Table A6.5- 7: **Summary of long term toxicity.**

Study type	Species	Route	Test material, Dose levels	NOEL	Reference
Carcinogenicity	Rat	Diet	Copper sulphate, 530, or 1600 ppm (= 27 or 80 mg Cu/kg bw/day) Potassium sodium copper chlorophyllin 0.1, 1 or 3% (= 2.7, 27 or 80 mg Cu/kg bw/day) Copper gluconate 1600 ppm (= 80 mg Cu/kg bw/day)	Not carcinogenic. NOEL for Potassium sodium copper chlorophyllin 3% (=80 mg Cu/kg bw/day) NOAEL for copper sulphate = 27 mg Cu/kg bw/day	**A6.5/01**
Carcinogen co-administration	Rat	Diet	*p*-dimethylaminobenzene at 0.9% diet with or without 0.5% copper acetate or 2% ferric citrate	Co-administration of copper markedly reduced the incidence of liver tumours caused by *p*-dimethylaminobenzene	Cross-reference: **A6.7/01**
Special study	Rat	Diet	Copper sulphate 2000 ppm Cu (= 200 mg Cu/kg bw/day)	Toxicity after 6 weeks, followed by regeneration of tissues and recovery by 15 weeks	**A6.5/02:** Haywood S (1980) The effect of excess dietary copper on the liver and kidney of the male rat. J. Comp. Path. 90: 217–232 (published).
Special study	Rat	Diet	Copper sulphate 3000, 4000, 5000 or 6000 ppm Cu (= 150, 200, 250 or 300 mg Cu/kg bw/day)	6000 ppm showed unsustainable liver damage by 6 weeks. Lower doses showed toxicity followed by regeneration of tissues and recovery	**A6.5/03:** Haywood S (1985) Copper toxicosis and tolerance in the rat I – changes in copper content of the liver and kidney. J. Path. 145: 149–158 (published).
Special study	Rat	Diet	Copper sulphate 3000 ppm Cu (= 250 mg Cu/kg bw/day) for 1 year Copper sulphate 3000 ppm Cu (= 250 mg Cu/kg bw/day) for 15 weeks, followed by 6000 ppm Cu for 3 weeks. Naïve rats given 6000 ppm after 15 weeks on control diet	Not carcinogenic after 1 year administration at 3000 ppm. Increasing the dose from 3000 ppm to 6000 ppm after 15 weeks showed no adverse effects. Treatment of naïve rats showed hepatocellular necrosis.	Cross-reference: **A6.7/02**

Section A6.6.1 *In-vitro* gene mutation study in bacteria

Annex Point IIA6.6

			Official use only
		65 REFERENCE	
65.1	**Reference**	[redacted] (1994): Study to determine the ability of copper II sulphate pentahydrate to induce mutation in five histidine-requiring strains of *Salmonella typhimurium*. [redacted]; Report no.: 456/31, June 21, 1994 (unpublished). Doc.No. 456/31	
65.2	**Data protection**	Yes	
65.2.1	Data owner	Spiess-Urania Chemicals GmbH, Hamburg, Germany	
65.2.2	Companies with letter of access	--	
65.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		66 GUIDELINES AND QUALITY ASSURANCE	
66.1	**Guideline study**	Yes OECD 471; EU method B14	
66.2	**GLP**	Yes (Certified laboratory)	
66.3	**Deviations**	No	
		67 MATERIALS AND METHODS	
67.1	**Test material**	Copper II sulphate pentahydrate	
67.1.1	Lot/Batch number	A668269350	
67.1.2	Specification	Not specified	
67.1.3	Purity	99.0 – 100.5 %	
67.1.4	Description	Blue crystalline solid	
67.1.5	Stability	Not stated	
67.2	**Study type**	Bacterial reverse mutation test	
67.2.1	Organism/cell type	*S. typhimurium:* TA 1535, TA 1537, TA 98, TA 100, TA 102	
67.2.2	Deficiencies / Proficiencies	Histidine requiring strains	?
67.2.3	Metabolic activation system	S9 mix Mammalian liver post-mitochondrial fraction (S9) prepared from male Sprague-Dawley rats induced with Aroclor 1254.	
67.2.4	Negative control	Vehicle only	

Section A6.6.1 *In-vitro* gene mutation study in bacteria

Annex Point IIA6.6

67.2.5	Positive control	TA 98: 2-nitrofluorene (without metabolic activation) TA100, TA1535: Sodium azide (without metabolic activation) TA1537: 9-aminoacridine (without metabolic activation) TA102: Glutaraldehyde (without metabolic activation) At least 1 strain: 2-Aminoanthracene (with metabolic activation)
67.3	**Application of test substance**	
67.3.1	Concentrations	Two independent experiments were performed using triplicate plates for each group: Exp. 1: 1.6, 8, 40, 200, and 1000 µg test item/plate (corresponding to 0.40722 – 254.51 µg Cu II/plate) Exp. 2: 50, 100, 200, 400, and 800 µg test item/plate (corresponding to 12.725 – 203.61 µg CU II/plate)
67.3.2	Way of application	The test substance was dissolved in sterile purified water. The platings were achieved by the following sequence of additions to 2.5 mL molten agar at 46°C: 0.1 mL bacterial culture 0.1 mL test agent solution 0.5 mL 10 % S-9 mix or buffer solution followed by rapid mixing and pouring on to Minimal Davis agar plates. When set, the plates were inverted and incubated at 37°C in the dark for 3 days. As the results of the first experiment were negative, treatments in the presence of S-9 mix in experiment 2 included a pre-incubation step, where the quantities of test chemical or control solution, bacteria and S-9 mix were mixed and incubated for 1 hour at 37°C before the addition of 2.5 mL molten agar at 46°C followed by the normal plate-incorporation procedure.
67.3.3	Pre-incubation time	None (Experiment 1) 1 hour at 37°C (Experiment 2, with metabolic activation)
67.3.4	Other modifications	None
67.4	**Examinations**	Number of revertant colonies and background lawn
		68 RESULTS
68.1	**Genotoxicity**	
68.1.1	Without metabolic activation	No The results are presented in Table A6.6.1- 1 and Table A6.6.1- 2.
68.1.2	With metabolic activation	No The results are presented in Table A6.6.1- 1 and Table A6.6.1- 2.

Section A6.6.1 *In-vitro* gene mutation study in bacteria

Annex Point IIA6.6

68.2	**Cytotoxicity**	Yes Evidence of toxicity was observed following all treatments at 1000 µg/plate in experiment 1 and at 800 µg/plate in experiment 2. In experiment 1, some evidence of toxicity was also observed following strain TA 102 treatments at 200 µg/plate in the presence of S9 mix only. In experiment 2, some treatments in the presence of S9 mix at test doses below 800 µg/plate also produced evidence of toxicity which was attributed to the use of a pre-incubation step.
		69 APPLICANT'S SUMMARY AND CONCLUSION
69.1	**Materials and methods**	The mutagenic potential of Copper II sulphate pentahydrate was tested in five strains of *Salmonella typhimurium* according to OECD 471 and EU method B14 .
69.2	**Results and discussion**	All solvent control values were within the range of the historical control; positive control chemicals induced large increases in revertant colonies in the appropriate strains and less than 5 % of plates were lost. No Copper II sulphate pentahydrate treatment, either in the absence or presence of S9 mix, gave rise to a statistically significant increase in revertant numbers when the data were analysed at the 1 % level using Dunnett's test.
69.3	**Conclusion**	Copper II sulphate pentahydrate is not genotoxic under the conditions of the test. The extrapolation from copper sulphate to other copper compounds is considered not be restricted in any way, since the moiety of interest is the copper ion itself, which may be expected to be released from both compounds during passage of the GI tract after oral uptake. Despite the somewhat limited bioavailability for poorly soluble copper compounds, the extrapolation from the readily bioavailable copper sulphate will only lead to a more conservative but nevertheless valid assessment.
69.3.1	Reliability	1
69.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	12/01/2005
Materials and Methods	3.2.2 – It is normal to have HIS requiring strains of S Typhimurium. This statement has nothing to do in the section "Deficiencies / Proficiencies" Agree with the applicant's version
Results and discussion	Agree with the applicant's version
Conclusion	Agree with the applicant's version

Reliability	1
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.1- 1: Relative reverse mutation rates in *S. typhimurium* after treatment with Copper II sulphate pentahydrate (experiment 1)

Concentration [µg per plate]	Relative reverse mutation rates[1]										Comments
	– S9					+ S9					
	TA98	TA100	TA1535	TA1537	TA102	TA98	TA100	TA1535	TA1537	TA102	
Historical control values mean (range)	24 (7–41)	107 (59–154)	12 (1-24)	10 (1-19)	285 (168-402)	29 (9–50)	124 (65-183)	16 (1-32)	11 (1-21)	306 (126-485)	All control values were within the range of the historical control.
Control (mean number of revertant colonies)	23.0 ± 4.6	104.4 ± 11.7	16.0 ± 2.5	16.2 ± 2.2	260.6 ± 23.7	26.6 ± 4.6	122.8 ± 6.1	24.8 ± 4.3	16.6 ± 1.3	345 ± 26.3	
1.6	0.7	-	1.1^	1.1^	1.1^	1.2^	-	0.9	0.9	1.0^	Evidence of toxicity was observed following all treatments at 1000 µg/plate. Some evidence of toxicity was also observed following strain TA 102 treatments at 200 µg/plate in the presence of S9 mix only.
8.0	1.0	1.0^	0.9	1.1	1.1	1.0	1.1^	0.9	0.9	1.0	
40	0.6	1.0	1.1	1.0	1.0	0.8	1.0	0.9	1.0^	0.9	
200	0.8	0.9	1.0	1.1	0.8	0.9	0.8	0.8	0.9	0.6	
1000	0.4	0.7	0.9	0.7	0.4	0.7	0.5	0.6	0.6	0.6	
2-nitrofluorene (50.0)	47.2	-	-	-	-	-	-	-	-	-	
Sodium azide (2.0)	-	6.2	30.1	-	-	-	-	-	-	-	
9-aminoacridine (50.0)	-	-	-	48.5	-	-	-	-	-	-	
Glutaraldehyde (25.0)	-	-	-	-	2.2	-	-	-	-	-	
2-aminoanthracene (5.0)	-	-	-	-	-	47.3	17.1	10.4	-	-	

