Section A6.8.1		**Teratogenicity Study**
Annex Point IIA6.8.1		*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.2/04		**A6.8.1(04), Teratogenicity of copper**
221.3.1.1 males	Parent	*give critical effect and concentration* Not applicable.
221.3.1.2 females	Parent	*give critical effect and concentration* No dose-related effects were observed in any of the species tested..
221.3.1.3	F1 males	*give critical effect and concentration* No dose-related effects were observed in male offspring (F_1 generation) of dosed females in any of the species tested.
221.3.1.4	F1 females	*give critical effect and concentration* No dose-related effects were observed in female offspring (F_1 generation) of dosed females in any of the species tested.
221.3.1.5	F2 males	*give critical effect and concentration* No dose-related effects were observed in male descendents (F_2 generation) of dosed females in any of the species tested.
221.3.1.6	F2 females	*give critical effect and concentration* No dose-related effects were observed in female descendents (F_2 generation) of dosed females in any of the species tested.
221.3.2 NO(A)EL		Non-entry field
221.3.2.1 males	Parent	*give concentration* Not applicable.
221.3.2.2 females	Parent	*give concentration* Rat and Hamster : The NOAEL following intrauterine exposure is estimated to be $\geq$ 2.75 µg per day (see section 3.3.6). Rabbit : The NOAEL following intrauterine exposure is estimated to be $\geq$ 5.50 µg per day (see section 3.3.6).
221.3.2.3	F1 males	*give concentration* No dose-related effects were observed in male offspring (F_1 generation) of dosed females in any of the species tested.
221.3.2.4	F1 females	*give concentration* No dose-related effects were observed in female offspring (F_1 generation) of dosed females in any of the species tested.
221.3.2.5	F2 males	*give concentration* No dose-related effects were observed in male descendents (F_2 generation) of dosed females in any of the species tested.
221.3.2.6	F2 females	*give concentration* No dose-related effects were observed in female descendents (F_2 generation) of dosed females in any of the species tested.
221.3.3 Reliability		*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4* 2
221.3.4 Deficiencies		Yes. This study was not conducted and/or reported in compliance with GLP. When compared with generally accepted principles to be applied to reproductive toxicity and teratogenicity studies, it is also apparent that there were a number of methodological deviations, including the

Section A6.8.1	**Teratogenicity Study**
Annex Point IIA6.8.1	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.2/04	**A6.8.1(04), Teratogenicity of copper**

following:

- The toxicity of copper to reproduction / teratogenicity was assessed only after implantation of embryos in the Parent females. No copper was administered to males.
- Only a single 'dose level' was used. The dose received by parent females was estimated, not measured.
- F_1 and F_2 generations were not exposed to copper during their growth, mating and reproduction.
- Test and control groups generally contain fewer animals than recommended.
- Effects on the oestrus cycle were not assessed.
- Sperm parameters were not assessed.

Reporting deficiencies included the following:

- Information on the numbers of animals mated is deficient in some cases.
- Detailed information on survival of pups at birth and at weaning (hamsters and rabbits).

These deficiencies do not, however, 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 and that the results obtained are consistent with work published by other researchers. Furthermore, this research (including its methodology) was published in a peer-reviewed publication, and has therefore been subject to the prior scrutiny of experts in the field. The study has also been referenced in a number of reviews of the toxicity of copper to reproduction.

No internationally accepted guidelines are available that specifically address the objective of the research presented in this summary.

Overall, this is a well-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 the embryotoxic / teratogenic potential of copper. 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 [redacted]

Section A6.8.1	**Teratogenicity Study**
Annex Point IIA6.8.1	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.2/04	**A6.8.1(04), Teratogenicity of copper**
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.8.1 **Teratogenicity Study**
Annex Point IIA6.8.1 *Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.2/04 **A6.8.1(04), Teratogenicity of copper**

Table A6.8.1(04). Table for reproductive toxicity study (modify if appropriate)

If effects are found in one generation, the figures for the other generation(s) should be given as well (as shown as an example for mortality). Give only information on endpoints with effects, delete other endpoints.

Parameter		Genera-tion	control		Rat		Hamster		Rabbit	
			m	f	m	f	m	f	m	f
Mortality	incidence	P	0	0	0	0	0	0	0	0
		F_1	0	0	0	0	0	0	0	0
		F_2	0	0	0	0	0	0	0	0
		F_3	0	0	0	0	0	0	0	0
Food consumption	% of control	P	--*	--	--	--	--	--	--	--
		F		--	--	--	--	--	--	--
		F		--	--	--	--	--	--	--
Body weight gain	% of control	P	--	--	--	--	--	--	--	--
		F_1	100	100	98.0	98.1	99.6	98.4	100.6	110.0
		F_2	100	100	95.9	98.0	96.9	110.1	--	--
Clinical Observations *specify effects*	Incidence	P	0	0	0	0	0	0	0	0
		F_1	0	0	0	0	0	0	0	0
		F_2	0	0	0	0	0	0	0	0
		F_3	0	0	0	0	0	0	0	0
Organ weights	% of control									
OVARIES		P - -		--	--	--	--	--	--	--
		F_1	--	100	--	94.9	--	102.5	--	153.6
		F_2	--	100	--	94.7	--	98.3	--	--
Uteri		P	--	--	--	--	--	--	--	--
		F_1	--	100	--	88.6	--	85.3	--	144.6
		F_2	--	100	--	101.1	--	127.9	--	--

Section A6.8.1 **Teratogenicity Study**
Annex Point IIA6.8.1 *Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.2/04 **A6.8.1(04), Teratogenicity of copper**

Adrenals		**P**	--	--	--	--	--	--	--	--
		F_1	100	100	98.4	92.5	113.4	90.0	100.2	100.2
		F_2	100	100	95.9	104.7	122.1	101.5	--	--
Testes		**P**	--	--	--	--	--	--	--	--
		F_1	100	--	100.8	--	96.5	--	120.6	--
		F_2	100	--	101.2	--	113.2	--	--	--
Epididymi		**P**	--	--	--	--	--	--	--	--
		F_1	100	--	--	--	--	--	120.6	--
		F_2 --		--	--	--	--	--	--	--
Seminal vescicle		**P**	--	--	--	--	--	--	--	--
		F_1	100	--	89.6	--	122.0	--	117.3	--
		F_2	100	--	104.4	--	104.9	--	--	--
Ventral prostate		**P**	--	--	--	--	--	--	--	--
		F_1	100	--	86.7	--	123.1	--	--	--
		F_2	100	--	102.7	--	98.4	--	--	--
Pathology										
Histopathologic examination *specify effects*	Incidence									
		P	0	0	0	0	0	0	0	0
		F_1	0	0	0	0	0	0	0	0
		F_2	0	0	0	0	0	0	--	--

Copper Oxide

Reproductive Performance	Generation	Species					
		Rat		Hamster		Rabbit	
		Control	Test Animals	Control	Test Animals	Control	Test Animals
Female fertility index (No. pregnant animals/ No. females mated) x 100.	P	--	--	--	--	--	--
	F_1	68.75	86.8	100	75.0	60.0	66.67
	F_2	--	--	--	--	--	--
Male fertility index (No. males that became sires / No. males placed with females) x 100.	P	--	--	--	--	--	--
	F_1	85.7	93.3	60.0	70.0	--	--
	F_2	--	--	--	--	--	--
Mean number of implantation sites (range)	P	--	--	--	--	8.6	7.9
	F_1	--	--	--	--	--	7.5 (5-10)
	F_2 offspring of F_1 females	(BB ♀) 10.0 ± 1.1	(AA ♀) 12.8 ± 0.7	--	--	--	--
		(BB ♂) 11.1 ± 0.5	(AA ♂) 10.9 ± 0.5	--	--	--	--
	F_2 offspring of F_1 males	(B'B'♀) 11.5 ± 0.5	(A'A'♀) 10.0 ± 1.0	--	--	--	--
		(B'B' ♂) 10.7 ± 0.4	(A'A' ♂) 10.5 ± 0.3	--	--	--	--
Mean number of lost implantations (No. implantation sites – No. pups born alive).	P	--	--	--	--	24	48
	F_1	--	--	--	--	--	--
	F_2	--	--	--	--	--	--
Post-implantation loss ((No. implantation sites – No. pups born alive)/ No. implantation sites) x 100.	P	--	--	--	--	55.81	67.6
	F_1	--	--	--	--	--	--
	F_2	--	--	--	--	--	--
Duration of pregnancy (mean days)	P	22.5	23.0	16.5	17.0	33.1	31.7
	F_1	--	--	--	--	--	--
		F_2 - -	--	--	--	--	--
Live birth index (No. pups born alive / No. pups born) x 100.	P	100	100	100	100	100	100
	F_1	--	--	--	--	--	--
	F_2	--	--	--	--	--	--

Gestation index (No. females with live pups/ No. females pregnant) x 100.	P	100	100	100	100	100	100
	F_1	--	--	--	--	--	--
	F_2	--	--	--	--	--	--
Mean litter size (range)	P	8.6 ± 0.6	6.5 ± 0.7	6.7 (3-11)	6.9 (2-10)	6.0 (5-7)	3.8 (1-5)
	F_1	(B' ♂) 8.8 ± 0.7	(A' ♂) 9.3 ± 0.6	(B' ♂) 6.4 ± 1.2	(A' ♂) 8.0 ± 0.8	--	--
		(B ♀) 8.5 ± 0.9	(A ♀) 10.1± 0.5	(B ♀) 7.8 ± 0.9	(A ♀) 7.9 ± 0.8	--	--
	F_2 offspring of F_1 females	--	--	(BB ♀) 2.0 ± 1.0	(AA ♀) 7.8 ± 0.9	--	--
		--	--	(BB ♂) 7.9 ± 0.8	(AA ♂) 3.1 ± 0.6	--	--
	F_2 offspring of F_1 males	--	--	--	--	--	--
		--	--	--	--	--	--
Sex ratio (male/female)	F_1	28/32	40/38	19/21	42/34	--	--
		F_2 - -	--	--	--	--	--
Survival rate at birth (%)	F_1	100	100	100	100	--	--
	F_2	--	--	--	--	--	--
Survival rate at weaning (25 days old) (%)	F_1	93.3	98.7	--	--	84.2	82.4
	F_2	--	--	--	--	--	--

* No data

Table A6.8.1(04)-1

Table I. Effect of Copper Wire on Pregnancy and Parturition in Rats

Treatment group	No. of Animals	No. of litters born	No. of pups born ♀	No. of pups born ♂	Av. No. of pups ± S.E.	Gestation period (days)
Copper	12	12	38	40	6.5 ± 0.7*	23.0
Control	7	7	32	28	8.6 ± 0.6	22.5

* $p < 0.05$

1

Table A6.8.1(04)-2

Table II. Survival of F_1 Generation Rats at the Time of Weaning (25 days old)

Treatment group	Number of pups At birth ♀	♂	total	At weaning ♀	♂	total	Survival Rate (%)
Copper	38	40	78	38	39	77	98.7
Control	32	28	60	28	28	56	93.3

Table A6.8.1(04)-3

Table III. Organ Weights of F_1 Generation Rats

Treatment group	Sex[1]	No. of animals	B.W. gm ±S.E.	Organ weights, mg ± S.E. Ovaries	Uteri	Adrenals	Testes	Seminal vesicles	Ventral prostate
A (Copper)	♀	32	250.8 ±2.5	87.5 ±2.5	416.1 ±22.1	70.6 ±1.8			
B (Control)	♀	14	255.6 ±3.7	92.2 ±3.9	469.8 ±40.8	76.3 ±2.1			
A' (Copper)	♂	38	404.4 ±5.1			49.9 ±0.9	3486.1 ±40.4	762.1 ±19.2	592.9 ±23.4
B' (Control)	♂	20	412.7 ±9.7			50.7 ±1.6	3459.8 ±50.4	850.6 ±24.8	684.1 ±27.2

[1]Female rats were autopsied at the age of 125-130 days; male rats were autopsied at the age of 145-150 days.

