Section 6.3.1 Annex Point 6.3.1	Short-Term Repeat Dose Toxicity (Oral)	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [X]**	
Detailed justification:	The TGD 'Guidance on Data Requirements for Active Substances and Biocidal Products (version 4.3.1, April 2000) states that short term repeat dose toxicity tests are not required when an adequate sub-chronic toxicity study is available in a rodent. A sub-chronic repeat dose oral toxicity study, carried out in the rat and mouse is presented in Section 6.4. This, therefore, negates the need for a 28-day repeat dose oral toxicity study.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	29 Dec.2004	
Evaluation of applicant's justification	Agree with applicant's justification (Annex II A Directive 98/8/EC). A short-term repeated dose toxicity (28 days) is not required when a sub-chronic toxicity study is available in a rodent.	
Conclusion	Acceptable.	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE	
Date	*(specify) Give date of comments submitted*	

Section 6.3.1 Short-Term Repeat Dose Toxicity (Oral)

Annex Point 6.3.1

Evaluation of applicant's justification *Discuss if deviating from view of rapporteur member state*

Conclusion *Discuss if deviating from view of rapporteur member state*

Remarks

Section A6.3.2
Annex Point A6.3.2
IUCLID: 5.4/16

A6.3.2, Repeated Dose Toxicity (Dermal)

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.
If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [X]**

Limited exposure [ ] **Other justification [X]**

Detailed justification:

According to the Technical Notes for Guidance on data requirements for active substances and biocidal products, this study is required for active substances that have the following characteristics:

- A percutaneous study is required, where the potential dermal exposure is significant and route-to-route extrapolation is not possible.
- However, a percutaneous study may be necessary where it is justified that dermal route is more appropriate or specific effects of concern are different from the effects seen in the studies in other routes.

This study is usually required when the dermal route of exposure is significant and the compound is known to be toxic by the dermal route and can penetrate through intact skin. The need to conduct this study with either copper (II) oxide or copper carbonate must therefore be questioned as although the dermal route of exposure is the most significant route of exposure in professional wood preservation use, there is no evidence to indicate that either salt can cause toxicity or indeed pass through intact skin. Acute dermal toxicity studies showed no toxic effects up to and including the highest dose tested (See Section 6.1.2). It is also possible to calculate the route-to-route exposure from available oral toxicity studies and using dermal penetration studies (see Section 6.2) as there are no specific effects observed following dermal exposure of both salts in animals. Therefore an accurate and realistic determination of dermal toxicity can be derived from available sub-chronic oral exposure studies, permissible systemic copper levels and *in vitro* dermal penetration studies on copper sulphate and insoluble copper compounds (NTP, 1993; SANCO, 2003; [redacted]).

Section A6.3.2 **Annex Point A6.3.2** **IUCLID: 5.4/16**	**A6.3.2, Repeated Dose Toxicity (Dermal)**
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29 Dec.2004
Evaluation of applicant's justification	Agree with applicant's justification. Comment: Quoted references aren't detailed and not introduced in IUCLID like SANCO 2003 [redacted]
Conclusion	**Acceptable.**
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specij)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.3.3
Annex Point A6.3.3
IUCLID: 5.4/17

A6.3.3, Repeated Dose Toxicity, Inhalation

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.
If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [ ]**

Limited exposure [X] **Other justification [X]**

Detailed justification:

According to the Technical Notes for Guidance on data requirements for active substances and biocidal products, these studies are required for active substances that have the following characteristics:

- For volatile substances and gases (vapour pressure > 1 x 10-2 Pa)
- In cases where inhalation exposure is significant, an inhalation study is required instead of an oral study
- In some cases (e.g. aerosols and dusts/particulate matter) studies by the inhalation route should be required in addition to studies by the oral route.

Copper compounds are not volatile substances or gases and in fact have negligible vapour pressures.

Although inhalation exposure could be considered relatively high at a wood preservative treatment plants as calculated from the TnG on human health (Doc IIB), engineering controls significantly reduce or even eliminate plant operator exposure to the product.

A plant treating 15,000 m3 wood per year would emit 0.0003kg = 0.3 mg copper per year into the air from the vacuum system. Because this figure is so low, emissions from the plant vacuum system are considered to be insignificant.

After the treatment cycle is finished and the cylinder door is opened there may be a few seconds of aerosol emission from the vessel and are quickly dispersed. This is of a transient nature and vessels and operating procedures do not result in the generation of aerosols. Measurements made in the NIOSH Technical Report, 1983 show copper levels of <1.5µg Cu / m3 air (i.e. <LOD) when sampled adjacent to the cylinder door opening. Similar levels are recorded

Section A6.3.3 Annex Point A6.3.3 IUCLID: 5.4/17	A6.3.3, Repeated Dose Toxicity, Inhalation
	elsewhere. This emission route is considered insignificant for the purposes of this environmental exposure assessment. Connell and Hughes (1998) also showed there was no elevation in atmospheric copper levels as the door of a treatment vessel opened when using copper azole wood preservative. These engineering controls would be in place because of the existence of a long-established Operator Exposure Limit for copper dust (inhalation) in many European Countries. Some of these are presented below: DE-MAK 1 mg/m^3 (total dust) SE-LEVL 1 mg/m^3 (as Cu, total dust); 0.2 mg/m^3 (as Cu, respirable dust) UK-LTEL 1 mg/m^3 (as Cu, dusts and mists) UK-STEL 2 mg/m^3 (as Cu, dusts and mists) Due to the established operator exposure limits and the monitoring data above, it would appear unnecessary to conduct new animal studies when established levels have been in place in the workplace for many years. No significant inhalation exposure will occur to passer-by at the treatment plant or the general public through use of treated timber.
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29 Dec.2004
Evaluation of applicant's justification	Agree with applicant's justification.
Conclusion	Acceptable.
Remarks	

Section A6.3.3 A6.3.3, Repeated Dose Toxicity, Inhalation

Annex Point A6.3.3
IUCLID: 5.4/17

COMMENTS FROM OTHER MEMBER STATE *(specij)*

Date *Give date of comments submitted*

Evaluation of applicant's justification *Discuss if deviating from view of rapporteur member state*

Conclusion *Discuss if deviating from view of rapporteur member state*

Remarks

Section A 6.4.1	**Repeated dose toxicity in the Rat**
Annex Point 6.4 .1	*Specify section no. and heading, route and species*
IUCLID: 5.4/04	**A6.4.1(01), Subchronic Oral Toxicity Test**

Official use only

1 REFERENCE

1.1 Reference

Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages) If necessary, copy field and enter other reference(s).

[redacted]

1.2 Data protection

No

(indicate if data protection is claimed) 1.2.1 Data owner *Give name of company* – Not applicable

1.2.2 Criteria for data protection

Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:

Not applicable

2 GUIDELINES AND QUALITY ASSURANCE

2.1 Guideline study

No - The method was developed by the US NTP specifically for the purposes of this study

(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")

2.2 GLP

Yes

(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)

2.3 Deviations

See Section 5.5.5

(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y") X

3 MATERIALS AND METHODS

In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values depending on the true methodological parameters.

3.1 Test material

Copper sulphate

or give name used in study report

3.1.1 Lot/Batch number

List lot/batch number if available

533344

3.1.2 Specification

Not reported

(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):

3.1.2.1 Description

If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution) Blue, crystalline solid X

3.1.2.2 Purity

Give purity in % of active substance

Not reported

Section A 6.4.1	**Repeated dose toxicity in the Rat**
Annex Point 6.4 .1	*Specify section no. and heading, route and species*
IUCLID: 5.4/04	**A6.4.1(01), Subchronic Oral Toxicity Test**
3.1.2.3 Stability	*Describe stability of test material* Stable at room temperature
~~**3.2 Test Animals**~~	~~Non-entry field~~
3.2.1 Species	Rat
3.2.2 Strain	F344/N
3.2.3 Source	[redacted]
3.2.4 Sex	Male and Female
3.2.5 Age/weight at study initiation	Test animals were approximately 6 weeks old at study initiation. Male mean bodyweights ranged from 119-120 g, mean female bodyweights ranged from 105-107 g
3.2.6 Number of animals per group	*Give number* *specify, if there are differences for example for treatment and recovery groups* In the base study, groups of 10 animals per sex were tested at each dose level. A supplementary study was carried out on 10 males and females per sex per dose for haematology and clinical chemistry evaluations on Days 5 and 21 (all surviving base-study rats were also subject to the same examinations on test termination – Day 92).
3.2.7 Control animals	Yes
3.3 Administration/ Exposure	Oral *(fill in respective route in the following, delete other routes)*
3.3.1 Duration of treatment	92 Days
3.3.2 Frequency of exposure	*ad libitum* for 7-days a week
3.3.3 Postexposure period	
3.3.4 Oral	
Preparation of active ingredient in feed	Copper sulphate was mixed with NIH-07 Open Formula Diet in meal form. Homogeneity analysis were conducted on the copper sulphate feed mixture using inductively coupled plasma-atomic emission spectroscopy. Samples taken prior to study initiation and twice during the study, confirmed homogeneity between feed mixtures.
3.3.4.1 Concentration in vehicle	Feed mix was available *ad libitum* throughout the study period. 0 (control), 500, 1000, 2000, 4000 or 8000 ppm were administered to the test organisms in feed. Doses were based on a preliminary 2-week feed study.
3.3.4.2 Duration of exposure	92-Days
3.3.4.3 Controls	Yes –vehicle only
3.4 Examinations	*Non entry field*
3.4.1 Observations	*Non entry field*
3.4.1.1 Clinical signs	*yes/no (give time periods for observation)* Yes – test animals were observed weekly for clinical signs

Section A 6.4.1	**Repeated dose toxicity in the Rat**	
Annex Point 6.4 .1	*Specify section no. and heading, route and species*	
IUCLID: 5.4/04	**A6.4.1(01), Subchronic Oral Toxicity Test**	
3.4.1.2 Mortality	*yes/no (give time periods for observation)* Yes – test animals were observed twice daily for mortality/morbidity.	
3.4.2 Body weight	*yes/no (give time periods for determinations)* Yes - Individual bodyweights were recorded prior to the start of the study, on Day 1 and weekly thereafter.	
3.4.3 Food consumption	*yes/no (give time periods for determinations)* Yes – test animals were observed once weekly for food consumption.	
3.4.4 Water consumption	*yes/no (give time periods for determinations)* Not reported	
3.4.5 Ophthalmoscopic examination	*yes/no (give time periods for examinations)* See histological examinations	
3.4.6 Haematology	Yes number of animals: taken from all supplementary animals and base-study rats. Blood samples were collected from the retroorbital sinus time points: Supplementary rats - Day 5 and 21, Base study rats – Day 92 and test termination Parameters: hematocrit , haemoglobin concentration, erythrocyte count, reticulocytes, nucleated erythrocytes, mean cell volume and haemoglobin, concentration, platelets and leukocyte count and differential.	
3.4.7 Clinical Chemistry	Yes number of animals: taken from all supplementary animals and base-study rats time points: Supplementary rats - Day 5 and 21, Base study rats – Day 92 and test termination Parameters: alanine aminotransferase , alkaline phosphatase, 5'-nucleotidase, sorbitol dehydrogenase, bile salts, total protein, albumin, creatinine and urea nitrogen.	
3.4.8 Urinalysis	Yes number of animals: taken from all supplementary animals and base-study rats time points: Supplementary rats - Day 5 and 21, Base study rats – Day 92 and test termination Parameters: creatinine, glucose, protein, asparate aminotransferase, N-acetyl-**β**-D-glucosaminidase, volume and specific gravity.	X
3.4.9 Tissue Metal Level Analysis	Yes Number of animals: Plasma and tissue samples (liver, kidney and testis) were collected from all surviving male base-study rats Time Points: Day 92 - copper, zinc, magnesium and calcium analysis. Blood samples (2 ml) were collected from the retroorbital sinus and placed into 3 ml Vacutainer® tubes containing EDTA. The samples were centrifuged and the separated plasma collected. To prepare for analysis, samples were weighed to the nearest 0.1 mg, digested in a nitric acid-perchloric acid mixture and heated until evolution of nitric acid was complete. The residue was then dissolved in 10% perchloric acid solution and an aliquot removed for analysis by ICP-AES. Metal concentrations were determined by comparing the instrument response to the digested tissues to spiked tissue standards.	
3.5 Sacrifice and pathology	Non entry field	

Section A 6.4.1	**Repeated dose toxicity in the Rat**	
Annex Point 6.4 .1	*Specify section no. and heading, route and species*	
IUCLID: 5.4/04	**A6.4.1(01), Subchronic Oral Toxicity Test**	
3.5.1 Organ Weights	Yes organs: liver, kidneys, adrenals, testes, uterus, ovaries, thymus, spleen, brain, heart	X
3.5.2 Gross and histopathology	Yes Number of animals: Complete necropsies were performed on all animals in the control and high dose groups and on all other animals that died early Time point: See above Parameters: adrenal glands, brain (three sections), esophagus, eyes (if grossly abnormal) femur with marrow, gross lesions, heart, intestines (large: cecum, colon, rectum: small: duodenum, jejunum. Ileum), kidneys, liver, lung/mainstream bronchi, lymph nodes (mandibular, mesenteric) mammary gland, nasal cavity and turbinates (three sections), ovaries, pancreas, parathyroid glands, pharynx (if grossly abnormal), pituitary gland, preputial or clitoral glands, prostate gland, salivary glands, spinal cord/sciatic nerve (if neurological signs were present), spleen, stomach (forestomach, glandular stomach), testes (with epididymis) thymus, thyroid gland, trachea, urinary bladder and uterus	
3.5.3 Other examinations	Non entry field	
3.5.3.1 Supplemental histological examination	To characterise the distribution of copper in the liver and kidney, section of both organs from selected male and females were stained for copper using the rhodanine method. In order to determine the nature of the proteinaceous droplets (see in previous study on rats) sections from selected animals were stained for carbohydrate (PAS method), protein (Mallory-Heidenhain method), lipofuscin (AFIP method) and **α**-2-microglobulin (immunochemistry). Liver sections from the same rats were stained for lipofuscin, and kidney and liver sections from rats of both sections were examined by transmission electron microscopy. Perl's stain for iron was used to stain sections of spleen from rats in all groups.	
3.5.3.2 Sperm morphology and vaginal cytology	Sperm morphology and vaginal cytology evaluations were performed on rats from the 0, 500, 200 and 4000 ppm groups (10 animals per sex and dose group). The method employed was as follows: National Toxicology Program (NTP) 1987. Technical Protocol for Sperm Morphology and Vaginal Cytology Evaluations in Toxicity Testing for Rats and Mice, 10/31/82 version. Research Triangle Park, N.C. Females: 12 days prior to sacrifice, the vaginal vaults of 10 individuals per dose group were lavaged and the aspirated lavage fluid and cells stained with Toluidine Blue. Relative numbers of leukocytes, nucleated epithelial cells and large squamous epithelial cells were determined and used to ascertain estrous cycle stage. Males: Sperm motility was evaluated at necropsy. The left testis and epididymis were weighed, the tail of the epididymis was removed from the epididymis body and weighed. Test yolk was applied to slides and a small incision made in the cauda. The sperm effluxing from the incision were dispersed in the buffer on the slides, and the number of motile and non-motile spermatozoa counted for five microscopic fields per slide. Following motility determination, each left cauda were placed in phosphate buffered saline solution for sperm density determination with a hemacytometer	

Section A 6.4.1	**Repeated dose toxicity in the Rat**
Annex Point 6.4 .1	*Specify section no. and heading, route and species*
IUCLID: 5.4/04	**A6.4.1(01), Subchronic Oral Toxicity Test**

3.6	**Statistics**	The following statistical procedures were followed; Dunnet, C.W. 1955. A multiple comparison procedure for comparing several treatments with a control. J. Am. Stat. Assoc. 50, 1095-1121 Williams, D. A. 1971. Biometrics, 27, 103-117 Williams, D.A. 1972. The comparison of several dose levels with a zero dose control. Biometrics 28, 519-531 Shirley, E. 1977. A nonparametric equivalent of William's test for contrasting increasing dose levels of a treatment. Biometrics 33, 386-389 Dun, O.J. 1964. Multiple comparisons using rank sums. Technometrics 6, 241-252 Jonckheere, A.R. 1954. A distribution free k-sample test against ordered alternatives. Biometrika, 41, 133-145 Dixon & Massay 1951 Introduction to Statistical Analysis, McGraw-Hill Book Co.	X

4 RESULTS AND DISCUSSION

(Describe findings. If appropriate, include table. Sample tables are given below.)

4.1	**Observations**	Non entry field
4.1.1	Clinical signs	*no effects / describe effects* No clinical signs of toxicity could be directly attributed to cupric sulphate consumption in any male or female group. For further details please refer to Table A6_4-5
4.1.2	Mortality	*no mortalities at any dose/concentration level / describe significant effects referring to data given in results table* Except for one female that was accidentally killed, all rats survived to the end of the study. For further details please refer to TableA6_4.5
4.2	**Body weight gain**	*no effects / describe significant effects referring to data given in results table* Final mean bodyweights of test organisms were lower than those of the controls for male rats in the 500, 4000 and 8000 ppm groups and for female rats in the 8000 ppm group. These differences were most pronounced in males in the high dose (8000 ppm). For further details please refer to Table A6_4.5
4.3	**Food consumption and compound intake**	*no effects / describe significant effects referring to data given in results table* For male and female rats in the 500, 1000, 2000 and 4000 ppm groups, average daily food consumption was similar to that of the controls. However, food consumption by both sexes in the 8000 ppm dose groups was below that of the controls. Despite this, the average daily compound consumption increased proportionally with increasing concentrations of copper sulphate in the feed. For further details please refer to Table A6_4.5

Section A 6.4.1	**Repeated dose toxicity in the Rat**
Annex Point 6.4 .1	*Specify section no. and heading, route and species*
IUCLID: 5.4/04	**A6.4.1(01), Subchronic Oral Toxicity Test**

4.4	**Neurotoxicity**	Determination of neurotoxicity was not part of this study. Information on neurotoxicity is presented in TNG Summary 6.9 and IULICD 5.9 [redacted]
4.5	**Ophtalmoscopic examination**	Not reported. See Section 3.5.2
4.6	**Blood analysis**	*Non entry field*
4.6.1 Haematology		*no effects / describe significant effects referring to data given in results table*

Significant changes in haematology parameters were noted in both sexes at all time points. At Day 5, significant increases in hematocrit (HCT) and hemoglobin (HGB) concentrations were noted in high dose male and female rats. By Day 21, these parameters were significantly decreased for male rats in the two highest dose groups (4000 and 8000 ppm) and female rats in the three highest dose groups. At Day 92, HCT and HGB concentrations were significantly decreased in males in the two highest dose groups and in females in the highest dose group. At Day 5, significant increases in erythrocyte (RBC) counts were noted in males in the two highest dose groups and in the high dose females; on Day 92, the only significant increase in RBC count was noted in the high-dose males. In both sexes, in the two highest dose groups, significant decreases in reticulocytes counts were noted on Day 5. By Day 21, reticulocyte counts in males and females in the same dose groups were significantly greater than those of the controls; at Day 92, this parameter was significantly increased in high dosed males. The only significant change noted in nucleated erythrocytes was a marginal decrease in high dose males at Day 5.

On Day 5, mean cell volume (MCV) values were significantly decreased in males in the two highest dose groups and in females in the highest dose group; mean cell hemoglobin (MCH) values were also significantly decreased for males in the two highest dose groups. At Days 21 and 92, decreases in MCV and MCH were noted in both sexes in the three highest dose groups, and all decreases were significant with the exception of the Day 92 MCH values for females receiving 4000 ppm. The only significant changes in mean cell hemoglobin concentrations were increases noted on Day 21 in high dose females and in males in the two highest dose groups.

At Days 5 and 21, significant increases in platelet counts were noted in males and females in the three highest dose groups; the Day 5 platelet count for males in the 1000 ppm group was also significantly increased compare to the controls. At Day 92, increases in platelet counts were noted for both sexes in the two highest dose groups, but this was only significant for males.

Leukocyte counts were increased at all time points in both sexes in the two highest dose groups, with significant increases occurring at Day 5 in high-dose males, at Day 21 in males in the 4000 ppm dose group, and at Day 92 in high-dose males and females: leukocyte count was also significantly increased at Day 21 in males receiving 2000 ppm copper sulphate. Significant increases in lymphocytes were noted at Day 5 in high dose males, at Day 21 in males receiving 2000 or 4000 ppm copper

sulphate, and at Day 92 in high dose females. The only other significant change in haematology parameters was an increase in segmented neutrophils at Day 92 in high dose male rats.

For further details please refer to Table A6_4-1

4.6.2 Clinical chemistry

no effects / describe significant effects referring to data given in results table

Significant changes in serum chemistry parameters occurred in male and female rats at all time point in the two highest groups. Alanine aminotransferase activities were significantly increased at all time points in both sexes in the two highest dose groups; and was significantly increased at Day 92 in males receiving 1000 or 2000 ppm. At Days 5 and 21, decreases in alkaline phosphate activities were noted in both sexes in the two highest dose groups; except for Day 21 in males in the 4000 ppm group, all these decreases were significant. Changes in sorbitol dehydrogenase (SDH) were limited to Days 21 and 92. At both of these time points, SDH activates were significantly elevated in males in the two highest dose groups and in high dose females; significant increases in SDH activities were also noted at Day 92 in males in the 2000 ppm group and females in the 4000 ppm group. When compared to the control values, 5'necleotidase was significantly decrease in highdose females at Days 5 and 21 and in high dose males at Day 5; at Day 92, however, this parameter was significantly increased in males receiving 4000 and 8000 ppm cupric sulphate.

At Day 5, slight increases in bile salts were noted in males in the three highest dose groups; however, female bile salts were decreased for all treated groups, with significant decreases in the 1000 and 8000 ppm groups. By Day 21, no significant changes were noted in females, but significant increases were noted in males in the two highest dose groups. At Day 92, significant increases in bile salts were noted in high-dose males and in females receiving 2000 or 4000 ppm copper sulphate.

At all time points, total protein was significantly decreased in high dose males and in females in the 4000 and 8000 ppm dose groups; at Days 5 and 21, total protein was also significantly decreased in males and females receiving 4000 and 2000 ppm copper sulphate respectively. At Days 5 and 21, decreases in albumin concentrations were noted in both sexes at the three highest doses, all of these were significant, excluding the Day 21 for males receiving 2000 ppm. At Day 92, this parameter was significantly decreased in high dose males and females in the two highest groups.

Urea nitrogen (UN) was significantly increased for both sexes in the two highest groups at Day 5, and by Day 21, this was significantly increased in males in the three highest dose groups and females in the highest dose group. At Day 92, UN was significantly elevated in the high-dose males and females as well as females receiving 1000, 2000 or 4000 ppm copper sulphate. The only significant change in creatinine was an increased noted in high dose females on Day 92.

For further information please refer to Table A6_4-2

4.6.3 Urinalysis

no effects / describe significant effects referring to data given in results table

Significant changes in urinalysis parameters were noted in supplemental study rats at Days 19 and in base study Day 90. Significant increases in urinary aspirate aminotransferase (AST) activities, occurred at Days 19

Section A 6.4.1 **Repeated dose toxicity in the Rat**

and 90 in both sexes in the highest dose groups. Increases in this parameter also occurred at both time points in male and female rats in the 4000 ppm groups. A few significant increases in AST activities occurred in animals in the lower dose groups (500 to 2000 ppm). Significant increases in N-acetyl-**β**-D-glucosaminidase activities were noted in both sexes in the highest dose group on Day 90; at this time point, increases also occurred in males and females in the 4000 ppm groups. Glucose output was significantly increased at Day 19 in males in the 2000 ppm group and at Day 90, this parameter was significantly elevated in males in the two highest dose groups. A significant decrease in protein output was noted in the high dose males at Day 19, however, the Day 90 elevation in base study rats, this parameter was significantly increased relative to the controls in males in the two highest dose groups. No significant changes in glucose or protein output were noted in females at either time point.

Please refer to Table A6_4-3 for further information.

4.7 Sacrifice and pathology

Non entry field

4.7.1 Organ weights

no effects / describe significant effects referring to data given in results table

Significant changes in absolute organ weights were limited to males and females in the high dose groups and included decreases in absolute brain, heart, kidney, liver, lung and thymus weights in males and absolute kidney weight in females. Generally, relative organ weights for treated groups were similar to those of the controls or increased with decreasing mean body weights in the two highest dose groups (4000 and 8000 ppm).

For further information please refer to Table A6_4-5

4.7.2 Gross and histopathology

no effects / describe significant effects referring to data given in results table

Gross lesions were present in the forestomach of both sexes receiving copper sulphate at concentrations of 2000 ppm or greater. The limiting ridge that forms the junction of the forestomach squamous mucosa with the glandular gastic mucosa appeared enlarged in all rats in the 4000 and 8000 ppm dose groups.

Histopathological findings that correspond to the gross lesions consisted of minimal to moderate hyperplasia of the squamous mucosa at the site of the limiting ridge. This lesion was characterised by a thickening and increased folding of the squamous mucosa; hyperkeratosis was also a component of the squamous cell hyperplasia. The increased incidence and severity of this lesion were dose related. When this lesion was more severe, there was often an increase in the number of inflammatory cells and/or edema in the lamina propria of the limiting ridge. There was no evidence or erosion/ulceration and no lesions were present in other areas of the squamous mucosa.

Other histopathological findings were present in the liver and kidney in both sexes. There was a dose related increase in the incidence and severity of chronic-active inflammation in the liver of male and female rats. This lesion was present in most rats in the 4000 and 8000 ppm

Copper Oxide

groups and in one male in the 2000 ppm group and was characterised by multiple foci of a mixture of mononuclear inflammatory cells, primarily macrophages. These foci of inflammation occurred primarily in the periportal portion of the hepatic lobules. Necrosis of one to several hepatocytes was often observed adjacent to or within the foci of inflammation.

Chemical related cytoplasmic alteration was present in the kidneys of male and female rats at doses of 2000 ppm and greater. This lesion was morphologically similar in both sexes but was less severe in females. A few droplets were also present in the tubule lumina of female rats. In treated male rats, the protein droplets were much larger and more numerous than those in the control males or in the treated females, and many large droplets were present in the tubule lumina. These droplets stained strongly positive for protein but were negative by iron, PAS and acid-fast staining methods. Results of **α**-2-microglobulin staining of kidney sections from male and female control and high dose rats were inconclusive. While the kidneys of male rats stained positive for **α**-2- microglobulin, there were no clear qualitative differences in staining between treated and control rats. Also present in the kidneys of rats in the high dose groups was minimal nuclear enlargement in renal tubule cells. Degeneration of the renal tubule epithelium was present in three females in the 8000 ppm group.

4.8 Other *Non entry field*

The results of the analysis indicated that copper accumulated in the liver and kidney in a dose related manner and was accompanied by an accumulation of zinc in these tissues. Copper concentrations were significantly increased in the kidney and liver of rats in all treated groups. Copper levels were also significantly elevated in the plasma and testis of rats in the three highest dose groups. Significant increases in zinc concentration in the kidney and liver were noted in animals in the three highest dose groups, and concentrations of calcium in plasma were significantly decreased in the 4000 and 8000 ppm groups. Significant increases in magnesium were noted in the kidney and plasma of rats receiving 2000 ppm copper sulphate as well as in the plasma of rats receiving 8000 ppm copper sulphate.