[1] results are expressed as ratio: Mean number of revertant colonies per treated plate / Mean number of revertant colonies per control plate
- not tested
^ represents maximum increase over control

Table A6.6.1- 2: Relative reverse mutation rates in *S. typhimurium* after treatment with Copper II sulphate pentahydrate (experiment 2)

Concentration [µg per plate]	Relative reverse mutation rates[1]										Comments
	– S9					+ S9					
	TA98	TA100	TA1535	TA1537	TA102	TA98	TA100	TA1535	TA1537	TA102	
Historical control values mean (range)	24 (7–41)	107 (59–154)	12 (1-24)	10 (1-19)	285 (168-402)	29 (9–50)	124 (65-183)	16 (1-32)	11 (1-21)	306 (126-485)	All control values were within the range of the historical control.
Control (mean number of revertant colonies)	33.4 ± 1.3	132.2 ± 11.78	19.8 ± 2.9	10.2 ± 1.9	333.8 ± 12.9	42.4 ± 7.6	169.4 ± 11.3	20.0 ± 4.7	11.0 ± 2.5	416.6 ± 15.6	
50	1.0	0.9	0.9	0.7	0.9	0.8	0.9	1.3^	0.6	0.9	Evidence of toxicity was observed following all treatments at 800 µg/plate. Some treatments in the presence of S9 mix at test doses below 800 µg/plate also produced evidence of toxicity which was attributed to the use of a pre-incubation step..
100	0.8	0.9	1.0	0.8	0.8	0.5	0.7	1.1	0.5	0.8	
200	0.8	0.9	0.8	0.7	0.7	0.4	0.6	1.1	0.6	0.6	
400	0.7	0.8	0.9	0.4	0.7	0.5	0.5	0.7	0.7	0.5	
800	0.6	0.5	0.7	0.6	0.6	0.1	0.4	0.7	0.6	0.4	
2-nitrofluorene (50.0)	34.7	-	-	-	-	-	-	-	-	-	
Sodium azide (2.0)	-	5.1	27.3	-	-	-	-	-	-	-	
9-aminoacridine (50.0)	-	-	-	122.3	-	-	-	-	-	-	
Glutaraldehyde (25.0)	-	-	-	-	1.8	-	-	-	-	-	
2-aminoanthracene (5.0)	-	-	-	-	-	33.9	5.2	-	-	-	

[1] results are expressed as ratio: Mean number of revertant colonies per treated plate / Mean number of revertant colonies per control plate
- not tested
^ represents maximum increase over control

Section A6.6.2 Annex Point IIA6.6	In-vitro cytogenicity in mammalian cells	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The performance of an *in vitro* cytogenicity test in mammalian cells is redundant because there is a higher level *in vivo* study performed with Copper II sulphate pentahydrate available for extrapolation (Riley 1994, refer to Section A6.6.4).	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	12/01/2005	
Evaluation of applicant's justification	Agree with applicant's version	
Conclusion	Agree with applicant's version	
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 IIA6.6	In-vitro gene mutation in mammalian cells	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The performance of an *in vitro* gene mutation test in mammalian cells is redundant because there is a higher level *in vivo* study performed with Copper II sulphate pentahydrate available for extrapolation (Ward 1994, refer to Section A6.6.5).	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	12/01/2005	
Evaluation of applicant's justification	Agree with applicant's version	
Conclusion	Agree with applicant's version	
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 *In vivo* mammalian micronucleus test

Annex Point IIA6.6

			Official use only
		70 REFERENCE	
70.1	**Reference**	[redacted] (1994): Copper II sulphate pentahydrate: induction of micronuclei in the bone marrow of treated mice. [redacted]; Report no.: 456/33, July 07, 1994 (unpublished).	
		Doc.No. 456/33	
70.2	**Data protection**	Yes	
70.2.1	Data owner	Spiess-Urania Chemicals GmbH	
70.2.2	Companies with letter of access	--	
70.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		71 GUIDELINES AND QUALITY ASSURANCE	
71.1	**Guideline study**	Yes	
		EU method B.12	
71.2	**GLP**	Yes	
		(Certified laboratory)	
71.3	**Deviations**	No	X
		72 MATERIALS AND METHODS	
72.1	**Test material**	Copper II sulphate pentahydrate	
72.1.1	Lot/Batch number	A668269 350	
72.1.2	Specification	Not stated	
72.1.3	Purity	99 – 100.5 %	
72.1.4	Description	Blue crystalline substance	
72.1.5	Stability	Not stated	
72.1.6	Maximum tolerable dose	A range-finding test was conducted at concentrations in a range of 142.8 to 2000 mg/kg using groups of 3 males and 3 females. The LD_{50} two days after the second dose was calculated at 745 mg/kg (*2). A dose equivalent to 50-80 % of the LD_{50} was considered acceptable as a maximum dose level, thus 447 mg/kg was chosen as the upper dose for the micronucleus assay.	
72.2	**Test animals**		
72.2.1	Species	Mouse	
72.2.2	Strain	CD-1 mice	
72.2.3	Source	[redacted]	
72.2.4	Sex	Male and female	
72.2.5	Age/weight at study initiation	Age: 35-42 days Body weight: m 24-30 g, f 21-26 g	

Section A6.6.4 *In vivo* mammalian micronucleus test

Annex Point IIA6.6

72.2.6	Number of animals per group	15 males and 15 females	
72.2.7	Control animals	Yes	
		10 males and 10 females (vehicle control), 5 males and 5 females (positive control, single administration)	
72.3	**Administration/ Exposure**	Oral	
72.3.1	Number of applications	Two	
72.3.2	Interval between applications	Dosed once daily for 2 consecutive days	
72.3.3	Post-exposure period	24 or 48 hours	
		(24 hours for the positive control)	
72.3.4	Type	By gavage	
72.3.5	Concentration	447 mg/kg bw/day	
		corresponding to 113.76 mg Cu/kg bw/day	
72.3.6	Vehicle	Purified water	
72.3.7	Concentration in vehicle	22.35 mg/mL	
72.3.8	Total volume applied	20 ml/ kg bw	
72.3.9	Controls	Purified water (vehicle control)	
		Cyclophosphamide (positive control, 80 mg/kg)	
72.4	**Examinations**		
72.4.1	Mortality	Yes	
72.4.2	Tissue	Bone marrow	
		Number of animals:	5 per sex at each of 2 time points
		Time points:	24, 48 hours after treatment
		Type of cells:	Bone marrow cells
		Parameters:	PCE/NCE ratio
		Number of cells:	at least 1000 cells
		Parameters:	frequency of micronucleated PCE
		Number of cells:	at least 2000 cells
72.5	**Further remarks**	None	

Section A6.6.4 *In vivo* mammalian micronucleus test

Annex Point IIA6.6

		73 RESULTS	
73.1	**Mortality**	In the test item group, 5 male out of 15 and 2 females out of 15 died prior to sampling, indicating that it would not have been practicable to administer the test agent at an appreciably higher dose.	X
73.2	**Tissue examination**	The heterogeneity χ^2 test provided evidence of acceptable variability in the number of micro-nucleated PCE between animals within each group. The incidence of micro-nucleated PCE in the vehicle control was within the range of the historical control. At least 8 animals out of each group at both sampling times were available for analysis and the positive control chemical induced a statistically significant increase in the frequency of micro-nucleated PCE. Thus, the assay was considered to be valid. Decreased PCE/NCE ratios were observed in mice treated with Copper II sulphate pentahydrate at the 24 hour sampling time, indicating cellular toxicity and evidence of test substance penetration into the bone marrow. At 48 hours, PCE/NCE ratios in the test substance group were similar to those in vehicle controls. The numbers of micro-nucleated PCE seen at both sampling times were similar to those seen in controls and were not significantly different by χ^2 analysis. The results are summarised in Table A6.6.4- 1.	
73.3	**Genotoxicity**	No	
73.4	**Other**	No	
		74 APPLICANT'S SUMMARY AND CONCLUSION	
74.1	**Materials and methods**	Copper II sulphate pentahydrate was assayed *in vivo* in a mouse bone marrow micronucleus test at a single dose level according to EU method B.12.	
74.2	**Results and discussion**	Decreased PCE/NCE ratios were observed in mice treated with Copper II sulphate pentahydrate at the 24 hour sampling time. At 48 hours, PCE/NCE ratios in the test substance group were similar to those in vehicle controls. For both sampling times, the test substance induced no significant effect on the number of micro-nucleated PCE when compared to the vehicle control.	
74.3	**Conclusion**	Copper II sulphate pentahydrate was **not genotoxic** under the conditions of this test. The extrapolation from copper sulphate to other copper compounds is considered not be restricted in any way, since the moiety of interest is the copper ion itself, which may be expected to be released from both compounds during passage of the GI tract after oral uptake. Despite the somewhat limited bioavailability for poorly soluble copper compounds, the extrapolation from the readily bioavailable copper sulphate will only lead to a more conservative but nevertheless valid assessment.	
74.3.1	Reliability	1	
74.3.2	Deficiencies	No	

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	12/01/2005
Materials and Methods	Agree with applicant's version
Results and discussion	2.3 – Only one dose tested in the main study. Considering the substance, the test can, however, be consider as valid. Agree with applicant's version Rem. 4.1 - From the study, 4 out of 15 females died (1 due to dosing error, 1 before 24h sampling time, 2 before 48h sampling time).
Conclusion	Agree with applicant's version
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.4- 1: Results of the micronucleus test with Copper II sulphate pentahydrate (*in vivo*)