Table A6.8.1(04)-4

Table IV. Organ Weights of F_2 Generation Rats

Treatment group*	Sex[1]	No. of Animals	B.W. gm ±S.E.	Organ weights, mg ± S.E. Ovaries	Uteri	Adrenals	Testes	Seminal Vesicles	Ventral Prostat
AA	♀	10	248.5 ±4.6	86.5 ±1.5	456.3 ±32.4	74.8 ±1.6			
BB	♀	6	251.8 ±6.7	88.7 ±7.1	411.6 ±30.9	71.6 ±2.1			
A'A'	♀	8	261.1 ±3.7	84.4 ±5.3	393.3 ±28.1	76.0 ±1.8			
B'B'	♀	6	268.0 ±8.1	91.8 ±4.5	429.1 ±28.8	72.4 ±2.5			
AA	♂	10	384.1 ±11.8			48.6 ±2.5	3477.1 ±39.1	727.8 ±52.2	550.1 ±27.0
BB	♂	15	391.6 ± 9.0			50.6 ±2.1	3403.8 ±61.0	735.9 ±33.9	526.5 ±31.8
A'A'	♂	8	376.5 ±16.7			49.2 ±1.1	3481.0 ±41.4	820.7 ±21.5	590.8 ±31.3
B'B'	♂	8	401.2 ±6.5			51.4 ±2.8	3468.9 ±66.3	747.6 ±44.1	584.9 ±30.2

NOTE: Legend for table given on following page.

Legend for Table IV.

* AA -- offspring born to F_1A (Copper)

BB -- offspring born to F_1B (Control)

A'A' -- offspring born to F_1A' (Copper)

B'B' -- offspring born to F_1B' (Control)

AA -- offspring born to F_1A (Copper)

BB -- offspring born to F_1B (Control)

A'A' -- offspring born to F_1A (Copper)

B'B' -- offspring born to F_1B' (Control)

[1] Female rats were autopsied at the age of 125-130 days; male rats were autopsied at the age of 145-150 days.

Table A6.8.1(04)-5

Table V. Effect of Copper Wire on Pregnancy and Parturition in Hamsters

Treatment group	No. of animals	No. of litters born	No. of pups born ♀	No. of pups born ♂	Av. No. of pups (range)	Gestation period(days)
Copper	11	11	34	42	6.9 (2-10)	17.0
Control	6	6	21	19	6.7 (3-11)	16.5

Table A6.8.1(04)-6

Treatment group	Sex[1]	No. of animals	B. W. gm ± S.E.	Organ Weights, mg ± S.E. Ovaries	Uteri	Adrenals	Testes	Seminal Vesicles	Ventral prostate
A (Copper)	♀	23	129.3 ±3.5	32.8 ±1.6	361.5 ±38.9	15.3 ±0.8			
B (Control)	♀	11	131.4 ±7.0	32.0 ±1.5	423.8 ±41.7	17.0 ±0.6			
A' (Copper)	♂	32	129.5 ±3.1			27.9 ±0.9	3192.0 ±27.1	493.5 ±25.4	98.7 ±8.9
B' (Control)	♂	14	130.0 ±2.9			24.6 ±2.9	3307.7 ±107.6	404.5 ±17.4	80.2 ±7.7

[1] Female hamsters were autopsied at the age of 145-150 days; male hamsters were autopsied at the age of 155-160 days.

Table A6.8.1(04)-7

Table VII. Organ Weights of F_2 Generation Hamsters

Treatment group*	Sex[1]	No. of animals	B. W. gm ± S.E.	Organ weights, mg ± S.E.					
				Ovaries	Uteri	Adrenals	Testes	Seminal vesicles	Ventral prostate
AA	♀	15	129.4 ±8.5	35.1 ±1.6	421.9 ±29.7	16.7 ±0.8			
BB	♀	14	116.1 ±6.2	32.9 ±1.4	339.3 ±28.5	16.0 ±0.9			
A'A'	♀	15	130.8 ±6.6	33.8 ±2.0	308.7 ±40.4	16.9 ±0.5			
B'B'	♀	12	120.2 ±10.3	37.2 ±2.6	231.8 ±55.7	17.1 ±0.9			
AA	♂	17	118.7 ±3.3			26.1 ±0.9	3219.2 ±97.1	485.9 ±34.4	247.4 ±64.9
BB	♂	11	111.1 ±4.0			22.8 ±0.7	3064.7 ±199.7	397.0 ±26.0	229.1 ±9.8
A'A'	♂	20	131.8 ±2.4			30.9 ±1.1	3675.1 ±45.1	533.5 ±43.0	169.0 ±10.1
B'B'	♂	7	147.4 ±5.9			23.9 ±1.5	3025.5 ±91.3	575.2 ±55.8	194.0 ±11.1

NOTE: Legend for table given on following page.

Legend for Table VII.

* AA -- offspring born to F_1A (Copper)

BB -- offspring born to F_1B (Control)

A'A' -- offspring born to F_1A' (Copper)

B'B' -- offspring born to F_1B' (Control)

AA -- offspring born to F_1A (Copper)

BB -- offspring born to F_1B (Control)

A'A' -- offspring born to F_1A' (Copper)

B'B' -- offspring born to F_1B' (Control)

[1] Female hamsters were autopsied at the age of 145-150 days; male hamsters were autopsied at the age of 155-160 days.

Table A6.8.1(04)-8

Table VIII. Effect of Copper Wire on Pregnancy and Parturition in Rabbits

Treatment group	No. animals	No. implantation sites (Lap. D_7)	No. litters born	No. pups born (%)	No. pups alive at weaning	Av. No. pups (range)	Gestation period (days)
Copper	9	71	6	23 (32.4)	19 (82.4)	3.8 (1-5)	31.7
Control	5	43	3	19 (44.2)	16 (84.2)	6.0 (5-7)	33.1

Table A6.8.1(04)-9

Table IX. Organ Weights of F_1 Generation Rabbits

Treatment group	Sex[1]	No. of animals	B.W. gm ± S.E.	Organ weights, mg ± S.E. Ovaries	Uteri	Adrenals	Testes	Epididymi	Seminal Vesicle
A* (Cu)	♀	5	3810.0 ±285.6	794.4 ±107.9	5844.7 ±269.2	371.5 ±17.1			
B**(Control)	♀	6	3462.5 ±78.8	517.3 ±70.1	3904.8 ±223.9	370.9 ±43.8			
A***(Cu)	♂	9	3422.7 ±113.5			368.3 ±32.8	5469.0 ±314.3	1867.3 ±93.9	996.4 ±101.1
B****(Control)	♂	4	3406.3 ±73.9			367.7 ±54.3	4566.4 ±494.1	1547.8 ±227.8	849.1 ±89.3

* Two female rabbits were autopsied at the age of 6 months and were not included here.

** Three female rabbits were autopsied at the age of 3 months and were not included here.

*** One male rabbit was autopsied at the age of 6 months and was not included here.

**** Three male rabbits were autopsied at the age of 3 months and were not included here.

[1] Both female and male rabbits were autopsied at the age of 8 1/2 months.

Figure A6.8.1(04)-1

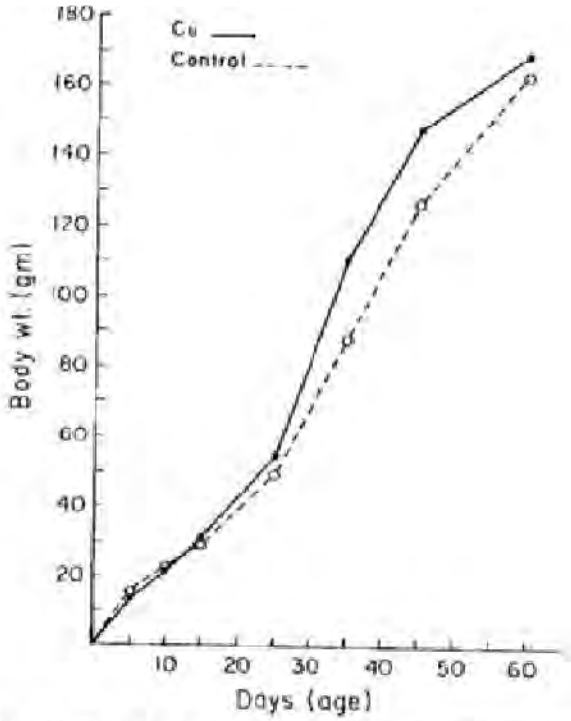


Fig. 1. Growth Rates of F_1 Generation Female Rats

Figure A6.8.1(04)-2

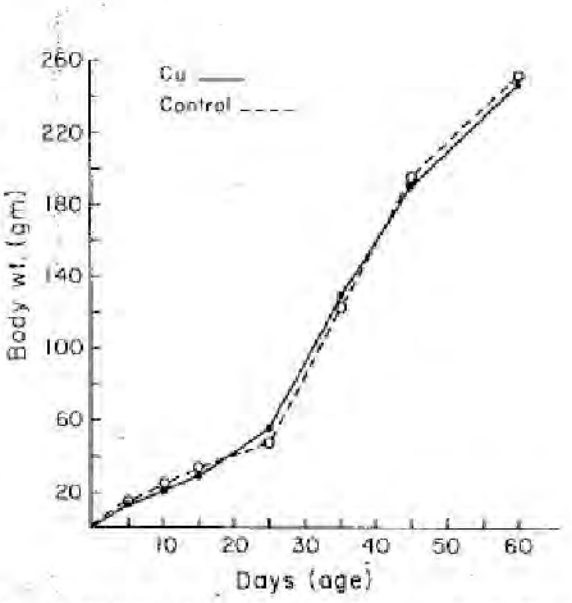
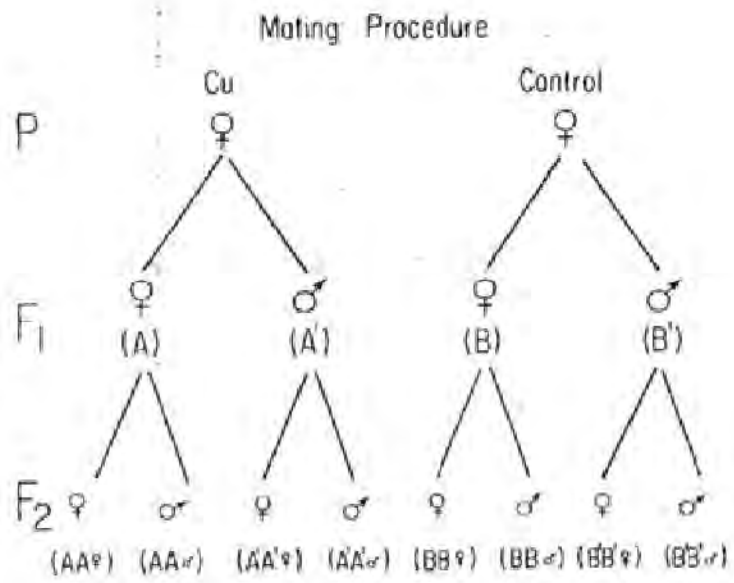


Fig. 2. Growth Rates of F_1 Generation Male Rats

Figure A6.8.1(04)-3



Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/01	**A6.8.2(01), Toxicity of copper to fertility**

Official use only

222 REFERENCE

222.1 Reference

Lecyk, M. (1980). Toxicity of $CuSO_4$ in mice embryonic development. Zoologica Poloniae, 28(2): 101-105 (published).