4.9 Tissue Metal Level Analysis

For further information please refer to Table A6_4-4

A summary of nonneoplastic lesions is presented in the attached document Table A6_4-6

Liver and kidneys of rats were stained for the presence of copper. Positive staining in liver sections was limited to 4000 and 8000 ppm. At 8000 ppm, staining in the liver had a clear periportal to midzonal distribution and consisted of a few to numerous (10-20) red granules of 1-2 mm in the cytoplasm of hepatocytes. In addition there was minimal staining of the cytoplasm in some of the cells in the inflammatory foci. At 4000 ppm, staining of the hepatocytes was limited to the periportal area and there was a marked reduction in the number of cells stained and the number of granules per cell.

4.10 onneoploastic lesions

Kidney sections also stained positive for copper only in the two highest dose groups. Staining consisted of red granules in the cytoplasm of the renal tubule epithelium and a diffuse or stippled red staining of the

4.11 Supplemental histological examination

		protein droplets in the cytoplasm and the tubule lumen. However, many of these (especially in the 4000 ppm group) did not stain positive for copper. Positive staining of the kidney tubule cells was limited to the cortex; there was not staining in the medullary rays outer and inner medulla. Sections of hear and spleen showed no positive stained in any dose group. Sections of spleen from 4 rats per dose group were evaluated for iron. In the 8000 ppm groups there was only a few iron-positive granules in the cytoplasm of macrophages in the red pulp. The reduction in ironpositive material in the spleens from the 2000 and 4000 ppm groups was much less prominent than the 8000 ppm group, but a minimal decrease was evident compared to the controls. Transmission electron microscopy of the livers of both sexes showed that within the cytoplasm of hepatocytes in the periportal area, there was degenerative changes consisting of increased numbers of secondary lysosomes, many of which were enlarged and contained clear, non-staining crystalline structures and electron-dense material. Kidneys had mild to marked increases in the number and size of electron dense protein droplets in the cytoplasm of the proximal convoluted tubule epithelium. In addition to changes in the size and number, many droplets in the kidneys of male rats had irregular crystalline shapes	
4.12	Sperm Morphology and Vaginal Cytology	There were no significant findings in males or females. See attached Table A6_4-7	
		5 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	*Give guidelines and describe/discuss deviations from test guidelines or, in case of non-guideline study, briefly describe method*	
		The aim of the study was to examine the effect of copper sulphate (0, 500, 1000, 2000, 4000 or 8000 ppm) administered to male and female B6C3F1 mice in feed for 13 weeks. The test organisms were observed throughout the study for signs of clinical toxicity, mortality, bodyweight changes and food consumption. Throughout the study blood and urine samples were collected to determine haematology, clinical chemistry and urinalysis parameters and tissue metal level, At the end of the study period all animals were sacrificed and subject to pathological examinations to determine any histological, sperm morphology or vaginal cytology abnormalities. The study was conducted to a methodology developed by the US National Toxicology Programme specifically for the test. The study was conducted in accordance with GLP.	X X
5.2	**Results and discussion**	*Summarize relevant results; discuss dose-response relationship.* Hematological, clinical chemistry and urinalysis evaluations of rats revealed variable chemical-related changes that were, for the most part, restricted to the 4000 and 8000 ppm groups. Increases in serum alanine aminotransferase and sorbitol dehydrogenase activities in both sexes were indicative of hepatocellular damage, as were increases in 5'-nucleotidase and bile salts in males. Decreases in mean cell volume, hematocrit and haemoglobin indicated the development of a microcytic anaemia, while increases in reticulocyte numbers at the same time points suggested a compensatory response to the anaemia by the bone marrow. Increases in urinary glucose and N-acetyl-**β**-Dglucosaminidase (a lysosome enzyme) and asparate aminotransferase (a	

Section A 6.4.1	**Repeated dose toxicity in the Rat**
Annex Point 6.4 .1	*Specify section no. and heading, route and species*
IUCLID: 5.4/04	**A6.4.1(01), Subchronic Oral Toxicity Test**

	cytosolic enzyme) were suggestive of renal tubule epithelial damage. Dose related increases in copper occurred in all male rat tissues examined. These increases were accompanied by increases in zinc in the liver and kidney. Plasma calcium was significantly reduced in the 4000 and 8000 ppm groups, and there was a trend towards reduction in calcium in the kidney and testis as well. In the 8000 ppm group, plasma magnesium was significantly increased relative to the controls. Rats in the three highest dose groups had hyperplasia and hyperkeratosis of the forestomach, inflammation of the liver and increases in the number and size of protein droplets in the epithelial cytoplasm and the lumina of the proximal convoluted tubules. Many of the droplets in the male kidneys were large and had irregular crystalline shapes. These droplets stained strongly positive for protein but were negative for iron, PAS, and acid-fast (lipofuscin) staining methods. **A**-2-microglobulin was present in the droplets of male rats, but there was no dose-related qualitative difference in the content of this protein. In the 4000 and 8000 ppm groups, copper was distributed in a periportal to midzonal pattern in the liver and was restricted to the cytoplasm of the proximal convoluted tubule epithelium in the kidney. Copper was present in some, but not all, of the protein droplets. Transmission electron microscopy of the livers of rats of each sex revealed increases in the number of secondary lysosomes in hepatocytes in the periportal area.	
5.3 Conclusion	*Non entry field*	
5.3.1 LO(A)EL	*Give critical effect and dose/concentration, if necessary separately for males and females* The LOAEL for forestomach lesions was 2000 ppm for both males and females. The LO(A)EL for liver damage was 2000 ppm for males and 4000 ppm for females. The LO(A)EL for kidney damage was 2000 ppm for males and 1000 ppm for females.	
5.3.2 NO(A)EL	*Give dose/concentration, if necessary separately for males and females* The NO(A)EL for forestomach lesions was 1000 ppm for both males and females. The NO(A)EL for liver damage was 1000 ppm for males and 2000 ppm for females. The NO(A)EL for kidney damage was 1000 ppm for males and 500 ppm for females.	
5.3.3 Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4*	X
5.3.4 Deficiencies	Yes The study deviated from 'Directive 88/302/EEC B.26 Subchronic 90-Day Oral Toxicity Study in Rodents' as follows; • No additional top dose group or control animals group were	

included in the study for observation of recovery from toxic effects after the treatment period.

- Ophthalomological examinations were only carried out where the eyes showed clinical signs of gross abnormalities. General eye examinations of the control and high dose group were not carried out.

- Sensory activity and signs of neurotoxicity were not determined towards the end of the study. The study was conducted prior to this requirement being included in the guidelines. However, signs of reproductive toxicity were included in the test methodology. See Section 6.4.14.

- Heamatological examinations did not include a measure of blood clotting time/potential.

- It was not reported if animals were fasted overnight prior to blood sampling.

- Determinations of plasma or serum did not include sodium, potassium or total cholesterol analysis.

- Histopathological examinations did not include the aorta.

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 29 Dec.2004

Reference

- In Reference (1.1) : NIH Publication 93 3 **93-3352.**
- In paragraph Deviations (2.3) : See Section 5.5.5 **5.3.4.**

Section A 6.4.1	**Repeated dose toxicity in the Rat**
Annex Point 6.4 .1	*Specify section no. and heading, route and species*
IUCLID: 5.4/04	**A6.4.1(01), Subchronic Oral Toxicity Test**
Materials and Methods	Agree with applicant's version. **Comments:** • Test material (3.1): copper sulphate copper(II) sulphate pentahydrate (according to CAS number). • The purity of the active substance (3.1.2.2) was reported in the study : 99%. • Control animals (3.2.7): Yes: **10 males and 10 females.** • Concentration in vehicle (3.3.4.2): doses in mg/kg body weight/day must be indicated: 0, 8, 18, 34, 67 & 138 mgCu/kg body weight/day. • Urinalysis (3.4.8) time points : supplementary rats – D5 and D21 **D19**, Base study rats – Day 92 and test termination D90. • Organ Weights (3.5.1) : organs: liver, kidneys, adrenals, testes, uterus, ovaries, thymus, spleen, brain, heart **and lungs.** • Statistics (3.6) : It misses the reference of Morrison, 1976 (multivariate analysis of variance). • Materials and Methods (5.1): The aim of the study was to examine the effect of copper sulphate **pentahydrate** (0, 500, 1000, 2000, 4000 or 8000 ppm) administered to male and female B6C3F1 mice **F344/N rats** in feed for 13 weeks.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. LO(A)EL: 2000 ppm (34 mgCu/kg bw/day) for males and 1000 ppm (18 mgCu/kg bw/day) for females. NO(A)EL: 1000 ppm (18 mgCu/kg bw/day) for males and 500 ppm (8 mgCu/kg bw/day) for females.
Reliability	2 Some important results or studies as additional top dose group, ophthalmologic examinations, sensory activity, signs of neurotoxicity are missing.
Acceptability	Acceptable This sub-chronic repeated dose oral toxicity study in the rat is realised with cupric sulphate pentahydrate and not with copper oxide.
Remarks	• In results of significant haematology effects (Table A6 4-1): it misses some parameters like Mean cell volume, mean cell haemoglobin or platelet counts... however discussed in the results. • Errors in the tables were corrected in red and bold.

Table A6_4-2. Results of Significant Clinical Chemistry Effects

(Use this or similar table, if relevant effects occur and if time sequence is important. Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

Parameter	Control 0 ppm			500 ppm			1000 ppm			2000 ppm			4000 ppm			8000 ppm		
	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92
Males																		
Alanine aminotransferase	-	-	-	-	-	-	-	-	↑	-	-	↑	↑	↑	↑	↑	↑	↑
Alkaline phosphate	-	-	-	-	-	-	-	-	-	-	-	-	↓	-	-	↓	↓	-
Sorbital dehydrogenase	-	-	-	-	-	-	-	-	-	-	-	↑	-	↑	↑	-	↑	↑
5'nucleotidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	↓	-	↑
Bile salts	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	-	↑	↑
Total protein	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓	-	↓	↓	↓
Albumin concentrations	-	-	-	-	-	-	-	-	-	↓	-	-	↓	↓	-	↓	↓	↓
Urea nitrogen	-	-	-	-	-	-	-	-	-	-	↑	-	↑	↑	-	↑	↑	↑
Females																		

Alanine aminotransferase	-	-	-	-	-	-	-	-	-	-	-	-	↑	↑	↑	↑	↑	↑
Alkaline phosphate	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓	-	↓	↓	-
Sorbital dehydrogenase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	↑	↑
5'nucleotidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓	-
Bile salts	-	-	-	-	-	-	↓	-	-	-	-	↑	-	-	↑	↓	-	-
Total protein	-	-	-	-	-	-	-	-	-	↓	↓	-	↓	↓	↓	↓	↓	↓
Albumin concentrations	-	-	-	-	-	-	-	-	-	↓	↓	-	↓	↓	↓	↓	↓	↓
Urea nitrogen	-	-	-	-	-	-	-	-	↑	-	-	↑	↑	-	↑	↑	↑	↑
Creatinine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑

Table A6_4-5. Results of repeated dose toxicity study

Parameter	Control 0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm	dose-response +/-

Copper Oxide

	m[a]	1[a]	m[a]	1[a]	m[a]	1[a]	m[a]	1[a]	m[a]	1[a]	m[a]	1[a]	m[a]	1'[a]
number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10		
Mortality	0	0	0	0	0	1	0	0	0	0	0	0	-	-
clinical signs*	0	0	0	0	0	1	0	0	0	0	0	0	-	-
body weight (grams) (initial: final)	119 : 362	106 : 193	120 : 335	106 : 196	119 : 360	105 : 199	119 : 354	107 : 196	120 : 338	107 : 188	119 : 275	106 : 179	+	+
Final weight relative to controls (%)	-	-	92	101	99	103	98	101	93	97	76	93	+	+
food consumption (g/day)	16.3	11.1	16.6	11.0	17.0	11.3	16.5	11.3	16.5	10.8	14.4	10.1	+	+
Compound consumption (mg/kg/day)	-	-	32	34	64	68	129	135	259	267	551	528	+	+
Organ weight														
Brain	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Heart	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Right kidney	-	-	-	-	-	-	-	-	-	-	↓	↓	+	
Liver	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Lungs	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Right testis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymus	-	-	-	-	-	-	-	-	-	-	↓	-	+	-

** specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

a *give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased*

organ weights reported in absolute weight

	COMMENTS FROM ... *(specij)*
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	

Table A6_4-1. Results of Significant Haematology Effects

(Use this or similar table, if relevant effects occur and if time sequence is important. Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

Parameter	Control 0 ppm			500 ppm			1000 ppm			2000 ppm			4000 ppm			8000 ppm		
	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92
Males																		
Hematocrit concentrations	-	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓	↑	↓	↓
Haemoglobin concentrations	-	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓	↑	↓	↓
Erythrocyte count	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	-	↑	-	↑
Reticulocyte count	-	-	-	-	-	-	-	-	-	-	-	-	↓	↑	-	↓	↑	↑
Nucleated erythrocytes	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↓	-	-
Leukocyte counts	-	-	-	-	-	-	-	-	-	-	↑	-	-	↑	-	↑	-	↑
Segmented neutrophils	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑

Parameter	Control 0 ppm			500 ppm			1000 ppm			2000 ppm			4000 ppm			8000 ppm		
	Day 5	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**
Females																		
Hematocrit concentrations	-	-	-	-	-	-	-	-	-	-	↓	-	-	↓	-	↑	↓	↓
Haemoglobin concentrations	-	-	-	-	-	-	-	-	-	-	↓	-	-	↓	-	↑	↓	↓
Erythrocyte count	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	-
Reticulocyte count													↓	↑		↓	↑	
Leukocyte count																		↑

* $p < 0,05$

Give only those parameters which are changed in at least one dose group compared to control. Usually only statistically significant effects

Depending on number of parameters changed one table each for Haematology, Clinical Chemistry, Urinalysis

Table A6_4-2. Results of Significant Clinical Chemistry Effects

(Use this or similar table, if relevant effects occur and if time sequence is important. Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

Parameter	Control 0 ppm			500 ppm			1000 ppm			2000 ppm			4000 ppm			8000 ppm		
	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92	Day 5	Day 21	Day 92
Males																		
Alanine aminotransferase	-	-	-	-	-	-	-	-	↑	-	-	↑	↑	↑	↑	↑	↑	↑
Alkaline phosphate	-	-	-	-	-	-	-	-	-	-	-	-	↓	-	-	↓	↓	-
Sorbital dehydrogenase	-	-	-	-	-	-	-	-	-	-	-	↑	-	↑	↑	-	↑	↑
5'nucleotidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	↓	-	↑
Bile salts	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	-	↑	↑
Total protein	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓	-	↓	↓	↓

Albumin concentrations	-	-	-	-	-	-	-	-	-	↓	-	-	↓	↓	-	↓	↓	↓
Urea nitrogen	-	-	-	-	-	-	-	-	-	-	↑	-	↑	↑	-	↑	↑	↑
Females																		

Copper Oxide

Parameter	**Control 0 ppm**			**500 ppm**			**1000 ppm**			**2000 ppm**			**4000 ppm**			**8000 ppm**		
	Day 5	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**	**Day 5**	**Day 21**	**Day 92**
Alanine aminotransferase	-	-	-	-	-	-	-	-	-	-	-	-	↑	↑	↑	↑	↑	↑
Alkaline phosphate	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓	-	↓	↓	-

Sorbital dehydrogenase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑	-	↑	↑
5'nucleotidase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↓	↓
Bile salts	-	-	-	-	-	-	↓	-	-	-	-	-	-	-	↑	↓	-	-
Total protein	-	-	-	-	-	-	-	-	-	↓	↓	-	↓	↓	↓	↓	↓	↓
Albumin concentrations	-	-	-	-	-	-	-	-	-	↓	↓	-	↓	↓	↓	↓	↓	↓
Urea nitrogen	-	-	-	-	-	-	-	-	↑	-	-	↑	↑	-	↑	↑	↑	↑
Creatinine	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	↑

Table A6_4-3. Results of Significant Urinalysis Effects

(Use this or similar table, if relevant effects occur and if time sequence is important. Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

Parameter	**Control 0 ppm**		**500 ppm**		**1000 ppm**		**2000 ppm**		**4000 ppm**		**8000 ppm**	
Males	**Day 19**	**Day 90**	**Day 19**	**Day 90**	**Day 19**	**Day 90**	**Day 19**	**Day 90**	**Day 19**	**Day 90**	**Day 19**	**Day 90**
Urinary aspartate aminotransferase	-	-	-	-	-	-	-	-	↑	↑	↑	↑
N-acetyl-**β**-D-glucosaminidase	-	-	-	-	-	-	-	-	-	↑	-	↑
Glucose output	-	-	-	-	-	-	↑	-	-	↑	-	↑
Protein output	-	-	-	-	-	-	-	-	-	↑	↓	↑
Females			-	-	-	-	-	-	-	-	-	-

Copper Oxide

Parameter	Control 0 ppm		500 ppm		1000 ppm		2000 ppm		4000 ppm		8000 ppm	
Males	**Day 19**	**Day 90**	**Day 19**	**Day 90**	**Day 19**	**Day 90**	**Day 19**	**Day 90**	**Day 19**	**Day 90**	**Day 19**	**Day 90**
Urinary aspartate aminotransferase	-	-	↑	-	-	↑	-	↑	↑	↑	↑	↑
N-acetyl-β-D-glucosaminidase	-	-	-	-	-	-	-	↑	-	↑	-	↑
Glucose output	-	-	-	-	-	-	-	-	-	-	-	-
Protein output	-	-	-	-	-	-	-	-	-	-	-	-

* $p < 0,05$

Give only those parameters which are changed in at least one dose group compared to control. Usually only statistically significant effects

Depending on number of parameters changed one table each for Haematology, Clinical Chemistry, Urinalysis

Table A6_4-4. Results of Significant Tissue Metal Concentrations Effects from Male Rats

(Use this or similar table, if relevant effects occur and if time sequence is important. Give either symbols for increases or decreases (↑↓) or abbreviations inc., dec. Only if more information is needed, give figures or percentages.)

Parameter	Control 0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Copper						
Kidney	-	↑	↑	↑	↑	↑
Liver	-	↑	↑	↑	↑	↑
Plasma	-	-	-	↑	↑	↑
Testis	-	-	-	↑	↑	↑
Calcium						
Kidney	-	-	-	-	-	-
Liver	-	-	-	-	-	-
Plasma	-	-	-	-	↓	↓
Testis	-	-	-	-	-	-
Magnesium						

Copper Oxide

Parameter	Control 0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Kidney	-	-	-	↑	-	-
Liver	-	-	-	-	-	-
Plasma	-	-	-	↑	-	↑
Testis	-	-	-	-	-	-
Zinc						
Kidney	-	-	-	↑	↑	↑
Liver	-	-	-	↑	↑	↑
Plasma	-	-	-	-	-	-
Testis	-	-	-	-	-	-

* $p < 0,05$

Give only those parameters which are changed in at least one dose group compared to control. Usually only statistically significant effects

Depending on number of parameters changed one table each for Haematology, Clinical Chemistry, Urinalysis

Table A6_4-5. Results of repeated dose toxicity study

Parameter	Control 0 ppm		500 ppm		1000 ppm		2000 ppm		4000 ppm		8000 ppm		dose-response +/-	
	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]
number of animals examined	10	10	10	10	10	10	10	10	10	10	10	10		
Mortality	0	0	0	0	0	1	0	0	0	0	0	0	-	-
clinical signs*	0	0	0	0	0	1	0	0	0	0	0	0	-	-
body weight (grams) (initial: final)	119 : 362	106 : 193	120 : 335	106 : 196	119 : 360	105 : 199	119 : 354	109 : 196	120 : 338	107 : 188	119 : 275	106 : 179	+	+
Final weight relative to controls (%)	-	-	92	101	99	103	98	101	93	97	76	93	+	+
food consumption (g/day)	16.3	11.1	16.6	11.0	17.0	11.3	16.5	11.3	16.5	10.8	14.4	10.1	+	+
Compound consumption (mg/kg/day)	-	-	32	34	64	68	129	135	259	267	551	528	+	+
Organ weight														
Brain	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Heart	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Right kidney	-	-	-	-	-	-	-	-	-	-	↓	↓	+	
Liver	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Lungs	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Right testis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymus	-	-	-	-	-	-	-	-	-	-	↓	-	+	-

** specify effects; for different organs give special findings in the order organ weight, gross pathology and microscopic pathology if there are effects*

[a] *give number of animals affected/total number of animals, percentage, or just ↑ or ↓ for increased or decreased*

organ weights reported in absolute weight

Table A6_4-6a Summary of the Incidence of Nonneoplastic Lesions in Male Rats.

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Survivors						
Terminal sacrifice	10	10	10	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Liver	(10)		(10)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	1 (10%)		1 (10%)	1 (10%)		
Inflammation, chronic active				1 (10%)	10 (100%)	10 (100%)
Pancreas	(10)					(10)
Atrophy	2 (20%)					1 (10%)
Stomach, forestomach	(10)	(1)	(10)	(10)	(10)	(10)
Hyperplasia				10 (100%)	10 (100%)	10 (100%)
Stomach, glandular	(10)		(10)	(10)	(10)	(10)
Mineralization			1 (10%)			
Cardiovascular System						
Heart	(10)					(10)
Inflammation, chronic active	10 (100%)					5 (50%)
Endocrine System						
Pituitary gland	(10)					(10)
Cyst						1 (10%)
General Body System						
None						
Genital System						
Epididymis	(10)					(10)
Inflammation, chronic active	1 (10%)					
Preputial gland	(10)					(10)
Inflammation, chronic active	7 (70%)					6 (60%)
Prostate	(10)					(10)
Inflammation, chronic active	1 (10%)					1 (10%)
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active	1 (10%)					
Special Senses System						
None						
Urinary System						
Kidney	(10)	(9)	(10)	(10)	(10)	(10)
Cytoplasmic alteration				5 (50%)	10 (100%)	10 (100%)
Nephropathy	10 (100%)	9 (100%)	10 (100%)	8 (80%)	9 (90%)	6 (60%)
Proximal convoluted renal tubule karyomegaly						10 (100%)

Number of animals examined microscopically at site and number of animals with lesion.

Table A6_4-6b Summary of the Incidence of Nonneoplastic Lesions in Female Rats.

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Disposition Summary						
Animals initially in study	10	10	10	10	10	10
Early deaths						
Accidently killed			1			
Survivors						
Terminal sacrifice	10	10	9	10	10	10
Animals examined microscopically	10	10	10	10	10	10
Alimentary System						
Intestine small, jejunum	(10)		(1)			(10)
Inflammation, acute	1 (10%)					
Liver	(10)	(1)	(2)	(10)	(10)	(10)
Hepatodiaphragmatic nodule	2 (20%)	1 (100%)	2 (100%)	2 (20%)		
Inflammation, chronic active					6 (60%)	10 (100%)
Inflammation, focal	2 (20%)					
Mesentery		(1)				
Fat, necrosis		1 (100%)				
Pancreas	(10)		(1)			(10)
Atrophy	1 (10%)					1 (10%)
Stomach, forestomach	(10)		(10)	(10)	(10)	(10)
Cyst epithelial inclusion						1 (10%)
Hyperplasia				7 (70%)	10 (100%)	10 (100%)
Cardiovascular System						
Heart	(10)					(10)
Inflammation, chronic active						1 (10%)
Endocrine System						
None						
General Body System						
None						
Genital System						
Clitoral gland	(10)		(1)			(10)
Inflammation, chronic active	9 (90%)					10 (100%)
Ovary	(10)	(1)	(1)			(10)
Cyst		1 (100%)				
Hematopoietic System						
None						
Integumentary System						
None						

Table A6_4-6b Summary of the Incidence of Nonneoplastic Lesions in Female Rats (cont.).

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm
Musculoskeletal System						
None						
Nervous System						
Brain	(10)		(1)			(10)
Gliosis	1 (10%)					
Respiratory System						
Lung	(10)					(10)
Inflammation, chronic active	1 (10%)					1 (10%)
Special Senses System						
None						
Urinary System						
Kidney	(10)	(10)	(10)	(10)	(10)	(10)
Cyst	1 (10%)					
Cytoplasmic alteration			1 (10%)	9 (90%)	10 (100%)	10 (100%)
Mineralization		1 (10%)				
Nephropathy			1 (10%)	1 (10%)		2 (20%)
Pigmentation						2 (20%)
Proximal convoluted renal tubule, karyomegaly						10 (100%)
Renal tubule, degeneration						3 (30%)

[1] Number of animals examined microscopically at site and number of animals with lesion.