Treatment group (mg/kg bw x 2)	Sampling time [h]	Sex	Mean ratio PCE/NCE	Group mean frequency of micronucleated PCE (per 1000) per sex	per treatment group
Historical control[1]	24 and 48	male female	1.06 (0.67-1.83) 1.08 (0.70-1.45)	0.51 (0-1.29) 0.47 (0-1.10)	
Vehicle control	24	male female	1.07 1.20	0.40 0.30	0.35
	48	male female	1.44 0.83	0.38 0.30	0.33
Test item	24	male female	0.70 0.84	0.60 0.40	0.50
	48	male female	1.12 1.32	0.50 0.40	0.45
Positive control[2]	24	male female	0.52 0.48	26.87 29.27	28.07

[1] Average of group means from 26 consecutive studies at October 23, 1992 (data from 24 and 48 hour sampling times are combined)
[2] administered as a single dose

Section A6.6.5 *In vivo* test of unscheduled DNA synthesis in rat liver

Annex Point IIA6.6

Official use only

		75 REFERENCE
75.1	**Reference**	[redacted] (1994): Copper II sulphate pentahydrate: measurement of unscheduled DNA synthesis in rat liver using an *in vivo/in vitro* procedure. [redacted]; Report no.: 456/32, July 20, 1994.
		Doc.No. 456/32
75.2	**Data protection**	Yes
75.2.1	Data owner	Spiess-Urania Chemicals GmbH, Hamburg, Germany
75.2.2	Companies with letter of access	--
75.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.
		76 GUIDELINES AND QUALITY ASSURANCE
76.1	**Guideline study**	No
		The conduct of the study was consistent in all important aspects to method B.39 (2000/32/EC).
76.2	**GLP**	Yes
		(Certified laboratory)
76.3	**Deviations**	No
		77 MATERIALS AND METHODS
77.1	**Test material**	Copper II sulphate pentahydrate
77.1.1	Lot/Batch number	A668269 350
77.1.2	Specification	Not stated
77.1.3	Purity	99 – 100.5 %
77.1.4	Description	Blue crystalline substance
77.1.5	Stability	Not stated
77.1.6	Maximum tolerable dose	2000 mg/kg bw (based on an initial range-finding test)
77.2	**Test animals**	
77.2.1	Species	Rat
77.2.2	Strain	Wistar
77.2.3	Source	[redacted]
77.2.4	Sex	Male
77.2.5	Age/weight at study initiation	Age: 41-51 days Body weight: 189-254 g
77.2.6	Number of animals per group	6

Section A6.6.5 *In vivo* test of unscheduled DNA synthesis in rat liver

Annex Point IIA6.6

77.2.7	Control animals	Yes	
77.3	**Administration/ Exposure**	Oral	
77.3.1	Number of applications	One	
77.3.2	Interval between applications	Not applicable	
77.3.3	Post-exposure period	12-14 hours (experiment 1)	
		2-4 hours (experiment 2)	
77.3.4	Type	By gavage	
77.3.5	Concentration	632.5, 2000 mg/kg bw (corresponding to 161, 509 mg Cu II/kg bw)	
77.3.6	Vehicle	Purified water	
77.3.7	Concentration in vehicle	63.25, 200 mg/mL	
77.3.8	Total volume applied	10 mL/ kg bw	
77.3.9	Controls	Purified water (vehicle control)	
		2-Acetamidofluorene (suspended in corn oil, positive control, 75 mg/kg)	
		Dimethylnitrosamine (suspended in water, positive control, 10 mg/kg)	
77.4	**Examinations**		
77.4.1	Clinical signs	Yes	
77.4.2	Tissue	Mammalian liver cells	
		Number of animals:	5 per group
		Time points:	12-14 hours (exp. 1); 2-4 hours (exp. 2)
		Type of cells:	Hepatocytes (treated with [^{3}H] thymidine)
		Parameters:	Cytoplasmic and nuclear grain count
		Number of cells:	100 cells per animal
77.5	**Further remarks**	None	

Section A6.6.5 *In vivo* test of unscheduled DNA synthesis in rat liver

Annex Point IIA6.6

		78 RESULTS
78.1	**Clinical signs**	No deaths were observed in an initial range finding study at doses of up to 2000 mg/kg bw. Lethargy was observed at or above 250 mg/kg bw. During the main test, deaths occurred in the 2000 mg/kg bw group of experiment 2 and animals of a spare dose group were included to obtain hepatocytes from a total of 5 animals.
78.2	**Tissue examination**	The group mean net grain count was less than 0 in the vehicle control group. The positive control chemicals 2-AAF and DMN induced increases in the group mean net grain count of 5 or more and the percent of cells in repair was above 50 %. Thus, the experiment was considered to be valid. Following treatment with Copper II sulphate pentahydrate at doses of up to 2000 mg/kg bw the group mean net nuclear grain counts (NG) were well below the value of 5 NG required for a positive response. In addition, no more than 1.0 % cells were seen in repair. The results are summarised in Table A6.6.5- 1.
78.3	**Genotoxicity**	No
78.4	**Other**	No
		79 APPLICANT'S SUMMARY AND CONCLUSION
79.1	**Materials and methods**	Copper II sulphate pentahydrate was administered orally to male rats at 632.5 or 2000 mg/kg bw. Unscheduled DNA synthesis was investigated in hepatocytes isolated approximately 12 – 14 or 2 – 4 hours after dosing. Although not a guideline study, the conduct of the study was consistent in all important aspects to method B.39 (2000/32/EC).
79.2	**Results and discussion**	Following treatment with Copper II sulphate pentahydrate at doses of up to 2000 mg/kg bw the group mean net nuclear grain counts (NG) were well below the value of 5 NG required for a positive response. In addition, no more than 1.0 % cells were seen in repair.
79.3	**Conclusion**	Copper II sulphate pentahydrate was **not genotoxic** under the conditions of this test. The extrapolation from copper sulphate to other copper compounds is considered not be restricted in any way, since the moiety of interest is the copper ion itself, which may be expected to be released from both compounds during passage of the GI tract after oral uptake. Despite the somewhat limited bioavailability for poorly soluble copper compounds, the extrapolation from the readily bioavailable copper sulphate will only lead to a more conservative but nevertheless valid assessment.
79.3.1	Reliability	1
79.3.2	Deficiencies	No

Evaluation by Competent Authorities	
Use separate "evaluation boxes" to provide transparency as to the comments and views submitted	
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)

Date	12/01/2005
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version
Reliability	2
Acceptability	Acceptable
Remarks	
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.6.5- 1: Group mean net grain count values

Sampling time [hours]	Dose (mg/kg bw)	Net nuclear grain count (NG) mean	SD	Net grain count of cells in repair mean	SD	Percent of cells in repair (NG ≥ 5) mean	SD
12 – 14	0	-1.3	0.6	0	-	-	-
	632.5	-1.3	0.3	10.2	6.4	0.6	0.9
	2000	-1.0	0.3	5.5	0.9	1.0	1.0
	2-AAF[1] (75)	12.7	0.9	13.7	0.8	90.0	4.0
2 – 4	0	-2.2	0.3	0	-	-	-
	632.5	-2.2	0.2	0	-	-	-
	2000	-3.2	0.5	0	-	-	-
	DMN[1] (10)	17.2	2.8	17.3	2.7	99.6	0.9
Historical control	vehicle control	-1.5	1.4	-	-	0.7	0.9
	(range)	(-7.2 – 0)				(0 – 3.6)	
	2-AAF	11.2	3.2	-	-	80.7	12.9
	(range)	(5.4 – 18.7)				(45.6 – 98.4	
	DMN	11.5	4.3	-	-	79.7	14.9
	(range)	(5.6 – 21.9)				(51.8 – 98.8)	

[1] positive control

Section A6.6.6

Annex Point IIA6.6.6

Genotoxicity *in vivo*
Mouse
Bone marrow chromosome aberration assay
Micronucleus assay
Sperm abnormality assay

			Official use only
		80 REFERENCE	
80.1	**Reference**	BHUNYA, S.P., PATI, P.C. (1987): Genotoxicity of an Inorganic Pesticide, Copper Sulphate in Mouse *in vivo* Test System. *Cytologia* 52, 801- 808 Doc.No.: 00620B-IIA-666	
80.2	**Data protection**	No	
80.2.1	Data owner	published data	
80.2.2	Companies with letter of access	--	
80.2.3	Criteria for data protection	No data protection claimed	
		81 GUIDELINES AND QUALITY ASSURANCE	
81.1	**Guideline study**	No	
81.2	**GLP**	No	
81.3	**Deviations**	Only three animals per group were used; no positive control group	
		82 MATERIALS AND METHODS	
82.1	**Test material**	Copper Sulphate, analytical grade	
82.1.1	Lot/Batch number	not stated	
82.1.2	Specification	$CuSO_4*5H_2O$ (BHD)	
82.1.2.1	Description	not stated	
82.1.2.2	Purity	not stated	
82.1.2.3	Stability	not stated	
82.1.2.4	Maximum tolerable dose	20 mg/kg body weight	
82.2	**Test Animals**		
82.2.1	Species	mouse	
82.2.2	Strain	swiss	
82.2.3	Source	not stated	
82.2.4	Sex	sex not stated	X
82.2.5	Age/weight at study initiation	10 -12 weeks old, average body weight of 15 g	X
82.2.6	Number of animals per group	3 animals per group in all three tests	