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).

222.2 Data protection

No.
(indicate if data protection is claimed)

222.2.1 Data owner

Give name of company

Public domain.

222.2.2 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.

222.2.3 Criteria for data protection

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

No data protection claimed.

223 GUIDELINES AND QUALITY ASSURANCE

223.1 Guideline study

No. This was a non-regulatory study carried out to determine the effect of $CuSO_4$, added to the food of pregnant female mice, on the development of their offspring.

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

223.2 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)

223.3 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")

224 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.

224.1 Test material

Cu^{2+} as $CuSO_4$

or give name used in study report

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

Not stated.

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/01	**A6.8.2(01), Toxicity of copper to fertility**

224.1.2 Specification		Deviating from specification given in section 2 as follows *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):*
224.1.2.1	Descriptio n	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* $CuSO_4$ was added to the diet as an aqueous solution.
224.1.2.2	Purity	*Give purity in % active substance* [redacted]
224.1.2.3	Stability	*Describe stability of test material.* Not stated.

Non-entry field

224.2 Test Animals

224.2.1 Species	Mouse
224.2.2 Strain	C_{57}BL and DBA
224.2.3 Source	Institute of Immunology and Experimental Therapy, Polish Academy of Sciences.
224.2.4 Sex	Male and female
224.2.5 Age/weight at study initiation	Experimental animals were sexually mature at study initiation.

224.2.6 Number of animals per group

Group	*Number of females treated*	
	Strain C_{57}BL	**Strain DBA**
Control	21	17
1	10	10
2	18	10
3	7	14
4	10	10
5	22	18
6	18	20

Give number, specify, if there are differences for example for treatment and recovery groups

224.2.7 Control animals	Yes
224.2.8 Mating period	Not stated

224.3 Administration/ Exposure

Oral

Fill in respective route in the following, delete other routes

224.3.1 Duration of exposure

Female test animals were continuously exposed to the test substance in their diet from one month prior to mating until they were sacrificed on the 19th day of pregnancy. Males were also fed the appropriate test diet prior to mating.

rat/mouse: day 6-15 post mating

X

Section A6.8.2 **Fertility Study**

Annex Point IIA6.8.2 *Specify section no., heading, route and species as appropriate*

IUCLID: 5.8.1/01 **A6.8.2(01), Toxicity of copper to fertility**

	Hamster:	day 6-14	post mating
	rabbit:	day 6-18	post mating
	or other		

224.3.2 Postexposure period: None. Females were killed on day 19 of pregnancy.

Oral

224.3.3 Type: In food

224.3.4 Concentration: food consumption per day ..... ad libitum.

Group	*g $CuSO_4$/ kg food*	
	Strain C_{57}BL	**Strain DBA**
Control	0	0
1	0.5	0.5
2	1.0	1.0
3	1.5	1.5
4	2.0	2.0
5	3.0	3.0
6	4.0	4.0

224.3.5 Vehicle: Aqueous solution

224.3.6 Concentration in vehicle: Not stated.

224.3.7 Total volume applied: Not stated.

224.3.8 Controls: Plain diet.

No entry field

224.4 Examinations

224.4.1 Body weight: Not stated.

224.4.2 Food consumption: Not stated.

224.4.3 Clinical signs: Not stated.

224.4.4 Examination of uterine content: Gravid uterine weight not stated.
Number of corpora lutea not stated
Number of implantations not stated..

224.4.5 Examination of foetuses: No entry field

224.4.5.1 General: Litter size, Nr. of living foetuses, Nr. of dead foetuses, foetal weight, Nr. of abnormal foetuses.

224.4.5.2 Skelet: Yes

224.4.5.3 Soft tissue: Yes

224.5 Further remarks None.

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/01	**A6.8.2(01), Toxicity of copper to fertility**

225 RESULTS AND DISCUSSION

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

225.1 Maternal toxic Effects

No effects / describe significant effects referring to data in results table; give concentrations of test substance resulting in toxic effects if any

Maternal toxic effects were not reported.

225.2 Teratogenic / embryotoxic effects

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

$CuSO_4$ doses in the range 0.5 to 1.0 g per kg feed had no harmful effects on the embryonic growth of mice, and may even have stimulated growth to some extent. This is indicated by the absence of foetal abnormality and slightly higher weights of foetuses than those of the controls (**Tables A6.8.2(01)-2a** and **A6.8.2(01)-2b** for C57BL and DBA strains, respectively). Adverse effects on the foetus were recorded only in the foetuses of females fed a diet containing 3 or 4 g $CuSO_4$/kg food, where greater mortality rates and decreased litter weights were observed (**Tables A6.8.2(01)-2a** and **A6.8.2(01)-2b** for C57BL and DBA strains, respectively).

Developmental malformations were seen in a number of foetuses from the top two dose groups. In $_{C57BL}$ mice from the 4 g $CuSO_4$/kg food group, a thoracic wall hernia was found in one foetus, hydrocephalis in another, and coalescence of two adjacent thoracic vertebrae and ribs in a third. The last lumbar vertebra was included in the sacral region of a single $_{C57BL}$ foetus from the 3 g $CuSO_4$/kg food group (**Table A6.8.2(01)-3a**). In the foetuses of DBA mice from the 4 g $CuSO_4$/kg food group, two had encephaloceles, and another two showed inclusion of a half of the last lumbar vertebra in the sacral region. Two DBA strain foetuses from the 3g $CuSO_4$/kg food group had unilateral coalescence of adjacent ribs (**Table A6.8.2(01)-3b**).

225.3 Other effects *Describe any other significant effects*

None reported.

226 APPLICANT'S SUMMARY AND CONCLUSION

226.1 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 $CuSO_4$ added to the diet of pregnant mice on the development of their offspring. The study was not designed to follow an internationally accepted guideline, and was not carried out or reported in compliance with GLP.

Sexually mature male and female mice of the $_{C57BL}$ and DBA strains were divided into six experimental groups and one control group. Animals in the experimental groups were then fed diets containing, 0.5, 1.0, 1.5, 2.0, 3.0 or 4.0 g of $CuSO_4$ per kg of feed *ad libitum* for one month. The diet was prepared by crushing the standard "Murigran" diet and mixing it with aqueous solutions of $CuSO_4$. Control animals received the standard diet only. Male and female animals of each group were held in separate cages during this period.

Section A6.8.2 **Fertility Study**

Annex Point IIA6.8.2 *Specify section no., heading, route and species as appropriate*

IUCLID: 5.8.1/01 **A6.8.2(01), Toxicity of copper to fertility**

After one month, the males and females of each group were paired, and the females continued to receive the appropriate diet through the first 19 days of the resulting pregnancy. The number of pregnant females in each treatment group were as follows:

Group	*Number of females treated*	
	Strain C57BL	**Strain DBA**
Control	21	17
1	10	10
2	18	10
3	7	14
4	10	10
5	22	18
6	18	20

On the 19th day of pregnancy, females were killed and living and dead foetuses were removed, counted and weighed. Half the foetuses of each group were examined by Wilson's method. The other half were stained with alizarin red S in 1% KOH and cleared in glycerin.

226.2 Results and discussion

Summarize relevant results; discuss dose-response relationship.

$CuSO_4$ in the diet at concentrations of 0.5 and 1.0 g per kg feed had no apparent adverse effects on mouse embryonic growth. Indeed, the slightly higher weights of foetuses in groups receiving up to 2.0 g $CuSO_4$/kg food (when compared to controls) may indicate that supplementation of the diet with $CuSO_4$ stimulated growth to some extent (**Tables A6.8.2(01)-2a** and **A6.8.2(01)-2b** for C57BL and DBA strains, respectively).

Adverse effects were recorded in the highest dose groups. Foetuses of females fed a diet containing 3 or 4 g $CuSO_4$/kg food, appeared to have markedly higher mortality rates and decreased litter weights, when compared to controls (**Tables A6.8.2(01)-2a** and **A6.8.2(01)-2b** for C57BL and DBA strains, respectively).

Developmental malformations were seen in a number of foetuses from females fed diets containing 3 or 4 g $CuSO_4$/kg food. In $_{C57BL}$ mice from the 4g $CuSO_4$/kg food group, a thoracic wall hernia was found in one foetus; a hydrocephalis in a second, and coalescence of two adjacent thoracic vertebrae and ribs in a third. The last lumbar vertebra was included in the sacral region of a single $_{C57BL}$ foetus from the 3 g $CuSO_4$/kg food group (**Table A6.8.2(01)-3a**).

In foetuses of DBA mice from the 4 g $CuSO_4$/kg food group, two had encephaloceles, and another two had inclusion of a half of the last lumbar vertebra in the sacral region. Two DBA foetuses from the group fed 3 g $CuSO_4$/kg had unilateral coalescence of adjacent ribs (**Table A6.8.2(01)-3b**).