Table A6_4-7 Summary of the Reproductive Evaluations in Male and Female Rats

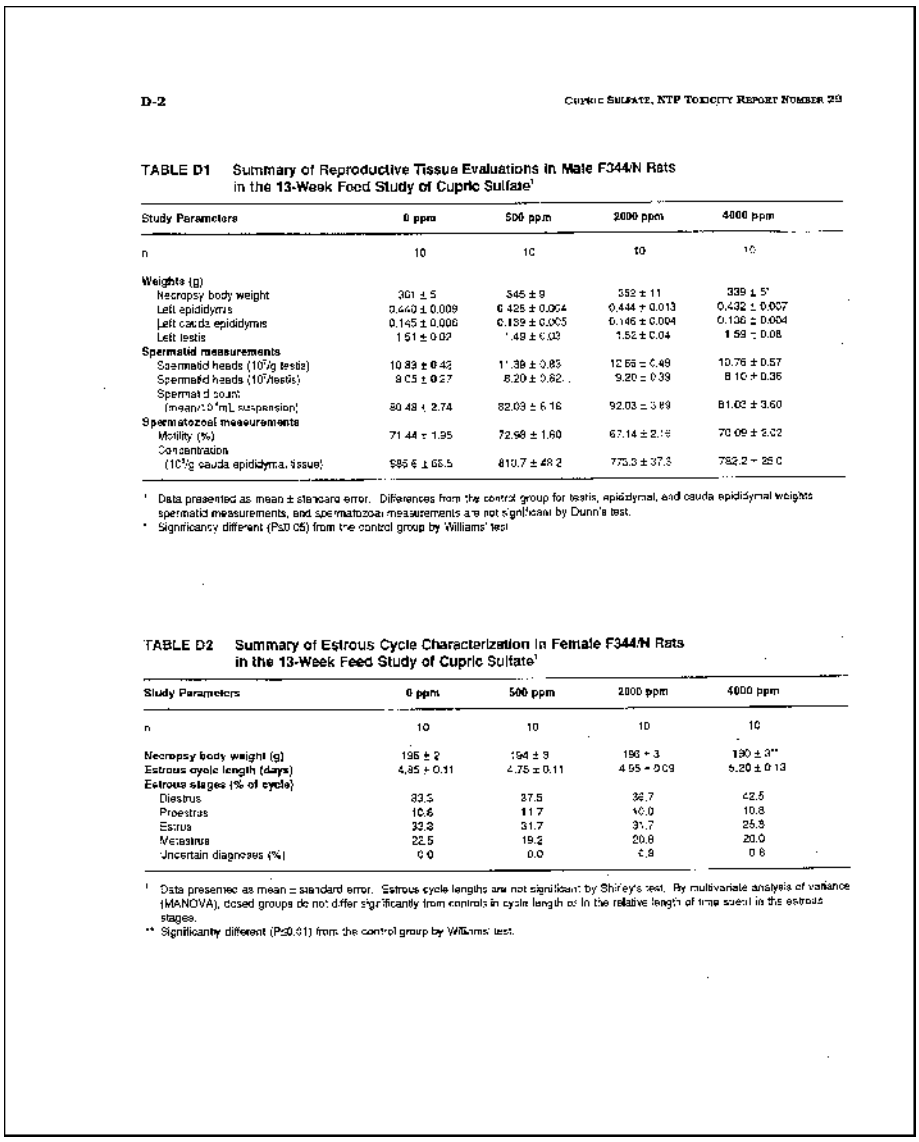

TABLE D1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	500 ppm	2000 ppm	4000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	361 ± 5	345 ± 9	352 ± 11	339 ± 5*
Left epididymis	0.440 ± 0.009	0.425 ± 0.004	0.444 ± 0.013	0.432 ± 0.007
Left cauda epididymis	0.145 ± 0.006	0.139 ± 0.005	0.146 ± 0.004	0.136 ± 0.004
Left testis	1.51 ± 0.02	1.49 ± 0.03	1.52 ± 0.04	1.53 ± 0.08
Spermatid measurements				
Spermatid heads (10^7/g testis)	10.83 ± 0.42	11.38 ± 0.83	12.55 ± 0.49	10.76 ± 0.57
Spermatid heads (10^7/testis)	8.05 ± 0.27	8.20 ± 0.62	9.20 ± 0.39	8.10 ± 0.36
Spermatid count (10^6/mL suspension)	80.48 ± 2.74	82.03 ± 6.16	92.03 ± 3.89	81.03 ± 3.60
Spermatozoal measurements				
Motility (%)	71.44 ± 1.95	72.98 ± 1.60	67.14 ± 2.19	70.09 ± 3.02
Concentration (10^3/g cauda epididymal tissue)	985.6 ± 68.5	810.7 ± 48.2	775.3 ± 37.3	782.2 ± 25.0

[1] Data presented as mean ± standard error. Differences from the control group for testis, epididymal, and cauda epididymal weights, spermatid measurements, and spermatozoal measurements are not significant by Dunn's test.
* Significantly different (P≤0.05) from the control group by Williams' test

TABLE D2 Summary of Estrous Cycle Characterization in Female F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	500 ppm	2000 ppm	4000 ppm
n	10	10	10	10
Necropsy body weight (g)	195 ± 2	194 ± 3	193 ± 3	190 ± 3**
Estrous cycle length (days)	4.85 ± 0.11	4.75 ± 0.11	4.55 ± 0.09	5.20 ± 0.13
Estrous stages (% of cycle)				
Diestrus	33.5	37.5	36.7	42.5
Proestrus	10.5	11.7	10.0	10.8
Estrus	32.3	31.7	31.7	25.3
Metestrus	22.5	19.2	20.8	20.0
Uncertain diagnoses (%)	0.0	0.0	0.8	0.8

[1] Data presented as mean ± standard error. Estrous cycle lengths are not significant by Shirley's test. By multivariate analysis of variance (MANOVA), dosed groups do not differ significantly from controls in cycle length or in the relative length of time spent in the estrous stages.
** Significantly different (P≤0.01) from the control group by Williams' test.

Section A6.4.1
Annex Point A6.4.1
IUCLID: 5.4/03

A6.4.1, Subchronic Oral Toxicity Test in the Dog

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.
If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [X]**

Limited exposure [ ] **Other justification [X]**

Detailed justification:

This data waiver has been constructed using the reviews and expert judgement expressed by [redacted]

Concentrations of copper in body fluids tend to be lower that they are in cells (Table 6.4_1) with the exception of bile (the major route of copper excretion) and cerebrospinal fluid. The data on organ copper contents are generally consistent among vertebrates (Table 6.4_1). Some species-specific exceptions include the dog and sheep, where liver concentrations are higher. Although it has been suggested that the high dog values are due to a high copper liver diet, the dog is also peculiar in terms of its albumin (which is normally involved in delivery of copper to the liver).

Albumin is well known as the most abundant protein in vertebrate blood plasma and interstitial fluids. A rather acid protein (pI 4.7) of 68,000 Da, it is involved in the transport of numerous substances, from tryptophan, fatty acids and bilirubin to drugs and metal ions. These various substrates mostly occupy different positions on the protein. It is also well recognised that albumin binds copper and has a role in copper transport. The interactions of copper with albumin and amino acids (relating to transport) have been reviewed in detail by [redacted]

In contrast to the copper in ceruplasmin, copper on albumin is readily exchangeable with that on other ligands, particularly amino acids that may be present in serum and other body fluids ([redacted] It also binds additional copper atoms, but with lower affinity. In the presence of histidine, which itself forms a tight His_2-Cu complex (K_D 10^{-17} [redacted] His_2-Cu is in equilibrium with albumin-Cu through intermediary, ternary, albumin-Cu-His complex,

Cu-His2 t Cu-His t albumin-Cu-His t albumin-Cu

which may in fact be the prominent *in vivo* species. The dissociation constant for the ternary complex has been measured as being 10-22 M [redacted] Other authors have reported that a ternary complex between albumin, copper and cysteine may also be formed *in vivo* [redacted]

The high-affinity copper binding site is situated at the amino terminus [redacted] and involves primarily the histidine position 3. This histidine is essential for tight binding, a matter that was largely proved by studies in the dog. In this species (and in the pig, [redacted] the crucial histidine has been replaced by tyrosine and the albumin does not have the same high affinity for copper [redacted] It does have several lower affinity sites, however (as do other albumins). [redacted] has proposed that the lower affinity of canine albumin for copper may contribute to the unusually high levels of copper in dog liver. This would occur through more rapid uptake occurring in the liver in the absence of high-affinity albumin binding that would slow down copper uptake in plasma. This is consistent with the data of [redacted] who showed that livers of mongrel dogs had copper concentrations of 82 ± 29 mg/g wet weight (n=10) and when compared to other species, was more than ten times that of most species tested. All other tissues were in the standard range of copper concentrations when compared to other species tested. Dog liver rapidly took up copper administered intravenously, but dogs were unable to tolerate the kinds of repeated doses of copper that can be given to rats. This suggests that albumin in blood functions as a buffer for excess copper in humans and rats that might otherwise cause intracellular organ toxicity, making the dog an unsuitable species for studying the sub-chronic and chronic effects of copper within regulatory studies.

The only limitation to this argument is that the pig and chicken both of which have "normal" liver copper concentrations (Table 6.4_1) also have albumin with no histidine in the third position. This implies that another factor in the dog, such as the ability to excrete excess copper in the bile, is also limiting.

This hypothesis has been investigated by [redacted]

Section A6.4.1
Annex Point A6.4.1
IUCLID: 5.4/03

A6.4.1, Subchronic Oral Toxicity Test in the Dog

[redacted]. They suggested that the high liver values of dogs and sheep were the result of a reduced capacity for biliary copper excretion ([redacted]

Not all mammals are as tolerant of copper as humans, rodents, poultry and pigs, which can chronically tolerate many times their usual daily intakes. In some breeds of sheep, copper accumulates quite readily. Sheep are more sensitive to high copper intakes ([redacted] the same is true for dogs, and for Bedlington terriers in particular [redacted] The latter were thought of as models for Wilson disease. The defect these species (sheep and dogs) appear to have in common with Wilson disease is a reduced capacity for biliary copper excretion. As a result, toxic concentrations of copper accumulate, particularly in the liver.

Therefore, sub-chronic (90-day) and chronic (1 year) studies in the dog can be waived as the dog is an unsuitable animal model for studying copper toxicity in relation to man.

For information and completeness, a study in beagle dogs was conducted in 1972. Groups of 6-8 males and females were given a diet containing 0, 0.012%, 0.06% and 0.24% copper gluconate for 6-12 months (equivalent to approximately 0, 0.42, 2.1 and 8.4 mgCu/kg bw/day). This study was conducted by [redacted] and reported by WHO, 1998. The study included a detailed examination of haematological biochemical and urinalysis parameters, and tissue copper concentrations in kidney, liver and spleen. Detailed necropsy, histopathology and organ weight information was also provided.

No effect on mortality or body weight gain was observed. Physical examinations, haematology, urinalysis and most blood biochemical analysis revealed no effect of the compound except in two of the 12 dogs in the highest dose level which showed elevated levels of serum GPT, however, this was reversible and the elevated levels were considered not toxicologically significant by the WHO task force. No compound related gross or microscopic pathologic lesions or changes in organ weight were observed. At 6 and 12 months, there was a dose-dependent increase in copper level in kidney, liver and spleen. Liver biopsy from 4 animals at 0, 4, and 12 weeks after withdrawal of 12 months dosing (8.4 mg/kg bw/day) showed some reversibility of liver

Section A6.4.1 Annex Point A6.4.1 IUCLID: 5.4/03	A6.4.1, Subchronic Oral Toxicity Test in the Dog
	copper levels. [redacted] and only a summary provided by WHO, 1988 is now available.
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29 Dec.2004
Evaluation of applicant's justification	Agree with applicant's version. Comment: • This is consistent with the data of [redacted] who showed that livers of mongrel dogs had copper concentrations of 82 ~~mg/g~~ **µg/g** wet weight (n=10) and when compared to other species, was more than ten times that of most species tested. • Quoted references aren't submitted in the dossier or included in IUCLID like: [redacted]
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specij)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.4_1. Copper Concentrations in Tissues of Adult Humans and Animals: Major Organs[a]

	Copper Concentration[b] (µg/g)				
Tissue/organ	Human	Rat	Pig	Mouse	Other
Kidney	12 ± 7 (19)	7.9 ± 5.5 (14)	7.3 ± 4.5 (4)	4.4 ± 1.1 (3)	5.8, 7.9 (2) (chick) 6.9, 10 (2) (dog)
Liver	6.2 ± 0.8 (9)	4.6 ± 1.1 (23)	5.2 ± 0.7 (5)	4.1[c], 4.7[d] (2)	3.0, 2.9 (2) (chicken) 67 ± 23 (5) (dog)[e]
Brain	5.2 ± 1.1 (10)	3.1 ± 1.2 (10)	3.9 ± 1.5 (4)	4.0 ± 2.1 (4)	
Heart	4.8 ± 1.9 (14)	4.8[f], 6.2 (2)	4.6 (1)		4.6 (1) (cow)
Bone	4.1 ± 1.3 (8)	2.5 ± 0.6 (3)	1.4, 2.4 (2)		4.4 (1) (sheep)
Stomach	2.2 ± 0.7 (7)		1.6 (1)		
Intestine	1.0, 3.0 (2)	1.7, 2.1 (2)		1.7 (1)	
Spleen	1.5 ± 0.4 (14)	2.3 ± 2.2 (8)	1.4 ± 0.3 (4)	1.2[c], 4.2 (2)	2.3, 4.2 (2) (dog)
Lung	1.3 ± 0.4 (11)	1.8 ± 0.6 (5)	1.2, 1.4 (2)	3.9 (1)	2.6 (1) dog
Blood	1.11 ± 0.13 (5)				
Plasma	1.13 ± 0.15 (70)	1.28 ± 0.26 (12)	1.75 ± 0.43 (11)	0.38[c] (1)	0.42 ± 0.20 (9) (chicken)
Muscle	0.9 ± 0.3 (7)	1.0 ± 0.4 (5)	1.0 ± 0.6 (3)		3.7 (1) (cow) 0.5-0.9 (3) (beef)[g]
Skin	0.8 ± 0.4 (9)	1.7 ± 0.8 (4)	1.0, 1.5 (2)		0.4 (1) (cow)
Adipose	0.2, 0.3 (2)	0.35 (1)	0.8, 0.7 (2)	2.4 (1)	1.2 (1) (whale)

[a] [redacted]

[b] Mean ± SD of reported mean values for number of reports in parentheses.

[c] [redacted]

[e] Based on recent data, [redacted] believes that the high values reflect the high Cu content of dog food, which contains a great deal of liver [redacted] However, other factors, such as albumim, may be involved. See Chapter 4, Section 4.1.2.

[f] [redacted]

Section A6.4.2
Annex Point A6.4.2
IUCLID: 5.4/18

A6.4.2, Subchronic Dermal Toxicity Test

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.
If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [ ]**

Limited exposure [ ] **Other justification [X]**

Detailed justification:

According to the Technical Notes for Guidance on data requirements for active substances and biocidal products, this study is required for active substances that have the following characteristics:

- A percutaneous study is required, where the potential dermal exposure is significant and route-to-route extrapolation is not possible.
- However, a percutaneous study may be necessary where it is justified that dermal route is more appropriate or specific effects of concern are different from the effects seen in the studies in other routes.

This study is usually required when the dermal route of exposure is significant and the compound is known to be toxic by the dermal route and can penetrate through intact skin. The need to conduct this study with either copper (II) oxide or copper carbonate must therefore be questioned as although the dermal route of exposure is the most significant route of exposure in professional wood preservation use, there is no evidence to indicate that either salt can cause toxicity or indeed pass through intact skin. Acute dermal toxicity studies showed no toxic effects up to and including the highest dose tested (See Section 6.1.2). It is also possible to calculate the route-to-route exposure from available oral toxicity studies and using dermal penetration studies (see Section 6.2) as there are no specific effects observed following dermal exposure of both salts in animals. Therefore an accurate and realistic determination of dermal toxicity can be derived from available sub-chronic oral exposure studies, permissible systemic copper levels and *in vitro* dermal penetration studies on copper sulphate and insoluble copper compounds (NTP, 1993; SANCO, 2003; [redacted]).

Section A6.4.2 **Annex Point A6.4.2** **IUCLID: 5.4/18**	**A6.4.2, Subchronic Dermal Toxicity Test**
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29 Dec.2004
Evaluation of applicant's justification	Agree applicant's justification. Comment: Quoted references aren't detailed and not introduced in IUCLID like SANCO 2003 [redacted].
Conclusion	**Acceptable**
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specij)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.4.3
Annex Point A6.4.3
IUCLID: 5.4/19

A6.4.3, Repeated Dose Toxicity, Inhalation

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.
If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [ ]**

Limited exposure [X] **Other justification [X]**

Detailed justification:

According to the Technical Notes for Guidance on data requirements for active substances and biocidal products, these studies are required for active substances that have the following characteristics:

- For volatile substances and gases (vapour pressure > 1 x 10-2 Pa)
- In cases where inhalation exposure is significant, an inhalation study is required instead of an oral study
- In some cases (e.g. aerosols and dusts/particulate matter) studies by the inhalation route should be required in addition to studies by the oral route.

Copper compounds are not volatile substances or gases and in fact have negligible vapour pressures.

Although inhalation exposure could be considered relatively high at a wood preservative treatment plants as calculated from the TnG on human health (Doc IIB), engineering controls significantly reduce or even eliminate plant operator exposure to the product. X

A plant treating 15,000 m3 wood per year would emit 0.0003kg = 0.3 mg copper per year into the air from the vacuum system. Because this figure is so low, emissions from the plant vacuum system are considered to be insignificant.

After the treatment cycle is finished and the cylinder door is opened there may be a few seconds of aerosol emission from the vessel and are quickly dispersed. This is of a transient nature and vessels and operating procedures do not result in the generation of aerosols. Measurements made in the NIOSH Technical Report, 1983 show copper levels of <1.5µg Cu / m3 air (i.e. <LOD) when sampled adjacent to the cylinder door opening. Similar levels are recorded

Section A6.4.3 Annex Point A6.4.3 IUCLID: 5.4/19	A6.4.3, Repeated Dose Toxicity, Inhalation
	elsewhere. This emission route is considered insignificant for the purposes of this environmental exposure assessment. Connell and Hughes (1998) also showed there was no elevation in atmospheric copper levels as the door of a treatment vessel opened when using copper azole wood preservative. These engineering controls would be in place because of the existence of a long-established Operator Exposure Limit for copper dust (inhalation) in many European Countries. Some of these are presented below: DE-MAK 1 mg/m^3 (total dust) SE-LEVL 1 mg/m^3 (as Cu, total dust); 0.2 mg/m^3 (as Cu, respirable dust) UK-LTEL 1 mg/m^3 (as Cu, dusts and mists) UK-STEL 2 mg/m^3 (as Cu, dusts and mists) Due to the established operator exposure limits and the monitoring data above, it would appear unnecessary to conduct new animal studies when established levels have been in place in the workplace for many years. No significant inhalation exposure will occur to passer-by at the treatment plant or the general public through use of treated timber.
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29 Dec.2004
Evaluation of applicant's justification	Agree with applicant's justification.
Conclusion	Acceptable.
Remarks	

Section A6.4.3 A6.4.3, Repeated Dose Toxicity, Inhalation

Annex Point A6.4.3
IUCLID: 5.4/19

COMMENTS FROM OTHER MEMBER STATE *(specij)*

Date *Give date of comments submitted*

Evaluation of applicant's justification *Discuss if deviating from view of rapporteur member state*

Conclusion *Discuss if deviating from view of rapporteur member state*

Remarks

Sections A6.5 & A6.7	**Combined Chronic toxicity/Carcinogenicity**
Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate*
IUCLID 5.4/11 & 5.7/01	**A6.5(01) & A6.7(01), Combined Chronic toxicity/Carcinogenicity of copper**

		Official use only
	182 REFERENCE	
1.1 Reference	*Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages) If necessary, copy field and enter other reference(s).* [redacted]	
1.2 Data protection	No *(indicate if data protection is claimed)*	
1.2.1 Data owner	*Give name of company* Public domain.	
182.1.1 Companies with letter of access	*Give name of company/companies which have the right to use these data on behalf of the data owner (see TNsG in support of AnnexVI)* Letter of access not required.	
182.1.2 Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* No data protection claimed.	
	183 GUIDELINES AND QUALITY ASSURANCE	
183.1 Guideline study	No. This was a non-regulatory study to determine whether a high level of Cu would have an inhibitory effect on the induction of neoplasia by acetylaminofluorene (AAF) or dimethylnitrosamine (DMN) and to determine whether the incidence of neoplasia would be increased or whether neoplasms would appear earlier in rats fed a diet low in Cu. *(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*	
183.2 GLP	No. This was a non-regulatory study. Furthermore, GLP was not compulsory at the time the study was performed. *(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)*	
183.3 Deviations	Yes. Refer to section 5.3.6 for a general discussion of deviations and deficiencies. *(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")*	X
	184 MATERIALS AND METHODS	
	In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.	

Sections A6.5 & A6.7	Combined Chronic toxicity/Carcinogenicity	
Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate*	
IUCLID 5.4/11 & 5.7/01	**A6.5(01) & A6.7(01), Combined Chronic toxicity/Carcinogenicity of copper**	
184.1 Test material	Cu^{2+} as copper sulphate ($CuSO_4$) Acetylaminofluorene (AAF) Dimethylnitrosamine (DMN) *or give name used in study report*	
184.1.1 Lot/Batch number	Not stated. *List lot/batch number if available*	
184.1.2 Specification	Deviating from specification given in section 2 as follows *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):*	
184.1.3 Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Refer to section 2.1.	
184.1.4 Purity	*Give purity in % active substance* Not stated.	
184.1.5 Stability	*Describe stability of test material* Not stated.	X
184.2 Test Animals	Non-entry field	
184.2.1 Species	Rat	
184.2.2 Strain	Sprague-Dawley	
184.2.3 Source	An un-named commercial supplier.	
184.2.4 Sex	Male.	
184.2.5 Age/weight at study initiation		
184.2.6 Number of animals per group	Not stated.	

Diet	Other treatment	No. of rats
Copper-deficient (1 ppm Cu).	Control	50
	DMN	74
	AAF	55
Excess copper (800 ppm Cu).	Control	58
	DMN	102
	AAF	65

Sections A6.5 & A6.7	**Combined Chronic toxicity/Carcinogenicity**	
Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate*	
IUCLID 5.4/11 & 5.7/01	**A6.5(01) & A6.7(01), Combined Chronic toxicity/Carcinogenicity of copper**	
184.2.6.1 at interim sacrifice	After 90 days of feeding, 5 rats from each diet group were killed. Thereafter, each 30 days and additional 5 animals from each group were killed.	
184.2.6.2 at terminal sacrifice	All surviving animals were killed at study termination.	
184.2.7 Control animals	Refer to section 2.2.6. Control animals received the basal diet containing the appropriate amount of $CuSO_4$.	X
184.3 Administration/ Exposure	Oral in the diet. *Fill in respective route in the following, delete other routes*	
184.3.1 Duration of treatment	9 months.	
184.3.2 Interim sacrifice(s)	Refer to section 2.2.6.1.	X
184.3.3 Final sacrifice	Refer to section 2.2.6.2.	X
184.3.4 Frequency of exposure	7 days a week.	
184.3.5 Postexposure period	None.	
	Oral	
184.3.6 Type	$CuSO_4$ was administered in the diet. DMN was administered in the drinking water. AAF was administered in the diet.	
184.3.7 Concentration	The purified basal diet (Cu-deficient) contained 1 ppm Cu. The excess Cu diet contained 800 ppm Cu as $CuSO_4$. DMN was added to drinking water at a concentration of 50 ppm. AAF was added to the diets at a concentration of 0.06%.	
184.3.8 Vehicle	Basal diet.	
184.3.9 Concentration in vehicle	Refer to section 3.3.7.	
184.3.10 Total volume applied	Not applicable.	
184.3.11 Controls	Controls received either basal diet only (Cu deficient) or 800 ppm Cu as $CuSO_4$.	
184.4 Examinations		
184.4.2 Body weight	Yes.	
184.4.3 Food consumption	No. 184.4.4	
Water consumption	No.	
184.4.5 Clinical signs	Yes.	
184.4.6 Macroscopic investigations	Yes.	

Sections A6.5 & A6.7	**Combined Chronic toxicity/Carcinogenicity**
Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate*
IUCLID 5.4/11 & 5.7/01	**A6.5(01) & A6.7(01), Combined Chronic toxicity/Carcinogenicity of copper**

184.4.7 Ophthalmoscopic examination	No.	
184.4.8 Haematology	No.	
184.4.9 Clinical Chemistry	No.	
184.4.10 Urinalysis	No.	
184.4.11 Pathology	Yes.	
184.4.11.1 Organ Weights	Yes	
	from:	at interim sacrifice (3, 5 and 7 months), at terminal sacrifice.
	Organs:	Liver; enlarged neoplastic kidneys.
	Other:	None.
184.4.11.2 Histopathology	Yes	
	From:	All dose groups
	From:	at interim sacrifice (at 90 days and each 30 days thereafter), at terminal sacrifice.
	Organs:	Spleen, kidneys, lungs, heart, thyroid gland, adrenal gland, duodenum and pancreas.
	Other:	None.
184.4.12 Other examinations	*E.g. enzyme induction, cell proliferation, reversibility of effects*	
	Concentrations of Cu in non-neoplastic and neoplastic hepatic and renal tissues were determined.	
184.5 Statistics	Simple statistical methods were applied, as appropriate.	
184.6 Further remarks	Liver and kidney Cu concentrations were determined by atomic absorption spectrophotometry. Copper analyses were carried out on 5 g pooled samples of liver. The analyses were run in triplicate and precautions were taken to prevent Cu-contamination of the tissues.	

185 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

185.1 Results	*No effects / describe significant effects referring to data in results table* Non-entry field.
185.1.2 Body weight	Mean bodyweight data are summarised in **Figure A6.5(01) & A6.7(01)-1**. Rats fed the Cu-deficient control diet had the highest mean bodyweights. The mean weights of the Cu-deficient-DMN group were well below those of Cu-deficient control rats. The excess-Cu control and excess-Cu DMN groups had similar mean weights, approximately 120 g below the mean weight of the Cu-deficient control group after 6 months. AAF was markedly toxic and the mean weights of rats fed either of the AAF diets were markedly below those of the Cu-deficient

control, with the excess-Cu-AAF having the lowest mean weights of the various groups.

185.1.3 Mortality

After 3 months, the mortality rate in the six groups varied from 2% to 69% (**Table A6.5(01) & A6.7(01)-1**). Deaths were lowest in the Cudeficient control and greatest in the excess-Cu-DMN group. These data indicate that excess Cu, DMN and AAF were all toxic and increased the number of deaths over that in the Cu-deficient control group. At the termination of the experiment, the lowest mortality (16%) was observed in the Cu-deficient control group and the highest in the excess-CuDMN group. This group, which had the highest mortality during the first 3 months (69%) had the fewest deaths later.

185.1.4 Organ weights

Liver weights expressed as a percentage of body weight were similar for all groups except those receiving AAF (**Table A6.5(01) & A6.7(01)-2**). Of these, Cu-deficient-AAF animals generally had higher liver weights than those in the excess-Cu-AAF group. Some of this increase in size was due to the presence of neoplasms.

185.1.5 Copper determinations

The Cu content of grossly non-neoplastic hepatic tissue from rats fed Cu-deficient diets did not vary greatly, although values for the group fed AAF were generally lower than for other groups (**Table A6.5(01) & A6.7(01)-3**). The Cu content of livers from the rats fed excess-Cu diets with carcinogens was greater than that found in the excess-Cu control rats.

The Cu content of neoplastic hepatic tissue from rats receiving Cu-deficient carcinogenic diets was similar to that in grossly normal tissue (**Table A6.5(01) & A6.7(01)-4**). In the two groups of rats which were fed the excess-Cu-AAF diet and had grossly separable neoplasms, the neoplastic tissue contained less Cu than the non-neoplastic tissue from the same animal.

Renal neoplasms were observed grossly only in the Cu-deficient-DMN rats and the concentrations of Cu were lower in these neoplasms than in the non-neoplastic renal tissue. The Cu concentration of this latter tissue was somewhat lower than that found in the kidneys of the Cudeficient control rats (**Table A6.5(01) & A6.7(01)-5**). The Cu concentration of large neoplasms was lower than that found in small neoplasms (under 22 g).

185.1.6 Macroscopic investigations

Liver: Livers of rats fed the Cu-deficient-DMN diet for 3 or 4 months varied in appearance from those that were grossly normal to those with severe macroscopic changes and fusion of lobes. Some were tan-coloured and slightly swollen. Abnormal features of livers from rats fed this diet for 5-8 months varied in severity and included: swelling, colour variation, presence of clear cysts, haematocysts and/or neoplasms.

Grossly visible lesions of the livers were observed at the monthly samplings in Cu-deficient-AAF rats. Abnormalities observed after 3 months included discoloration, enlargement and presence of focal pale areas. After 4 months, a few clear cysts were also present. Later, livers

Sections A6.5 & A6.7	**Combined Chronic toxicity/Carcinogenicity**
Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate*
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were pale, cystic and markedly enlarged, and neoplasms ranging in size from pin-point nodules to 3 cm in diameter were observed in all lobes.

Livers from Cu-deficient and excess-Cu control rats were grossly normal. Livers from excess-Cu-DMN rats were either normal or slightly off-colour after 3 and 4 months. Few further changes were observed after 5 and 6 months, except for prominent capsular vessels. Cysts, swollen lobes and haematocysts occurred in livers of rats fed for 7 months. Livers from the 4 rats killed after 8 months were more severely affected; haematocysts were observed in 2 livers and a neoplasm was found in one other.

Livers of excess-Cu-AAF rats had a striking appearance in one rat at month 3 that was consistently present in one or more livers at the other autopsy periods; the hepatic surface was converted into a mass of small nodules. This was more marked on the visceral surface. In addition, clear cysts were present peripherally after 5 months. Increased hepatic size, cysts and small white foci also appeared after 6 months. Neoplasms were larger after 7 months, and all livers from rats fed for 8 months had clear cysts, neoplasms and capsular nodularity, although there still was some variation in the severity of gross alterations.