Section A6.6.6 Annex Point IIA6.6.6		Genotoxicity *in vivo* Mouse Bone marrow chromosome aberration assay Micronucleus assay Sperm abnormality assay	
82.2.7	Control animals	yes	
82.3	**Administration/ Exposure**	oral, intraperitoneal, subcutaneous	X
82.3.1	Number of applications	5 applications	X
82.3.2	Interval between applications	24 h	X
82.3.3	Postexposure period	6, 24, 48 h for acute treatment 24 h for chronic treatment	X
		Oral	
82.3.4	Type	Usually gavage	
82.3.5	Concentration	20 mg/kg bw	
82.3.6	Vehicle	Moistened with glass double distilled water	
82.3.7	Concentration in vehicle	not stated	
82.3.8	Total volume applied	20 mg $CuSO_4$/kg bw	
82.3.9	Controls	Vehicle (glass double distilled water)	
		Intraperitoneal/subcutaneous	
82.3.10	Vehicle	glass double distilled water	
82.3.11	Concentration in vehicle	not stated	
82.3.12	Total volume applied	not stated	
82.3.13	dose applied	5, 10, 20 mg/kg bw	
82.3.14	Substance used as Positive Control	glass double distilled water	
82.3.15	Controls	Vehicle (glass double distilled water)	

Section A6.6.6 **Annex Point IIA6.6.6**		**Genotoxicity *in vivo*** **Mouse** **Bone marrow chromosome aberration assay** **Micronucleus assay** **Sperm abnormality assay**	
82.4	**Examinations**		
82.4.1	Clinical signs	No	
82.4.2	Tissue	Bone marrow chromosome aberration assay Sperm abnormality assay Micronucleus assay	
		Number of animals: all animals	
		Number of cells: 4600 cells	
		Time points: 6, 24, 48, 120 h after treatment	
		Type of cells: bone marrow cells	
		spermatogonia	X
		Parameters: Frequency of chromosomal aberrations (chromatid and isochromatid gaps, isochromatid breaks, fragments, double minutes, exchanges and rings)	
		Incidence of sperm abnormality	
82.5	**Further remarks**	--	
		83 RESULTS AND DISCUSSION	
83.1	**Clinical signs**	not stated	
83.2	**Haematology / Tissue examination**	The results of the tissue examination are given in • Table A6.6.6-1: Micronucleus test • Table A6.6.6-2: Sperm abnormalities • Table A6.6.6-3: Chromosome aberration	
83.3	**Genotoxicity**	Effect dose not stated	
83.4	**Other**	See table A6.6.6-1, A6.6.6-2 and A6.6.6-3	

Section A6.6.6

Annex Point IIA6.6.6

Genotoxicity *in vivo*
Mouse
Bone marrow chromosome aberration assay
Micronucleus assay
Sperm abnormality assay

		84 APPLICANT'S SUMMARY AND CONCLUSION	
84.1	**Materials and methods**	Mutagenicity of copper sulphate was evaluated in vivo by chromosome aberration, sperm abnormality and micronucleus tests in mice. For the three tests different doses (5,10 and 20 mg/kg bw) were administered intraperitoneal, oral and subcutaneous.	
84.2	**Results and discussion**	Dose, route and time influenced significantly the frequency of chromosome aberration, incidence of micronucleus and sperm abnormality. The relative sensitivity of three assays are: sperm abnormality > chromosome aberration > micronuclei formation. No statement is given on signs of mortality, The PCE//NCE ratio was increased in comparison to control. Disregarding "gaps", the effect of treatment is only marginal, and the test result may be considered as ambiguous.	X
84.3	**Conclusion**	Results indicated that copper sulphate solution administered by intra-peritoneal injection caused mutagenic activity in bone marrow cells and in sperm. Dose, route and time influenced significantly the frequency of chromosomal aberration, incidence of micronucleus and sperm abnormality. The extrapolation from copper sulphate to copper hydroxide is considered not to be restricted in any way, since the moiety of interest is the copper ion itself, which may be expected to be released from both compounds during passage of the GI tract after oral uptake. Despite the somewhat limited bioavailability for poorly soluble copper compounds, the extrapolation from the readily bioavailable copper sulphate will only lead to a more conservative but nevertheless valid assessment.	X
84.3.1	Reliability	3	X
84.3.2	Deficiencies	Yes several deficiencies render this a publication of limited validity	X

Section A6.6.6 **Annex Point IIA6.6.6**	**Genotoxicity *in vivo*** **Mouse** **Bone marrow chromosome aberration assay** **Micronucleus assay** **Sperm abnormality assay**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	12/01/2005
Materials and Methods	3.2.4 and 3.2.5 Average body weight of 25g. Male mice of sperm abnormality assay (!) 3.3 – Experimental procedure is not clearly reported in the sections 3.3.x Three different experiments are reported in this publication. - Bone marrow chromosome aberration assay: Acute treatment with 20 mg/kg i.p. on three animals with fixation times of 6, 24 and 48 h. Two other doses (10 mg/kg and 5 mg/kg) were administered to 2 more animals with fixation time of 24 h. The highest dose was also given orally or subcutaneously to 2 animals with fixation time of 24 h. Chronic administration of 5x4 mg/kg was also performed on one animal (24 h between each injection and sacrifice 24 h after the last treatment). - Sperm abnormality assay: 3 animals/dose were treated with 20 mg/kg, 10 mg/kg and 5 mg/kg. Doses were fractionated into 5 equal parts and injected i.p. 5 times with 24 h between each dose. Animals were sacrificed **35 days** after the first injection. Sperms were collected from caudae epididymides, and 500 sperms were examined per animal for sperm abnormalities. - Micronucleaus assay: each dose (20, 10, 5 mg/kg) was injected i.p. twice at an interval of 24 h and the animals were sacrificed 6 h after the second injection. 3.4.2 – Spermatogonies are the target cells of this study but spermatozoids are the observed cells at the end of the observation period of 35 days.
Results and discussion	Quite low doses, mortality is not expected at these levels. Results and experimental data is not enough reported to have a clear opinion of these experiments.
Conclusion	Some concerns are raised by this series of studies. These results are not confirmed by other studies (mutagenicity and reproductive toxicity). Moreover insufficient reporting weaken the validity of the results. But these effects cannot be disregarded and should be kept in mind when assessing other studies.
Reliability	4

Section A6.6.6 **Annex Point IIA6.6.6**	**Genotoxicity *in vivo*** **Mouse** **Bone marrow chromosome aberration assay** **Micronucleus assay** **Sperm abnormality assay**
Acceptability	Not acceptable Other data exist, no further testing is required.
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 A6.6.6-1: Mouse bone marrow findings (Micronucleus test and lysis) induced by $CuSO_4$

State mean ± standard deviation state individual numbers for critical findings		control group	low dose 5 mg/kg	mid dose 10 mg/kg	high dose 20 mg/kg
Number of cells evaluated		**3000**	**3000**	**3000**	**3000**
Sampling time (h)		**6 h**	**6 h**	**6 h**	**6 h**
Number of erythrocytes	**normochromatic erythrocytes with micronuclei**	**0.10 ± 0.05**	**0.66 ± 0.27**	**1.03 ± 0.72**	**1.46 ± 0.72**
	polychromatic erythrocytes with micronuclei	**0.20 ± 0.05**	**1.30 ± 0.47**	**1.80 ± 0.94**	**2.06 ± 0.72**
	poly- and normochromatic erythrocytes with micronuclei	**0.15 ± 0.05**	**0.98 ± 0.27**	**1.41 ± 0.27**	**1.76 ± 1.44**
Ratio of erythrocytes	**polychromatic with micronuclei/ normochromatic with micronuclei**	**0.88**	**1.1**	**1.1**	**1.1**
Immature white cells with MN		**0.06 ± 0.03**	**0.4 ± 0.27**	**0.88 ± 0.27**	**1.23 ± 0.72**
Nuclei in lysis		--	**0.2 ± 0.05**	**0.30 ± 0.15**	**0.46 ± 0.27**

Table A6.6.6-2: Incidence of sperm abnormality in mice by $CuSO_4$ treated intraperitoneal

Dose [mg/kg]	No. of animals / no. of sperm studies	No. of abnormal sperms	Mean [%]	'Z' value
20	**3 / 1500**	**231**	**15.40 ± 0.81**	**17.09**
10	**3 / 1500**	**166**	**11.60 ± 0.98**	**12.97**
5	**3 / 1500**	**87**	**5.80 ± 1.41**	**6.59**
Control	**3 / 3000**	**62**	**2.06 ± 0.54**	--

The result is statistically relevant when $Z \geq 1.96$

Table A6.6.6-3: Frequency of chromosomal aberration induced in bone marrow cells by $CuSO_4$ administered through different routes

Dose [mg/kg]	Route	Interval of fixation	No. of cells studied	Chro-matid gap	Chro-matid break	Isochro-matid gap	Frag-ments	Double minutes	Ex-change/ ring	Total	Aber-ration [%]
Acute											
20	**i.p.**	**6**	**300**	**4**	**3**	**1**	**1**	**2**	**1**	**12**	**4.00 ± 0.57**
20	**i.p.**	**24**	**300**	**6**	**5**	**1**	**3**	--	--	**15**	**5.00 ± 1.15**
20	**i.p.**	**48**	**300**	**9**	**2**	**1**	**1**	--	--	**13**	**4.33 ± 0.33**
10	**i.p.**	**24**	**300**	**11**	--	--	**2**	--	**1**	**14**	**4.66 ± 0.33**
5	**i.p.**	**24**	**300**	**8**	**3**	--	**1**	--	--	**12**	**4.00 ± 1.00**
Chronic											
5 x 4	**i.p.**	**120**	**300**	**9**	--	**1**	**1**	**1**	--	**12**	**4.00 ± 0.57**
Composite											
Control	**i.p.**	--	**1000**	**5**	**2**	--	--	--	--	7	**0.70 ± 0.26**
20	**or**	**24**	**300**	**8**	--	--	**2**	**2**	--	**12**	**4.00 ± 0.57**
Control	**or**	**24**	**600**	**3**	**1**	--	--	--	--	**4**	**0.66 ± 0.33**
20	**sc**	**24**	**300**	**9**	**2**	--	**1**	**1**	**1**	**14**	**4.66 ± 0.88**
Control	**sc**	**24**	**600**	**4**	--	--	--	--	--	**4**	**0.66 ± 0.33**

i.p. = intraperitoneal, or = oral, sc = subcutaneous

Section A6.6.7 Annex Point IIA6.6.7	Further testing if metabolites of concern are formed in mammals	
	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [ ]	**Technically not feasible [ ]** **Scientifically unjustified [X]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	The performance of further genotoxicity testing is considered to be not required since no metabolites are formed by Copper hydroxide.	
	Evaluation by Competent Authorities	
	EVALUATION BY RAPPORTEUR MEMBER STATE	
Date	12/01/2005	
Evaluation of applicant's justification	Agree with applicant's version	
Conclusion	Agree with applicant's version	
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 A6.7 Carcinogenicity