226.3 Conclusion No entry field

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/01	**A6.8.2(01), Toxicity of copper to fertility**

226.3.1 LO(A)EL maternal toxic effects	*Give critical effect and dose/concentration* Not reported	
226.3.2 NO(A)EL maternal toxic effects	*Give dose/concentration, if necessary separately for males and females* Not reported	
226.3.3 LO(A)EL embryotoxic / teratogenic effects	*Give critical effect and dose/concentration* 3 g $CuSO_4$/kg diet	
226.3.4 NO(A)EL embryotoxic / teratogenic effects	*Give dose/concentration* 2 g $CuSO_4$/kg diet	
226.3.5 Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4* 2	X
226.3.6 Deficiencies	Yes This study was not conducted and/or reported in compliance with GLP. When compared with generally accepted principles to be applied to teratogenicity studies, as set out in OECD guideline 414, it is also apparent that experimental details/results were poorly reported in places, including: • Housing and feeding conditions of test animals; • Information on the age and weight of test animals; • In several dose groups, the number of pregnant animals was smaller than recommended by the guideline (16 animals). • In the absence of information on the weight of test animals and the weight of treated diet consumed, it was not possible to accurately determine the dose received on a mg/kg bodyweight basis. • No information on maternal toxicity was presented in the report. • No post-mortem information was presented in the report for dams. • No information was presented on: the weight of gravid uteri; the number of corpora lutea; degrees of resorption of dead foetuses. • The sex ratio of live foetuses was not reported. These deficiencies do not, however, necessarily compromise the validity of the data reported, or the author's interpretation of that data, given that the study was not carried out for regulatory purposes and that the information that did appear in the report was clearly presented. Furthermore, this research (including its methodology) was published in a peer-reviewed publication, and has therefore been subject to the prior scrutiny of experts in the field. In addition this report has been included in a number of expert reviews of the embryotoxic/teratogenic potential of copper. Overall, this is a well-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 the embryotoxic / teratogenic potential of copper. A reliability indicator of 2 has been assigned on this basis.	X

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/01	**A6.8.2(01), Toxicity of copper to fertility**
	(If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/01	**A6.8.2(01), Toxicity of copper to fertility**

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

EVALUATION BY RAPPORTEUR MEMBER STATE

Date [redacted]

Materials and Methods [redacted]

Results and discussion [redacted]

Conclusion [redacted]

Reliability [redacted]

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/01	**A6.8.2(01), Toxicity of copper to fertility**

Acceptability	[redacted]
Remarks	

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.8.2 **Fertility Study**

Annex Point IIA6.8.2 *Specify section no., heading, route and species as appropriate*

IUCLID: 5.8.1/01 **A6.8.2(01), Toxicity of copper to fertility**

Table A6.8.2(01)-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Modify if necessary and give historical data if available

Parameter	control data		low dose	medium dose	high dose	dose-response + / -
	historical	study				
Number of dams examined						
Clinical findings during application of test substance						
Mortality of dams *state %*						
Abortions						
Body weight gain *day 0-x, day 0-y, day x-y, day 0-end of test,*						
Food consumption						
Water consumption *if test substance is applied with drinking water*						
Pregnancies *pregnancy rate or*						
Necropsy findings in dams dead before end of test						

Table A6.8.2(01)-2a. Table for Teratogenic effects (separate data for all dosage groups) Litter response (Caesarean section data) for $_{C57BL}$ stock Modify if necessary and give historical data if available

Parameter	Concentration of $CuSO_4$ in food (g/kg)							dose-response + / -
	0 (control)	0.5	1.0	1.5	2.0	3.0	4.0	
Corpora lutea state total/number of dams								
Implantations state total/number of dams								
Resorptions state total/number of dams								
total number of fetuses								
pre-implantation loss state %								
post-implantation loss state %								
total number of litters								
Mean fetuses / litter (± SD)	3.09 (±0.83)	4.60 (±1.64)	4.50 (±1.15)	4.42 (±1.13)	4.20 (±1.39)	2.50 (±1.01)	1.94 (±0.80)	
No. of live fetuses / dose group (%)	65 (83.1)	46 (89.2)	81 (86.5)	31 (87.1)	42 (78.6)	55 (72.8)	35 (71.5)	
No. of dead fetuses / dose group (%)	11 (16.9)	5 (10.8)	11 (13.5)	4 (12.9)	9 (21.4)	15 (27.2)	10 (28.5)	
Mean fetus weight in g (± SD)	1.10 (±0.15)	1.35 (±0.18)	1.22 (±0.13)	1.14 (±0.12)	1.25 (±0.24)	1.00 (±0.14)	0.99 (±0.10)	
placenta weight (mean) [g]								
crown-rump length (mean) [mm]								
Fetal sex ratio [state ratio m/f]								

Table A6.8.2(01)-2b. Table for Teratogenic effects (separate data for all dosage groups) Litter response (Caesarean section data) for DBA stock
Modify if necessary and give historical data if available

Parameter	Concentration of $CuSO_4$ in food (g/kg)							dose-response + / -
	0 (control)	0.5	1.0	1.5	2.0	3.0	4.0	
Corpora lutea state total/number of dams								
Implantations state total/number of dams								
Resorptions state total/number of dams								
total number of fetuses								
pre-implantation loss state %								
post-implantation loss state %								
total number of litters								
Mean fetuses / litter (± SD)	4.47 (±1.46)	5.40 (±1.17)	5.10 (±1.19)	4.14 (±1.93)	4.10 (±0.87)	3.11 (±1.27)	2.70 (±1.26)	
No. of live fetuses / dose group (%)	76 (84.3)	54 (90.8)	51 (88.3)	58 (82.8)	41 (83.0)	56 (75.0)	45 (70.4)	
No. of dead fetuses / dose group (%)	12 (15.7)	5 (9.2)	6 (11.7)	10 (17.2)	7 (17.0)	14 (25.0)	16 (29.6)	
Mean fetus weight in g (± SD)	0.96 (±0.16)	1.24 (±0.12)	1.19 (±0.18)	1.17 (±0.20)	1.13 (±0.11)	1.11 (±0.10)	1.09 (±0.18)	
placenta weight (mean) [g]								
crown-rump length (mean) [mm]								
Fetal sex ratio [state ratio m/f]								

Table A6.8.2(01)-3a. Table for Teratogenic effects (separate data for all dosage groups) Examination of the fetuses (C_{57}BL stock) Modify if necessary and give historical data if available

Parameter	control data	0.5	1.0	1.5	2.0	3.0	4.0	dose-response +/-
Number of external malformations* (%)	0	0	0	0	0	0	1 (2.8)	+
Number of external anomalies* (%)	0	0	0	0	0	0	0	-
Number of skeletal malformations* (%)	0	0	0	0	0	1 (1.8)	1 (2.8)	+
Number of skeletal anomalies* (%)	0	0	0	0	0	0	0	-
Number of skeletal variants* (%)	0	0	0	0	0	0	0	-
Number of visceral malformations* (%)	0	0	0	0	0	0	1 (2.8)	+
Number of visceral anomalies* (%)	0	0	0	0	0	0		-
Number of variants visceral* (%)	0	0	0	0	0	0	0	-

Table A6.8.2(01)-3b. Table for Teratogenic effects (separate data for all dosage groups) Examination of the fetuses (DBA stock) Modify if necessary and give historical data if available

Parameter	control data	0.5	1.0	1.5	2.0	3.0	4.0	dose-response +/-
Number of external malformations* (%)	0	0	0	0	0	0	2 (3.7)	+
Number of external anomalies* (%)	0	0	0	0	0	0	0	-
Number of skeletal malformations* (%)	0	0	0	0	0	2 (3.7)	2 (3.7)	+
Number of skeletal anomalies* (%)	0	0	0	0	0	0	0	-

Number of skeletal variants* (%)	0	0	0	0	0	0	0	-
Number of visceral malformations* (%)	0	0	0	0	0	0	0	-
Number of visceral anomalies* (%)	0	0	0	0	0	0	0	-
Number of variants visceral* (%)	0	0	0	0	0	0	0	-

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/02	**A6.8.2(02), Toxicity of copper to fertility**

		Official use only
	227 REFERENCE	
227.1 Reference	Aulerich, R.J., Ringer, R.K., Bleavins, M.R. and Napolitano, A. (1982). Effects of Supplemental Dietary Copper on Growth, Reproductive Performance and Kit Survival of Standard Dark Mink and the Acute Toxicity of Copper to Mink, **55(2):** 337 - 343 (published). *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).*	X
227.2 Data protection	No. *(indicate if data protection is claimed)*	
227.2.1 Data owner	*Give name of company* Public domain.	
227.2.2 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.	
227.2.3 Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation in support of AnnexVI) and delete the others:* No data protection claimed.	
	228 GUIDELINES AND QUALITY ASSURANCE	
228.1 Guideline study	No. This was a non-regulatory study carried out to investigate the role of Cu on growth in the physiology and nutrition of mink (including an evaluation of the effects of the supplemental Cu on reproduction and early kit growth and survival). *(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*	
228.2 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)*	
228.3 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")*	
	229 MATERIALS AND METHODS	
229.1 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 $CuSO_4 5H_2O$ *or give name used in study report*	

Section A6.8.2 **Fertility Study**

Annex Point IIA6.8.2 *Specify section no., heading, route and species as appropriate*

IUCLID: 5.8.1/02 **A6.8.2(02), Toxicity of copper to fertility**

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

Not stated.

229.1.2 Specification

Deviating from specification given in section 2 as follows
(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):

229.1.2.1 Description

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

$CuSO_4$ was added to the diet as an aqueous solution.

229.1.2.2 Purity

Give purity in % active substance X

[redacted]

229.1.2.3 Stability

Describe stability of test material.

Not applicable to metals.
Non-entry field.

229.2 Test Animals

229.2.1 Species

Mink.

229.2.2 Strain

Standard dark mink.

229.2.3 Source

Not stated.

229.2.4 Sex

Male and female

229.2.5 Age/weight at study initiation

Male mink kits had mean body weights in the range 625 – 709 g (SE 36 g) at study initiation.

female mink kits had mean body weights in the range 517 – 563 g (SE 24 g) at study initiation.

229.2.6 Number of animals per group

Numbers of animals in treatment groups at study initiation were as follows:

Cu Supplement	*Number of animals per group*	
	Male	Female
Control	12	12
25 ppm Cu	12	12
50 ppm Cu	11	12
100 ppm Cu	12	12
200 ppm Cu	12	12

Only four males from each treatment group were eventually mated with the twelve females from the corresponding group.

Give number, specify, if there are differences for example for treatment and recovery groups

229.2.7 Control animals

Yes

229.2.8 Mating period

The females were mated with males within their respective dietary groups for a 20 day period between March 1 and 20, 1980.

229.3 Administration/ Exposure

Oral

Fill in respective route in the following, delete other routes

Section A6.8.2	**Fertility Study**	
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*	
IUCLID: 5.8.1/02	**A6.8.2(02), Toxicity of copper to fertility**	
229.3.1 Duration of exposure	Test animals received diets supplemented with copper for the period July 6 1979 to June 27, 1980 (1 year). This included a nine month period prior to mating, the whole of gestation and at least six weeks after whelping (whelping took place between April 15 and May 15, 1980).	
	rat/mouse: day 6-15 post mating	X
	Hamster: day 6-14 post mating	
	Rabbit: day 6-18 post mating	
	or other	
229.3.2 Postexposure period	None.	
	Oral	
229.3.3 Type	In food	
229.3.4 Concentration	food consumption per day ..... ad libitum.	
	Dietary groups were (1) basal diet, no supplemental Cu (control); (2) basal diet plus 25 ppm Cu from copper sulphate; (3) basal diet plus 50 ppm Cu from copper sulphate; (4) basal diet plus 100 ppm Cu from copper sulphate; (5) basal diet plus 200 ppm Cu from copper sulphate;	
	The control diet contained 60.6 ppm Cu. No Cu was detected in drinking water by atomic absorption spectrophotometry.	X
229.3.5 Vehicle	Aqueous solution	
229.3.6 Concentration in vehicle	Not stated.	
229.3.7 Total volume applied	Not stated.	
229.3.8 Controls	Basic diet.	
229.4 Examinations	No entry field	
229.4.1 Body weight	Yes.	
229.4.2 Food consumption	Not stated.	
229.4.3 Clinical signs	Not stated.	
229.4.4 Examination of uterine content	No.	
	Number of corpora lutea not stated. Number of implantations not stated. Or other	
229.4.5 Examination of foetuses	No entry field	

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/02	**A6.8.2(02), Toxicity of copper to fertility**

229.4.5.1 General Number of kits whelped alive and dead; average number of kits whelped per female whelped and per female mated. Refer to section 3.5 for other factors assessed.