Kidney: Grossly enlarged kidneys with neoplasms were seen after 5 months in Cu-deficient-DMN rats. The kidneys of 4 of the 5 rats had neoplasms of various sizes. After 6 months, neoplasms were present in all 5 rats. Grossly apparent neoplasms were present in 3 of the 5 rats examined after 7 months. Only one renal neoplasm was obvious at autopsy in 5 rats killed after 8 months. Three of the 13 rats on this treatment which died during the study had grossly apparent renal neoplasms.

Other organs: Abnormalities other than alterations of the liver and kidneys observed at autopsy in Cu-deficient-DMN rats included pale, expanding masses in the lungs of 2 rats. Grossly detectable neoplasms were observed in the lungs of excess-Cu-DMN rats after 7 and 8 months.

Neoplasms at locations other than the liver were most numerous in Cu-deficient-AAF rats. After 5 months, 3 rats had grossly obvious neoplasms in one or more of the following locations: ventral throat area, middle of side, groin area and base of ear. After 6 months, neoplasms were noted in the lungs of 2 rats and in the spleen of another. At month 7, neoplasms were present in the ventral thorax, spleen, abdomen, perianal region, base of ear, right rear leg and small intestine.

Fewer extrahepatic neoplasms were found in excess-Cu-AAF rats. Those that occurred werelocated at the base of the ear, along the lateral abdomen and in the lungs.

No gross abnormalities were observed in the urinary bladder of animals in any group.

185.1.7 Histopathology

Liver: Commonly occurring non-neoplastic lesions in the livers of carcinogen-treated rats included biliary-ductule cell hyperplasia, proliferation of biliary ducts and the presence of haematocysts. Many

of the proliferated biliary ducts were dilated and some were markedly enlarged, accounting for the clear cysts noted at autopsy. The cystic ducts had an epithelial lining of simple squamous to low cuboidal cells and when multiple were separated by a fine connective-tissue stroma.

The incidence rates of hepatic neoplasms and other lesions observed in the rats fed the various diets are summarized in **Table A6.6Ø-6**. Lesions listed as transitional nodules were localized groups of hepatocytes differing in staining intensity from the surrounding parenchyma but showing only minimal deviation of nuclear morphology and no compression of the surrounding parenchyma, those classed as hepatomas were larger foci of hepatocytes showing changes in nuclear morphology and causing compression of the surrounding parenchyma, and the hepatocellular carcinomas were large, highly cellular neoplasms showing marked alterations in nuclear and cytoplasmic morphology, containing areas of necrosis and blood cysts and invading blood and lymph vessels. In addition to hepatomas and hepatocellular carcinomas, a fibrosarcoma and cholangiocarcinoma of the liver were observed in rats of the Cu-deficient-DMN group. Hepatomas, hepatocellular carcinomas, cholangiomas and one cholangiocarcinoma were observed in the livers of rats fed the Cudeficient-AAF diet. Lung metastases of hepatocellular carcinomas were observed in the AAF-fed groups. The Cu level of the diet appeared to have little or no effect on the incidence rate of hepatic neoplasms.

Kidney: Fibrosarcomas, adenomas and adenocarcinomas were observed in kidneys of Cu-deficient-DMN rats. Emboli of tumour cells from a renal fibrosarcoma were observed in the lung. One fibrosarcoma was found in a kidney from a rat fed the Cu-deficient control diet. No renal neoplasms were observed either grossly or microscopically in the rats from other groups killed for autopsy. One renal adenoma was observed in a rat that died after 7 months on the excess-Cu-DMN treatment.

Other organs: Neoplasms in locations other than liver and kidneys included those of the lung, spleen, skin and -intestine. The neoplasms observed included adnexal gland adenocarcinomas, keratoacanthomas, splenic lymphoma, alveolar-cell adenomas and adenocarcinomas, adenocarcinoma arising from the epithelium of the intestinal mucosa, squamous cell carcinomas of the skin and lungs, fibrosarcoma of the dermis and a rhabdomyosarcoma. Incidences of these neoplasms were less in rats receiving excess Cu and a carcinogen (**Table A6.5(01) & A6.7(01)-6**).

185.2 Discussion

No effects / describe significant effects referring to data in results table

Body weight & mortality: The Cu-deficient diet (1 ppm) was adequate to sustain normal growth. However, the excess-Cu diet was toxic.

DMN was toxic at the level given, mean body weights being reduced and mortality increased compared with the Cu-deficient control group. The combination of carcinogen and excess Cu appeared not to be additive, although total mortality was slightly greater in the excess-Cu

group. The mortality rate after 3 months was less in both the DMN groups and it appeared that rats surviving the early toxic effects developed some tolerance.

AAF produced a greater reduction in weight than DMN. The administration of AAF and excess Cu had an additive toxic effect, as this diet markedly decreased body-weight gains, producing the lowest means in the experimental groups.

Mortality over the experimental period did not vary greatly between the Cu-deficient-AAF and the excess-Cu-AAF groups. This illustrates one of the differences in the response to the two carcinogens. Excess Cu in the diet markedly increased the mortality rate after DMN administration but appeared to have little effect when AAF was fed.

Organ weights: Enlargement of the liver was restricted, with few exceptions, to the AAF-fed rats. DMN administration slightly increased the mean liver weight expressed as a percentage of body weight. Livers from AAF-fed rats were generally enlarged, some greatly. The rats receiving AAF had the lowest body weights and little body fat. These factors, combined with the presence of many cysts and neoplasms, account for the high liver-weight values observed in the AAF-fed groups.

Cu determinations: The Cu content of livers from rats fed the Cudeficient diets did not vary greatly. However, the hepatic Cu concentration of excess-Cu control rats was less than the mean concentrations for the two carcinogen-treated groups. Thus, the carcinogens appeared to increase the retention of Cu by the liver in animals receiving the excess Cu diet. The Cu levels of non-neoplastic and grossly neoplastic hepatic tissue from rats fed the Cu-deficient diet and treated with DMN or AAF were similar, but the Cu content of nonneoplastic hepatic tissue from rats fed the excess-Cu diet with AAF was greater than that of neoplastic tissue.

The Cu content of the renal tissues decreased in the following order: normal tissue in Cu-deficient control rats, non-neoplastic tissue in Cudeficient-DMN rats, DMNinduced small neoplasms and DMN-induced large neoplasms. Part of the lower Cu content may be due to the tissue composition of the neoplasms; fibrosarcomas are composed of connective tissue known to have a low Cu content.

Macroscopic and microscopic investigations: The incidence of hepatic neoplasms in DMN-treated rats was similar for the Cu-deficient and excess-Cu groups. DMN-induced renal neoplasms were found to originate from the epithelium of the renal tubules and the connective tissue of the interstitium. Those originating in the interstitial cells were classified as fibrosarcomas on the basis of morphology and staining reactions. Seventeen rats killed for autopsy had one or more neoplasms, including 12 fibrosarcomas, seven adenomas and two adenocarcinomas.

Organs with neoplasms induced by AAF included the liver, spleen, lung, skin, muscle, pancreas and intestine, although neoplasms were uncommon in the last three organs. The numbers of hepatic neoplasms

in AAF-treated rats on the Cu-deficient and excess-Cu diets were similar and it appeared that the concentration of Cu had no effect upon the incidence of hepatic neoplasms. However, the latency period may have been slightly increased, as hepatocellular carcinomas and metastases occurred 1 month later in the excess-Cu group.

The incidence of extrahepatic neoplasms in rats killed for autopsy was 40% in the Cu-deficient-AAF group, but only 17% of the rats fed the excess-Cu-AAF diet had neoplasms outside the liver. When the extrahepatic tumours from rats found dead after receiving the AAF treatment for at least 3 months are combined with those from rats killed for autopsy, the difference in incidence of neoplasms between Cu-deficient and excess-Cu groups was decreased (31% vs. 23%) but the data suggest that the Cu supplement acted to reduce the number of extrahepatic neoplasms.

185.3 Time to tumours *For dermal route and skin tumours: give mean time until appearance of tumour or time until appearance of first tumour or other measure*

Tumours were evident in rats treated with the carcinogens AAF and DMN from the time of the first interim sacrifice at 3 months.

185.4 Other *Describe any other significant effects*

None.

186 APPLICANT'S SUMMARY AND CONCLUSION

186.1 Materials and methods *Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines*

A study was carried out to determine whether a high level of Cu would have an inhibitory effect on the induction of neoplasia by acetylaminofluorene (AAF) or dimethylnitrosamine (DMN) and to determine whether the incidence of neoplasia would be increased, or whether neoplasms would appear earlier in rats fed a diet low in Cu.

Six experimental groups of Sprague-Dawley rats were included in this study. Three groups were fed a basal diet containing 1 ppm Cu ("Cu-deficient diet") and a further 3 groups received the basal diet supplemented with $CuSO_4$ to give a Cu concentration of 800 ppm ("excess-Cu diet"). Within each of these two dietary regimens, one group received DMN in the drinking water and the other received AAF in the diet. Groups without these carcinogens served as controls. The initial number of animals used in each group was as follows: Cu-deficient control, 50 rats; Cu-deficient-DMN, 74 rats; Cu-deficientAAF, 55 rats; excess-Cu-control, 58 rats; excess-Cu-DMN, 102 rats; excess-Cu-AAF, 65 rats. The numbers in each group varied because preliminary studies showed that higher DMN concentrations were toxic.

DMN was added to the drinking water for 6 months at a concentration of 50 ppm for 4 days out of every 8. Similarly, AAF was added to the diets for 6 months at a concentration of 0.06% for 4 days out of every 8.

After 90 days, 5 rats from each diet group were killed. Each 30 days thereafter, an additional 5 animals from each group were killed. Spleen,

kidneys, lungs, heart, thyroid gland, adrenals, duodenum and pancreas were taken from each animal and fixed in 10% formalin. The liver was divided into 2 portions; one of which was retained for analysis of Cu content; the other was fixed in formalin. Liver and enlarged neoplastic kidneys were weighed prior to fixation. Fixed tissues were processed, sectioned and stained with H&E for histological examination.

Liver and kidney Cu concentrations were determined by atomic absorption spectrophotometry. The analyses were run in triplicate and precautions were taken to prevent Cu-contamination of the tissues.

186.2 Results and discussion

Summarize relevant results; discuss dose-response relationship.

Growth response: Rats fed the Cu-deficient control diet consistently had the highest mean bodyweights. Mean weights of other groups decreased in the following sequence: Cu-deficient-DMN; excess-Cu control and excess-Cu DMN had similar mean weights; Cu-deficient AAF; excess-Cu-AAF. AAF was considered to be markedly toxic.

Mortality: After 3 months, mortality in the 6 groups was as follows: Cu-deficient control, 2%; Cu-deficient-DMN, 38%; Cu-deficient-AAF, 15%; excess-Cu control, 33%; excess-Cu-DMN, 69%; excess-Cu-AAF, 39%. At study termination, the lowest mortality (16%) was seen in Cu-deficient controls and the highest in the excess-Cu-DMN group (57%). Excess Cu, DMN and AAF were all toxic at the levels administered. Excess Cu in the diet markedly increased the mortality rate after DMN administration but appeared to have little effect when AAF was fed.

Organ weights: Liver weights expressed as percentage of body weight were similar for all groups except those receiving AAF, for which elevated values were obtained. Of these, Cu-deficient-AAF animals generally had higher liver weights than those in the excess-Cu-AAF group. Some of this increase was due to the presence of neoplasms.

Copper determinations:

Liver: The Cu content of non-neoplastic hepatic tissue from rats fed Cu-deficient diets did not vary greatly, although values for the group fed AAF were generally lower than for the other groups (mean Cu contents at study termination were 4.5, 3.9 and 2.8 ppm for the control, DMN and AAF groups, respectively). The Cu content of livers from rats fed excess-Cu diets with DMN and AAF was, however, greater than that found in the excess-Cu control rats (mean Cu contents at study termination were 244, 394 and 354 ppm for the control, DMN and AAF groups, respectively). The carcinogens therefore appeared to increase retention of Cu by the liver in animals receiving the excess-Cu diet.

The Cu content of neoplastic hepatic tissue from rats receiving Cu-deficient carcinogenic diets was similar to that in grossly normal tissue. In rats fed the excess-Cu-AAF diet and that had grossly separable neoplasms, neoplastic tissue contained less Cu (347 and 163 ppm at 5 and 8 months, respectively) than non-neoplastic tissue (418 and 294 ppm, respectively) from the same animal.

Kidney: Gross renal neoplasms were observed only in the Cu-deficient-DMN rats and the concentrations of Cu were lower in these neoplasms

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Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate*
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than in non-neoplastic tissue. The Cu concentration of this latter tissue was somewhat lower than that in the kidneys of Cu-deficient control rats. The Cu concentration of large neoplasms was lower than that of small neoplasms. This was attributed in part to the composition of neoplasms containing connective tissue known to have low Cu content.

Macroscopic investigations:

Liver: Livers from control rats fed both Cu-deficient and excess-Cu diets were grossly normal.

The incidence of hepatic neoplasms in DMN-treated rats was similar for the Cu-deficient and excess-Cu groups. Livers of rats fed the Cu-deficient-DMN diet for 3 or 4 months varied in appearance from those that were grossly normal to those with severe macroscopic changes. Some were tan-coloured and slightly swollen. Features of livers from rats fed this diet for 5-8 months included: swelling, colour variation, presence of clear cysts, haematocysts and/or neoplasms. Livers from excess-Cu-DMN rats were either normal or slightly off-colour after 3 and 4 months. Few further changes were observed after 5 and 6 months, except for prominent capsular vessels. Cysts, swollen lobes and haematocysts occurred in livers of rats fed for 7 months. Livers from 4 rats killed after 8 months were more severely affected; haematocysts were observed in 2 livers and a neoplasm in one other.

Gross hepatic lesions were observed at monthly samplings in Cu-deficient-AAF rats. At 3 months, these included discoloration, enlargement and presence of focal pale areas. After 4 months, a few clear cysts were also present. Later, livers were pale, cystic and enlarged. Neoplasms of varying size were found in all lobes. At 3 months, the surface of the liver of one rat fed the excess-Cu-AAF diet was converted into a mass of nodules. This was also seen in one or more livers at the other autopsy periods, and was more marked on the visceral surface. Clear cysts were also present peripherally after 5 months. Increased hepatic size, cysts and small white foci appeared after 6 months. Neoplasms were larger after 7 months, and all livers at 8 months had clear cysts, neoplasms and capsular nodularity.

The numbers of hepatic neoplasms in AAF-treated rats on the Cu-deficient and excess-Cu diets were similar and it appeared that the concentration of Cu had no effect upon the incidence of hepatic neoplasms. However, the latency period may have been slightly increased, as hepatocellular carcinomas and metastases occurred 1 month later in the excess-Cu group.

Kidney: Grossly enlarged kidneys with neoplasms were seen after 5 months in Cu-deficient-DMN rats. The kidneys of 4/5 rats had neoplasms of various sizes. After 6 months, neoplasms were present in all 5 rats. Grossly apparent neoplasms were present in 3/5 rats examined after 7 months. Only one renal neoplasm was obvious at autopsy in 5 rats killed after 8 months. 3/13 rats on this treatment which died during the study had grossly apparent renal neoplasms.

Other organs: Abnormalities observed at autopsy in Cu-deficient-

Sections A6.5 & A6.7	Combined Chronic toxicity/Carcinogenicity
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DMN rats included pale, expanding masses in the lungs of 2 rats. Grossly detectable neoplasms were observed in the lungs of excess-CuDMN rats after 7 and 8 months.

Neoplasms at locations other than the liver were most numerous in Cudeficient-AAF rats. After 5 months, 3 rats had grossly obvious neoplasms in one or more of the following locations: ventral throat area, middle of side, groin area and base of ear. After 6 months, neoplasms were noted in the lungs of 2 rats and in the spleen of another. At month 7, neoplasms were present in the ventral thorax, spleen, abdomen, perianal region, base of ear, right rear leg and small intestine.

Fewer extrahepatic neoplasms were found in excess-Cu-AAF rats (17% compared with 40% in the excess-Cu-AAF). Those that occurred werelocated at the base of the ear, along the lateral abdomen and in the lungs. It was considered that the Cu supplement acted to reduce the number of extrahepatic neoplasms.

No gross abnormalities were observed in the urinary bladder of animals in any group.

Histopathology:

Liver: Commonly occurring non-neoplastic lesions in the livers of carcinogen-treated rats included biliary-ductule cell hyperplasia, proliferation of biliary ducts and the presence of haematocysts.

Transitional nodules were localized groups of hepatocytes showing only minimal deviation of nuclear morphology and no compression of the surrounding parenchyma. Hepatomas were larger foci of hepatocytes showing changes in nuclear morphology and causing compression of the surrounding parenchyma. Hepatocellular carcinomas were large, highly cellular neoplasms showing marked alterations in nuclear and cytoplasmic morphology, containing areas of necrosis and blood cysts and invading blood and lymph vessels. In addition to hepatomas and hepatocellular carcinomas, a fibrosarcoma and cholangiocarcinoma were observed in Cu-deficient-DMN rats. Hepatomas, hepatocellular carcinomas, cholangiomas and one cholangiocarcinoma were observed in livers of Cu-deficient-AAF rats. The Cu level of the diet appeared to have no effect on the incidence rate of hepatic neoplasms.

Kidney: Fibrosarcomas, adenomas and adenocarcinomas were seen in kidneys of Cu-deficient-DMN rats. One fibrosarcoma was found in a kidney from a rat fed the Cu-deficient control diet. No renal neoplasms were observed either grossly or microscopically in the rats from other groups killed for autopsy. One renal adenoma was observed in a rat that died after 7 months on the excess-Cu-DMN treatment.

Other organs: Neoplasms in locations other than liver and kidneys included those of the lung, spleen, skin and -intestine. The neoplasms observed included adnexal gland adenocarcinomas, keratoacanthomas, splenic lymphoma, alveolar-cell adenomas and adenocarcinomas, adenocarcinoma arising from the epithelium of the intestinal mucosa, squamous cell carcinomas of the skin and lungs, fibrosarcoma of the dermis and a rhabdomyosarcoma. The incidences of these neoplasms

Sections A6.5 & A6.7	Combined Chronic toxicity/Carcinogenicity
Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate*
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	were less in rats receiving excess Cu and a carcinogen.
186.3 Conclusion	Microscopic examination of tissue samples confirmed the following: *Liver:* livers from excess-Cu control rats confirmed the occurrence of liver necrosis and transitional nodules in 3/32 and 1/32 animals, respectively. Neither of these lesions was found in the livers of animals fed a Cu-deficient diet. Exposure to DMN and AAF increased the incidence of liver necrosis and transitional nodules, and each induced a similar incidence of liver tumours in rats fed both the Cu-deficient and excess-Cu diets. It was concluded that the Cu level of the diet had no effect on the incidence of hepatic neoplasms. *Kidney:* In the DMN group, 17/30 rats on the Cu-deficient diet had kidney tumours compared with 0/29 given excess Cu. There were no kidney tumours in the AAF-treated groups. *Other organs:* The incidence of AAF-induced extra-hepatic tumours was apparently reduced by the excess-Cu diet (5/30, compared with 11/27 in the Cu-deficient group).
186.3.2 Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4* 2
186.3.3 Deficiencies	Yes This study was not conducted and/or reported in strict compliance with the principles of GLP. There were also a number of deficiencies in the methodology used, when compared with the requirements of currently accepted guidelines for the conduct of carcinogenicity studies (e.g. OECD 451), including the following: • The test substance was not characterised in detail; • Only males were used, rather than both sexes; • Only two $CuSO_4$ test concentrations were used; • No blood sampling was reported for adversely affected animals; • The range of tissues examined macroscopically was limited; • The range of organ weights reported was limited; • The range of organs examined microscopically was limited; • The duration of the study was 9 months (24 months recommended). However, these deficiencies do not necessarily compromise the validity of the data generated, or the author's interpretation of that data, given that the study was not carried out for regulatory purposes. Furthermore, the research was published in a peer-reviewed journal, and has therefore been subject to the prior scrutiny of experts in the field. It has also been referred to in reviews of the carcinogenicity of copper. Overall, this is an adequately-reported study, and its findings are considered to make a valuable contribution to the 'weight of evidence' approach that has been adopted for the purposes of the current review of

Sections A6.5 & A6.7	Combined Chronic toxicity/Carcinogenicity
Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate*
IUCLID 5.4/11 & 5.7/01	**A6.5(01) & A6.7(01), Combined Chronic toxicity/Carcinogenicity of copper** copper carcinogenicity. A reliability indicator of 2 has been assigned on this basis. *(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 Dec.2004
Guidelines and quality assurance	In paragraph Deviations (2.3): Refer to section 5.3.6 **5.3.3.**
Materials and Methods	Agree with applicant's version. **Comments:** • The purity and impurities of the active substance were not reported (3.1.4), so we cannot compare with notified substance. • Control animals (3.2.7): Refer to section 2.2.6. **3.2.6**. Control animals received the basal diet containing the appropriate amount of $CuSO_4$ **(1ppm copper).** • Interim sacrifice(s) (3.3.2): Refer to section 2.2.6.1. **3.2.6.1.** • Final sacrifice (3.3.3): Refer to section 2.2.6.2. **3.2.6.2.**
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version. Furthermore, there are an important number of deficiencies in methodology used in this publication, when compared with requirements of currently accepted guidelines for the conduct of combined chronic toxicity/carcinogenicity studies (e.g. OECD 453). Besides those noted in the paragraph 5.3.3, it also misses the weight and age of the individual rats, a control satellite group, urinalysis, clinical chemistry as well as neurological and ocular clinical signs.
Reliability	2
Acceptability	Not acceptable. Not really a key study. Could be summarized in the waiving proposal for cancerogenicity.
Remarks	Error in table was corrected in red and bold.

Table 3. Table A6.5(01) & A6.7(01)-3. Copper content of non-neoplastic liver tissue from rats fed copper-deficient and excess-copper diets with DMN or AAF treatment for 3-8 months.

Duration of treatment (months)	Diet...	Copper content (ppm)*					
		Copper-deficient			Excess-copper		
	Other treatment...	Control	DMN	AAF	Control	DMN	AAF
3		4.6	3.2	3.1	314	380	412
4		4.2	3.5	2.9	234	460	372
5		4.3	4.5	2.0	236	432	418
6		4.1	4.4	2.2	200	270	312
7		5.0	4.2	2.6	236	383	314
8		4.8	3.8	3.9	---	438	294
Mean		4.5	3.9	2.8	244	394	354

DMN – 50 ppm dimethylnitrosamine in drinking-water for 4-day periods alternating with distilled water. AAF – 0.06% acetylaminofluorene in the diet for 4-day periods alternating with control diet. * One pooled sample was analysed for each group at each time.

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	

Table A6.5(01) & A6.7(01)-1. Mortality in groups of rats fed copper-deficient or excess-copper diets with DMN or AAF treatment for 3-9 months.

Mortality (%)							
	Diet	Copper-deficient			Excess-copper		
Duration of treatment (months)	OTHER TREATMENT...	Control	DMN	AAF	Control	DMN	AAF
	No of rats*.....	50	74	55	58	102	65
3		2	38	15	33	69	39
4		2	41	15	40	71	39
5		8	45	20	43	71	40
6		10	49	35	43	71	40
7		16	53	48	45	72	43
8		16	53	51	45	72	51
9		16	57	--	45	--	54
	Change in % mortality over 6-month period	14	19	36	12	3	15

DMN – 50 ppm dimethylnitrosamine in drinking-water for 4-day periods alternating with distilled water. AAF – 0.06% acetylaminofluorene in the diet for 4-day periods alternating with control diet. * Total no. of rats started on diet.

Table A6.5(01) & A6.7(01)-2. Liver weight of rats fed copper-deficient or excess-copper diet with DMN or AAF treatment and killed for autopsy after treatment for 3-9 months.

Mean liver weight (% of body weight)*			
Experimental group	Month 3	5	7
Copper-deficient diet:			
Control	3.2 (3.0-3.6)	2.9 (2.7-3.0)	3.1 (2.8-3.4)
+ DMN	3.6 (3.2-4.2)	3.6 (1.3-3.8)	3.2 (2.7-3.1)
+ AAF	6.3 (4.8-8.5)	16.2 (13.4-18.8)**	12.8 (9.4-16.1)**
Excess-copper diet:			
Control	4.0 (3.5-4.9)	3.3 (2.9-3.8)	3.3 (3.0-3.5)
+ DMN	4.1 (2.9-4.7)	3.7 (2.7-5.1)	3.7 (3.1-4.3
+ AAF	5.9 (5.2-7.0)	6.4 (5.5-7.2)**	9.3 (6.4-12.1)**

DMN – 50 ppm dimethylnitrosamine in drinking-water for 4-day periods alternating with distilled water. AAF – 0.06% acetylaminofluorene in the diet for 4-day periods alternating with control diet. * With ranges of value in parentheses.
** Large liver weights were due to the presence of hepatomas or hepatocellular carcinomas.

Table 3. Copper content of non-neoplastic liver tissue from rats fed copper-deficient and excess-copper diets with DMN or AAF treatment for 3-8 months.

Duration of treatment (months)	Diet...	Copper content (ppm)*					
		Copper-deficient			Excess-copper		
	Other treatment...	Control	DMN	AAF	Control	DMN	AAF
3		4.6	3.2	3.1	314	380	412
4		4.2	3.5	2.9	234	460	372
5		4.3	4.5	2.0	236	432	418
6		4.1	4.4	2.2	200	270	312
7		5.0	4.2	2.6	236	383	314
8		4.8	3.8	3.9	---	438	294
Mean		4.5	3.9	2.8	244	394	354

DMN – 50 ppm dimethylnitrosamine in drinking-water for 4-day periods alternating with distilled water. AAF – 0.06% acetylaminofluorene in the diet for 4-day periods alternating with control diet. * One pooled sample was analysed for each group at each time.

Table A6.5(01) & A6.7(01)-4. Copper content of selected hepatic neoplasms compared with that of non-neoplastic hepatic tissue from rats fed copper-deficient and excess-copper diets with DMN and AAF treatment

Experimental group	Duration of treatment (months)	Copper content (ppm) of	
		Non-neoplastic tissue	Neoplastic tissue
Copper-deficient diet: + DMN	4	3.5	4.2
	6	4.4	4.4
+ AAF	5	2.0	1.9
	6	2.2	2.6
	7	2.6	2.6
	8	3.9	2.7
Excess-copper diet: + AAF	5	418	347
	8	294	163

DMN – 50 ppm dimethylnitrosamine in drinking-water for 4-day periods alternating with distilled water. AAF – 0.06% acetylaminofluorene in the diet for 4-day periods alternating with control diet.

Table A6.5(01) & A6.7(01)-5. Copper content of selected renal samples from rats fed a copper-deficient diet (1 ppm) with or without DMN treatment.

Experimental group	Tissue	Copper content (ppm) of renal tissues at month			
		5	6	7	8
Copper-deficient diet:					
Control	Normal	8.1	7.6	9.3	9.4
+ DMN	Non-neoplastic	7.3	7.0	7.2	7.1
	Small neoplasms	5.5	2.6	2.7	---
	Large (>22g) neoplasms	2.4	2.0	1.8	2.0

DMN – 50 ppm dimethylnitrosamine in drinking-water for 4-day periods alternating with distilled water.