Annex Point IIA6.7

	JUSTIFICATION FOR NON-SUBMISSION OF DATA	Official use only
Other existing data [X]	**Technically not feasible [ ]** **Scientifically unjustified [ ]**	
Limited exposure [ ]	**Other justification [ ]**	
Detailed justification:	In subchapter 6.7 of the TNsG on data requirements according to Directive EC98/8/EEC, carcinogenicity testing is required for one rodent and one other mammalian species. The carcinogenicity study should identify the carcinogenicity potential of the substance in laboratory animals in order to facilitate the extrapolation of potential risks to humans. The studies must be sufficient to establish the species specificity and organ specificity of tumours induced, to establish the dose-response relationship and for non-genotoxic carcinogens to identify doses eliciting no adverse effects (threshold dose). However, the applicant is of the opinion that the conduct and submission of a conventional carcinogenicity study is not required, for the following reasons: (1)Copper is an essential micronutrient, and its use, and incorporation in many enzyme systems in the human has been researched in great depth. The absorption, distribution and excretion of copper is described in Section A6.2, using data from several species, including the human. Sections A6.5 and A6.7 contain summaries of several long-term animal studies from peer-reviewed journals in the public domain. None of these meets exactly the requirements of the guideline B.30/32/33, but they do show conclusively that copper has no carcinogenic activity. Also, there is no need to perform additional animal studies, because there are human data. (2) Two rare genetic diseases of copper in the human provide evidence that copper is not carcinogenic following systemic absorption. These are Wilson's disease (WD) and Menkes' disease (MD): Wilson's disease is a defect in the ATPase for copper transport ATP7B (or WND), expressed mainly in the liver (LEEMING, N.M., 2003; reference A6.2/01), resulting in faulty copper transport, impaired incorporation of copper into ceruloplasmin, impaired copper biliary excretion, and copper accumulation in the liver and brain. Frequency in the human population is stated as 1 in 300,000 live births. Hepatic copper levels range from 200 to 800 µg/g dry weight (normal range 20 to 50 µg/g), and patients present with hepatic cirrhosis and fatty infiltration of the liver. Urinary copper is much higher than normal (as in rats given sufficiently high oral doses to cause liver toxicity). Treatment is by chelation therapy using D-penicillamine, such that intestinal absorption is reduced, and chelated copper complexes are excreted in the urine, and liver and body levels are kept below levels at which liver disease occurs. Zinc therapy (orally as zinc sulphate) acts to induce excess metallothionein in the intestinal cells. Metallothionein has a stronger affinity for copper than zinc. The copper remains bound in the gut cells, which are then sloughed off, and the copper is lost. In the second or third decade of the disease, neurological symptoms can occur. Copper accumulation in the brain causes degeneration of the basal ganglia, resulting in defective movement, slurred speech, difficulty	X

Supprimé : [

in swallowing, facial and other muscular spasms, dystonia and poor motor control. Depression and schizophrenia have been reported. Copper may also be deposited in the cornea (Kayser-Fleischer rings).

Menkes disease is an X-linked copper deficiency disease that is usually fatal in early childhood. It is usually present in males, but has been recorded in eight females (cases have been cited where genetic translocation was noted in a female). The frequency in the human population is stated as 1 in 100,000 to 1 in 250,000 live births. The symptoms result from a defect in the MNK protein, producing an inability to export copper from cells, particularly from the basal membrane of the small intestine, where copper is absorbed (see LEEMING, N.M., 2003; reference A6.2/01). This leads to very high concentrations of copper in sloughed intestinal cells, but the failure to export the 'absorbed' copper to the bloodstream results in an effective copper deficiency for the rest of the body. The disease shows progressive mental retardation, hypothermia, seizures, poor muscle tone, feeding difficulties, jaundice, diarrhoea and a general failure to thrive. There are abnormalities of connective tissue with deformities of the skull, long bones and ribs. The hair is abnormal with a wiry texture and a spiral twist.

Both diseases result from genetic defects where the subject is unable to produce respectively the copper ATPases ATP7B and ATP7A. These are members of the human cation-transporting P-type ATPase family. The P-type ATPases are a large group of membrane proteins that utilise the energy of ATP hydrolysis to transport various ions across cell membranes. During the catalytic cycle the γ-phosphate of ATP is transferred to the invariant aspartic acid residue within the nucleotide-binding site of ATPase with the formation of acylphosphate intermediate: this property distinguishes the P-type ATPases from other cation-transporting pumps. Over 100 P-type ATPases have been described. The loci of the encoding genes have been identified for both WD and MD. Both pump copper across cell membranes. The MD pump (ATP7A) is the pump that actually moves copper through the basal membrane of the intestinal epithelial cells so that copper enters the hepatic portal system where it binds to albumin, transcuprein and histidine to reach the liver. In the MD subject, ATP7A is inactive, and copper from the diet accumulates in the intestinal epithelial cells, bound to induced metallothionein. The presence of copper within the cell induces the production of more metallothionein, and the copper-metallothionein complex accumulates during the life of the cell. When the cells are sloughed off into the intestinal lumen, as is the normal course of events, the cells and the copper within them are excreted in the faeces, and the copper is lost to the body. Subjects with Menkes' disease can still absorb small amounts of copper. Copper accumulates in fibroblasts and in the kidney of Menkes' disease subjects, but there is no evidence of increased incidence of cancer in these tissues either. Menkes' disease is effectively a disease of copper deficiency. In terms of risk assessment of copper in the normal human, the accumulation of copper in the intestinal epithelium on Menkes' subjects can be considered as the equivalent of an excessive oral dose of copper to the epithelial cells. Carcinogens of the intestine may act by irritation or some other means to cause proliferation of the intestinal epithelium that eventually results in hyperplasia and tumour formation. MD subjects do not suffer from increased incidence of cancer of the intestine. This

shows conclusively that excess copper in the intestinal cells does not cause cancer or long-term toxicity in that tissue.

Wilson's disease (WD) involves the other ATPase previously referred to, ATP7B. In normal humans, this enzyme is primarily active in hepatocytes. It is involved in the trans-Golgi network (TGN). Copper absorbed by the hepatocyte via the inbound membrane pump hCTR1 (human copper transporter protein 1, see LEEMING, N.M., 2003; reference A6.2/01) and is bound to metallothionein within the cell. It may be bound by ATP7B to ceruloplasmin (a protein that binds up to 6 copper ions tightly and transports them to various tissues for use, including the brain. If there is excess copper in the hepatocyte, ATP7B is induced to traffic to vesicular compartments (lysosomes) and directly to the apical membrane, where copper is secreted from the cell bound to a trypsin-independent fragment of ceruloplasmin and excreted in the bile. In WD, ATP7B is inactive and the absorbed copper accumulates in the hepatocytes bound to metallothionein. The bile of WD subjects does not contain copper. In the hepatocyte, excess copper may accumulate in mitochondria, in the cytoplasm and in lysosomes, bound to metallothionein. Eventually the cell's copper storage capacity is exceeded. Mitochondrial damage occurs and eventually the hepatocyte dies, whence the cell contents are released to the circulation, depositing copper in extrahepatic tissues.

Wilson's disease thus leads to massive accumulation of copper in the liver. The disease usually manifests in late adolescence, and is ultimately fatal if not treated, but death is from liver failure, not from cancer. Treatment involves administration of penicillamine, which forms a copper complex capable of urinary excretion. There is no evidence of increased incidence of liver cancer in WD subjects. This shows that even massive accumulation of copper in the target organ, the liver, does not result in cancer in the human. Accumulation of copper leads to cell death, but this is only in the presence of excessive copper concentrations, brought about by a genetic condition resulting in the disruption of the natural homeostatic mechanisms for copper. It should be noted that Wilson's disease is genetic, and the accumulation of copper and resulting liver failure occur under the natural levels of copper in the diet, not as a result of exposure to excessive levels of copper in the environment. However, the accumulation of copper in the liver may be taken as a model for accumulation of excess copper in a toxicity study, and the conclusion drawn that chronic high liver levels do not result in increased incidence of cancer.

As with short-term toxicology it is considered appropriate to present data on the active substance, the copper ion, rather than the formulated or technical materials. A metabolism/bioequivalence study has been performed to demonstrate that the ion, as present in the form of cupric sulphate pentahydrate, is similarly bioavailable to the five forms defended, such that data from studies with the pentahydrate, and other forms that liberate the copper ion, may be used in the risk assessment process (HIMMELSTEIN, M., 2004; reference A6.2/02).