229.4.5.2 Skeleton No.

229.4.5.3 Soft tissue No.

229.5 Further remarks Factors assessed: number of females whelped vs. number mated; average gestation period; % kit mortality, birth to 4 weeks; litter mass; average kit weight at birth and at 4 weeks.

230 RESULTS AND DISCUSSION

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

230.1 Maternal toxic Effects

No effects / describe significant effects referring to data in results table; give concentrations of test substance resulting in toxic effects if any

There was no evidence of maternal toxic effects in any treatment group prior to whelping (**Table A6.8.2(02)-1**). See comments in section 4.2 for comments on possible adverse effects during lactation.

230.2 Teratogenic / embryotoxic effects

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

Except for the trend toward greater kit mortality between birth and 4 weeks of age and the reduced litter mass at weaning with increased Cu supplementation, the characteristics measured were generally within the normal range for mink. Gestation length and kit weight at birth were not adversely affected by the dietary treatments (**Table A6.8.2(02)-2**).

It was considered that the greater kit mortality during the nursing period and the reduced litter mass at weaning suggest that the higher levels of supplemental Cu may have had an adverse effect on lactation.

230.3 Other effects *Describe any other significant effects*

None reported.

231 APPLICANT'S SUMMARY AND CONCLUSION

231.1 Materials and methods

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

A study carried out to investigate the role of Cu on growth in the physiology and nutrition of mink (including an evaluation of the effects of the supplemental Cu on reproduction and early kit growth and survival). The study was not designed to follow an internationally accepted guideline, and was not carried out or reported in compliance with GLP.

A study was carried out in which 120 standard dark mink kits were assigned to five groups (each containing 12 males and 12 females) and placed on the following dietary treatments *ad libitum*: (1) basal diet, no supplemental Cu (control); (2) basal diet plus 25 ppm Cu; (3) basal diet plus 50 ppm Cu; (4) basal diet plus 100 ppm Cu; (5) basal diet plus 200 ppm Cu. In all cases the Cu was derived from $CuSO_4.5H_2O$. The control diet contained 60.6 ppm Cu; no Cu was detected in drinking water by atomic absorption spectrophotometry.

X

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/02	**A6.8.2(02), Toxicity of copper to fertility**

	Littermates were divided among the various groups in an effort to minimise genetic influence on reproduction and response to the dietary treatments. For the first six months of the study, the animals were housed individually in open-sided sheds in mink growing cages with additional nest boxes. During the remainder of the study, the mink were kept in breeder cages with attached nest boxes. After approximately nine months on the supplemented diets, the females were mated with four males from within their respective dietary groups (the other 8 males were retained for alternative investigations). All matings were confirmed by the presence of motile sperm in vaginal smears taken immediately after mating. During the whelping period, the mated females were checked daily for evidence of whelping. The kits were counted and weighed on the day of birth and at 4 weeks of age. Body weight comparisons were made with initial body weight as a covariate. Differences between means were determined using Dunnett's test on the adjusted treatment means.
231.2 Results and discussion	*Summarize relevant results; discuss dose-response relationship.* The reproductive performance of the female mink fed the Cu-supplemented diets is summarised in **Table A6.8.2(02)-3**. Except for the trend towards greater kit mortality between birth and 4 weeks of age and the reduced litter mass at weaning with increased Cu supplementation, the characteristics measured were within the normal range for mink. Length of gestation and kit weight at birth were not adversely affected by the dietary treatments. The greater kit mortality during the nursing period and the reduced litter mass at weaning suggest that the higher levels of supplemental Cu may have had an adverse effect on lactation (i.e. these findings were due to an adverse maternal effect at Cu supplementation rates of 100 and 200 ppm).
231.3 Conclusion	No entry field
231.3.1 LO(A)EL maternal toxic effects	*Give critical effect and dose/concentration* Effect on lactation, resulting in increased kit mortality and reduced litter mass; 100 ppm Cu.
231.3.2 NO(A)EL maternal toxic effects	*Give dose/concentration, if necessary separately for males and females* 50 ppm Cu.
231.3.3 LO(A)EL embryotoxic / teratogenic effects	*Give critical effect and dose/concentration* 100 ppm Cu. Adverse effects due to maternal toxicity – see section 5.3.1.
231.3.4 NO(A)EL embryotoxic / teratogenic effects	*Give dose/concentration* 50 ppm Cu.
231.3.5 Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4* 2
231.3.6 Deficiencies	Yes This study was not conducted and/or reported in compliance with GLP.

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/02	**A6.8.2(02), Toxicity of copper to fertility**

When compared with generally accepted principles to be applied to embryotoxicity/teratogenicity studies, as set out in OECD guideline 414, it is also apparent that experimental details/results were poorly reported in places, including:

- Housing and feeding conditions of test animals;
- The number of pregnant animals was smaller than recommended by the guideline (16 animals).
- In the absence of information on the weight of treated diet consumed, it was not possible to accurately determine the dose received on a mg/kg bodyweight basis.
- No post-mortem information was presented in the report for dams.
- The sex ratio of live foetuses was not reported.

It should be noted that the study was not designed to address endpoints such as examination of uterine contents and the condition of foetuses during gestation.

These deficiencies do not, however, necessarily compromise the validity of the data reported, or the author's interpretation of that data, given that the study was not carried out for regulatory purposes and that the information that did appear in the report was clearly presented. Furthermore, this research (including its methodology) was published in a peer-reviewed publication, and has therefore been subject to the prior scrutiny of experts in the field. In addition this report has been included in a number of expert reviews of the embryotoxicity/teratogenicity potential of copper.

Overall, this is a well-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 the embryotoxic / teratogenic potential of copper. 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.)

Section A6.8.2 **Fertility Study**

Annex Point IIA6.8.2 *Specify section no., heading, route and species as appropriate*

IUCLID: 5.8.1/02 **A6.8.2(02), Toxicity of copper to fertility**

	Evaluation by Competent Authorities
	Use separate "evaluation boxes" to provide transparency as to the comments and views submitted
	EVALUATION BY RAPPORTEUR MEMBER STATE
Date	[redacted]
Reference	[redacted]
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	

COMMENTS FROM ...

Date *Give date of comments submitted*

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1/02	**A6.8.2(02), Toxicity of copper to fertility**

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 A6.8.2(02)-1. Table for Teratogenic effects (separate data for all dosage groups)

Maternal effects

Modify if necessary and give historical data if available

Parameter	Concentration of $CuSO_4$ in food (ppm)					dose-response + / -
	Control	25	50	100	200	
Number of dams examined	12	11	12	12	12	-
Clinical findings during application of test substance						
Mortality of dams *state %*	0	0	0	0	0	-
Abortions	--*	--	--	--	--	--
Body weight gain (g) *Initial weight; at 8 weeks; at 20+ weeks*	517; 909; 1074	563; 937; 994	545; 947; 1068	536; 947; 1065	534; 965; 1058	-
Food consumption	--	--	---	--	--	--
Water consumption *if test substance is applied with drinking water*	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	-
Pregnancies *pregnancy rate*	11/12	6/11	12/12	8/12	10/12	-
Average gestation *days*	47.2	46.7	49.6	49.4	46.7	-
Necropsy findings in dams dead before end of test	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	-

* Not reported

Table A6.8.2(02)-2. Table for Teratogenic effects (separate data for all dosage groups)
Litter response (Caesarean section data)

Modify if necessary and give historical data if available

Parameter	Concentration of $CuSO_4$ in food (ppm)					dose-response + / -
	Control	25	50	100	200	
Corpora lutea *state total/number of dams*	--*	--	--	--	--	--
Implantations *state total/number of dams*	--	--	--	--	--	--
Resorptions *state total/number of dams*	--	--	--	--	--	--
total number of fetuses	77	38	70	54	63	-
pre-implantation loss state %	--	--	--	--	--	--
post-implantation loss state %	--	--	--	--	--	--
total number of litters	11	6	12	8	10	-
No. of kits whelped alive / dose group	69	33	64	50	57	-
No. of kits whelped dead / dose group	8	5	6	4	6	-
Mean No. kits / female whelped	7.0	6.3	5.8	6.8	6.3	
Mean No. kits / female mated	6.4	3.5	5.8	4.5	5.3	
Mean kit weight (g) (± SE) *At birth; at 4 weeks*	8.8 ± 0.2; 136.9 ± 1.4	9.7 ± 0.3; 143.0 ± 3.7	9.4 ± 0.2; 133.3 ± 2.7	8.5 ± 0.2 116.4 ± 7.1	8.3 ± 0.3; 143.3 ± 4.8	-
Mean litter mass (g)	704.6	666.5	582.3	474.8	580.5	-
placenta weight *(mean)* [g]	--	--	--	--	--	--
crown-rump length ***(mean)*** [mm]	--	--	--	--	--	--
Fetal sex ratio *[state ratio m/f]*	--	--	--	--	--	--
% Kit mortality birth to 4 weeks	12	9	19	38	32	

Table A6.8.2(02)-3

TABLE 5. REPRODUCTIVE PERFORMANCE OF FEMALE MINK FED THE CONTROL DIET OR DIETS THAT CONTAINED SUPPLEMENTAL COPPER AND THE AVERAGE WEIGHT, LITTER MASS[a] AND SURVIVAL OF THEIR KITS

Dietary treatment	No. ♀'s whelped/ no. mated	Avg gestation[b], d	No. kits whelped		Avg no. kits whelped/♀		% kit mortality birth to 4 wk	Avg ± SE kit weight, g		Litter ma g
			Alive	Dead	Whelped	Mated		At birth	At 4 wk	
Control	11/12	47.2	69	8	7.0	6.4	12	8.8 ± .2[c]	136.9 ± 1.4[c]	704.6
25 ppm supplemental Cu	6/11	46.7	33	5	6.3	3.5	9	9.7 ± .3[c]	143.0 ± 3.7[c]	666.5
50 ppm supplemental Cu	12/12	49.6	64	6	5.8	5.8	19	9.4 ± .2[c]	133.3 ± 2.7[c]	582.3
100 ppm supplemental Cu	8/12	49.4	50	4	6.8	4.5	38	8.5 ± .2[c]	116.4 ± 7.1[d]	474.8
200 ppm supplemental Cu	10/12	46.7	57	6	6.3	5.3	12	8.3 ± .3[c]	143.3 ± 4.8[c]	580.5

[a]Average kit body weight gain between birth and 4 wk of age X the average number of kits raised per lactating female.

[b]Based on date of final mating.

[c,d]Body weight means for each age followed by different superscripts differ (P<.05).