TableA6.5(01) & A6.7(01)-6. Incidence of hepatic lesions and neoplasms in rats fed copper-deficient and excess-copper diets with DMN or AAF treatment

and killed at monthly intervals for autopsy.

		Incidence (%)* of						
Experimental group	**Total no. of rats killed**	**Liver necrosis**	**Transitional nodules**	**Hepatomas**	**Hepatocellular Carcinomas**	**Metastases**	**Kidney neoplasms**	**Other neoplasms**
Copper deficient:								
Control	42	0.0	0.0	0.0	0.0	0.0	2.4	0.0
+ DMN	30	30.8	76.7	23.3	10.0	0.0	56.7	30.0
+ AAF	27	22.2	100.0	92.6	40.7	3.7	0.0	40.0
Excess-copper diet:								
Control	32	9.4	3.1	0.0	0.0	0.0	0.0	0.0
+ DMN	29	55.2	82.8	27.6	13.8	0.0	0.0	24.1
+ AAF	30	30.0	100.0	90.0	30.0	10.0	0.0	16.7

DMN – 50 ppm dimethylnitrosamine in drinking-water for 4-day periods alternating with distilled water.
AAF – 0.06% acetylaminofluorene in the diet for 4-day periods alternating with control diet. *
Percentage of rats affected

Figure A6.5(01) & A6.7(01)-1

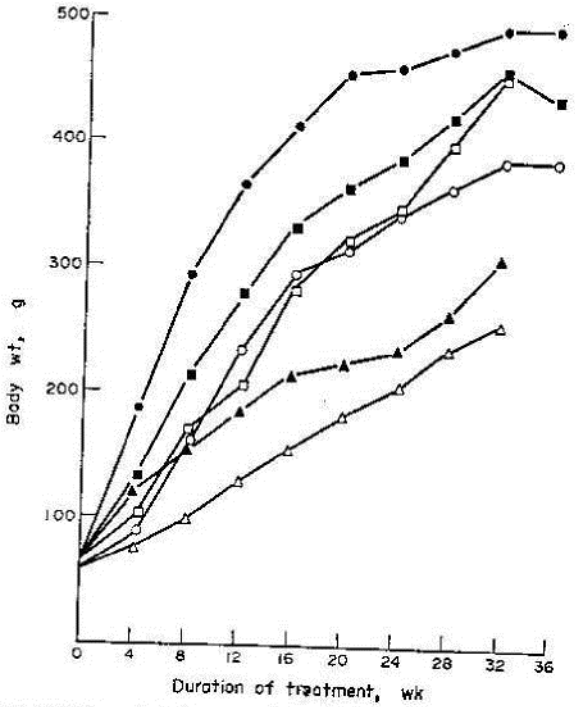

FIG. 1. Mean body weights of male rats fed copper-deficient (●——●; ■——■; ▲——▲) and excess-copper (○——○; □——□; △——△) diets and treated with DMN (■; □), AAF (▲; △) or no carcinogen (controls: ●; ○).

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

		Official use only
	187 REFERENCE	
1.1 Reference	*Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages) If necessary, copy field and enter other reference(s).* [redacted]	
1.2 Data protection	No *(indicate if data protection is claimed)*	
1.2.1 Data owner	*Give name of company* Public domain.	
1.2.2 Companies with letter of access	*Give name of company/companies which have the right to use these data on behalf of the data owner (see TNsG in support of AnnexVI)* Letter of access not required.	
1.2.3 Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* No data protection claimed.	
	188 GUIDELINES AND QUALITY ASSURANCE	
188.1 Guideline study	No. This was a non-regulatory study designed to investigate the effects of oral $CuSO_4$ on the incidence of 7,12-dimethylbenz(**α**)anthracene (DMBA) induced ovarian tumours, tumours of the breast and lymphomas in C57BL/6J mice and of tumours of the lung in strain A mice. *(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*	
188.2 GLP	No. This was a non-regulatory study. Furthermore, GLP was not compulsory at the time the study was performed. *(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)*	
188.3 Deviations	Yes. Refer to section 5.3.6 for a general discussion of deviations and deficiencies. *(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")*	X
	189 MATERIALS AND METHODS	
189.1 Test material	*In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.* Cu^{2+} as $CuSO_4$ *or give name used in study report*	

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

189.1.1 Lot/Batch number	*List lot/batch number if available*	
189.1.2 Specification	Deviating from specification given in section 2 as follows *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):*	
189.1.3 Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Copper sulphate ($CuSO_4.5H_2O$).	
189.1.4 Purity	*Give purity in % active substance* Reagent grade, Baker and Adamson.	X
189.1.5 Stability	*Describe stability of test material* Not stated.	
189.2 Test Animals	Non-entry field	
189.2.1 Species	Mouse	
189.2.2 Strain	C57BL/6J mice (59 intact virgins and 65 pseudopregnant females) were used to investigate the incidence of ovarian tumours, tumours of the breast and lymphomas. Strain A mice (50 animals bred by brother-sister mating) were used to investigate tumours of the lung.	
189.2.3 Source	[redacted]	
189.2.4 Sex	Female.	
189.2.5 Age/weight at study initiation	*Experiment A:* 4 – 6 months. *Experiment B:* 12 – 15 weeks. *Experiment C:* 12 – 16 weeks. *Experiment D:* Not stated.	
189.2.6 Number of animals per group	*Experiment A:* Five C57BL/6J virgins were injected i.v. with 0.75 mg DMBA. Five C57BL/6J virgins were injected i.v. with 0.75 mg DMBA and received $CuSO_4$ in drinking water. *Experiment B:* Eleven C57BL/6J virgins were injected i.v. with 0.75 mg DMBA. Eleven C57BL/6J virgins were injected i.v. with 0.75 mg DMBA and received $CuSO_4$ in drinking water. *Experiment C:* Ten strain A virgins were injected i.v. once with 0.75 mg DMBA and, 12 days later, with 0.5 mg DMBA i.p. Nine strain A virgins received 0.75 mg DMBA i.v. , 0.5 mg DMBA i.p. and $CuCO_4$ in their drinking water. *Experiment D:* Nineteen C57BL/6J pseudopregnant females received 6 skin paintings of 0.5 ml of a 0.5% DMBA solution in olive oil at biweekly intervals.	

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	
	Eighteen C57BL/6J pseudopregnant females received 6 DMBA skin paintings and $CuSO_4$ in the drinking water.
189.2.6.1 at interim sacrifice	Not applicable.
189.2.6.2 at terminal sacrifice	Refer to section 3.2.6.
189.2.7 Control animals	Experiment A: Five C57BL/6J mice. Experiment B: Ten untreated C57BL/6J mice and 12 C57BL/6J mice fed $CuSO_4$. Experiment C: Nineteen untreated strain A mice and 12 strain A mice fed $CuSO_4$. Experiment D: Eleven untreated C57BL/6J mice and 17 $CuSO_4$-fed pseudopregnant mice.
189.3 Administration/ Exposure	DMBA: dermal, intraperitoneal and intravenous. $CuSO_4$: Oral (in drinking water). *Fill in respective route in the following, delete other routes*
189.3.1 Duration of treatment	Experiment A: Terminated 74 weeks after DMBA treatment. Experiment B: Terminated 44 weeks after DMBA treatment. Experiment C: Terminated 33 weeks after first DMBA application. Experiment D: Terminated 50 weeks after first skin painting with DMBA.
189.3.2 Interim sacrifice(s)	None.
189.3.3 Final sacrifice	Refer to section 3.3.1.
189.3.4 Frequency of exposure	*Experiment A:* A single DMBA injection was administered i.v. to test animals. Relevant groups were started on $CuSO_4$ treatment 2 weeks before administration of DMBA. Feeding of $CuSO_4$ continued throughout the entire experimental period. These animals had access to the $CuSO_4$ solution *ad libitum*. *Experiment B:* A single DMBA injection was administered i.v. to test animals. Relevant groups were started on $CuSO_4$ treatment 2 weeks before administration of DMBA. Feeding of $CuSO_4$ continued throughout the entire experimental period. These animals had access to the $CuSO_4$ solution *ad libitum*. *Experiment C:* DMBA was administered once i.v. and, 12 days later, once i.p. Relevant groups were started on $CuSO_4$ treatment 2 weeks before the first application of DMBA. Feeding of $CuSO_4$ continued throughout the entire experimental period. These animals had access to the $CuSO_4$ solution *ad libitum*. *Experiment D:* Six dermal paintings of DMBA were administered at biweekly intervals. Relevant groups were started on $CuSO_4$ treatment 2 weeks before the

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

	first application of DMBA. Feeding of $CuSO_4$ continued throughout the entire experimental period. These animals had access to the $CuSO_4$ solution *ad libitum*.	
189.3.5 Postexposure period	None.	
	Oral	
189.3.6 Type	$CuSO_4$ was administered in drinking water.	
189.3.7 Concentration	$CuSO_4$ was dissolved in water to a concentration of 198 mg/l (approximately 50 mg Cu^{2+}/l). Treatment water was supplied *ad libitum*.	
189.3.8 Vehicle	Tap water.	
189.3.9 Concentration in vehicle	$CuSO_4$ was dissolved in water to a concentration of 198 mg/l (approximately 50 mg Cu^{2+}/l).	
189.3.10 Total volume applied	Not stated.	
189.3.11 Controls	Vehicle (water).	
	Dermal	
189.3.12 Area covered	Not stated.	
189.3.13 Occlusion	Not stated.	
189.3.14 Vehicle	Olive oil.	
189.3.15 Concentration in vehicle	5 mg/ml	
189.3.16 Total volume applied	(0.5%). 0.5 ml.	
189.3.17 Duration of exposure	Not stated.	
189.3.18 Removal of test substance	Not stated. *give solvent, detergent*	
189.3.19 Controls	Untreated.	
	Intraperitoneal/Intravenous/Intratracheal instillation	
189.3.20 Vehicle	For parenteral administration, a fatty emulsion of DMBA was produced in 1.2% w/w lecithin, 0.3% w/v poloxalkol, 15% cottonseed oil and water.	X
189.3.21 Concentration in vehicle	0.5% w/v DMBA.	X
189.3.22 Total volume applied	0.1 or 0.15 ml.	
189.3.23 Controls	Untreated.	

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

189.4 Examinations			
189.4.1 Body weight	No.		
189.4.2 Food consumption	No.		
189.4.3 Water consumption	No.		
189.4.4 Clinical signs	No.		
189.4.5 Macroscopic investigations	Not reported.		
189.4.6 Ophthalmoscopic examination	No.		
189.4.7 Haematology	No.		
189.4.8 Clinical Chemistry	No.		
189.4.9 Urinalysis	No.		
189.4.10 Pathology	No.		
189.4.10.1 Organ Weights	No.		
189.4.11 Histopathology	Yes.		
	from:	all dose groups	
	from:	all surviving animals and all animals that died during the study.	
	Organs:	Thymus, liver, kidneys, spleen, ovaries.	X
Other examinations	*E.g. enzyme induction, cell proliferation, reversibility of effects*		
189.5 Statistics	Vaginal smears for investigation of effects on the incidence of oestrus. Chi-square test and Wilcoxon ranking test were applied as appropriate.		
189.6 Further remarks	Pseudopregnant females refers to virgin mice housed together with vasectomised males. Vasectomy was performed under pentobarbital anaesthesia (70 mg/kg). Each group consisted of 3-4 virgins and 1-2 vasectomised males per cage.		
	190 RESULTS AND DISCUSSION		
	Describe findings. If appropriate, include table. Sample tables are given below.		
190.1 Results	*No effects / describe significant effects referring to data in results table*		
	Non-entry field.		
190.1.1 Experiments A & B	The results of Experiments A and B are shown in **Table A6.5(02) & A6.7(02)-1**.		
	Histopathology:		
	A single application of 0.75 mg DMBA caused a high incidence of ovarian tumours in C75BL/6J virgin mice. These tumours varied in size from 8 – 15 mm in diameter, and were classified histologically as granulosa cell tumours. Mice receiving the combination of DMBA and		

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

	$CuSO_4$ showed a lower incidence of ovarian tumours than those treated with DMBA alone. Histologically, the ovaries of all mice injected with DMBA showed similar precancerous changes, as evidenced by the destruction of oocytes and loss of follicular structure. However, addition of $CuSO_4$ to the diet appears to delay progression of precancerous lesions to frank ovarian tumours. Feeding of $CuSO_4$ to DMBA-treated females appeared to increase the incidence of lymphomas in Experiment A, but not in Experiment B. *Other examinations:* The incidence of oestrus, 20 – 22 weeks after DMBA application, was significantly elevated ($P<0.25$, chi-square test) to 60% oestrus in DMBA-treated females, compared to 51% for solvent controls and 50% for $CuSO_4$ controls.
190.1.2 Experiment C	The results of Experiment C are shown in **Table A6.5(02) & A6.7(02)-2** *Histopathology:* The feeding of $CuSO_4$ had no effect on the incidence of DMBAinduced adenomas of the lung. However, the total number of all tumours observed in the group treated with DMBA and $CuSO_4$ was only 8, compared to 16 in the group receiving DMBA only. *Other examinations:* $CuSO_4$ added to the diet appeared to prolong the survival of DMBA-treated mice ($P<0.025$).
190.1.3 Experiment D	The results of Experiment D are shown in **Table A6.5(02) & A6.7(02)-3** *Histopathology*: Animals that received both $CuSO_4$ and DMBA had a greater cumulative number of breast tumours than those receiving DMBA only. No effort was made to count skin tumours, as many non-malignant lesions were also observed after skin-painting with DMBA. *Other examinations*: When $CuSO_4$ was added to the diet of DMBA-treated mice, the mean survival time increased to 25 weeks in comparison with 21 weeks for animals treated only with DMBA ($P<0.05$, Wilcoxon ranking test).
190.2 Discussion	*No effects / describe significant effects referring to data in results table* This study was carried out to investigate the incidence of DMBAinduced tumours in mice kept on a diet supplemented with $CuSO_4$. It was shown in Experiments A and B that one injection of 0.75 mg DMBA induced ovarian tumours in nearly all C57BL/6J virgin females within 44 weeks. Conversely, $CuSO_4$ added to the diet of DMBAtreated females appeared to reduce the incidence of ovarian tumours and to prevent the increased incidence in oestrus observed in DMBAtreated females. However, all ovaries of mice treated with DMBA + $CuSO_4$ showed pre-cancerous changes, indicating that $CuSO_4$ had no

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

effect on the initiation step of DMBA oncogenesis. Instead, it appeared that the greater availability of copper in the body delayed the full expression of the carcinogenic lesions induced by DMBA.

In Experiment A, it was observed that the incidence of lymphomas were greater in DMBA + $CuSO_4$-treated mice than in those receiving DMBA only. However, this finding could not be repeated in subsequent experiments (Experiment B), and it was concluded that $CuSO_4$ had no effect on the induction of lymphomas by DMBA.

$CuSO_4$ did not alter the incidence of adenomas of the lung in DMBA-treated strain A females (Experiment C).

The increased incidence of breast tumours observed in $CuSO_4$-fed pseudopregnant C57BL/6J mice receiving DMBA skin paintings (Experiment D) may have been related to the prolonged survival observed in this group, compared to animals treated only with DMBA skin paintings. The increased survival in strain A mice treated with DMBA + $CuSO_4$, compared to animals receiving DMBA only, is unexplained (Experiment C).

No toxic effects were observed in otherwise untreated mice fed $CuSO_4$ at the concentration used in these experiments.

190.3 Time to tumours *For dermal route and skin tumours: give mean time until appearance of tumour or time until appearance of first tumour or other measure*

The cumulative number of breast tumours occurring over time in pseudopregnant females treated with 6 skin paintings of DMBA are shown in **Table A6.5(02) & A6.7(02)-3**.

190.4 Other *Describe any other significant effects*

None.

191 APPLICANT'S SUMMARY AND CONCLUSION

191.1 Materials and methods *Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines*

A study was carried out to investigate the effects of oral $CuSO_4$ on the incidence of 7,12-dimethylbenz(**α**)anthracene (DMBA) induced ovarian tumours, tumours of the breast and lymphomas in C57BL/6J mice and of tumours of the lung in strain A mice. The study was divided into four separate experiments, designated A, B, C and D.

In all cases, $CuSO_4$ was dissolved in drinking water at a concentration of 198 mg/l (equivalent to approximately 50 mg Cu^{2+}/l). $CuSO_4$-treated animals had access to the solution *ad libitum* over the entire experimental period.

Experiment A: $CuSO_4$ was administered in the drinking water of 5 female mice (C57BL/6J) aged 4 – 6 months. Two weeks after commencement of copper treatment, the mice received an intravenous (i.v.) injection of 0.75 mg dimethylbenz(**α**)anthracene (DMBA), a known carcinogen. A second group of 5 mice received DMBA alone. Five untreated mice served as controls. The experiment was terminated

Annex Points IIA6.5 & IIA6.7 **IUCLID: 5.4/12 & 5.7/02**	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**

74 weeks after DMBA treatment.

Experiment B: $CuSO_4$ was administered in the drinking water of 11 female mice (C57BL/6J) aged 12 – 15 weeks. After commencement of copper treatment, the mice received an i.v. injection of 0.75 mg DMBA. A second group of 11 mice received DMBA alone. Ten untreated mice and 12 mice receiving $CuSO_4$ served as controls. The experiment was terminated 44 weeks after DMBA treatment.

Experiment C: $CuSO_4$ was administered in the drinking water of 9 female mice (strain A) aged 12 – 16 weeks. After commencement of the copper treatment, the mice received an i.v. injection of 0.75 mg DMBA and, 12 days later, an intraperitoneal (i.p.) injection of 0.5 mg DMBA. Ten other mice received 0.75 mg DMBA i.v., and 0.5 mg DMBA i.p only. Nineteen untreated mice and 12 mice receiving $CuSO_4$ served as controls. The experiment was terminated 33 weeks after the first DMBA treatment.

Experiment D: $CuSO_4$ was administered in the drinking water of eighteen pseudopregnant C57BL/6J female mice (i.e. virgins housed with vasectomised males), each of which also received 6 dermal applications of 0.5 ml of a 0.5% DMBA solution in olive oil at biweekly intervals. A separate group of 19 pseudopregnant females received dermal applications of DMBA, but did not receive $CuSO_4$ in their drinking water. Eleven untreated mice and 17 pseudopregnant mice receiving $CuSO_4$ served as controls. The experiment was terminated 50 weeks after the first DMBA treatment.

Animals in all experiments were observed daily. All mice found dead and those sacrificed were subject to post-mortem evaluation. Sections of the liver, lung, kidney, spleen, thymus, ovaries and all tumour-like structures were fixed in 10% formalin in phosphate buffer at pH 7.4. Specimens were embedded in wax, sectioned for light microscopy and stained by haematoxylin and eosin. Vaginal smears were also taken and stained with Wright’s stain.

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	
191.2 Results and discussion	*Summarize relevant results; discuss dose-response relationship.* *Experiments A and B:* The incidences of ovarian tumours in Experiment A after 76 weeks were 0/5, 4/5, and 0/5 in the untreated controls, DMBA-treated mice and DMBA plus Cu-treated mice, respectively. The incidences of these tumours in Experiment B after 46 weeks were 0/10, 0/12, 11/11 and 6/11 in the untreated controls, copper-treated mice, DMBA-treated mice and DMBA/copper treated mice respectively. The results of these two experiments suggested that $CuSO_4$ may inhibit DMBA-induced tumour development. The incidences of lymphomas in Experiment A were 0/5, 1/5, and 5/5 in the untreated controls, DMBA-treated mice and DMBA plus Cu treated mice respectively. Although these results implied that incidence of lymphomas were greater in DMBA plus $CuSO_4$-treated mice than in those receiving DMBA only, this finding could not be repeated in Experiment B (incidences of lymphoma 1/10, 2/12, 3/11 and 3/11 in the untreated controls, Cu-treated mice, DMBA-treated mice and DMBA plus Cu-treated mice, respectively). It was therefore concluded that $CuSO_4$ had no effect on the induction of lymphomas by DMBA. *Experiment C:* Tumour incidence in the 12 mice given $CuSO_4$ alone (1 breast tumour, 2 lymphomas and no lung or ovarian tumours) was similar to that in the 19 untreated controls (2 lymphomas, no breast, lung or ovarian tumours). $CuSO_4$ had no effect on the incidence of DMBA-induced lung adenomas (incidence 4/9 in DMBA plus Cutreated mice and 4/10 in mice treated with DMBA only), although it appeared to prolong the survival of DMBA-treated mice (mean survival 28 weeks compared with 19 weeks in mice treated with DMBA only), and to slightly reduce the total number of tumours seen, as compared with mice given DMBA only. *Experiment D:* No information was given on the tumour incidence in mice given $CuSO_4$ alone. However, mice given DMBA plus $CuSO_4$ had a greater number of mammary tumours (9 tumours amongst an original group of 18) than those given DMBA alone (5 tumours amongst an original group of 19). This increase was attributed to the greater longevity of Cu-treated mice. No toxic effects were observed in otherwise untreated mice fed $CuSO_4$ at the concentration used in these four experiments.
191.3 Conclusion	DMBA was injected or administered by skin paintings to C57BL/6J and to strain A female mice kept on a diet supplemented with $CuSO_4$. It was found that $CuSO_4$ had no effect on the incidence of DMBAinduced adenomas of the lung, lymphomas and breast tumours. $CuSO_4$ did not prevent the induction of pre-cancerous lesions in the ovary, but may have delayed the development of granulosa cell tumours.
191.3.1 Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4*

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

191.3.2 Deficiencies	Yes This study was not conducted and/or reported in strict compliance with the principles of GLP. There were also a number of deficiencies in the methodology used, when compared with the requirements of currently accepted guidelines for the conduct of carcinogenicity studies (e.g. OECD 451), including the following: • The test substance was not characterised in detail; • The number of animals per test group was smaller than recommended; • Only a single $CuSO_4$ test concentration was used; • The range of tissues examined macroscopically was limited; • The range of tissues examined microscopically was limited; • Body and organ weights were not reported; • No blood sampling was reported for adversely affected animals; • Feed and water consumption were not reported; • Study duration was shorter than recommended (33 – 74 weeks, rather than the recommended 2 years). However, these deficiencies do not necessarily compromise the validity of the data generated, or the author's interpretation of that data, given that the study was not carried out for regulatory purposes. Furthermore, the research was published in a peer-reviewed journal, and has therefore been subject to the prior scrutiny of experts in the field. It has also been referred to in reviews of the carcinogenicity of copper. Overall, this is an adequately-reported study, and its findings are considered to make a valuable contribution to the 'weight of evidence' approach that has been adopted for the purposes of the current review of copper carcinogenicity. A reliability indicator of 2 has been assigned on this basis. *(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)*

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

Evaluation by Competent Authorities
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 30 Dec.2004

Guidelines and quality assurance In paragraph Deviations (2.3): Refer to section 5.3.6 **5.3.2.**

Materials and Methods Agree with applicant's version.

Comments:

- The purity and impurities of the active substance were not reported (3.1.4), so we cannot compare with notified substance.
- Vehicle (3.3.20): w/w lecithin, 0.3% w/v w/w poloxalkol, 15% cottonseed oil and water.
- Concentration in vehicle (3.3.21): 0.5% w/v w/w DMBA.
- Histopathology/ Organs (3.4.11): Thymus, liver, kidneys, spleen, ovaries **+ lungs**.

Results and discussion Agree with applicant's version.

Conclusion Agree with applicant's version.

Furthermore, there are an important number of deficiencies in methodology used in this publication, when compared with requirements of currently accepted guidelines for the conduct of combined chronic toxicity/carcinogenicity studies (e.g. OECD 453). Besides those noted in the paragraph 5.3.2, it also misses a control satellite group.

Reliability 2

Acceptability Not acceptable.

Not really a key study. Could be summarized in the review proposal for cancerogenicity.

Remarks Errors in table were corrected in red and bold.

Table A6.5(02) & A6.7(02)-1 Effect of Oral Copper Sulphate on Incidence of DMBA-induced Tumours in C57BL/6J Female Mice.

	Number of Mice	Survival Weeks	Mice With Tumours: Ovary	Lymphomas	Other Tumou
Experiment A					
Controls	5	74	0/5	0/5	--
DMBA i.v.#	5	47-74	4/5	1/5	1 papilloma (sk
DMBA i.v.# + $CuSO_4$ **	5	52-67	0/5	5/5	--
Experiment B					
Controls	10	44*	0/10	1/10	--
$CuSO_4$ **	12	44*	0/12	2/12	--
DMBA i.v.#	11	44*	11/11	3/11	--
DMBA i.v.# + $CuSO_4$ **	11	44*	6/11	3/11	1 leukaemia

* Mice were killed
\# 0.75 mg DMBA i.v.
** $CuSO_4$ in the drinking water (50 mg Cu^{2+}/litre)

Copper Oxide

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

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(02) & A6.7(02), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/12 & 5.7/02	

Table A6.5(02) & A6.7(02)-1 Effect of Oral Copper Sulphate on Incidence of DMBA-induced Tumours in C57BL/6J Female Mice.

	Number of Mice	Survival Weeks	Mice With Tumours		
			Ovary	Lymphomas	Other Tumours
Experiment A					
Controls	5	74*	0/5	0/5	--
DMBA i.v.#	5	47-74	4/5	1/5	1 papilloma (skin)
DMBA i.v.# + $CuSO_4$	5	52-67	0/5	5/5	--
Experiment B					
Controls	10	44*	0/10	1/10	--
$CuSO_4$ **	12	44*	0/12	2/12	--
DMBA i.v.#	11	44*	11/11	3/11	--
DMBA i.v.# + $CuSO_4$	11	44*	6/11	3/11	1 leukaemia

* Mice were killed

0.75 mg DMBA i.v.

** $CuSO_4$ in the drinking water (50 mg Cu^{2+}/litre)

Table A6.5(02) & A6.7(02)-2 Effect of Oral Copper Sulphate on Incidence of DMBA-induced Tumours in C57BL/6J Female Mice (Experiment C).

Groups	Number of Mice	Survival Weeks	Mice With Tumours		
			Lung	Ovary	Other Tumours
Controls	19	33*	0/19	0/19	2 lymphomas
$CuSO_4$ **	12	33*	0/12	0/12	2 lymphomas 1 breast tumour
DMBA i.v.#	10	19	4/10	5/10	2 lymphomas 2 breast tumours 1 hepatoma 2 papillomas (skin)
DMBA i.v.# + $CuSO_4$ **	9	28##	4/9	3/9	1 lymphoma

* Mice were killed

** $CuSO_4$ in the drinking water (50 mg Cu^{2+}/litre)

0.75 mg DMBA i.v.

$P < 0.025$ compared to group 3 (Wilcoxon ranking test)

Table A6.5(02) & A6.7(02)-3 Effect of Oral Copper Sulphate on Incidence of Breast Tumours in Pseudopregnant Females Treated with 6 Skin Painitings of DMBA (Experiment D).