(3) No data have been presented on the mouse. Short-term studies on the mouse show that the mouse is much more tolerant of higher doses of copper than the rat (HÉBERT, C.D., 1993; reference A6.4.1/01), and that the mouse does not show the histological changes in the liver and kidney that are seen in the rat. As the rat shows lower short term NOELs than

Section A6.7 Carcinogenicity

Annex Point IIA6.7

	the mouse, it is logical to assume that the NOELs from long term mouse studies would be higher than in the rat. Therefore, data from long term mouse studies would not be used in setting values such as the ADI. As stated previously, there are human diseases that lead to chronic, lethal accumulations of copper in target tissues, but no evidence for tumour formation. In summary, submission of further carcinogenicity studies on animals are considered unnecessary, in view of the argumentation set forth above, and the studies cited and summarised further below.	
Undertaking of intended 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	17/01/2005	
Evaluation of applicant's justification	Agree with applicant's version But it can remain some concerns if inhalation exposure of copper carbone dusts could occur. (see previous comments on chronic toxicity document IIIA 6.5)	
Conclusion	Acceptable if it is demonstrated that inhalation exposure is negligible.	
Remarks		
	COMMENTS FROM ...	
Date		
Evaluation of applicant's justification		
Conclusion		
Remarks		

Section A6.7 Carcinogenicity in rats

Annex Point IIA6.7

			Official use only
		The following studies are considered to contain further information concerning carcinogenicity and are thus presented as supportive data.	
		85 REFERENCE	
85.1	**Reference**	A6.7/01: Doc.No. 00620B-IIA-67a HOWELL, J.S. (1958): The effect of copper acetate on *p*-dimethylamino-azobenzene carcinogenesis in the rat; Br. J. Cancer 12, p594-610.	
85.2	**Data protection**	No	
85.2.1	Data owner	published data	
85.2.2	Companies with letter of access	--	
85.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		86 GUIDELINES AND QUALITY ASSURANCE	
86.1	**Guideline study**	No Special investigation of the effect of copper acetate on DMAB carcinogenesis, which does not meet exactly the requirements of method B32 (88/303/EEC).	
86.2	**GLP**	No The study was conducted prior to implementation of GLP.	
86.3	**Deviations**	Not applicable	
		87 MATERIALS AND METHODS	
87.1	**Test material**	(i) *p*-dimethylaminoazobenzene (DMAB) (ii) copper acetate (iii) ferric citrate	
87.1.1	Lot/Batch number	Not stated	
87.1.2	Specification	Not specified	
87.1.3	Purity	Not stated	
87.1.4	Description	(i) dry crystalline (ii), (iii) powder	
87.1.5	Stability	DMAB was mixed with maize and copper acetate and stored for 2 month. Determination of DMAB by column chromatography showed that DMAB was stable in the diet mixture and did not underwent chemical alteration.	
87.2	**Test animals**		
87.2.1	Species	Rat	
87.2.2	Strain	Exp. A: entirely out-bred laboratory stock, not further specified Exp. B: Birmingham strain (Laboratory Animals Bureau Catalogue of Uniform Strains, No. 626, 1953), a heterozygous strain	

Section A6.7 Carcinogenicity in rats

Annex Point IIA6.7

87.2.3	Source	Not specified
87.2.4	Sex	Male and female
87.2.5	Age/weight at study initiation	Age: 2 to 6 month Body weight: not stated
87.2.6	Number of animals per group	5 males and 5 females
87.2.7	Control animals	No
87.3	**Administration/ Exposure**	Oral
87.3.1	Duration of treatment	Exp. A: lifespan Exp. B: until presence of palpable liver tumours and subsequent sacrifice
87.3.2	Frequency of exposure	Daily
87.3.3	Post-exposure period	None
87.3.4	Type	In food
87.3.5	Concentration	DMAB: 0.09 % w/w Copper acetate: 0.5 % Ferric citrate: 2.0 % The test design is outlined in Table A6.7- 1.
87.3.6	Vehicle	Finely ground maize or standard laboratory diet (Thompson diet, Heygate and Sons)
87.3.7	Concentration in vehicle	DMAB: 0.09 % w/w Copper acetate: 0.5 % Ferric citrate: 2.0 %
87.3.8	Total volume applied	The amount of food provided was calculated on the basis of 10 g per rat per day.
87.3.9	Controls	Not applicable
87.4	**Examinations**	
87.4.1	Body weight	Yes (regularly in experiment B)
87.4.2	Food consumption	Yes (at intervals in experiment B)
87.4.3	Water consumption	No
87.4.4	Clinical signs	Not stated
87.4.5	Macroscopic investigations	After the 3rd month of treatment all animals were examined at approx. 14 day intervals for the presence of palpable liver tumours.
87.4.6	Ophthalmoscopic examination	No
87.4.7	Haematology	No
87.4.8	Clinical chemistry	No

Section A6.7 Carcinogenicity in rats

Annex Point IIA6.7

87.4.9	Urinalysis	No
87.5	**Pathology**	
87.5.1	Organ weights	Yes (Experiment B) All spleens were weighed during post-mortem examination.
87.5.2	Histopathology	Yes from: all animals Organs: liver, spleen and any other tissue which showed pathological changes
87.5.3	Other examinations	In experiment A rats were subjected to liver biopsy under anaesthesia. They were biopsied in rotation at monthly intervals from the second to the tenth months of the experiment. During experiment B, liver function was assessed by means of the bromsulphalein excretion test.
87.6	**Statistics**	Not stated
		88 RESULTS
88.1	**Body weight**	In experiment B, all animals gained weight during the study. Animals in group 6 showed initial loss of weight during the 1st month of treatment most likely due to abstention of food.
88.2	**Food consumption**	Food consumption was determined at intervals in experiment B. Over periods of one month the quantity of food consumed by the animals was estimated by weighing the residues daily, without determination of food spillage and individual variations in consumption. However, it was found that the animals in the various dietary groups consumed roughly the same amounts of food.

Section A6.7 Carcinogenicity in rats

Annex Point IIA6.7

88.3	**Macroscopic investigations**	The minimum induction period was set at 6 month of administration, since the first animal to develop tumours in both experiments died during the 6th month. Animals that died before this time have been excluded since they were considered not 'at risk'. During experiment A, animals fed with a diet containing copper acetate and ferric citrate showed lower incidences of liver tumours (4 animals with liver tumours out of 8 rats for the standard diet), especially when incorporated into a maize diet (0 rats with liver tumours out of 8 animals). In addition, survival was 2.25 month (maize) or 0.4 month (standard diet) longer for diets with copper acetate than for respective diets without copper acetate and the time to first tumour was also increased. These findings were confirmed by the results of group 5 to 7 of experiment B. Group 6 receiving copper acetate in the diet showed lower incidences of tumours, a longer time to first tumour and an increased average time to death than animals of the other two groups which received no copper acetate in the diet. The results of the alternating feeding experiments (groups 8 to 11) were more difficult to interpret, as the lower incidence of tumours may also have been influenced by lower DMAB consumption. The results are presented in Table A6.7- 1.
88.4	**Organ weights**	During experiment B all spleens were weighed at post-mortem examination. Differences in splenic weight were significant ($0.02 > p > 0.01$) for the directly comparable groups 5 (2.3 g) and 6 (1.1 g), with spleens of animals on DMBA-maize diet without copper acetate being about 1 g heavier than the mean weight of the spleen in group 6 receiving DMBA in maize diet with copper acetate. Enlarged spleens were also observed for animals receiving the alternated diets, with group 9 and 10 receiving the smallest quantity of copper and the highest quantity of DMBA showing the most enlarged spleens.
88.5	**Histopathology**	

Section A6.7 Carcinogenicity in rats

Annex Point IIA6.7

88.5.1	Liver	In experiment A rats were subjected to liver biopsy under anaesthesia. They were biopsied in rotation at monthly intervals from the second to the tenth months of the experiment. The incidence of liver changes are summarised in Table A6.7- 2. The results showed that hepatic injury developed and progressed rapidly in groups 1 and 2 feed on diets without copper acetate. Animals of group 4 (maize diet with copper acetate) showed a very considerable retardation in the severity of the lesions and a considerable prolongation in the time required to produce them. No animals with regenerative hyperplasia or any degree of cirrhosis were observed in this group and it was not until the eighth month that marked changes were observed, consisting of fusiform cells and chronic inflammatory cells tending to encircle lobules. Liver histopathology in animals dying without developing tumours during both experiments revealed that the microscopic changes in the DMAB, copper treated rats were of the same nature as those which result from DMAB alone, but there was delay in the rate of development and progression of the lesions especially marked during the first 12 month of treatment.
88.5.2	Spleen	Gross and microscopic changes of the spleen typically induced by DMAB were also observed in copper acetate treated rats, but the changes in these animals were always much less advanced than in those animals given DMAB without copper acetate.
88.6	**Time to tumours**	The results are presented in Table A6.7- 1.
		89 APPLICANT'S SUMMARY AND CONCLUSION
89.1	**Materials and methods**	During experiment A, groups of 5 male and 5 female rats received the known carcinogen *p*-dimethylaminoazobenzene in either standard laboratory diets or maize supplemented with ferric acid and copper acetate for their whole lifespan. Liver biopsies were performed regularly. Experiment B was performed to confirm the inhibitory effect of copper acetate. Groups of 5 male and 5 female rats received DMAB in maize with or without ferric acid or copper acetate. In addition, groups with alternating feeding were included to reduce the likelihood of copper acetate interfering with DMAB absorption in the gut. The animals were sacrificed when palpable liver tumours were observed. Spleen weights were determined and histopathology of liver and spleen was conducted. The study does not exactly meet the requirements of method B32 (88/303/EEC), since it was a special investigation of the effect of copper acetate on DMAB carcinogenesis.
89.2	**Results and discussion**	Copper, when added to rat diets containing the known carcinogen p-dimethylaminobenzene significantly reduced the incidence of liver tumours, and delayed the onset of histological changes leading to cirrhosis and hyperplasia.