Section A 6.8.2	**Repeated dose toxicity in the Rat**	
Annex Point 6.8.2	*Specify section no. and heading, route and species*	
IUCLID: 5.8.1/03	**A6.8.2(03), Subchronic Oral Toxicity Test**	

			Official use only
		1 REFERENCE	
1.3	**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).* Hébert, C.D., 1993. NTP Technical Report on toxicity studies of cupric sulphate (CAS No. 7758-99-8) administered in drinking water and feed to F344/N rats and B6C3F1 mice. National Toxicology Program, Toxicity Report Series No. 29, United States Department of Health and Human Services (NIH Publication 93-3) (published)	X
1.4	**Data protection**	No *(indicate if data protection is claimed)* 1.4.1 Data owner *Give name of company* – Not applicable	
1.4.2	Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* Not applicable	
		6 GUIDELINES AND QUALITY ASSURANCE	
6.3	**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")*	
6.4	**GLP**	Yes *(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)*	
6.5	**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
		7 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.	
7.3	**Test material**	Copper sulphate *or give name used in study report*	X
7.3.1	Lot/Batch number	*List lot/batch number if available* 533344	
7.3.2	Specification	Not reported *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):*	
7.3.2.1	Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Blue, crystalline solid	
7.3.2.2	Purity	*Give purity in % of active substance* [redacted]	X

Section A 6.8.2	**Repeated dose toxicity in the Rat**	
Annex Point 6.8.2	*Specify section no. and heading, route and species*	
IUCLID: 5.8.1/03	**A6.8.2(03), Subchronic Oral Toxicity Test**	
7.3.2.3 Stability	*Describe stability of test material* Stable at room temperature	
7.4 Test Animals	Non-entry field	
7.4.1 Species	Rat	
7.4.2 Strain	F344/N	
7.4.3 Source	Simonsen Laboratories, Gilroy, California, USA	
7.4.4 Sex	Male and Female	
7.4.5 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	
7.4.6 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).	
7.4.7 Control animals	Yes	X
7.5 Administration/ Exposure	Oral *(fill in respective route in the following, delete other routes)*	
7.5.1 Duration of treatment	92 Days	
7.5.2 Frequency of exposure	*ad libitum* for 7-days a week	
7.5.3 Postexposure period	None	
7.5.4 Oral		
7.5.4.1 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.	
7.5.4.2 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.	X
7.5.4.3 Duration of exposure	92-Days	
7.5.4.4 Controls	Yes –vehicle only	
7.6 Examinations	*Non entry field*	
7.6.1 Observations	*Non entry field*	
7.6.1.1 Clinical signs	*yes/no (give time periods for observation)* Yes – test animals were observed weekly for clinical signs	

Section A 6.8.2	**Repeated dose toxicity in the Rat**
Annex Point 6.8.2	*Specify section no. and heading, route and species*
IUCLID: 5.8.1/03	**A6.8.2(03), Subchronic Oral Toxicity Test**

7.6.1.2 Mortality	*yes/no (give time periods for observation)* Yes – test animals were observed twice daily for mortality/morbidity.	
7.6.2 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.	
7.6.3 Food consumption	*yes/no (give time periods for determinations)* Yes – test animals were observed once weekly for food consumption.	
7.6.4 Water consumption	*yes/no (give time periods for determinations)* Not reported	
7.6.5 Ophthalmoscopic examination	*yes/no (give time periods for examinations)* See histological examinations	
	7.6.6 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.	
7.6.7 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.	
7.6.8 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
7.6.9 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.	
7.7 Sacrifice and pathology	Non entry field	

Section A 6.8.2	**Repeated dose toxicity in the Rat**
Annex Point 6.8.2	*Specify section no. and heading, route and species*
IUCLID: 5.8.1/03	**A6.8.2(03), Subchronic Oral Toxicity Test**

7.7.1 Organ Weights	Yes organs: liver, kidneys, adrenals, testes, uterus, ovaries, thymus, spleen, brain, heart	X
7.7.2 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	
7.7.3 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.	
7.7.3.1 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 6.8.2	**Repeated dose toxicity in the Rat**
Annex Point 6.8.2	*Specify section no. and heading, route and species*
IUCLID: 5.8.1/03	**A6.8.2(03), Subchronic Oral Toxicity Test**

7.8	**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

8 RESULTS AND DISCUSSION

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

8.3	**Observations**	Non entry field
8.3.1	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 A6.8.2(03)-5
8.3.2	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 TableA6.8.2(03).5
8.4	**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 A6.8.2(03).5
8.5	**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

Section A 6.8.2	**Repeated dose toxicity in the Rat**
Annex Point 6.8.2	*Specify section no. and heading, route and species*
IUCLID: 5.8.1/03	**A6.8.2(03), Subchronic Oral Toxicity Test**

		refer to Table A6.8.2(03)-5
8.6	**Ophtalmoscopic examination**	Not reported. See Section 3.5.2
8.7	**Blood analysis**	*Non entry field*
8.7.1	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 A6.8.2(03)-1

Section A 6.8.2	**Repeated dose toxicity in the Rat**
Annex Point 6.8.2	*Specify section no. and heading, route and species*
IUCLID: 5.8.1/03	**A6.8.2(03), Subchronic Oral Toxicity Test**

8.7.2 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 A6.8.2(03)-2
8.7.3 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 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 A6.8.2(03)-3 for further information.
8.8 Sacrifice and pathology	*Non entry field*
8.8.1 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 A6.8.2(03)-5
8.8.2 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 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.

8.9 Other

Non entry field

4.8 Tissue Metal Level Analysis

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.

For further information please refer to Table A6.8.2(03)-4

8.9 Nonneoploastic lesions

A summary of nonneoplastic lesions is presented in the attached document Table A6.8.2(03)-6

8.10 Supplemental histological examination

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.

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 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	
8.11	Sperm Morphology and Vaginal Cytology	There were no significant findings in males or females. See attached Table A6.8.2(03)-7	
		9 APPLICANT'S SUMMARY AND CONCLUSION	
9.3	**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
9.4	**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 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.	
9.5 Conclusion	*Non entry field*	
9.5.1 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.	
9.5.2 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.	
9.5.3 Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4*	X
9.5.4 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 [redacted]

Reference • [redacted]

Section A 6.8.2	**Repeated dose toxicity in the Rat**
Annex Point 6.8.2	*Specify section no. and heading, route and species*
IUCLID: 5.8.1/03	**A6.8.2(03), Subchronic Oral Toxicity Test**

Materials and Methods

[redacted]

Results and discussion

[redacted]

Conclusion

[redacted]

Reliability

[redacted]

Acceptability

[redacted]

Remarks

- [redacted]

[redacted]

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]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[a]	m[a]	f[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

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 A6.8.2(03)-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 A6.8.2(03)-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 A6.8.2(03)-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 A6.8.2(03)-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 A6.8.2(03)-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 A6.8.2(03)-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%)					8 (80%)
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				3 (30%)	10 (100%)	10 (100%)
Nephropathy	10 (100%)	9 (100%)	10 (100%)	8 (80%)	9 (90%)	5 (50%)
Proximal convoluted renal tubule, karyomegaly						10 (100%)

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

Table A6.8.2(03)-6b Summary of the Incidence of Nonneoplastic Lesions in Female Rats.

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	6000 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 A6.8.2(03)-6b Summary of the Incidence of Nonneoplastic Lesions in Female Rats (cont.).

	0 ppm	500 ppm	1000 ppm	2000 ppm	4000 ppm	6000 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%)

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

Table A6.8.2(03)-7 Summary of the Reproductive Evaluations in Male and Female Rats

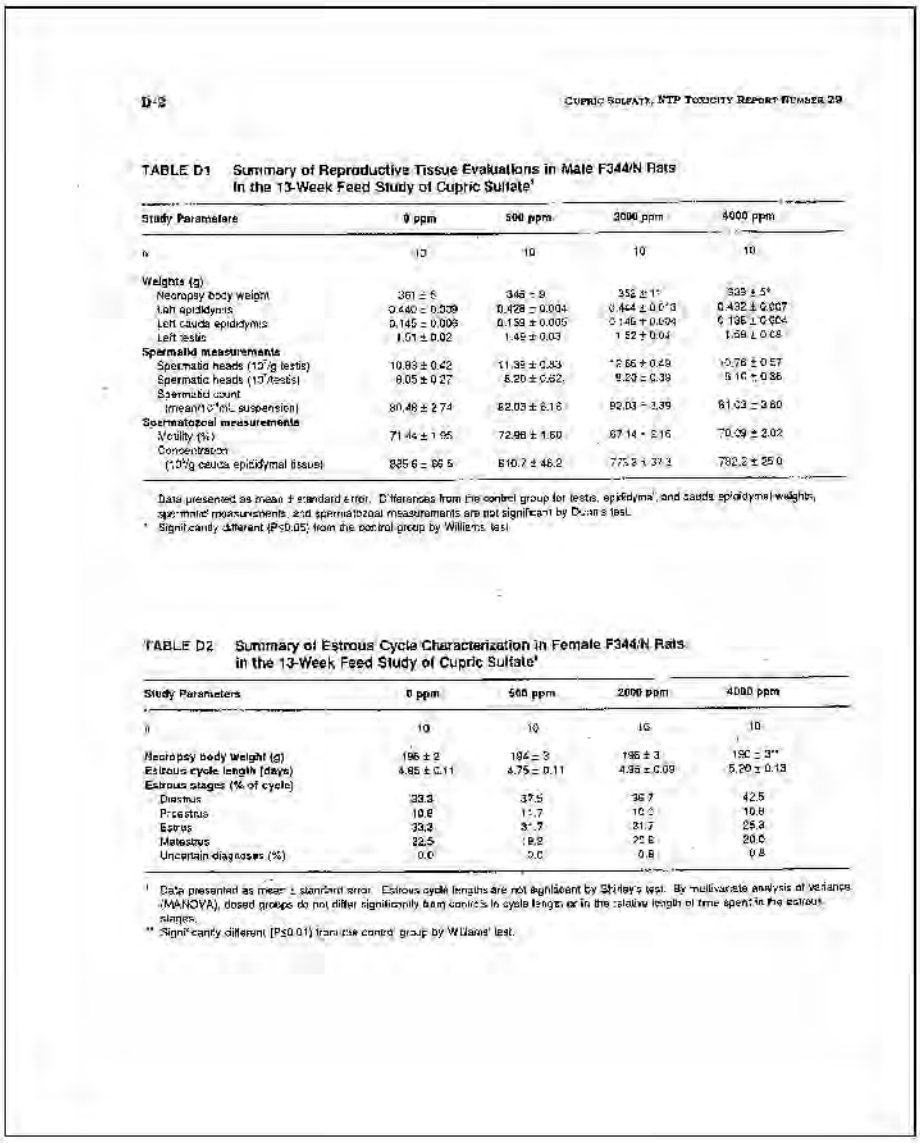


TABLE D1 Summary of Reproductive Tissue Evaluations in Male F344/N Rats in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	500 ppm	2000 ppm	4000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	361 ± 6	348 ± 9	352 ± 11	339 ± 5*
Left epididymis	0.440 ± 0.009	0.428 ± 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	1.48 ± 0.03	1.52 ± 0.04	1.58 ± 0.03
Spermatid measurements				
Spermatid heads (10⁷/g testis)	10.83 ± 0.42	11.39 ± 0.53	10.66 ± 0.49	10.76 ± 0.57
Spermatic heads (10⁷/testis)	8.05 ± 0.27	8.20 ± 0.62	8.23 ± 0.39	8.16 ± 0.86
Spermatid count (mean/10⁶/mL suspension)	80.48 ± 2.74	82.03 ± 6.16	82.03 ± 3.89	81.63 ± 3.60
Spermatozoal measurements				
Motility (%)	71.44 ± 1.65	72.98 ± 1.60	67.14 ± 2.16	70.29 ± 2.02
Concentration (10⁶/g cauda epididymal tissue)	825.6 ± 66.5	810.7 ± 48.2	775.3 ± 37.3	782.2 ± 25.0