Weeks After Treatment	Group 3 DMBA#		Group 4 DMBA i.v.# + $CuSO_4$*	
	Survivors	Cumulative number of breast tumours	Survivors	Cumulative number of breast tumours
0	19	0	18	0
16	17	2	18	2
20	11	2	17	6
25	8	4	9	6
30	4	4	7	6
40	0	5	2	9

Last skin painting with DMBA 10 weeks after start of experiment

***** $CuSO_4$ in the drinking water (50 mg Cu_{2+}/litre)

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of**
IUCLID: 5.4/13 & 5.7/03	**copper**

			Official use only
		192 REFERENCE	
1.1	**Reference**	*Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages) If necessary, copy field and enter other reference(s).* [redacted]	
1.2	**Data protection**	No *(indicate if data protection is claimed)*	
1.2.1 Data owner		*Give name of company* Public domain.	
1.2.2 Companies with letter of access		*Give name of company/companies which have the right to use these data on behalf of the data owner (see TNsG in support of AnnexVI)* Letter of access not required.	
1.2.3 Criteria for data protection		*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* No data protection claimed.	
		193 GUIDELINES AND QUALITY ASSURANCE	
193.1 Guideline study		No. This was a non-regulatory study designed to investigate the chronic toxicity on rats of potassium sodium copper chlorophyllin, copper sulphate (anhydrous) and copper gluconate. For the purposes of this summary, only information relevant to the chronic toxicity of copper sulphate is presented herein. *(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*	
193.2 GLP		No. This was a non-regulatory study. Furthermore, GLP was not compulsory at the time the study was performed. *(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)*	
193.3 Deviations		Yes. Refer to section 5.3.6 for a general discussion of deviations and deficiencies. *(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")*	X
		194 MATERIALS AND METHODS	
		In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.	

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/13 & 5.7/03	
194.1 Test material	Cu^{2+} as copper sulphate ($CuSO_4$). *or give name used in study report*
194.1.1 Lot/Batch number	Not stated. *List lot/batch number if available*
194.1.2 Specification	Deviating from specification given in section 2 as follows *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):*
194.1.3 Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Refer to section 2.1.
194.1.4 Purity	*Give purity in % active substance* Not stated.
194.1.5 Stability	*Describe stability of test material* Not stated.
194.2 Test Animals	Non-entry field
194.2.1 Species	Rat
194.2.2 Strain	Sprague-Dawley
194.2.3 Source	Not stated.
194.2.4 Sex	Male and female.

X

Initial bodyweights of weanling rats were as follows:

194.2.5 Age/weight at study initiation

Group	Males (grams)	Females (grams)
Controls	81 ± 2.3	73 ± 2.3
0.135% $CuSO_4$ in the diet (530 ppm Cu).	72 ± 3.4	67 ± 3.3
1.406% $CuSO_4$ in the diet (1600 ppm Cu).	71 ± 9.3	73 ± 2.2

Changes in bodyweight over the course of the study are shown in **Table A6.5(03) & A6.7(03)-1**.

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/13 & 5.7/03	

194.2.6 Number of animals per group

Group	Males	Females
Controls	23	25
0.135% $CuSO_4$ in the diet (530 ppm Cu)	25	25
1.406% $CuSO_4$ in the diet (1600 ppm Cu)	23	25

194.2.6.1 at interim sacrifice: Refer to **Table A6.5(03) & A6.7(03)-2**.

194.2.6.2 at terminal sacrifice: Not stated.

194.2.7 Control animals: Control animals received the basal diet only.

194.3 Administration/ Exposure: Oral in the diet.
Fill in respective route in the following, delete other routes

194.3.1 Duration of treatment: All surviving animals of all groups were sacrificed at weeks forty to forty-four.

194.3.2 Interim sacrifice(s): None.

194.3.3 Final sacrifice: Refer to section 2.3.1.

194.3.4 Frequency of exposure: 7 days a week

194.3.5 Postexposure period: None.

Oral

194.3.6 Type: $CuSO_4$ was administered in the diet.

194.3.7 Concentration: Not applicable.

194.3.8 Vehicle: Basal diet.

194.3.9 Concentration in vehicle: Treatment group animals received diets containing Cu at one of the following concentrations:
1600 ppm Cu as $CuSO_4$ (1.406% $CuSO_4$).
530 ppm Cu as $CuSO_4$ (0.135% $CuSO_4$).

X

194.3.10 Total volume applied: Not stated.

194.3.11 Controls: Controls received basal diet only.

194.4 Examinations

194.4.1 Body weight: Yes.

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/13 & 5.7/03	

194.4.2 Food consumption	Yes.		
194.4.3 Water consumption	No.		X
194.4.4 Clinical signs	Yes.		
194.4.5 Macroscopic investigations	Yes.		
194.4.6 Ophthalmoscopic examination	No.		
194.4.7 Haematology	Yes		
	Number of animals:	Not stated.	
	Time points: Unspecified intervals.		
	Parameters:	Routine examinations.	
	Other:	Oxygen carrying capacity.	
194.4.8 Clinical Chemistry	No		
	Number of animals:	None.	
	Time points: None.		
	Parameters: None.		
	Other:	None.	
194.4.9 Urinalysis	Yes		
	Number of animals:	Not stated.	
	Time points: Unspecified intervals.		
	Parameters:	Routine examinations.	
	Other:	None.	
194.4.10 Pathology	Yes.		
194.4.10.1 Organ Weights	Yes		
	from:	at interim sacrifice, at terminal sacrifice	
	Organs:	Liver, kidneys, testes, seminal vesicles, uterus, ovaries, spleen, brain, heart, lungs, stomach, brain.	
	Other:	None.	
194.4.10.2 Histopathology	Yes		
	from:	High dose group animals sacrificed at 30 – 35 weeks and also on the liver, kidneys and testes of animals receiving the lower level after 40 – 44 weeks.	
	Organs:	Spleen, adrenals, small intestine, large intestine, stomach, sciatic nerve, kidney, liver, testes, ovaries.	
	Other:	None.	

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of**
IUCLID: 5.4/13 & 5.7/03	**copper**

194.4.11 Other examinations *E.g. enzyme induction, cell proliferation, reversibility of effects*

Tissue-stored copper and iron were determined in the liver, kidneys and some spleens of animals from all groups.
Simple statistical methods were applied, as appropriate.

194.5 Statistics

194.6 Further remarks

In order to maintain a reasonably consistent ratio of copper sulphate intake per gram of animal weight over the duration of the study, a movable percentage in the diet was maintained. During the first 14 days on test when food intake is highest per gram of animal weight, 25% of the stated concentrations were fed; during the second 14 days, 50% of the stated concentrations were fed; thereafter for the balance of the study, 100% of the stated concentrations were fed.

195 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

195.1 Results

No effects / describe significant effects referring to data in results table

Non-entry field.

195.1.1 Body weight

The growth of animals on the high level of $CuSO_4$ was adversely affected by treatment (**Table A6.5(03) & A6.7(03)-1**). This retardation became readily discernible at the 26th week, when the male control animals and the animals receiving 530 ppm Cu weighed at least 50% more than those animals upon the 1600 ppm Cu intake.

195.1.2 Blood & urine examinations.

All factors examined were within normal expected ranges, except blood nonprotein (NPN) nitrogen levels. High NPN (83 mg. %) was noted in males receiving 1600 ppm Cu. Males receiving 530 ppm Cu and females from both treatment groups were just above the expected range of 60 – 70 mg. % of NPN.

Gasometric determinations of the oxygen-carrying capacity of the blood compared satisfactorily with the haemoglobin value determined by the iron and acid haematin methods.

195.1.3 Organ weights

Other than stomachs of female animals in the 1600 ppm group, the average weight of the various organs per 100 g bodyweight were within the expected ranges, when compared with controls of the same age (**Table A6.5(03) & A6.7(03)-2**).

195.1.4 Gross pathology

The following findings were common in animals in the 1600 ppm group: Bronzed kidneys exhibiting sharp demarcation between the cortex and the medulla; bronzed or yellowish livers; hypertrophied ridges between the cardiac and peptic portions of the stomach, occasional ulcers and some blood; bloody mucous in the intestinal tract.

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of**
IUCLID: 5.4/13 & 5.7/03	**copper**
195.1.5 Histopathology	Histopathological studies were performed on the organs of test animals receiving the high level of $CuSO_4$ (sacrificed at 30 to 35 weeks), and also on the liver, kidney and testes of animals receiving the lower level of $CuSO_4$ (sacrificed at 40 to 44 weeks). The following organs were found to be normal in all the test and control animals: Spleen; adrenals; small intestine; large intestine; stomach; sciatic nerve. Kidney sections of animals receiving the high level of $CuSO_4$ showed minor changes which did not correlate well enough throughout the animals to draw a definite conclusion. Liver sections of animals receiving the high level of $CuSO_4$ showed well-defined abnormalities of a toxic nature in both males and females; their icteric pigmentation was increased and cytoplasmic staining properties were abnormal. Varying degrees of testicular degeneration were noted in both the high and low $CuSO_4$ treatment levels; the ovaries of the females were not noticeably affected to any degree. The kidneys, liver and testes of all the control animals were found to be normal.
195.1.6 Tissue-stored copper	The liver, kidneys and some spleens of animals from all groups were examined as to their total Cu and Fe content (**Table A6.5(03) & A6.7(03)-3**). Liver Cu averaged less than 2 mg/100 g of tissue in control animals. Animals receiving 530 ppm Cu as $CuSO_4$ for 40 weeks had Cu concentrations of 12.47 and 32.36 mg/100g liver in males and females, respectively. Those on the 1600 ppm diet for a similar duration had concentrations of 38.28 and 45.77 mg Cu/100g liver. Cu storage in the kidneys of animals receiving 530 ppm Cu was somewhat higher than that of control animals, while that in animals receiving 1600 ppm Cu was higher again.
195.2 Discussion	*No effects / describe significant effects referring to data in results table* This study confirmed that high doses of Cu as $CuSO_4$ cause metal toxicity in albino rats, increased storage of Cu (especially in the liver, kidney and spleen), damage to these organs, and high mortality.
195.3 Time to tumours	*For dermal route and skin tumours: give mean time until appearance of tumour or time until appearance of first tumour or other measure* No tumours were reported in any test animal.
195.4 Other	*Describe any other significant effects* None.

196 APPLICANT'S SUMMARY AND CONCLUSION

196.1 Materials and methods	*Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines* A study was carried out to investigate the chronic toxicity to rats of potassium sodium copper chlorophyllin, copper sulphate (anhydrous) and copper gluconate. However, for the purposes of this summary, only information relevant to the chronic toxicity and carcinogenicity of copper sulphate is presented.

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/13 & 5.7/03	

	Two groups of individually housed, weanling Sprague-Dawley rats received diets supplemented with anhydrous $CuSO_4$, giving dietary Cu concentrations of 530 ppm (0.135%) and 1600 ppm (1.147%). A third control group received the basal diet only. Each test group contained approximately 50 animals, equally divided between the sexes. In order to maintain a reasonably consistent ratio of copper sulphate intake per gram of animal weight over the duration of the study, a movable percentage in the diet was maintained. During the first 14 days on test when food intake was highest per gram of animal weight, 25% of the stated concentrations were fed; during the second 14 days, 50% of the stated concentrations were fed; thereafter for the balance of the study, 100% of the stated concentrations were fed. The maximum duration of the study was 44 weeks. The weight of each animal was determined weekly, as well as the amount of food and water consumed. Animals were individually inspected at least three times each week. An interim sacrifice was carried out at 33 weeks in which 4 animals from the control group and 4 animals from the group fed 1600 ppm Cu were sacrificed. The balance of the animals were continued in the study, and all surviving animals of all groups were sacrificed at 40 – 44 weeks. Factors investigated in this study included growth (weight gain); blood and urine examinations; organ weights; gross pathology; histopathology and tissue storage of Cu. After dry-ashing at 525°C, determination of tissue Cu content was by the diethylthiocarbamate procedure.	X
196.2 Results and discussion	*Summarize relevant results; discuss dose-response relationship.* *Bodyweight:* The growth of animals on the high level of $CuSO_4$ was adversely affected by treatment. This was readily discernible at the 26th week, when male control animals and animals receiving 530 ppm Cu weighed at least 50% more than animals on the 1600 ppm Cu intake. *Blood and urine examinations:* All factors examined were within normal expected ranges, except blood nonprotein (NPN) nitrogen levels, which were high (83 mg. %) in males receiving 1600 ppm Cu. Levels in males receiving 530 ppm Cu and females from both treatment groups were just above the expected range of 60 – 70 mg. % of NPN. *Organ weights:* Other than consistently elevated weights for stomachs of female animals in the 1600 ppm group, the average weights of the various organs per 100 g bodyweight were within the expected ranges, when compared with controls of the same age. Other organs examined were heart, lungs, liver, spleen, kidneys, uterus, ovaries, seminal vesicles, testes and brain. *Gross pathology:* The following findings were common in animals in the 1600 ppm group: Bronzed kidneys exhibiting sharp demarcation between the cortex and the medulla; bronzed or yellowish livers; hypertrophied ridges between the cardiac and peptic portions of the	

stomach, occasional ulcers and some blood; bloody mucous in the intestinal tract. No treatment-related adverse findings were reported for animals in either the control or 530 ppm treatment group. No grossly

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/13 & 5.7/03	

obvious neoplasms were reported. *Histopathology:*

Histopathology was performed on the organs of animals in the 1600 ppm group (sacrificed at 30 to 35 weeks), and also on the liver, kidney and testes of animals in the 530 ppm group (sacrificed at 40 to 44 weeks). The following organs were normal in all animals: Spleen; adrenals; small intestine; large intestine; stomach; sciatic nerve. The livers of animals in the 1600 ppm group showed well-defined abnormalities of a toxic nature in both males and females; icteric pigmentation was increased and cytoplasmic staining properties were abnormal. The kidneys of animals in the 1600 ppm group showed minor changes. Varying degrees of testicular degeneration were noted in both treatment groups; the ovaries of the females were not noticeably affected to any degree. The kidneys, liver and testes of all the control animals were found to be normal. No microscopic evidence of neoplasms was reported.

Tissue-stored copper:

Liver Cu averaged less than 2 mg/100 g of tissue in control animals. Animals in the 530 ppm group for 40 weeks had Cu concentrations of 12.47 and 32.36 mg/100g liver in males and females, respectively. Those in the 1600 ppm group for a similar duration had concentrations of 38.28 and 45.77 mg Cu/100g liver. Cu storage in the kidneys of animals receiving 530 ppm Cu was somewhat higher than control, while that in animals receiving 1600 ppm Cu was higher again.

196.3 Conclusion

The growth of rats receiving 1600 ppm Cu as $CuSO_4$ was adversely affected, although organ weights were apparently unaffected (other than markedly increased stomach weight in females). Well-defined abnormalities of a toxic nature were evident in rats of the 1600 ppm treatment group upon histological examination, and varying degrees of testicular degeneration was evident in animals from both the 530 ppm and the 1600 ppm groups. There were no reports of evidence of neoplasms in any treatment group.

196.3.1 Reliability

Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4

2

196.3.2 Deficiencies

Yes

This study was not conducted and/or reported in strict compliance with the principles of GLP. There were also a number of deficiencies in the methodology used, when compared with the requirements of currently accepted guidelines for the conduct of carcinogenicity studies (e.g. OECD 451), including the following:

- The test substance was inadequately characterised;
- Environmental controls were not described in detail;
- Only two $CuSO_4$ test concentration were used;
- The range of tissues reported upon was limited;

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7 **IUCLID: 5.4/13 & 5.7/03**	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of copper**

- Body and organ weights were not reported;
- The duration of the study was a maximum of 44 weeks;
- Microscopic investigations were carried out in a limited number of tissues in animals sacrificed at study termination.
- Experimental results were inadequately reported in some cases, e.g. haematology and urinalysis.

However, these deficiencies do not necessarily compromise the validity of the data generated, or the author's interpretation of that data, given that the study was not carried out for regulatory purposes. Furthermore, the research was published in a peer-reviewed journal, and has therefore been subject to the prior scrutiny of experts in the field. It has also been referred to in reviews of the carcinogenicity of copper.

Overall, this is an adequately-reported study, and its findings are considered to make a valuable contribution to the 'weight of evidence' approach that has been adopted for the purposes of the current review of copper carcinogenicity. A reliability indicator of 2 has been assigned on this basis.

(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

Sections A6.5 & A6.7 Combined Chronic toxicity/Carcinogenicity

Annex Points IIA6.5 & IIA6.7	*Specify section no., heading, route and species as appropriate* **A6.5(03) & A6.7(03), Combined Chronic toxicity/Carcinogenicity of copper**
IUCLID: 5.4/13 & 5.7/03	

Evaluation by Competent Authorities

Use separate "evaluation boxes" to provide transparency as to the comments and views submitted

EVALUATION BY RAPPORTEUR MEMBER STATE

Date 30 Dec.2004

Guidelines and quality assurance In paragraph Deviations (2.3): Refer to section 5.3.6 **5.3.2.**

Materials and Methods Agree with applicant's version.

Comments:

- The purity and impurities of the active substance were not reported (3.1.4), so we cannot compare with notified substance.
- Concentration in vehicle (3.3.9): 1600 ppm Cu as $CuSo_4$ (1.406 % **0.406 %** $CuSo_4$).
- Water consumption (3.4.3): No **YES**
- Materials and Methods (5.1): *Two groups of individually housed, weanling Sprague-Dawley rats received diets supplemented with anhydrous $CuSO_4$, giving dietary Cu concentrations of 530 ppm (0.135%) and 1600 ppm (1.147%).* **(0.406%)**

Results and discussion Agree with applicant's version.

Conclusion Agree with applicant's version.

There are an important number of deficiencies in methodology used in this publication, when compared with requirements of currently accepted guidelines for the conduct of combined chronic toxicity/carcinogenicity studies (e.g. OECD 453). Besides those noted in the paragraph 5.3.2, it also misses a control satellite group.

Reliability 2

Acceptability Acceptable.

Remarks Errors in table were corrected in red and bold.

Copper Oxide

Table A6.5(03) & A6.7(03)-1. Average Body Weights of Rats Receiving Copper Sulphate (grams)

Treatment Group	0 week	4th week	8th week	12th week	26th week	35th week
Females						
Controls	73 ± 2.3 g[a]	172 ± 3.2 g	204 ± 4.0 g	220 ± 3.9 g	261 ± 4.5 g	265 ± 4.3 g
Number of rats	25	24	24	24	24	24
530 ppm Cu	67 ± 3.3 g	154 ± 2.8 g	207 ± 3.5 g	232 ± 3.2 g	270 ± 3.5 g	260 ± 5.1 g
Number of rats	25	25	25	25	25	25
1600 ppm	**73 ± 2.2** g	**153 ± 3.4 g**	**198 ± 2.7 g**	**224 ± 3.1 g**	**220 ± 4.2 g**	**257 ± 3.6 g**
N	25	25	25	25	**24**	**20**
Males						
Controls	81 ± 2.3 g	218 ± 7.2 g	310 ± 6.2 g	382 ± 7.0 g	438 ± 17.3 g	459 ± 17.3 g
Number of rats	23	23	23	23	23	22
530 ppm Cu	72 ± 3.4 g	194 ± 6.5 g	279 ± 1.3 g	358 ± 5.8 g	425 ± 10.7 g	431 ± 3.7 g
Number of rats	25	25	25	25	24	23
1600 ppm	71 ± 9.3 g	174 ± 5.7 g	247 ± 6.3 g	280 ± 9.1 g	282 ± 10.6 g	335 ± 9.5 g
Number of rats	23	23	23	23	20	16

[a] Standard error.

[b] Depleted due to high mortality.

	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	

Table A6.5(03) & A6.7(03)-1. Average Body Weights of Rats Receiving Copper Sulphate (grams)

Treatment Group	0 week	4th week	8th week	12th week	26th week	35th week
Females						
Controls	73 ± 2.3 g[a]	172 ± 3.2 g	204 ± 4.0 g	220 ± 3.9 g	261 ± 4.5 g	265 ± 4.3 g
Number of rats	25	24	24	24	24	24
530 ppm Cu	67 ± 3.3 g	154 ± 2.8 g	207 ± 3.5 g	232 ± 3.2 g	270 ± 3.5 g	260 ± 5.1 g
Number of rats	25	25	25	25	25	25
1600 ppm	75 ± 2.5 g	170 ± 2.9 g	200 ± 3.1 g	235 ± 4.1 g	204 ± 3.8 g	182 ± 11.7 g
N	25	25	25	25	23	6[b]
Males						
Controls	81 ± 2.3 g	218 ± 7.2 g	310 ± 6.2 g	382 ± 7.0 g	438 ± 17.3 g	459 ± 17.3 g
Number of rats	23	23	23	23	23	22
530 ppm Cu	72 ± 3.4 g	194 ± 6.5 g	279 ± 1.3 g	358 ± 5.8 g	425 ± 10.7 g	431 ± 3.7 g
Number of rats	25	25	25	25	24	23
1600 ppm	71 ± 9.3 g	174 ± 5.7 g	247 ± 6.3 g	280 ± 9.1 g	282 ± 10.6 g	335 ± 9.5 g
Number of rats	23	23	23	23	20	16

[a] Standard error.

[b] Depleted due to high mortality.

Table A6.5(03) & A6.7(03)-2. Average Weight of Tissues, grams per 100 grams of Body Weight.

Group	N	Heart	Lungs	Liver	Spleen	Kidneys	Uterus (Seminal Vesicles)	Ovaries (Testes)	Stomach	Brain	Approx. Weeks on Test
Females											
Contols	9	0.317	0.500	3.214	0.203	0.717	0.274	0.038	0.615	0.656	42
530 ppm Cu	15	0.295	0.553	3.250	0.182	0.714	0.212	0.037	0.628	0.630	42
1600 ppm Cu	10	0.301	0.564	3.778	0.209	0.799	0.179	0.040	0.821	0.684	42
Males											
Contols	8	0.268	0.495	3.586	0.169	0.798	0.827	0.350	0.518	0.424	42
530 ppm Cu	12	0.282	0.487	3.674	0.189	0.792	0.666	0.357	0.585	0.423	42
1600 ppm Cu	6	0.301	0.488	4.072	0.198	0.889	0.839	0.405	0.686	0.505	42
Females											
Contols	4	0.336	0.770	3.524	0.188	0.753	0.230	0.039	0.645	0.668	33
1600 ppm Cu	4	0.333	0.569	3.767	0.185	0.670	0.135	0.024	0.795	0.669	33
Males											
Contols	4	0.301	0.713	3.556	0.173	0.777	0.923	0.359	0.531	0.479	33
1600 ppm Cu	4	0.297	0.518	3.492	0.176	0.720	0.700	0.255	1.061	0.572	33

Table A6.5(03) & A6.7(03)-3. Copper Content of Tissues of Rats Receiving Copper Sulphate, mg Cu/g tissue (wet basis).

Tissue	Control Diet		530 ppm Cu		1600 ppm Cu	
	Male	Female	Male	Female	Male	Female
Liver						
Av.	1.16	1.78	12.47	32.36	38.28	45.77
S.E.	0.31	0.39	2.52	14.6	13.85	5.18
N.	6	6	6	6	6	6
Kidney						
Av.	2.48	3.53	3.49	6.91	15.83	12.11
S.E.	0.20	0.33	0.54	0.48	6.21	4.80
N.	6	6	6	6	6	**6**
Spleen						
Av.	3.34	4.83	5.63	5.12	13.91	6.07
S.E.	0.63	0.33	1.5	1.3	7.50	1.72
N.	6	6	6	6	6	6

Section A6.5
Annex Point A6.5
IUCLID: 5.4/04

A6.5, Chronic Toxicity in the Dog

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.
If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [X]**

Limited exposure [ ] **Other justification [X]**

Detailed justification:

This data waiver has been constructed using the reviews and expert judgement [redacted]

Concentrations of copper in body fluids tend to be lower that they are in cells (Table 6.5_1) with the exception of bile (the major route of copper excretion) and cerebrospinal fluid. The data on organ copper contents are generally consistent among vertebrates (Table 6.5_1) Some species-specific exceptions include the dog and sheep, where liver concentrations are higher. Although it has been suggested that the high dog values are due to a high copper liver diet, the dog is also peculiar in terms of its albumin (which is normally involved in delivery of copper to the liver).

Albumin is well known as the most abundant protein in vertebrate blood plasma and interstitial fluids. A rather acid protein (pI 4.7) of 68,000 Da, it is involved in the transport of numerous substances, from tryptophan, fatty acids and bilirubin to drugs and metal ions. These various substrates mostly occupy different positions on the protein. It is also well recognised that albumin binds copper and has a role in copper transport. The interactions of copper with albumin and amino acids (relating to transport) have been reviewed in detail by [redacted]

In contrast to the copper in ceruplasmin, copper on albumin is readily exchangeable with that on other ligands, particularly amino acids that may be present in serum and other body fluids [redacted] It also binds additional copper atoms, but with lower affinity. In the presence of histidine, which itself forms a tight His_2-Cu complex (K_D 10^{-17} [redacted], His_2-Cu is in equilibrium with albumin-Cu through intermediary, ternary, albumin-Cu-His complex,

Cu-His2 t Cu-His t albumin-Cu-His t albumin-Cu

which may in fact be the prominent *in vivo* species. The dissociation constant for the ternary complex has been measured as being 10-22 M [redacted] Other authors have reported that a ternary complex between albumin, copper and cysteine may also be formed *in vivo* [redacted]

The high-affinity copper binding site is situated at the amino terminus [redacted] and involves primarily the histidine position 3. This histidine is essential for tight binding, a matter that was largely proved by studies in the dog. In this species (and in the pig, [redacted] the crucial histidine has been replaced by tyrosine and the albumin does not have the same high affinity for copper [redacted] It does have several lower affinity sites, however (as do other albumins). [redacted]) has proposed that the lower affinity of canine albumin for copper may contribute to the unusually high levels of copper in dog liver. This would occur through more rapid uptake occurring in the liver in the absence of high-affinity albumin binding that would slow down copper uptake in plasma. This is consistent with the data of [redacted] who showed that livers of mongrel dogs had copper concentrations of 82 ± 29 mg/g wet weight (n=10) and when compared to other species, was more than ten times that of most species tested. All other tissues were in the standard range of copper concentrations when compared to other species tested. Dog liver rapidly took up copper administered intravenously, but dogs were unable to tolerate the kinds of repeated doses of copper that can be given to rats. This suggests that albumin in blood functions as a buffer for excess copper in humans and rats that might otherwise cause intracellular organ toxicity, making the dog an unsuitable species for studying the sub-chronic and chronic effects of copper within regulatory studies.

The only limitation to this argument is that the pig and chicken both of which have "normal" liver copper concentrations (Table 6.5_1) also have albumin with no histidine in the third position. This implies that another factor in the dog, such as the ability to excrete excess copper in the bile, is also limiting.

This hypothesis has been investigated by [redacted]

Section A6.5
Annex Point A6.5
IUCLID: 5.4/04

A6.5, Chronic Toxicity in the Dog

[redacted] They suggested that the high liver values of dogs and sheep were the result of a reduced capacity for biliary copper excretion [redacted]

Not all mammals are as tolerant of copper as humans, rodents, poultry and pigs, which can chronically tolerate many times their usual daily intakes. In some breeds of sheep, copper accumulates quite readily. Sheep are more sensitive to high copper intakes [redacted] the same is true for dogs, and for Bedlington terriers in particular [redacted] The latter were thought of as models for Wilson disease. The defect these species (sheep and dogs) appear to have in common with Wilson disease is a reduced capacity for biliary copper excretion. As a result, toxic concentrations of copper accumulate, particularly in the liver.

Therefore, sub-chronic (90-day) and chronic (1 year) studies in the dog can be waived as the dog is an unsuitable animal model for studying copper toxicity in relation to man.