Section A6.7 Carcinogenicity in rats

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89.3	**Conclusion**	The design of the study did not permit assessment of tumour incidence of copper administered alone, and in the context of a risk assessment of the carcinogenic potential of copper the study (and the numerous other studies in the literature that also show a beneficial effect of copper when administered together with known carcinogens) must only be considered illustrative. However, if copper were to have any carcinogenic action either alone, or as a co-carcinogen, this type of study would certainly have shown an increased incidence of tumours, and an earlier onset. It did neither; the authors concluded that copper has a beneficial effect in reducing the action of the carcinogen. The study indicates that copper has no carcinogenic potential when administered in the diet. Other similar studies from the literature have not been included in this dossier as none of them meet the regulatory guideline for carcinogenicity, although most do show that the co-administration of copper with known carcinogens reduces the onset and/or incidence of the anticipated tumours, and where a copper-only control has been part of the design, there were no adverse effects of copper administration. The extrapolation from copper acetate to copper hydroxide is considered not be restricted in any way, since the moiety of interest is the copper ion itself, which may be expected to be released from both compounds during passage of the GI tract after oral uptake.
89.3.1	Reliability	2
89.3.2	Deficiencies	Yes Copper acetate was only investigated in combination with the carcinogen DMAB.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	10/06/05
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Data not suitable for cancer hazard assessment of copper carbonate
Reliability	3
Acceptability	Not acceptable
Remarks	Data available is sufficient to allow a hazard assessment of Copper carbonate by oral route. No more tests are needed.
	COMMENTS FROM ...
Date	

Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Table A6.7- 1: Tumour incidence

Group	Diet	Days on diet/week	No. surviving 6 month	Month to first tumour	No. of rats with liver tumour	Average induction time (month)	Average time to death (month)
Experiment A							
1	CP + Fe cit + DMAB	7	10	9	8	13.6 (9-16)	13.0 (9-16)
2	M + Fe cit + DMAB	7	9	6	8	11.25 (6-16)	11.0 (6-16)
3	CP + Fe cit + **Cu ac** + DMAB	7	8	11	4	14.0 (11-16)	12.5 (8-16)
4	M + Fe cit + **Cu ac** + DMAB	7	8	0	**0**	-	13.5 (10-16)
Experiment B							
5	M + DMAB	7	8	6	8	8.5 (6-10)	8.5 (6-10)
6	M + DMAB + **Cu ac**	7	8	18	1	-	13.3 (10-18)
7	M + DMAB + Fe cit	7	10	6	10	8.5 (6-12)	8.5 (6-12)
8	M + DMAB + Fe cit M + Cu ac	4 3	10	15	3	15	15.1 (8-19)
9	M + DMAB + Fe cit M + Cu ac	5 2	8	9	6	11.3 (9-15)	10.5 (8-15)
10	M + DMAB + Fe cit M + Cu ac	6 1	10	9	8	12 (9-15)	11.4 (9-15)
11	M + DMAB + Fe cit CP	4 3	9	15	5	16.2 (15-18)	14.1 (9-18)

CP = standard laboratory diet, M = maize, Fe cit = 2.0 % Ferric citrate, Cu ac = 0.5 % Copper acetate, DMAB = 0.09 % *p*-Dimethylaminoazobenzene
Remark: Groups 8 to 11 were included to reduce the likelihood of copper acetate interfering with DMAB absorption or of combining chemically with it in the gut. Therefore alternating feeding was devised.

Table A6.7- 2: Number of animals showing regenerative hyperplasia and cirrhosis of the liver upon biopsy (Experiment A)

Group	No. of biopsies	Regenerative hyperplasia	Degree of cirrhosis			
			Absent	Incipient	Early	Advanced
1	8	4	3	2	3	-
2	8	6	1	3	3	1
3	9	3	8	-	1	-
4	8	0	8	-	-	-

Section A6.7 Copper toxicosis and tolerance in rats

Annex Point IIA6.7

			Official use only
		90 REFERENCE	
90.1	**Reference**	A6.7/02: Doc.No. 00620B-IIA-67b HAYWOOD, S.; LOUGHRAN, M. (1985): Copper toxicosis and tolerance in the rat, II Tolerance – a liver protective adaptation. Liver 5, p267-275.	
90.2	**Data protection**	No	
90.2.1	Data owner	published data	
90.2.2	Companies with letter of access	--	
90.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.	
		91 GUIDELINES AND QUALITY ASSURANCE	
91.1	**Guideline study**	No Special investigation on copper toxicosis and tolerance in the rat, which does not meet exactly the requirements of methods B.32 or B.33 (88/303/EEC).	
91.2	**GLP**	No The study was conducted prior to implementation of GLP.	
91.3	**Deviations**	Not applicable	
		92 MATERIALS AND METHODS	
92.1	**Test material**	Copper sulphate	
92.1.1	Lot/Batch number	Not stated	
92.1.2	Specification	Not specified	
92.1.3	Purity	Not stated	
92.1.4	Description	Not stated	
92.1.5	Stability	Not stated	
92.2	**Test animals**		
92.2.1	Species	Rat	
92.2.2	Strain	Wistar	
92.2.3	Source	Not stated	
92.2.4	Sex	Male	
92.2.5	Age/weight at study initiation	Age: not specified (weanling rats of uniform age) Body weight: uniform weight, not specified	
92.2.6	Number of animals per group	Exp. 1: 4 per group and sacrifice time Exp. 2: 3 to 4 per group and sacrifice time Exp. 3: 16 per group	
92.2.7	Control animals	Yes	

Section A6.7 Copper toxicosis and tolerance in rats

Annex Point IIA6.7

92.3	**Administration/ Exposure**	Oral
92.3.1	Duration of treatment	Exp. 1: 15 weeks Exp. 2: 52 weeks Exp. 3: 3 weeks (pre-treatment for 15 weeks)
92.3.2	Interim sacrifice(s)	Exp. 1: 4 animals from each group sacrificed in intervals up to 6 weeks Exp. 2: groups of 3 to 4 rats were sacrificed at 15, 20, 29 or 52 weeks Exp. 3: 4 rats/ group sacrificed at 15 weeks (after pre-treatment)
92.3.3	Final sacrifice	Exp. 1: at 6 weeks (6000 mg/kg), at 15 weeks (other treatments) Exp. 2: up to 52 weeks Exp. 3: after 3 weeks of 'challenge'
92.3.4	Frequency of exposure	Daily
92.3.5	Post-exposure period	None
92.3.6	Type	In food
92.3.7	Concentration	Exp. 1: 3000, 4000, 5000, 6000 mg Cu/kg diet Exp. 2: 3000 mg Cu/kg diet Exp. 3: 6000 mg Cu/kg diet (animals previously fed control or 3000 mg Cu/kg diet for 15 weeks)
92.3.8	Vehicle	Powdered diet (Labsur animal diet, RHM Agricultur South Ltd.) containing 10-20 mg/kg of copper
92.3.9	Concentration in vehicle	Exp. 1: 3000, 4000, 5000, 6000 mg Cu/kg diet Exp. 2: 3000 mg Cu/kg diet Exp. 3: 6000 mg Cu/kg diet
92.3.10	Total volume applied	*ad libitum*
92.3.11	Controls	Vehicle only
92.4	**Examinations**	
92.4.1	Body weight	Yes (upon study termination)
92.4.2	Food consumption	No
92.4.3	Water consumption	No
92.4.4	Clinical signs	Yes
92.4.5	Ophthalmoscopic examination	No
92.4.6	Haematology	No
92.4.7	Clinical chemistry	No
92.4.8	Urinalysis	No
92.5	**Pathology**	

Section A6.7 Copper toxicosis and tolerance in rats

Annex Point IIA6.7

92.5.1	Organ weights	No
92.5.2	Histopathology	Yes from: all animals Organs: liver
92.5.3	Other examinations	Copper analysis of livers by means of an atomic absorption spectrophotometer.
92.6	**Statistics**	Student's t-test, Pearson Product-Moment correlation
		93 RESULTS
93.1	**Experiment 1**	Effects of different levels of copper supplementation on growth rate, liver copper content and hepatic pathology were investigated.
93.1.1	Growth rate and clinical condition	All animals on the copper-supplemented diets showed a severe reduction in growth rate compared to the control. Animals receiving 3000, 4000 or 5000 mg Cu/kg diet gained body weight during the study and appeared active and sleek upon termination of the experiment. Animals receiving 6000 mg Cu/kg diet lost body weight and appeared ruffled, lethargic and had diarrhoea. Therefore, they were sacrificed after 6 weeks of administration.
93.1.2	Liver copper content	The liver copper concentration of rats on the 3000 mg/kg copper diet increased to a peak concentration of 3986 ± 297 µg/g at 4 weeks and declined thereafter. A similar pattern of liver copper distribution was noted for rats on the 4000 or 5000 mg/kg copper diets, except that peak copper concentrations occurred at 3 weeks of administration. Maximum liver copper concentrations occurred at 2 weeks on the 6000 mg/kg copper diet and showed no decrease upon sacrifice at 6 weeks of administration.
93.1.3	Histopathology	Liver necrosis first occurred as randomly distributed necrotic foci progressing to a more diffuse form which occasionally involved whole lobules. The onset of liver necrosis varied with the magnitude of copper loading, and was succeeded by regeneration in animals on dietary copper up to 5000 mg/kg. Hepatocellular damage persisted in animals receiving the highest dietary level of copper.
93.2	**Experiment 2**	Effects of prolonged exposure to a high copper diet on growth rate and liver copper content were investigated. Animals receiving 3000 mg Cu/kg diet gained body weight steadily during weeks 15 to 52. Body weight approximated 80 % of that of the control group upon study termination. Liver copper concentrations decreased over the same time period from 1303 ± 68 µg/g at 15 weeks to 440 ± 44 µg/g at 52 weeks (control: 23 ± 4 µg, 15 and 52 weeks). There was a negative correlation between liver copper concentration and body weight.