Data presented as mean ± standard error. Differences from the control group for testis, epididymis, 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)	196 ± 2	194 ± 3	196 ± 3	190 ± 3**
Estrous cycle length (days)	4.85 ± 0.11	4.75 ± 0.11	4.88 ± 0.09	5.20 ± 0.13
Estrous stages (% of cycle)				
Diestrus	33.3	37.5	36.7	42.5
Proestrus	10.8	11.7	10.0	10.8
Estrus	33.3	37.7	31.7	25.3
Metestrus	22.5	13.2	21.6	20.0
Uncertain diagnoses (%)	0.0	0.0	0.8	0.8

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 A6.8.2 **Repeated dose toxicity in the Mouse**

Annex Point 6.8.2 *Specify section no. and heading, route and species*

IUCLID: 5.8.1/04 **A6.8.2(04), Subchronic Oral Toxicity Test**

			Official use only
		1 REFERENCE	
1.5	**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).* Hébert, C.D., 1993. NTP Technical Report on toxicity studies of cupric sulphate (CAS No. 7758-99-8) administered in drinking water and feed to F344/N rats and B6C3F1 mice. National Toxicology Program, Toxicity Report Series No. 29, United States Department of Health and Human Services (NIH Publication 93-3) (published)	X
1.6	**Data protection**	No *(indicate if data protection is claimed)*	
1.6.1	Data owner	*Give name of company* - Not applicable	
1.6.2	Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* Not applicable	
		10 GUIDELINES AND QUALITY ASSURANCE	
10.3	**Guideline study**	No - The method was developed by the US National Toxicology Programme 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")*	
10.4	**GLP**	Yes *(If no, give justification, e.g. state that GLP was not compulsory at the time the study was performed)*	
10.5	**Deviations**	See Section 5.3.5 *(If yes, describe deviations from test guidelines or refer to respective field numbers where these are described, e.g. "see 3.x.y")*	X
		11 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.*	
11.1	**Test material**	Copper sulphate *or give name used in study report*	X
11.1.1	Lot/Batch number	*List lot/batch number if available* 533344	
11.1.2	Specification	Not reported *(describe specification under separate subheadings, such as the following; additional subheadings may be appropriate):*	

Section A6.8.2	**Repeated dose toxicity in the Mouse**	
Annex Point 6.8.2	*Specify section no. and heading, route and species*	
IUCLID: 5.8.1/04	**A6.8.2(04), Subchronic Oral Toxicity Test**	
11.1.2.1 Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Blue, crystalline solid	
11.1.2.2 Purity	*Give purity in % of active substance* [redacted]	X
11.1.2.3 Stability	*Describe stability of test material* Stable at room temperature Non-entry field	
11.2 Test Animals		
11.2.1 Species	Mouse	
11.2.2 Strain	B6C3F1	
11.2.3 Source	Simonsen Laboratories, Gilroy, California, USA	
11.2.4 Sex	Male and Female	
11.2.5 Age/weight at study initiation	Test animals were approximately 6 weeks old at study initiation. Male mean bodyweights ranged from 20.9-21.6 g, mean female bodyweights ranged from 17.1-18.6 g	
11.2.6 Number of animals per group	*Give number* *specify, if there are differences for example for treatment and recovery groups* In the study, groups of 10 animals per sex were tested at each dose level.	
11.2.7 Control animals	Yes (10 males and 10 females)	
11.3 Administration/ Exposure	Oral *(fill in respective route in the following, delete other routes)*	
11.3.1 Duration of treatment	92 Days	
11.3.2 Frequency of exposure	*ad libitum* for 7-days a week	
11.3.3 Postexposure period	None	
11.3.4 <u>Oral</u>	*Non entry field*	
11.3.4.1 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.	
11.3.4.2 Concentration in vehicle	Feed mix was available *ad libitum* throughout the study period. 0 (control), 1000, 2000, 4000, 8000 or 16,000 ppm were administered to the test organisms in feed. Doses were based on a preliminary 2-week feed study.	X
11.3.4.3 Duration of exposure	92-Days	
11.3.4.4 Controls	Yes –vehicle only	
11.4 Examinations	*Non entry field*	
11.4.1 Observations	*Non entry field*	

Section A6.8.2	**Repeated dose toxicity in the Mouse**	
Annex Point 6.8.2	*Specify section no. and heading, route and species*	
IUCLID: 5.8.1/04	**A6.8.2(04), Subchronic Oral Toxicity Test**	
11.4.1.1 Clinical signs	*yes/no (give time periods for observation)* Yes – test animals were observed weekly for clinical signs	
11.4.1.2 Mortality	*yes/no (give time periods for observation)* Yes – test animals were observed twice daily for mortality/morbidity.	
11.4.2 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.	
11.4.3 Food consumption	*yes/no (give time periods for determinations)* Yes – test animals were observed once weekly for food consumption.	
11.4.4 Water consumption	*yes/no (give time periods for determinations)* Not reported	
11.4.5 Ophthalmoscopic examination	*yes/no (give time periods for examinations)* See histological examinations Section 3.5.2	
11.4.6 Haematology	No haematology parameters were investigated	
11.4.7 Clinical Chemisty	No clinical chemistry parameters were investigated	
11.4.8 Urinalysis	No urinalysis investigation was carried out	
11.5 Sacrifice and pathology	*Non entry field*	
11.5.1 Organ Weights	Yes organs: liver, kidneys, adrenals, testes, epididymides, uterus, ovaries, thymus, spleen, brain, heart	X
11.5.2 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.	X
11.5.3 Other examinations	*Non entry field*	
11.5.4 Supplemental histological examination	To characterise the distribution of copper in the liver and kidney, sections of both organs from selected males 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). Perl's stain for iron was used to stain sections of spleen from mice in all groups	
11.5.5 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	X

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. X

11.6 Statistics

The following statistical procedures were followed; X

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, McGrawHill Book Co.

12 RESULTS AND DISCUSSION

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

12.1 Observations *Non entry field*

12.1.1 Clinical signs *no effects / describe effects*

No clinical signs of toxicity, considered to be substance related, were observed in male or female mice during the course of the study.

12.1.2 Mortality *no mortalities at any dose/concentration level / describe significant*

Section A6.8.2	**Repeated dose toxicity in the Mouse**
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		effects referring to data given in results table No mice in any of the dose groups died or were killed before the end of the 13-week study.	
12.2	**Body weight gain**	*no effects / describe significant effects referring to data given in results table* Mice exhibited a dose-related growth depression which resulted in more severe body weight depression at higher dose levels. Final mean bodyweights and bodyweight gains were slightly lower than those of the control group for males in the 4000 ppm group and were significantly lower for males and females in the 8000 and 16,000 ppm groups. See Table A6.8.2(04)-1	
12.3	**Food consumption and compound intake**	*no effects / describe significant effects referring to data given in results table* For both sexes in all dose groups, the average daily feed consumption was similar to, or exceeded that of the controls. The average daily compound consumption increased proportionally with increasing concentrations of copper sulphate pentahydrate in the feed. See Table A6.8.2(04)-1	
12.4	**Opthalmoscopic examination**	No effects	X
12.5	**Blood analysis**	*Non entry field*	
12.5.1	Haematology	*no effects / describe significant effects referring to data given in results table* Not applicable	
12.5.2	Clinical chemistry	*no effects / describe significant effects referring to data given in results table* Not applicable	
12.5.3	Urinalysis	*no effects / describe significant effects referring to data given in results table* Not applicable	
12.6	**Sacrifice and pathology**	*Non entry field*	
12.6.1	Organ weights	*no effects / describe significant effects referring to data given in results table* Significant decreases in organ weights were noted for the heart and kidney of high dose (16,000 ppm) male mice and the thymus and kidney of the high dose females. In addition, dose related decreases in absolute liver weights were noted for males and females, with significant decreases occurring in both sexes in the 8000 and 16,000 ppm groups and the 4000 ppm group in males. Generally relative organ weights for males and females in all dosed groups were greater than that of the controls, and many of these increases were significant for the higher dose groups. These changes in absolute and relative organ weights could be attributed to the lower final mean weights of mice in the higher doses. See Table A6.8.2(04)-1.	
12.6.2	Gross and histopathology	*no effects / describe significant effects referring to data given in results table* Chemical related gross lesions were limited to the forestomach of seven	

male and four female mice in the 16,000 ppm groups. This lesion was characterised as a focal white discolouration of the squamous mucosa in the area of the limiting ridge where it forms a junction with the glandular gastric mucosa. Histopathological findings included minimal to mild squamous cell hyperplasia with hyperkeratosis of the forestomach mucosa at the site of the limiting ridge. This lesion was present in male and female mice receiving 4000 ppm test substance or greater. There was no evidence of inflammation or erosion/ulceration in the forestomach, and there was no increase in hyperplasia or hyperkeratosis in other portions of the forestomach mucosa. See attached Table A6.8.2(04)-2.

12.7 Other

Non entry field

12.7.1 Supplemental histological examination

The livers and kidneys of male mice in all groups and female mice in the control group and 16,000 ppm were stained for the presence of copper. Positive staining was limited to the livers of high-dose male and female mice. Staining was extremely minimal and consisted of only a few positive staining hepatocytes in the entire liver section. Hepatocytes staining positive for copper contained a maximum of approximately 10 red granules per cell. Due to limited number of cells stained, no distribution of copper was apparent. There was no staining of livers in the lower doses or in the controls, and no staining was present in the kidneys of any mice.

Because of the reduction in iron in the spleen of rats (see Rat Repeat Dose Toxicity), additional sections of spleen from four mice in each dosed and control group were stained for iron. There was no difference between dosed and control mice in the amount of iron-positive granules in the spleen.

12.7.2 Sperm Morphology and Vaginal Cytology

No significant findings were noted in males or females in any dose group. See attached Table A6.8.2(04)-3

13 APPLICANT'S SUMMARY AND CONCLUSION

13.1 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, 1000, 2000, 4000, 8000 or 16,000 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. At the end of the study period all animals were sacrificed and subject to pathological examinations, sperm morphology and vaginal cytology.

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.

13.2 Results and discussion

Summarize relevant results; discuss dose-response relationship.