For information and completeness, a study in beagle dogs was conducted in 1972. Groups of 6-8 males and females were given a diet containing 0, 0.012%, 0.06% and 0.24% copper gluconate for 6-12 months (equivalent to approximately 0, 0.42, 2.1 and 8.4 mgCu/kg bw/day). This study was conducted by [redacted] and reported by WHO, 1998. The study included a detailed examination of haematological biochemical and urinalysis parameters, and tissue copper concentrations in kidney, liver and spleen. Detailed necropsy, histopathology and organ weight information was also provided.

No effect on mortality or body weight gain was observed. Physical examinations, haematology, urinalysis and most blood biochemical analysis revealed no effect of the compound except in two of the 12 dogs in the highest dose level which showed elevated levels of serum GPT, however, this was reversible and the elevated levels were considered not toxicologically significant by the WHO task force. No compound related gross or microscopic pathologic lesions or changes in organ weight were observed. At 6 and 12 months, there was a dose-dependent increase in copper level in kidney, liver and spleen. Liver biopsy from 4 animals at 0, 4, and 12 weeks after withdrawal of 12 months dosing (8.4 mg/kg bw/day) showed some reversibility of liver

Section A6.5 Annex Point A6.5 IUCLID: 5.4/04	A6.5, Chronic Toxicity in the Dog
	copper levels. [redacted] and only a summary provided by WHO, 1988 is now available.
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* Not applicable
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	29 Dec.2004
Evaluation of applicant's justification	Agree with applicant's version. Comment: • This is consistent with the data of [redacted] who showed that livers of mongrel dogs had copper concentrations of 82 ± 29 mg/g **µg/g** wet weight (n=10) and when compared to other species, was more than ten times that of most species tested. • Quoted references aren't submitted in the dossier or included in IUCLID like: [redacted]
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.5_1. Copper Concentrations in Tissues of Adult Humans and Animals: Major Organs[a]

	Copper Concentration[b] (µg/g)				
Tissue/organ	Human	Rat	Pig	Mouse	Other
Kidney	12 ± 7 (19)	7.9 ± 5.5 (14)	7.3 ± 4.5 (4)	4.4 ± 1.1 (3)	5.8, 7.9 (2) (chick) 6.9, 10 (2) (dog)
Liver	6.2 ± 0.8 (9)	4.6 ± 1.1 (23)	5.2 ± 0.7 (5)	4.1[c], 4.7[d] (2)	3.0, 2.9 (2) (chicken) 67 ± 23 (5) (dog)[e]
Brain	5.2 ± 1.1 (10)	3.1 ± 1.2 (10)	3.9 ± 1.5 (4)	4.0 ± 2.1 (4)	
Heart	4.8 ± 1.9 (14)	4.8[f], 6.2 (2)	4.6 (1)		4.6 (1) (cow)
Bone	4.1 ± 1.3 (8)	2.5 ± 0.6 (3)	1.4, 2.4 (2)		4.4 (1) (sheep)
Stomach	2.2 ± 0.7 (7)		1.6 (1)		
Intestine	1.0, 3.0 (2)	1.7, 2.1 (2)		1.7 (1)	
Spleen	1.5 ± 0.4 (14)	2.3 ± 2.2 (8)	1.4 ± 0.3 (4)	1.2[c], 4.2 (2)	2.3, 4.2 (2) (dog)
Lung	1.3 ± 0.4 (11)	1.8 ± 0.6 (5)	1.2, 1.4 (2)	3.9 (1)	2.6 (1) dog
Blood	1.11 ± 0.13 (5)				
Plasma	1.13 ± 0.15 (70)	1.28 ± 0.26 (12)	1.75 ± 0.43 (11)	0.38[c] (1)	0.42 ± 0.20 (9) (chicken)
Muscle	0.9 ± 0.3 (7)	1.0 ± 0.4 (5)	1.0 ± 0.6 (3)		3.7 (1) (cow) 0.5-0.9 (3) (beef)[g]
Skin	0.8 ± 0.4 (9)	1.7 ± 0.8 (4)	1.0, 1.5 (2)		0.4 (1) (cow)
Adipose	0.2, 0.3 (2)	0.35 (1)	0.8, 0.7 (2)	2.4 (1)	1.2 (1) (whale)

Section A6.5 **A6.5 Chronic toxicity**
Annex Point IIA, VI. 6.5

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.
If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [ ]**

Limited exposure [ ] **Other justification [X]**

Detailed justification: All available studies on the carcinogenicity of copper are

Section A6.5 **A6.5 Chronic toxicity**
Annex Point IIA, VI. 6.5

public domain studies and therefore, taken in isolation are of limited value to ascertain the carcinogenic potential copper compounds. This is due to the fact that these studies are limited due to shorter exposure periods (<2 years) and group sizes being small. However, when the 3 studies summarised below are assessed on an overall balanced approach, the information from these studies does give useful information as to the carcinogenic potential of copper compounds (See Table 6.7_1)

These results indicate that copper sulphate and other copper salts do not appear to have carcinogenic potential even at very high dose levels of up to 120 mg Cu/kg/bw/day [redacted] The data in [redacted] are especially useful since positive control groups were added in this study and showed an induction of neoplasms in the rat, indicating that the exposure period (although not two years) was long enough for neoplasms to appear if you have a positive carcinogen. In addition, this study indicates that excess copper may have a protective effect on known carcinogens.

These animal carcinogenicity studies have been conducted with copper compounds. Short duration, small sample sizes and limited histopathologic examination limit the findings of the studies. Nevertheless, the findings of these studies do not raise concerns with respect to carcinogenic activity.

In addition, the available genotoxicity studies support the indication that copper compounds have no carcinogenic potential. The studies include Ames assays in *Salmonella typhimurium* on copper II sulphate pentahydrate; a micronucleus study on copper II sulphate pentahydrate and an unscheduled DNA synthesis ex vivo study in rat liver on copper II sulphate.

The Ames tests indicated that copper sulphate had no mutagenic activity ([redacted] No evidence of an increase in the incidence of micronuclei was detected in the mouse micronucleus study when mice were orally administered two doses of 447 mg/kg copper sulphate, 24 h apart ([redacted]). There was also no evidence of unscheduled DNA synthesis in the rat liver ([redacted]).

Section A6.5 **A6.5 Chronic toxicity**
Annex Point IIA, VI. 6.5

These studies are consistent and show a lack of *in vitro* mutagenic activity or *in vivo* clastogenic potential associated with soluble copper compounds. The results of these studies do not highlight a concern regarding the genotoxic potential of copper compounds.

Available data on the genotoxicity and carcinogenicity of copper and its compounds have been considered against EU classification criteria as contained in Annex VI of Directive 67/548/EEC. The available data for copper compounds do not meet the criteria requiring classification for carcinogenicity.

Chronic toxicity investigations in these studies, and in particular, in [redacted] indicate that, as in the 90- day rat study of NTP, 1993, the target organs for copper are the liver and kidney. These studies show substance-related adverse effects at concentrations of copper higher than the expected exposure levels resulting from copper-containing wood preservation for primary and secondary human exposure. In addition, the longer duration studies indicate that the adverse effects do not appear to become more severe over longer exposure periods (up to one year). This is probably due to the homeostatic control mechanisms present in animals which would regulate the uptake and excretion of copper on a daily basis (see Section 6.2 in Document IIA). As adverse effects are only observed at relatively high levels of copper outside the normal daily intake of copper for humans (up to 10 mg/day), new chronic studies extending over a 2 year time period is not expected to add further insight into the mechanisms of chronic toxicity of copper in humans.

From the exposure Section in Document IIC, it can be seen that the systemic exposure of primary and secondary populations to copper is significantly lower than the usual dietary intake of copper by these populations (2-3 mg/day). Therefore, there is no need to conduct new combined chronic/carcinogenicity studies to OECD guideline 451/453 when considering copper oxide or copper carbonate as active substances used in wood preservation unscheduled DNA synthesis in the rat liver ([redacted]

Undertaking of intended data submission [ ]

Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)

Section A6.5 Annex Point IIA, VI. 6.5	A6.5 Chronic toxicity
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 Dec.2004
Evaluation of applicant's justification	Agree with applicant's version.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.7_1 Summary of Carcinogenicity Data

Route	Species Strain Sex no/group	dose levels frequency of application	Tumours	Reference
Oral, diet 9 months	Rat, Sprague-Dawley, male 50 or 58 animals/group	1 ppm, 800 ppm (0.05, 40 mgCu/kg/bw/day)	Liver necrosis and transitional nodules in the liver (3/32) and transitional nodules in the liver (1/32) was observed at 40 mgCu/kg/bw/day whereas one kidney tumour (1/42) was observed in the low copper group (not thought significant). Decreased body weight gain and increased mortality were found in the high copper group. Exposure to known carcinogens increased the incidence of liver necrosis and transitional nodules and each induced a similar incidence of liver tumours in rats fed excess copper or copper-deficient diets. In the DMN group, 17/30 rats on the copper-deficient diet and kidney tumours compared to 0/29 given excess copper. The incidence of AAF-induced extrahepatic neoplasms was apparently reduced by the excess copper diet. (5/30 vs 11/27).	[redacted]
Oral drinking water 46 weeks	Mouse C57BL/6J, female 10-12 animals/group	198 mg/l (app. 10 mgCu/kg/bw/day)	The incidences of ovarian tumours after 46 weeks were 0/10, 0/12, 11/11 and 6/11 in the untreated controls, copper treated mice, DMBA-treated mice and DMBA-copper- treated mice respectively. This suggests that copper sulphate may possibly inhibit DMBA-induced tumour development. $CuSO_4$ had no effect on the incidence of DMBA-induced adenomas of the lung, lymphomas and breast tumours.	[redacted]
Oral diet, 30-44 weeks	Rat, Sprague-Dawley, male and female, 23- 26 animals/ group	0, 530 or 1600 ppm Cu (approx. 0, 27 or 80 mg Cu/kg b.w./day in males and 0, 40 or 120 mg Cu/kg b.w./day in females).	The growth of rats receiving 1600 ppm Cu as $CuSO_4$ was adversely affected, although organ weights were apparently unaffected (other than increased stomach weight in females). Well-defined abnormalities evident in the 1600 ppm treatment group included 'bronzed' kidneys, 'bronzed' or yellowish livers, hypertrophied ridges between cardiac and peptic portions of the stomach and blood in the intestinal tract. Histological examination revealed varying degrees of testicular degeneration in rats from both the 530 ppm and the 1600 ppm groups and effects on the liver were seen in both males and females. There were no reports of evidence of neoplasms in any treatment group.	[redacted]

Section A6.6.1 Genotoxicity in vitro

Annex Point IIA6.6.1 *Specify section no., heading, route and test system as appropriate*

IUCLID: 5.5/01 **A6.6.1(01), *In-vitro* Gene Mutation Study in Bacteria**

Official use only

197 REFERENCE

197.1 Reference

Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages)
If necessary, copy field and enter other reference(s).

[redacted]

197.2 Data protection

Yes

(indicate if data protection is claimed)

197.2.1 Data owner

Give name of company

[redacted]

197.2.2 Criteria for data protection

Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:

Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]

198 GUIDELINES AND QUALITY ASSURANCE

198.1 Guideline study

Yes - The study was carried out according to the following test guidelines;
OECD Guidelines 471
EC Directive 2000/32 Annex V Test B14
UKEMS Guidelines

(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")

198.2 GLP

Yes

(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)

198.3 Deviations

No

(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")

199 MATERIALS AND METHODS

In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.

199.1 Test material

Copper sulphate pentahydrate

or give name used in study report

199.1.1 Lot/Batch number *List lot/batch number if available*

A668269 350

Section A6.6.1	**Genotoxicity in vitro**
Annex Point IIA6.6.1	*Specify section no., heading, route and test system as appropriate*
IUCLID: 5.5/01	**A6.6.1(01), *In-vitro* Gene Mutation Study in Bacteria**

199.1.2 Specification		As given in section 2 *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):*
199.1.2.1	Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* blue crystalline solid
199.1.2.2	Purity	*Give purity in % active substance* 99.0 - 100.5%
199.1.2.3	Stability	*Describe stability of test material* Stable at room temperature
199.2 Study Type		*Select / delete as appropriate:* Ames test
199.2.1 Organism/cell type		*Select / delete as appropriate:* Salmonella typhimurium Strains TA98, TA100, TA1535, TA1537, TA102
199.2.2 Deficiencies / Proficiencies		*Select / delete as appropriate:* With the exception of strain TA102, these strains require biotin as well as histidine for growth. In strain TA102 the critical mutation in the histidine gene is located on a multicopy plasmid pAQ1. This strain is particularly sensitive to the activities of oxidative and cross-linking mutagens. The pKM101 plasmid derivatives (TA98, TA100 and TA102) have increased sensitivity to certain mutagens as the pKM101 codes for an error-prone DNA repair system.
199.2.3 Metabolic activation system		Tests were carried out in both the presence and absence of metabolic activation - Aroclor 1254-induced rat liver (Sprague-Dawley male rat) post-mitochondrial fraction (S-9 mix). *state species, organ, induction y/n, induction substance used, give short description*
199.2.4 Positive control		*give name of substance* Details of the positive controls are in Table A6.6.1_1 Positive Controls.
199.2.5 Negative control		Yes, tests carried out with purified water in quintuplicate both with and without metabolic activation
199.3 Administration / Exposure; Application of test substance		Non-entry field
199.3.1 Concentrations		*give concentrations of test substance* Following a range finding study, two experiments were carried out with concentrations of 1.6, 8, 40, 200 and 1000

X

Section A6.6.1	**Genotoxicity in vitro**
Annex Point IIA6.6.1	*Specify section no., heading, route and test system as appropriate*
IUCLID: 5.5/01	**A6.6.1(01), *In-vitro* Gene Mutation Study in Bacteria**

µg/l in experiment one and 50, 100, 200, 400 and 800 µg/l in experiment two. The tests were carried out in triplicate. X

199.3.2 Way of application *describe how test substance was applied and state solvent, e.g. "dissolved in medium", "as impregnation on paper discs" or other*

The test article was dissolved in sterile purified water.

199.3.3 Pre-incubation time Only Experiment two included a pre-incubation step for the tests with metabolic activation. The test substance (or control substance), bacteria and S-9 mix were mixed together and incubated for 1 hour at 37 °C before the addition of 2.5 ml molten agar at 46 °C. Plating of these treatments then proceeded as for normal plate-incorporation procedure.

199.3.4 Other modifications *e. g. addition of catalase, peroxidase or other enzymes*

Not applicable

199.4 Examinations *see tables in appendix for examinations and results*

199.4.1 Number of cells evaluated *give number (i.e. for micronucleus test, chromosome aberrations)*

Colonies were counted electronically using a Seescan Colony Counter and the background lawn inspected for signs of toxicity.

199.5 Statistical analysis The m-statistic was calculated to check that all the data were Poisson-distributed, and the Dunnett's test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis.

4 RESULTS AND DISCUSSION

Describe findings. If appropriate, include table. Sample tables are given below.

4.1 Genotoxicity *Non-entry field*

4.1.1 without metabolic activation No

There was no evidence of genotoxicity in either Experiment 1 or Experiment 2 in the absence of metabolic activation.

If yes, give concentrations with positive result

4.1.2 with metabolic activation No

There was no evidence of genotoxicity was observed in either Experiment 1 or Experiment 2 in the presence of metabolic activation.

If yes, give concentrations with positive result

4.2 Cytotoxicity Yes

Evidence of toxicity was observed in all Experiment 1 treatments of 1000 µg/plate. Some evidence of toxicity was also observed following strain TA102 treatments of 200 µg/plate in the presence of S-9 only.

Section A6.6.1	**Genotoxicity in vitro**
Annex Point IIA6.6.1	*Specify section no., heading, route and test system as appropriate*
IUCLID: 5.5/01	**A6.6.1(01), *In-vitro* Gene Mutation Study in Bacteria**

In Experiment 2, toxicity was observed following all treatments (with and without metabolic activation) of 800 µg/plate. Some treatments in the presence of S-9 at lower doses also produced evidence of toxicity.
The higher degree of toxicity observed with Experiment 2 treatments of S-9 was attributed to the use of a preincubation step, which allowed an enhanced exposure of the bacteria to the test article.

If yes, give concentrations with positive result

5. APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods *Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines*

Copper II sulphate pentahydrate was assayed for mutation in 5-histaidine requiring strains (TA98, TA100, TA1537 and TA102) of *Salmonella typhimurium*, both in the presence and absence of metabolic activation by Aroclor 1254-induced rat liver post-mitochondrial fraction (S-9) in 2 separate experiments. Following a range finding study, two experiments were carried out with concentrations of 1.6, 8, 40, 200 and 1000 µg/l in experiment one and 50, 100, 200, 400 and 800 µg/l in experiment two. The tests were carried out in triplicate. Both positive and negative controls were included. X X X X

The study complied with the following guidelines and was conducted in accordance with GLP;

OECD Guidelines 471
EC Directive 2000/32 Annex V Test B14 UKEMS Guidelines

5.2 Results and discussion *Summarize relevant results; discuss dose-response relationship.*

None of the dose concentrations in any of the test strains in either the absence or presence of S-9 resulted in an increase in revertant numbers that were statistically significant at the 1% level when analysed using a Dunnett's test. It was therefore concluded that copper II sulphate pentahydrate was unable to induce mutation in 5 strains of *S. typhimurium*, when tested at concentrations extending to the toxic range, in both the absence and presence of rat liver metabolic activation system.

5.3 Conclusion *Non entry field*

5.3.1 Reliability *Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4*

1

Section A6.6.1	**Genotoxicity in vitro**
Annex Point IIA6.6.1	*Specify section no., heading, route and test system as appropriate*
IUCLID: 5.5/01	**A6.6.1(01), *In-vitro* Gene Mutation Study in Bacteria**
5.3.2 Deficiencies	No *(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)*

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 Dec.2004
Materials and Methods	Agree with applicant's version. **Comments:** • Concentrations (3.3.1): the noted concentrations are not in µg/l but in **µg/plate**. • Materials and Methods (5.1): - Copper II sulphate pentahydrate was assayed for mutation in 5-~~histidine~~ **histidine** requiring strains (TA98, TA100, TA1537 and TA102 + **TA1535**) of *Salmonella typhimurium*... - the noted concentrations are not in µg/l but in **µg/plate**.
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	

Table 6.6.1_1 POSITIVE CONTROLS USED IN BACTERIAL REVERSE MUTATION ASSAY

CHEMICAL	STOCK* CONCENTRATION (µg/ml)	FINAL CONCENTRATION (µg/plate)	USE	
			STRAINS	S-9
2-nitrofluorene	500	50	TA98	-
Sodium azide	20	2	TA100 TA1535	-
9-aminoacridine	500	50	TA1537	-
Glutaraldehyde	250	25	TA102	-
2-aminoanthracene	50	5	At least one strain	+

* With the exception of sodium azide and glutaraldehyde, which were prepared in water, all stock solutions were prepared in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO) and stored in aliquots at 1-10 °C in the dark

Section A6.6.2 Annex Point 6.6.2 IUCLID: 5.5/02	A6.6.2, *In-vitro* Cytogenicity in Mammalian Cells	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Under normal physiological conditions, the concentration of free copper is extremely low *in vivo* and the majority of the copper is bound to ceruplasmin and albumin (See Section 6.2). In addition, cells contain high concentrations of potent antioxidants (e.g. glutathione). Therefore, the biological relevance of any *in vitro* observations would be uncertain where high concentration of the free ion would be available in cell culture growth medium. From reviews of public domain data (WHO, 1998; ACP, 1999), there is conflicting evidence regarding the activity of copper in cell based assays for genotoxicity, however, due to the relevance of such studies in determining the genotoxicity potential of copper it is considered not appropriate or applicable to use these studies for compounds where the free ion is the active substance. With due consideration to the above, two *in vivo* studies were conducted that follow internationally agreed guidelines and are conducted to GLP (See Section 6.6.4 and 6.6.5). These studies were negative and the existence of these two studies (with a quality criteria of 1) negates the need for *in vitro* mammalian cell gene mutation assays.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

Section A6.6.2 Annex Point 6.6.2 IUCLID: 5.5/02	A6.6.2, *In-vitro* Cytogenicity in Mammalian Cells
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 Déc.2004
Evaluation of applicant's justification	Applicant’s justification in the first paragraph is not acceptable but the justification of the second paragrah is correct.
Conclusion	Acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.3 Annex Point 6.6.3 IUCLID: 5.5/03	A6.6.3, *In-vitro* Gene Mutation Assay in Mammalian Cells	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [X]** **Other justification [ ]**	
Detailed justification:	Under normal physiological conditions, the concentration of free copper is extremely low *in vivo* and the majority of the copper is bound to ceruplasmin and albumin (See Section 6.2). In addition, cells contain high concentrations of potent antioxidants (e.g. glutathione). Therefore, the biological relevance of any *in vitro* observations would be uncertain where high concentration of the free ion would be available in cell culture growth medium. From reviews of public domain data (WHO, 1998; ACP, 1999), there is conflicting evidence regarding the activity of copper in cell based assays for genotoxicity, however, due to the relevance of such studies in determining the genotoxicity potential of copper it is considered not appropriate or applicable to use these studies for compounds where the free ion is the active substance. With due consideration to the above, two *in vivo* studies were conducted that follow internationally agreed guidelines and are conducted to GLP (See Section 6.6.4 and 6.6.5). These studies were negative and the existence of these two studies (with a quality criteria of 1) negates the need for *in vitro* mammalian cell gene mutation assays.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)* Not applicable	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	

Section A6.6.3 Annex Point 6.6.3 IUCLID: 5.5/03	A6.6.3, *In-vitro* Gene Mutation Assay in Mammalian Cells
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 Déc.2004
Evaluation of applicant's justification	Applicant’s justification in the first paragraph is not acceptable but the justification of the second paragrah is correct.
Conclusion	Acceptable
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.6.4 **Annex Point IIA6.6.4** **IUCLID: 5.6/01**	**Genotoxicity in vivo – Mouse Micronucleus Test** *Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)* **A6.4.4(01), In-vivo Mutagenicity Study**	
	200 REFERENCE	**Official use only**
200.1 Reference	*Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages) If necessary, copy field and enter other reference(s).* [redacted]	
200.2 Data protection	Yes *(indicate if data protection is claimed)*	
200.2.1 Data owner	[redacted]	
200.2.2 Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]	
	201 GUIDELINES AND QUALITY ASSURANCE	
201.1 Guideline study	Yes – the study following the following guidelines: EEC Annex V test B12. *(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*	X
201.2 GLP	Yes *(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)*	
201.3 Deviations	Yes Following test termination, test animals were sacrificed and the bone marrow extracted from both femurs of each test animal. However with one test animal (2338) only one femur was aspirated. This was not considered to have affected the outcome of the study. *(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")*	X
	202 MATERIALS AND METHODS *In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.*	
202.1 Test material	Copper sulphate *or give name used in study report*	X
202.1.1 Lot/Batch number	*List lot/batch number if available* A668269 350	
202.1.2 Specification	As given in section 2	

Section A6.6.4 **Annex Point IIA6.6.4** **IUCLID: 5.6/01**		**Genotoxicity in vivo – Mouse Micronucleus Test** *Specify section no., heading, route and species as appropriate* *Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)* **A6.4.4(01), In-vivo Mutagenicity Study**
		(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):
202.1.2.1	Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* blue crystalline substance
202.1.2.2	Purity	*Give purity in % active substance* 99-100.5 %
202.1.2.3	Stability	*Describe stability of test material* Stable at room temperature
202.1.2.4	Maximum tolerable dose	*usually the dose applied in single dose application* 338 mg/kg
202.2 Test Animals		Non-entry field
202.2.1 Species		Mouse
202.2.2 Strain		Out-bred CD-1
202.2.3 Source		[redacted]
202.2.4 Sex		Male and female
202.2.5 Age/weight at study initiation		Ages ranged from 35-42 days for both males and females. Bodyweight ranged from 24-30 g and 21-26 g for males and females respectively.
202.2.6 Number of animals per group		15 males and 15 females were treated with the test substance (this includes and additional 5 mice per sex to be used in the event of deaths among similarly dosed animals), 10 males and 10 females were treated with the negative control and 5 males and 5 females were treated with the positive control.
202.2.7 Control animals		Yes
202.3 Administration/ Exposure		Oral *Fill in respective route in the following, delete other routes*
202.3.1 Number of applications		2
202.3.2 Interval between applications		24 h
202.3.3 Postexposure period		Test animals were sacrificed at either 24 or 48 hours following the second dose administration.
		Oral
202.3.4 Type		By gavage
202.3.5 Concentration		Following a range finding study the test concentration was 447 mg/kg
202.3.6 Vehicle		Purified water

Section A6.6.4	**Genotoxicity in vivo – Mouse Micronucleus Test**
Annex Point IIA6.6.4	*Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)*
IUCLID: 5.6/01	**A6.4.4(01), In-vivo Mutagenicity Study**
202.3.7 Total volume applied	20 ml/kg
202.3.8 Controls	Cyclophosphamide (CPA) was dissolved in purified water at 4 mg/ml to serve as a positive control, and administered at 80 mg/kg with a dose volume of 20 ml/kg. The positive control was administered as a single dose. The negative control was purified water administered twice at the same sampling points as the test substance.
202.4 Examinations	Non entry field
202.4.1 Clinical signs	No
202.4.2 Tissue	Bone marrow
202.4.3 Number of animals and time points	Test substance and vehicle treated mice were sacrificed in groups of 5 male and 5 female after 24 or 48 hours; CPA mice were sacrificed after 24 hours.
202.4.4 Number of cells	Initially the relative proportions of polychromatic erthrocytes and normochromatic erythrocytes were determined until a total of at least 1000 cells had been analysed. Counting continued until at least 2000 polychromatic erythrocytes had been observed.
202.4.5 Type of cells	Erythrocytes in bone marrow
202.4.6 Parameters	Polychromatic/normochromatic erythrocytes ratio
	4 RESULTS AND DISCUSSION
	Describe findings. If appropriate, include table. Sample tables are given below.
4.0 Clinical signs	*No effects / describe significant effects referring to data in results table*
	Not reported

Section A6.6.4	Genotoxicity in vivo – Mouse Micronucleus Test
Annex Point IIA6.6.4 **IUCLID: 5.6/01**	*Specify section no., heading, route and species as appropriate* *Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)* **A6.4.4(01), In-vivo Mutagenicity Study**

No effects / describe significant effects referring to data in results table

Mice treated with copper II sulphate pentahydrate exhibited polychromatic/normochromatic erythrocytes (PCE/NCE) ratios which were decreased compared to concurrent vehicle controls at 24 hour sampling point. This is indicative of cellular toxicity and evidence of the test substance penetration into the bone marrow. Mice sampled at 48 hours after being treated with copper II sulphate pentahydrate exhibited ratios which were similar to those in the vehicle controls. The number of micronucleated PCE seen at both sampling times were similar to those seen in the controls and were not significantly different by x2 analysis.

The positive control induced a statistically significant increase in the frequency of micronucleated polychromatic erythrocytes

See Table A6.6.4_1 Summary of Group Mean Data.