Section A6.7 Copper toxicosis and tolerance in rats

Annex Point IIA6.7

93.3	**Experiment 3**	The effect of copper challenge on liver copper concentration and pathological response of copper-primed rats was investigated. Animals receiving the control diet prior to challenge with 6000 mg Cu/kg diet for 3 weeks appeared lethargic with ruffled coats. In contrast, animals receiving 3000 mg Cu/kg diet for 15 weeks prior to challenge with 6000 mg Cu/kg diet for 3 weeks were very active and did not appear to be affected by the additional challenge. Liver copper contents increased after challenge of un-primed animals while the copper contents in primed rats did not significantly alter. Concurrently no histopathological changes in livers of the primed rats were observed after challenge, while moderate to severe hepatocellular necrosis with an associated inflammatory response was observed after challenge of un-primed rats.
		94 APPLICANT'S SUMMARY AND CONCLUSION
94.1	**Materials and methods**	Three experiments were performed with male weanling Wistar rats. In the first experiment dietary levels of 3000, 4000, 5000 or 6000 mg Cu/kg diet and a concurrent vehicle control were administered for up to 15 weeks. In a second experiments rats received either control diet or 3000 mg/kg copper diets for up to 1 year. The third experiment investigated effects of copper challenge (6000 mg Cu/kg diet) in primed (3000 mg Cu/kg diet for 15 weeks) or un-primed (control diet) rats .Body weights, liver copper contents and histopathological changes of livers were determined during all experiments. The study does not exactly meet the requirements of methods B.32 or B.33 (88/303/EEC), since it was a special investigation on copper toxicosis and tolerance in rats.
94.2	**Results and discussion**	Animals treated with copper at 3000 ppm for one year showed no long-term evidence of liver toxicity: an adaptive response was shown similar to the earlier shorter study, and at 52 weeks, copper concentrations were lower than at 15 weeks. Animals previously treated with copper at 3000 ppm for 15 weeks that were then given 6000 ppm (double the dose) for three weeks did not show altered liver copper concentrations, whereas previously untreated rats of the same age and strain given 6000 ppm copper showed moderate to severe hepatocellular necrosis. The extrapolation from copper sulphate to copper hydroxide is considered not be restricted in any way, since the moiety of interest is the copper ion itself, which may be expected to be released from both compounds during passage of the GI tract after oral uptake. Despite the somewhat limited bioavailability for poorly soluble copper compounds, the extrapolation from the readily bioavailable copper sulphate will only lead to a more conservative but nevertheless valid assessment.
94.3	**Conclusion**	
94.3.1	Reliability	2
94.3.2	Deficiencies	Yes No haematological, clinical chemistry or urine parameters were recorded. Full gross necropsy and histopathology were not reported. Tumour incidences were also not reported.

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	10/06/05
Materials and Methods	Agree with applicant's version
Results and discussion	Agree with applicant's version
Conclusion	Data not suitable for cancer hazard assessment of copper carbonate
Reliability	3
Acceptability	Not acceptable
Remarks	Data available is sufficient to allow a hazard assessment of Copper carbonate by oral route. No more tests are needed.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	

Section A6.8.1 Range-finding study

Annex Point IIA6.8.1 Rabbit

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		95 REFERENCE
95.1	**Reference**	**A6.8.1/01: Doc.No. 00620B-IIA-681a** [redacted] (2003): Five copper substances – repeated dose toxicity and tolerability study in non-pregnant rabbits; [redacted]; Report no.: DuPont-11638, November 17, 2003.
95.2	**Data protection**	Yes
95.2.1	Data owner	Spiess-Urania Chemicals GmbH, Hamburg, Germany
95.2.2	Companies with letter of access	--
95.2.3	Criteria for data protection	Data submitted to the MS after 13 May 2000 on existing a.s. for the purpose of its entry into Annex I.
		96 GUIDELINES AND QUALITY ASSURANCE
96.1	**Guideline study**	No Range-finding study to determine the maximum tolerated dose with regard to clinical toxicity.
96.2	**GLP**	Yes Self-certified laboratory
96.3	**Deviations**	Not applicable
		97 MATERIALS AND METHODS
97.1	**Test material**	(i) Copper hydroxide (ii) Copper oxide (iii) Copper oxychloride (iv) Tribasic copper sulphate (v) Bordeaux mixture
97.1.1	Lot/Batch number	(i) 380-71-05 (ii) 280802 (iii) 27003B (iv) 471/2002 (v) 1/170
97.1.2	Specification	Not specified.
97.1.3	Purity	(i) 60.1 % copper (ii) 87 % copper (iii) 57 % copper (iv) 31.12 % copper (v) 27.38 % copper

Section A6.8.1 **Range-finding study**

Annex Point IIA6.8.1 **Rabbit**

97.1.4	Description	(i) light blue powder (ii) red-brown powder (iii) light green, fine homogeneous powder (iv) greenish-blue solid (v) green powder	
97.1.5	Stability	No evidence of instability was observed.	
97.2	**Test animals**		
97.2.1	Species	Rabbit	
97.2.2	Strain	New Zealand White	
97.2.3	Source	[redacted]	
97.2.4	Sex	Female (non-pregnant)	
97.2.5	Age/weight at study initiation	Age: 6 to 6.5 month Body weight: 3382 – 4116 g (day after arrival)	
97.2.6	Number of animals per group	Part 1: 2 to 4 Part 2: 2	
97.2.7	Control animals	No	
97.2.8	Mating period	Not applicable	
97.3	**Administration/ Exposure**	Oral	
97.3.1	Duration of exposure	Part 1: 14 days Part 2: 7 days	
97.3.2	Post-exposure period	None	
97.3.3	Type	By gavage	
97.3.4	Concentration	Part 1: 30 mg/kg bw/day for each test substance Part 2: 50/40 mg/kg bw/day for each test substance (lowered to 40 mg/kg bw/day on test day 2 on humane grounds)	X
97.3.5	Vehicle	0.5 % aqueous methylcellulose	
97.3.6	Concentration in vehicle	Not stated in the report.	X
97.3.7	Total volume applied	1 mL/ kg bw	
97.3.8	Controls	None	
97.4	**Examinations**		
97.4.1	Body weight	Yes (daily)	
97.4.2	Food consumption	Yes (daily)	
97.4.3	Clinical signs	Yes (daily)	

Section A6.8.1 **Range-finding study**

Annex Point IIA6.8.1 **Rabbit**

97.4.4	Gross pathology and histopathology	Yes Gross external and visceral examination was performed for all animals.
97.5	**Further remarks**	None
		98 RESULTS
98.1	**Clinical signs and necropsy**	No mortality or clinical signs were observed in the two animals administered with 30 mg Cu/kg bw/day as **copper hydroxide**. Upon necropsy ulceration and discolouration of the stomach lining was noted in one female. During part 2 of the study, one female died after administration of 50 mg Cu/kg bw/day. The surviving animal showed stomach ulceration upon necropsy. One female dosed with 30 mg Cu/kg bw/day as **copper oxide** showed transient diarrhoea. Necropsy observations of the two females of this dose group included discolouration and haemorrhages in the stomach linings. One animal died after administration at the 50 mg/kg level and necropsy revealed discoloration and thickening of the stomach lining. The survivor dosed at 40 mg/kg bw/day for the remaining 6 days showed no adverse clinical signs. Ulceration of the stomach lining was noted upon necropsy. Two of three females administered with **copper oxychloride** were found dead during part 1 of the study. The other female showed no adverse clinical signs. One of two females died after administration of the 50 mg/kg bw dose. The other female survived during part 2 of the study. Necropsy observations for both surviving animals included thickening and discoloration of and/or ulceration of the stomach lining. One of four females dosed with **tribasic copper sulphate** showed transient diarrhoea. It should be noted that two of these four females were inadvertently under-dosed by about 40 % for 8 days and one of the four was under-dosed for one day. Necropsy observations for three of four females included discolouration and/or ulcerations and/or haemorrhagic areas of the stomach lining. Similar observations were reported upon necropsy for the two females dosed in the 2nd part of the study. The two females dosed with **Bordeaux mixture** during part 1 of the study showed no adverse clinical signs or necropsy findings. One female died after administration with 50 mg Cu/kg bw and necropsy revealed discoloration and thickening of the stomach lining. Necropsy findings of the surviving high dose female included also discoloration and thickening in the stomach lining.

Section A6.8.1	Range-finding study
Annex Point IIA6.8.1	Rabbit

98.2	**Body weight and food consumption**	For all copper substances, the body weight change data for animals that survived until test termination indicated dose-related body weight losses during the first week of administration, concurrent to reduced food consumption. Animals generally appeared to recover from the second week of dosing onwards.
		99 APPLICANT'S SUMMARY AND CONCLUSION
99.1	**Materials and methods**	Female non-pregnant rabbits received 30 or 50/40 mg/kg bw/day (reduced to 40 mg/kg bw/day on day 2) of five copper test substances by gavage for 14 or 7 consecutive days, respectively. The study was designed as a range-finding study to determine the maximum tolerated dose relevant for dose selection in subsequent studies.
99.2	**Results and discussion**	The results of the study indicated that generally similar patterns of toxicity were evident for all copper substances tested. Animals surviving up to study termination showed a compound-related reduction in food consumption and subsequent reduced body weight losses during the first week of dosing. Animals generally appeared to recover from the second week of dosing onwards. Necropsy observations from all groups revealed apparent compound-related stomach lesions including haemorrhages, ulcerations, discoloration and/or thickening of the lining.
99.3	**Conclusion**	The general pattern and degree of inappetance and weight loss followed by recovery, and the observation of stomach ulceration at necropsy was considered sufficient to show that there were no major differences in the sensitivity of the rabbit to the five copper substances. Doses greater than 30 mg Cu/kg bw/day were considered unsustainable for repeat dosing studies. As there were no major differences between the five substances, further preliminary investigations would be performed on only one substance, copper hydroxide.
99.3.1	Reliability	2
99.3.2	Deficiencies	No

Evaluation by Competent Authorities	
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE (*)
Date	17/01/2005
Materials and Methods	3.3.4 – doses are expressed as mg **Cu**/kg bw 3.3.6 – concentrations were adjusted for copper content : 30 mg Cu/mL for exp 1 and 50/40 mg Cu/mL for exp 2
Results and discussion	Agree with applicant's version
Conclusion	Agree with applicant's version

Reliability	2
Acceptability	Acceptable
Remarks	This study is not really needed in this section as it is not a teratogenicity study. Only a summary in the main teratogenicity study would have been sufficient.
	COMMENTS FROM ...
Date	
Materials and Methods	
Results and discussion	
Conclusion	
Reliability	
Acceptability	
Remarks	