copper by liver hepatocytes (protecting nonhepatic tissues from copper overload), and reductant mechanisms existing within the hepatocytes to sequester copper ions in a stably bound state.
Test substance	:	Copper, not specified
Reliability	:	(4) not assignable Summary of toxicokinetic data, secondary literature

References:		**A6.2/09:** Aoyagi S, Baker DH (1993) Bioavailability of copper in analytical-grade and feed-grade inorganic copper sources when fed to provide copper at levels below the chick's requirement. Poultry Science 72, 1075-1083. **A6.2/10:** Baker DH (1999) Cupric oxide should not be used as a copper supplement for either animals or humans. Am. Soc. Nutri. Sci. J. Nutr. 129, 2278-2279.
In Vitro/in vivo	:	In vivo
Type	:	Absorption
Species	:	other: chick
Number of animals		
Males	:	
Females	:	
Doses		
Males	:	
Females	:	
Vehicle	:	
Result	:	Young chicks were fed a casein-soy protein concentrate basal diet (0.56 mg Cu/kg) containing graded levels of added Cu (0, 0.5, 1.0 mg/kg) from analytical grade CuSO4*5H2O, Cu2O, CuO, CuCO3*Cu(OH)2, CuCl and also from feed-grade CuSO4*5H2O and CuO. Weight gain, hematocrit, hemoglobin, plasma Cu, liver Cu, gall bladder (bile) Cu, and tendon lysine were assessed. The results indicated Cu bioavailability values (relative to the standard CuSO4*5H2O analytical grade, set at 100 per cent) in a range of 93.5 to 112.9 per cent for Cu2O, CuSO4*5H2O (feed grade) and CuCO3*Cu(OH)2. Relative bioavailability of Cu in CuCl was 142.5 per cent. CuO (analytical and feed grade) gave Cu bioavailability estimates not different from zero. The results indicate that when Cu levels are fed below the chick's requirement, bile Cu concentration is a sensitive indicator of net gut absorption of Cu.
Test substance	:	Cu from alytical grade CuSO4*5H2O, Cu2O, CuO, CuCO3*Cu(OH)2, CuCl and also from feed-grade CuSO4*5H2O and CuO.
Reliability	:	(2) valid with restrictions Acceptable, well documented publication which meets basic scientific principles

Section A6.3.1 **Repeated dose toxicity (oral)**

Annex Point IIA6.3 **Rat**

			Official use only
		45 REFERENCE	
45.1	**Reference**	Boyden, R., Potter, R., Elvehjem, C.A. (1937): Effect of feeding high levels of copper to albino rats. - *The Journal of Nutrition*, Vol. 15, no. 4., p. 397 – 402 Doc. no. URA 97-08740-051	
45.2	**Data protection**	No	
45.2.1	Data owner	published data	
45.2.2	Companies with letter of access		
45.2.3	Criteria for data protection	No data protection claimed	
		46 GUIDELINES AND QUALITY ASSURANCE	
46.1	**Guideline study**	No	
46.2	**GLP**	No	
46.3	**Deviations**	No	X
		47 MATERIALS AND METHODS	
47.1	**Test material**	copper sulfate	
47.1.1	Lot/Batch number	not stated	
47.1.2	Specification	not stated	
47.1.2.1	Description	not stated	
47.1.2.2	Purity	not stated	
47.1.2.3	Stability	not stated	
47.2	**Test Animals**		
47.2.1	Species	White rats	
47.2.2	Strain	not stated	
47.2.3	Source	not stated	
47.2.4	Sex	16 male, 14 female	
47.2.5	Age/weight at study initiation	21 days of age	
47.2.6	Number of animals per group	4 groups with 3 to 8 animals each	
47.2.7	Control animals	Yes	
47.3	**Administration/ Exposure**	Oral	