4.1	**Haematology / Tissue examination**	
4.2	**Genotoxicity**	No

If genotoxic give effect dose

Section A6.6.4 **Annex Point IIA6.6.4** **IUCLID: 5.6/01**		**Genotoxicity in vivo – Mouse Micronucleus Test** *Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)* **A6.4.4(01), In-vivo Mutagenicity Study**	
		5.0 APPLICANT'S SUMMARY AND CONCLUSION	
5.1	**Materials and methods**	*Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines* Copper II sulphate pentahydrate was assayed *in vivo* in a mouse bone marrow micronucleus test at a single dose level of 447 mg/kg (113.76 mg Cu/kg) for two consecutive days to groups of 5 male and 5 female mice sacrificed 24 or 48 hours after the second administration. Both negative (purified water) and positive controls (cyclophosphamide) were included in the study. The study was conducted in according to EEC Annex V test B12 guidelines and in compliance with GLP.	
5.2	**Results and discussion**	*Summarize relevant results; discuss dose-response relationship.* Slides from all dose and control groups sacrificed after 24 and 48 hours were analysed. Negative control mice exhibited normal ratios of PCE to NCE (normochromatic erythrocytes) and normal frequencies of micronucleated PCE within historical negative control ranges. Mice treated with copper sulphate exhibited ratios of PCE to NCE that were decreased compared to concurrent vehicle controls when sampled after 24 hours, which was taken as evidence of copper sulphate absorption into the bone marrow. The PCE/NCE ratios seen in animals sampled at 48 hours were similar to those seen in the vehicle controls. Mice treated with copper sulphate exhibited frequencies of micronucleated PCE which were similar to vehicle controls at all sampling times. There were no instances of statistically significant increases in micronucleus frequency for any group receiving the test chemical at either sampling point. It was concluded that copper sulphate did not induce micronuclei in the polychromatic erythrocytes of the bone marrow of mice treated with 447 mg/kg/day.	X
5.3	**Conclusion**	*Non entry field*	
5.3.1	Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4* 1	
5.3.2	Deficiencies	No *(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)*	

Section A6.6.4	**Genotoxicity in vivo – Mouse Micronucleus Test**
Annex Point IIA6.6.4	*Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)*
IUCLID: 5.6/01	**A6.4.4(01), In-vivo Mutagenicity Study**

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 Dec. 2004
Guidelines and quality assurance	• In paragraph guideline study (2.1): refer to EEC Annex V test B12. **We can add OECD Guidelines 474** • In paragraph Deviations (2.3): **Only one $CuSO_4$ test concentration was used.**
Materials and Methods	Agree with applicant's version. **Comment:** • Test material (3.1) Copper sulphate **pentahydrate**.
Results and discussion	Agree with applicant's version. **Comments (5.2):** • *Mice treated with copper sulphate exhibited ratios of PCE to NCE that were decreased compared to concurrent vehicle controls when sampled after 24 hours, which was taken as evidence of copper sulphate absorption into the bone marrow.* **This is also indicative of cellular toxicity.**
Conclusion	Agree with applicant's version.
Reliability	2 There is a deficiency in the methodology used, when compared with the requirements of currently accepted guidelines for the conduct of genotoxicity studies (e.g. EEC Annex V test B12): Only one $CuSO_4$ test concentration was used. If applicant considers that the substance has specific biological activities at low non-toxic doses and that highest dose may be defined as a dose that produces some indication of toxicity in the bone marrow, he must provide a justification.
Acceptability	Acceptable
Remarks	

Section A6.6.4	Genotoxicity in vivo – Mouse Micronucleus Test
Annex Point IIA6.6.4 **IUCLID: 5.6/01**	*Specify section no., heading, route and species as appropriate* *Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)* **A6.4.4(01), In-vivo Mutagenicity Study**

	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	

Table 6.6.4_1 Summary Of Group Mean Data

TREATMENT GROUP (mg/kg X2)	SAMPLING POINT (hours)	*SEX*	MEAN RATIO PCE/NCE	GROUP MEAN FREQUENCY OF MICRONUCLEATED PCE (per 1000)	
				PER SEX	PER TREATMENT GROUP
Vehicle control (purified water)	24	Male	1.07	0.40	0.35
		Female	1.20	0.30	
	48	Male	1.44	0.38	0.33
		Female	0.83	0.30	
447 (copper sulphate pentahydrate)	24	Male	0.70	0.60	0.50
		Female	0.84	0.40	
	48	Male	1.12	0.50	0.45
		Female	1.32	0.40	
Positive control (CPA – single dose only)	24	Male	0.52	26.87	28.07
		Female	0.48	29.27	

Section A6.6.4 **Genotoxicity in vivo – Unscheduled DNA Synthesis**

Annex Point IIA6.6.4

IUCLID: 5.6/02

Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)

A6.6.4(02), *In-vivo* Mutagenicity Study

Official use only

203 REFERENCE

203.1 Reference

Author(s), year, title, laboratory name, laboratory report number, report date (if published, list journal name, volume: pages) If necessary, copy field and enter other reference(s).

[redacted]

GLP, Unpublished.

203.2 Data protection Yes

(indicate if data protection is claimed)

203.2.1 Data owner *Give name of company*

[redacted]

203.2.2 Criteria for data protection *Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:*

Data submitted to the MS after 13 May 2000 on existing [a.s. / b.p.] for the purpose of its [entry into Annex I/IA / authorisation]

204 GUIDELINES AND QUALITY ASSURANCE

204.1 Guideline study No – The study was not conducted in accordance with any internationally recognised guidelines. However, the methods employed in the study are comparable to OECD Guidelines 486 "Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells *in vivo*".

(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")

204.2 GLP Yes

(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)

204.3 Deviations No

(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")

205 MATERIALS AND METHODS

In some fields the values indicated in the EC or OECD test guidelines are given as default values. Adopt, change or delete these default values as appropriate.

Test material Copper sulphate X

or give name used in study report

3.1.0 Lot/Batch number *List lot/batch number if available*

Section A6.6.4 **Annex Point IIA6.6.4** **IUCLID: 5.6/02**	**Genotoxicity in vivo – Unscheduled DNA Synthesis** *Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)* **A6.6.4(02), *In-vivo* Mutagenicity Study**
	A668269 350
205.1.1 Specification	As given in section 2 *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):*
205.1.1.1 Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Blue crystalline solid
205.1.1.2 Purity	*Give purity in % active substance* 99.0 - 100.5%
205.1.1.3 Stability	*Describe stability of test material* Stable at room temperature
205.1.1.4 Maximum tolerable dose	*usually the dose applied in single dose application* <2000 mg/kg
205.2 Test Animals	Non-entry field
205.2.1 Species	Rat
205.2.2 Strain	Wistar
205.2.3 Source	[redacted]
205.2.4 Sex	Male
205.2.5 Age/weight at study initiation	Test animals were 41-51 days old with a bodyweight range of 189-254 g.
205.2.6 Number of animals per group	6 animals
205.2.7 Control animals	Yes
205.3 Administration/ Exposure	Oral *Fill in respective route in the following, delete other routes*
205.3.1 Number of applications	1 *give reasons for more than one application*
205.3.2 Interval between applications	Not applicable
205.3.3 Postexposure period	12-14 hours for Experiment 1, 2-4 hours for Experiment 2
	Oral
205.3.4 Type	Gavage
205.3.5 Concentration	Following a range finding study dose concentrations were set at 632.5 mg/kg and 2000 mg/kg (equivalent to 161 or 509 mg Cu/kg) See Table A6.6.4_1 for further information.
205.3.6 Vehicle	Purified water

Section A6.6.4 **Annex Point IIA6.6.4** **IUCLID: 5.6/02**	**Genotoxicity in vivo – Unscheduled DNA Synthesis** *Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)* **A6.6.4(02), *In-vivo* Mutagenicity Study**
205.3.7 Concentration in vehicle	Not reported
205.3.8 Total volume applied	10 ml/kg
205.3.9 Controls	Purified water was used as the negative control. 7.5 mg/ml 2-Acetamidofluorene (2-AAF) suspended in corn oil was the positive control for the 12-14 hour experiment. 1.0 mg/ml dimethylnitrosamine (DMN) dissolved in purified water was used as the positive control for the 2-4 hour experiment. Both positive controls were dosed at 10 ml/kg giving achieved doses of 75 mg/kg and 10 mg/kg for the 12-14 and 2-4 hour experiments respectively. See Table A6.6.4_1 for further information.
205.4 Examinations	Non entry field
205.4.1 Clinical signs	No
205.4.2 Tissue	Liver
205.4.3 Number of animals	Cultures from 5 animals were taken
205.4.4 Number of cells	150,000 viable cells/ml
205.4.5 Time points	12-14 hours in Experiment 1, 2-4 hours in Experiment 2
205.4.6 Type of cells	hepatocytes from the liver
205.4.7 Parameters	After approximately 12-14 hours (experiment 1) or 2-4 hours (Experiment 2) after dose administration the animals were sacrificed and the livers perfused with collagenase to provide a primary culture of hepatocytes. Cultures were made from 5 animals in each dose group and were treated with [^{3}H] thymidine. Six slides were prepared with fixed hepatocytes and of these, 3 were dipped in photographic emulsion to prepare autoradiograms. Slides were examined microscopically after development of the emulsion and staining, and the net grain count (NG) and the number of grains present in the nucleus minus the mean number of grains in 3 equivalent areas of cytoplasm was determined for each of the at least 2 of the three slides from each animal in each dose group. **4 RESULTS AND DISCUSSION** *Describe findings. If appropriate, include table. Sample tables are given below.*

Section A6.6.4	**Genotoxicity in vivo – Unscheduled DNA Synthesis**
Annex Point IIA6.6.4 **IUCLID: 5.6/02**	*Specify section no., heading, route and species as appropriate* *Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)* **A6.6.4(02), *In-vivo* Mutagenicity Study**
4.1 Clinical signs	*No effects / describe significant effects referring to data in results table* Not reported
4.2 Haematology / Tissue examination	*No effects / describe significant effects referring to data in results table* Treatment with copper II sulphate pentahydrate at doses up to 2000 mg/kg yielded group mean net grain counts of less than 0, producing group mean net grain counts over the 2 experiments in the range of -1.0 to -3.2, well below the value of 5 net grain counts required for a positive response. No more than 1.0% of the cells were seen in repair at any dose of test substance. The data obtained indicate that oral treatment of male rats with 632.5 or 2000 mg/kg copper II sulphate pentahydrate did not result in increased unscheduled DNA synthesis in hepatocytes isolated approximately 12-14 or 2-4 hours after dosing. The positive control chemicals (2-AAF and DMN) induced increases in the group mean net grain count of 5 or more (12.7 and 17.2 respectively), and 50% or more of the cells (90% and 99.6% respectively) had net grain counts of 5 or more. This result showed that the test system was sensitive to 2 known DNA damaging agents requiring metabolism for their action and that the experiment was valid. The group mean net grain count for the vehicle-treated animals was less than 0 (-1.3 and -2.2 or Experiments 1 and 2 respectively). For further information on results, please refer to Table A6.6.4_2.
4.3 Genotoxicity	No *If genotoxic give effect dose*
4.4 Other	*Describe any other significant effects* Not applicable

5 APPLICANT'S SUMMARY AND CONCLUSION

5.1 Materials and methods	*Give concise description of method; give test guidelines no. and discuss relevant deviations from test guidelines* Copper II sulphate pentahydrate was tested for its ability to induced unscheduled DNA systhesis (UDS) in the livers of orally dosed male rats using an *in vivo/in vivo* procedure. Groups of 6 male rats were treated once with copper sulphate at 632.5 or 2000 mg/kg by oral gavage at a dose volume of 10 ml/kg. For the negative control, a further 6 male rats received purified water as a negative control at the same dose volume. Positive control animals for the12-14 hour experiment, 6 male rats were dosed orally with 75

Section A6.6.4	Genotoxicity in vivo – Unscheduled DNA Synthesis
Annex Point IIA6.6.4	*Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)*
IUCLID: 5.6/02	**A6.6.4(02), *In-vivo* Mutagenicity Study**

mg/kg 2-acetamidofluorene, suspended in corn oil. Dimethylmitrosamine, dissolved in purified water, was the positive control for the 2-4 hour experiment.

Approximately 12-14 hours (experiment 1) or 2-4 hours (Experiment 2) after dose administration the animals were sacrificed and the livers perfused with collagenase to provide a primary culture of hepatocytes. The net grain count, number of grains present in the nucleus minus the mean number of grains in 3 equivalent areas of cytoplasm were determined.

5.2 Results and discussion *Summarize relevant results; discuss dose-response relationship.*

Negative control animals gave a group mean net grain of less than 0 with no cells in repair. Group mean net grain values were increased by both positive controls to more than 5 with more than 50% of cells found to be in repair. This was consistent with historical control data.

Treatment with 632.5 or 2000 mg/kg copper sulphate pentahydrate (equivalent to 161 or 509 mg Cu/kg) did not produce a group mean net grain value greater than -1.0 nor were any more than 1.0% cells found in repair at either dose.

It was concluded that copper II sulphate pentahydrate has no genotoxic activity detectable in this test system under the experimental conditions employed.

5.3 Conclusion *Non entry field*

5.3.1 Reliability *Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4*

1

5.3.2 Deficiencies No

The study was not conducted according to an internationally recognised guideline although it is GLP compliant.
When compared with generally accepted principles to be applied in OECD Guidelines 486 Genetic Toxicology: DNA Damage and Repair/Unscheduled DNA Synthesis in Mammalian Cells *in vivo*, it is apparent that the study follows these guidelines and there are no apparent deficiencies.

(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

Section A6.6.4	**Genotoxicity in vivo – Unscheduled DNA Synthesis**
Annex Point IIA6.6.4	*Specify section no., heading, route and species as appropriate Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)*
IUCLID: 5.6/02	**A6.6.4(02), *In-vivo* Mutagenicity Study**

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	31 Déc.2004
Materials and Methods	Agree with applicant's version. Comments: • Test material (3.1) Copper sulphate **pentahydrate**.
Results and discussion	Agree with applicant's version.
Conclusion	Agree with applicant's version.
Reliability	2 There is a deficiency in the methodology used, when compared with the requirements of currently accepted guidelines for the conduct of genotoxicity studies (e.g. OECD Guidelines 486): Only males were used, rather than both sexes and no justification was provided.
Acceptability	Acceptable
Remarks	Error in table was corrected in red and bold.

Section A6.6.4 — **Genotoxicity in vivo – Unscheduled DNA Synthesis**

Annex Point IIA6.6.4

IUCLID: 5.6/02

Specify section no., heading, route and species as appropriate
Specify type of test (micronucleus test, cytogenetic in-vivo-test [chromosomal analysis], UDS in vivo or other special investigation)

A6.6.4(02), *In-vivo* Mutagenicity Study

EXPERIMENT: 2-4 HOUR SACRIFICE TIME

DOSE (mg/kg)	NET NUCLEAR GRAIN COUNT		NET GRAIN COUNT OF CELLS IN REPAIR		PERCENT OF CELLS IN REPAIR (Net Grain Count ≥5)	
	Mean	SD	Mean	SD	Mean	SD
0 water	-2.2	0.3	0	-	-	-
632.5 copper II sulphate pentahydrate	-2.2	0.2	0	-	-	-
2000 copper II sulphate pentahydrate	-3.2	0.5	0	-	-	-
10 DMN	17.2	2.8	17.3	2.7	99.6	0.9

	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	

TABLE A6.6.4_1 SUMMARY OF ADMINISTERED DOSES

TREATMENT	DOSE (mg/kg)	DOSE VOLUME (mg/kg)	NUMBER OF ANIMALS DOSED	
			EXPERIMENT 1 (12-14 HOURS)	EXPERIMENT 2 (2-4 HOURS)
Purified water	0	10	3+3	3+3
Copper II sulphate pentahydrate	632.5	10	3+3	3+3
Copper II sulphate pentahydrate	2000	10	3+3	3+3
2-AAF	75	10	3+3	-
DMN	10	10	-	3+3
Copper II sulphate pentahydrate	2000	10	2+1	0+3

A6.6.4_2 SUMMARY OF RESULTS

EXPERIMENT 1: 12-14 HOUR SACRIFICE TIME

DOSE (mg/kg)	NET NUCLEAR GRAIN COUNT		NET GRAIN COUNT OF CELLS IN REPAIR		PERCENT OF CELLS IN REPAIR (Net Grain Count ≥5)	
	Mean	SD	Mean	SD	Mean	SD
0 water	-1.3	0.6	0	-	-	-
632.5 copper II sulphate pentahydrate	-1.3	0.3	10.2	6.4	0.6	0.9
2000 copper II sulphate pentahydrate	-1.0	0.3	5.5	0.9	1.0	1.0
75 2-AAF	12.7	0.9	13.7	0.8	90.0	4.0

EXPERIMENT: 2-4 HOUR SACRIFICE TIME

DOSE (mg/kg)	NET NUCLEAR GRAIN COUNT		NET GRAIN COUNT OF CELLS IN REPAIR		PERCENT OF CELLS IN REPAIR (Net Grain Count ≥5)	
	Mean	SD	Mean	SD	Mean	SD
0 water	-2.2	0.3	0	-	-	-
632.5 copper II sulphate pentahydrate	-2.2	0.2	0	-	-	-
2000 copper II sulphate pentahydrate	-3.2	0.5	0	-	-	-
10 DMN	17.2	2.8	17.3	2.9	99.6	0.9

Section 6.6.5 **Annex Point 6.6.5**	**If negative in 6.6.4 but positive in-vitro tests then a second in-vivo study is required.**	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [ ]**	
Detailed justification:	The TGD for Guidance on Data Requirements for Active Substances and Biocidal Products (Version 4.3.2, October 2000) state that if a negative test exists in 6.6.4 but positive tests exist in some of the *in-vitro* tests then it is necessary to undertake a second *in-vivo* study to examine whether mutagenicity or evidence of DNA damage can be demonstrated in tissue other than bone marrow. The results presented in A6.6.1 (genotoxic *in-vitro* study), and A6.6.4 (*in vivo* micronucleus and unscheduled DNA synthesis) are all negative. There is, therefore, no need to provide any additional data under this section, A6.6.5.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
Date	**EVALUATION BY RAPPORTEUR MEMBER STATE** 31 Déc.2004	

Section 6.6.5 **Annex Point 6.6.5**	**If negative in 6.6.4 but positive in-vitro tests then a second in-vivo study is required.**
Evaluation of applicant's justification	Agree with applicant's justification. **Comment:** The TGD for Guidance on Data Requirements for Active Substances state that : - 6.6.4: if positive in 6.6.1, 6.6.2 or 6.6.3, then an *in vivo* mutagenicity will be required (bone marrow assay for chromosomal damage or a micronucleus test) - 6.6.5: if negative in 6.6.4 but positive in some of *in vitro* tests then it is necessary to undertake a second *in vivo* study to examine whether mutagenicity or evidence of DNA damage can be demonstrated in tissue other than bone marrow. **In vivo micronucleus could be considered as 6.6.4 and unscheduled DNA synthesis as 6.6.5.**
Conclusion	Acceptable
Remarks	Not all the studies available for genotoxicty of copper compounds are reported in the dossier. Other studies with positive results are not reported even in IUCLID. But given the database available no study is required.
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Copper Oxide

Section 6.6.6 **Annex Point 6.6.6**	**If positive in 6.6.4 a test to assess possible germ cell effects may be required**

	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [...]**	
Detailed justification:	The TGD data requirements stipulate that if a positive *in-vivo* result is obtained under Section 6.6.4 it is necessary to carry out a further study to assess possible germ cell effects. The data presented in Section 6.6.4 provide two negative *in-vivo* results and therefore a further study on the effects on germ cells is not required.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	31 Déc.2004	
Evaluation of applicant's justification	Agree with applicant's	
Conclusion	justification. Acceptable	
Remarks	Not all the studies available for genotoxicty of copper compounds are reported in the dossier. Other studies with positive results are not reported even in IUCLID. But given the database available no study is required.	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*	

Section 6.6.6 / Annex Point 6.6.6 — If positive in 6.6.4 a test to assess possible germ cell effects may be required

Date *Give date of comments submitted*

Evaluation of applicant's justification *Discuss if deviating from view of rapporteur member state*

Conclusion *Discuss if deviating from view of rapporteur member state*

Remarks

Section 6.6.7 Annex Point 6.6.7	Genotoxicity of metabolites of concern	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA *As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.* *If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable*	Official use only
Other existing data [X] **Limited exposure [ ]**	**Technically not feasible [ ]** **Scientifically unjustified [ ]** **Other justification [...]**	
Detailed justification:	In view of the negative *in-vitro* studies presented in A6.6.1 and the absence of metabolites of concern formed in mammals, it is not necessary to present any additional data under A6.6.7.	
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*	
	Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	31 Déc.2004	
Evaluation of applicant's justification	Agree with applicant's justification.	
Conclusion	Acceptable	
Remarks		
	COMMENTS FROM OTHER MEMBER STATE	
Date	*(specify) Give date of comments submitted*	

Section 6.6.7 Genotoxicity of metabolites of concern

Annex Point 6.6.7

Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.7
Annex Point IIA, VI. 6.7

A6.7 Carcinogenicity

JUSTIFICATION FOR NON-SUBMISSION OF DATA

Official use only

As outlined in the TNsG on data requirements, the applicant must always be able to justify the suggested exemptions from the data requirements. The justifications are to be included in the respective location (section) of the dossier.
If one of the following reasons is marked, detailed justification has to be given below. General arguments are not acceptable

Other existing data [ ] **Technically not feasible [ ]** **Scientifically unjustified [ ]**

Limited exposure [ ] **Other justification [X]**

Detailed justification:

All available studies on the carcinogenicity of copper are public domain studies and therefore, taken in isolation are of limited value to ascertain the carcinogenic potential copper compounds. This is due to the fact that these studies are limited due to shorter exposure periods (<2 years) and group sizes being small. However, when the 3 studies summarised below are assessed on an overall balanced approach, the information from these studies does give useful information as to the carcinogenic potential of copper compounds (See Table 6.7_1)

These results indicate that copper sulphate and other copper salts do not appear to have carcinogenic potential even at very high dose levels of up to 120 mg Cu/kg/bw/day [redacted]). The data in [redacted] are especially useful since positive control groups were added in this study and showed an induction of neoplasms in the rat, indicating that the exposure period (although not two years) was long enough for neoplasms to appear if you have a positive carcinogen. In addition, this study indicates that excess copper may have a protective effect on known carcinogens.

These animal carcinogenicity studies have been conducted with copper compounds. Short duration, small sample sizes and limited histopathologic examination limit the findings of the studies. Nevertheless, the findings of these studies do not raise concerns with respect to carcinogenic activity.

In addition, the available genotoxicity studies support the indication that copper compounds have no carcinogenic potential. The studies include Ames assays in *Salmonella typhimurium* on copper II sulphate pentahydrate; a

micronucleus study on copper II sulphate pentahydrate and an unscheduled DNA synthesis ex vivo study in rat liver on copper II sulphate.

The Ames tests indicated that copper sulphate had no mutagenic activity (No evidence of an increase in the incidence of micronuclei was detected in the mouse micronucleus study when mice were orally administered two doses of 447 mg/kg copper sulphate, 24 h apart There was also no evidence of unscheduled DNA synthesis in the rat liver (

These studies are consistent and show a lack of *in vitro* mutagenic activity or *in vivo* clastogenic potential associated with soluble copper compounds. The results of these studies do not highlight a concern regarding the genotoxic potential of copper compounds.

Available data on the genotoxicity and carcinogenicity of copper and its compounds have been considered against EU classification criteria as contained in Annex VI of Directive 67/548/EEC. The available data for copper compounds do not meet the criteria requiring classification for carcinogenicity.

Chronic toxicity investigations in these studies, and in particular, in indicate that, as in the 90-day rat study of NTP, 1993, the target organs for copper are the liver and kidney. These studies show substance-related adverse effects at concentrations of copper higher than the expected exposure levels resulting from copper-containing wood preservation for primary and secondary human exposure. In addition, the longer duration studies indicate that the adverse effects do not appear to become more severe over longer exposure periods (up to one year). This is probably due to the homeostatic control mechanisms present in animals which would regulate the uptake and excretion of copper on a daily basis (see Section 6.2 in Document IIA). As adverse effects are only observed at relatively high levels of copper outside the normal daily intake of copper for humans (up to 10 mg/day), new chronic studies extending over a 2 year time period is not expected to add further insight into the mechanisms of chronic toxicity of copper in

Section A6.7 Annex Point IIA, VI. 6.7	A6.7 Carcinogenicity
	humans. From the exposure Section in Document IIC, it can be seen that the systemic exposure of primary and secondary populations to copper is significantly lower than the usual dietary intake of copper by these populations (2-3 mg/day). Therefore, there is no need to conduct new combined chronic/carcinogenicity studies to OECD guideline 451/453 when considering copper oxide or copper carbonate as active substances used in wood preservation
Undertaking of intended data submission [ ]	*Give date on which the data will be handed in later (Only acceptable if test or study is already being conducted and the responsible CA has agreed on the delayed data submission.)*
	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	30 Nov.2004
Evaluation of applicant's justification	Agree with applicant's version.
Conclusion	Applicant's justification is acceptable.
Remarks	
	COMMENTS FROM OTHER MEMBER STATE *(specify)*
Date	*Give date of comments submitted*
Evaluation of applicant's justification	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Remarks	

Table 6.7_1 Summary of Carcinogenicity Data

Route	Species Strain Sex no/group	dose levels frequency of application	Tumours	Reference
Oral, diet 9 months	Rat, Sprague-Dawley, male 50 or 58 animals/group	1 ppm, 800 ppm (0.05, 40 mgCu/kg/bw/day)	Liver necrosis and transitional nodules in the liver (3/32) and transitional nodules in the liver (1/32) was observed at 40 mgCu/kg/bw/day whereas one kidney tumour (1/42) was observed in the low copper group (not thought significant). Decreased body weight gain and increased mortality were found in the high copper group. Exposure to known carcinogens increased the incidence of liver necrosis and transitional nodules and each induced a similar incidence of liver tumours in rats fed excess copper or copper-deficient diets. In the DMN group, 17/30 rats on the copper-deficient diet and kidney tumours compared to 0/29 given excess copper. The incidence of AAF-induced extrahepatic neoplasms was apparently reduced by the excess copper diet. (5/30 vs 11/27).	[redacted]
Oral drinking water 46 weeks	Mouse C57BL/6J, female 10-12 animals/group	198 mg/l (app. 10 mgCu/kg/bw/day)	The incidences of ovarian tumours after 46 weeks were 0/10, 0/12, 11/11 and 6/11 in the untreated controls, copper treated mice, DMBA-treated mice and DMBA-copper- treated mice respectively. This suggests that copper sulphate may possibly inhibit DMBA-induced tumour development. $CuSO_4$ had no effect on the incidence of DMBA-induced adenomas of the lung, lymphomas and breast tumours.	[redacted]
Oral diet, 30-44 weeks	Rat, Sprague-Dawley, male and female, 23- 26 animals/ group	0, 530 or 1600 ppm Cu (approx. 0, 27 or 80 mg Cu/kg b.w./day in males and 0, 40 or 120 mg Cu/kg b.w./day in females).	The growth of rats receiving 1600 ppm Cu as $CuSO_4$ was adversely affected, although organ weights were apparently unaffected (other than increased stomach weight in females). Well-defined abnormalities evident in the 1600 ppm treatment group included 'bronzed' kidneys, 'bronzed' or yellowish livers, hypertrophied ridges between cardiac and peptic portions of the stomach and blood in the intestinal tract. Histological examination revealed varying degrees of testicular degeneration in rats from both the 530 ppm and the 1600 ppm groups and effects on the liver were seen in both males and females. There were no reports of evidence of neoplasms in any treatment group.	[redacted]