There were no mortalities or signs of clinical toxicity observed in any of the test species during the duration of the study. Opthalmoscopic examinations revealed no abnormalities at any dose level tested. At gross pathology, significant decreases in heart and kidney weight were noted in the high dose males in the thymus and kidneys of high dose females. There was also a significant decrease in liver weights in both

Section A6.8.2	**Repeated dose toxicity in the Mouse**	
Annex Point 6.8.2	*Specify section no. and heading, route and species*	
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	sexes in the 8000 and 16000 ppm dose groups. Chemical related gross lesions were limited to the forestomach of 7 male and 4 females in the 16000 ppm dose group. Histopathological findings included minimal to mild squamous cell hyperplasia with hyperkeratosis of the forestomach mucosa at the site of the limiting ridge. Minimal positive staining for copper was present in the liver and was limited to the high-dose male and female mice. No significant findings were noted following examination of the sperm morphology and vaginal cytology.	
13.3 Conclusion	*Non entry field*	
13.3.1 LO(A)EL	*Give critical effect and dose/concentration, if necessary separately for males and females* 2000 ppm for males and females	X
13.3.2 NO(A)EL	*Give dose/concentration, if necessary separately for males and females* 1000 ppm for males and females	X
13.3.3 Reliability	*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4* 1	X
13.3.4 Deficiencies	*No/Yes (If yes, discuss the impact of deficiencies and implications on results. If relevant, justify acceptability of study.)* 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. • Haematological, clinical chemistry and urinalysis parameters were not investigated. • 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

Section A6.8.2	**Repeated dose toxicity in the Mouse**
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Date [redacted]

Reference • [redacted]

Materials and Methods • [redacted]

Results and discussion [redacted]

• [redacted]

Conclusion • [redacted]

Reliability [redacted]

Acceptability [redacted]

Section A6.8.2	**Repeated dose toxicity in the Mouse**
Annex Point 6.8.2	*Specify section no. and heading, route and species*
IUCLID: 5.8.1/04	**A6.8.2(04), Subchronic Oral Toxicity Test**

Remarks •

Table A6_4-1. Results of repeated dose toxicity study

Parameter	Control 0 ppm		1000 ppm		2000 ppm		4000 ppm		8000 ppm		16,000 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	0	0	0	0	0	0	0	-	-
clinical signs	0	0	0	0	0	0	0	0	0	0	0	0	-	-
body weight (grams) (initial: final)	21.6:32.9	17.7:28.5	20.9:32.6	18.6:28.5	21.0:31.1	17.2:27.8	20.9:29.5	17.1:26.9	21.1:29.0	18.3:25	21.6:26.8	17.8:21.7	+	+
Final weight relative to controls (%)	-	-	99	100	95	97	90	94	88	88	82	76	+	+
food consumption (g/day)	4.2	5.0	4.8	5.0	5.1	5.9	4.8	6.2	5.1	5.9	5.0	5.4	-	-
Compound consumption (mg/kg/day)	-	-	173	205	382	494	736	1048	1563	2106	3201	4157		
Organ weight														
Brain	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Right kidney	-	-	-	-	-	-	-	-	-	-	↓	↓	+	-
Liver	-	-	-	-	-	-	↓	-	↓	↓	↓	↓	+	+
Lungs	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Right testis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymus	-	-	-	-	-	-	-	-	-	-	-	↓	-	+

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 A6.8.2(04)-1. Results of repeated dose toxicity study

Parameter	Control 0 ppm		1000 ppm		2000 ppm		4000 ppm		8000 ppm		16,000 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	0	0	0	0	0	0	0	-	-
clinical signs	0	0	0	0	0	0	0	0	0	0	0	0	-	-
body weight (grams) (initial: final)	21.6:32.9	17.7:28.5	20.9:32.6	18.6:28.5	21.0:31.1	17.2:27.8	20.9:29.5	17.1:26.9	21.1:29.0	18.3:25	21.6:26.8	17.8:21.7	+	+
Final weight relative to controls (%)	-	-	99	100	95	97	90	94	88	88	82	76	+	+
food consumption (g/day)	4.2	5.0	4.8	5.0	5.1	5.9	4.8	6.2	5.1	5.9	5.0	5.4	-	-
Compound consumption (mg/kg/day)	-	-	173	205	382	494	736	1048	1563	2106	3201	4157		
Organ weight														
Brain	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Heart	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Right kidney	-	-	-	-	-	-	-	-	-	-	↓	-	+	-
Liver	-	-	-	-	-	-	↓	-	↓	↓	↓	↓	+	+
Lungs	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Right testis	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Thymus	-	-	-	-	-	-	-	-	-	-	-	↓	-	+

Table A6.8.2(04)-2a Summary of the Incidence of Non-Neoplastic Lesions in Male Mice

	0 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm	16,000 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)	(1)				(10)
Infarct		1 (100%)				
Stomach, forestomach	(10)	(10)	(10)	(10)	(10)	(10)
Hyperplasia				2 (20%)	6 (60%)	10 (100%)
Cardiovascular System						
None						
Endocrine System						
None						
General Body System						
None						
Genital System						
None						
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						

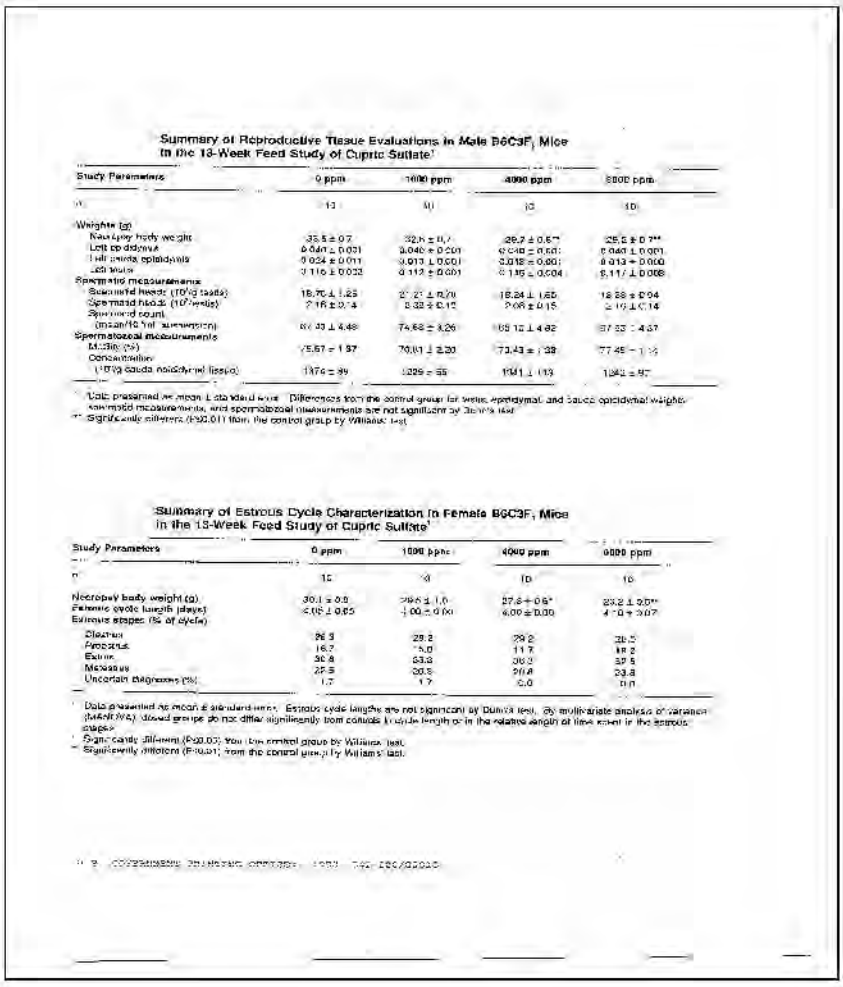
TABLE A6.8.2(04)-2B SUMMARY OF THE INCIDENCE OF NON-NEOPLASTIC LESIONS IN FEMALE MICE

In Female B6C3F1 Mice in the 13-Week Feed Study of Cupric Sulfate

	0 ppm	1000 ppm	2000 ppm	4000 ppm	8000 ppm	16,000 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
Alimentary System						
Stomach, forestomach	(10)		(10)	(10)	(10)	(10)
Hyperplasia				5 (50%)	8 (80%)	10 (100%)
Cardiovascular System						
Heart	(10)					(10)
Myocardium, mineralization	1 (10%)					
Endocrine System						
None						
General Body System						
None						
Genital System						
Clitoral gland	(10)					(8)
Cyst	1 (10%)					
Hematopoietic System						
None						
Integumentary System						
None						
Musculoskeletal System						
None						
Nervous System						
None						
Respiratory System						
None						
Special Senses System						
None						
Urinary System						
Urinary bladder	(10)					(10)
Transitional epithelium, mineralization	3 (30%)					

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

TABLE A6.8.2(04)-3 SUMMARY OF REPRODUCTIVE TISSUE EVALUATIONS IN MALE AND FEMALE MICE



Summary of Reproductive Tissue Evaluations in Male B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	1000 ppm	4000 ppm	8000 ppm
n	10	10	10	10
Weights (g)				
Necropsy body weight	35.5 ± 0.7	32.5 ± 0.7	29.2 ± 0.5**	25.5 ± 0.7**
Left epididymis	0.041 ± 0.001	0.040 ± 0.001	0.038 ± 0.001	0.040 ± 0.001
Left cauda epididymis	0.024 ± 0.001	0.023 ± 0.001	0.018 ± 0.001**	0.013 ± 0.000**
Left testis	0.116 ± 0.002	0.112 ± 0.001	0.105 ± 0.004*	0.117 ± 0.008
Spermatid measurements				
Suspended heads (10^6/g testis)	18.70 ± 1.23	21.27 ± 0.70	18.24 ± 1.55	18.38 ± 0.94
Spermatid heads (10^6/testis)	2.16 ± 0.14	2.33 ± 0.12	2.06 ± 0.15	2.19 ± 0.14
Spermatozoal measurements				
Spermatozoal count (mean/10 ml suspension)	67.33 ± 4.48	74.68 ± 4.26	68.12 ± 4.82	67.33 ± 4.67
Spermatozoal measurements				
Motility (%)	75.67 ± 1.97	70.61 ± 2.30	73.43 ± 1.38	77.45 ± 1.15
Concentration (10^6/g cauda epididymal tissue)	1376 ± 89	1229 ± 55	1341 ± 103	1042 ± 97*

[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≤0.01) from the control group by Williams' test

Summary of Estrous Cycle Characterization in Female B6C3F$_1$ Mice in the 13-Week Feed Study of Cupric Sulfate[1]

Study Parameters	0 ppm	1000 ppm	4000 ppm	8000 ppm
n	10	10	10	10
Necropsy body weight (g)	30.1 ± 0.8	29.6 ± 1.0	27.6 ± 0.6*	23.2 ± 0.5**
Estrous cycle length (days)	4.05 ± 0.05	4.00 ± 0.00	4.00 ± 0.00	4.10 ± 0.07
Estrous stages (% of cycle)				
Diestrus	36.3	29.3	29.3	36.5
Proestrus	16.7	15.0	11.7	18.3
Estrus	30.8	53.8	50.3	37.5
Metestrus	27.5	20.5	9.0	29.8
Uncertain diagnoses (%)	1.7	1.7	0.0	0.0

[1] Data presented as mean ± standard error. Estrous cycle lengths are not significant by Dunn'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≤0.05) from the control group by Williams' test.
** Significantly different (P≤0.01) from the control group by Williams' test.

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1(05)	**A6.8.2(05), Toxicity to fertility**

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	232 REFERENCE
232.1 Reference	Chang, C.C. And Tatum, H.J. (1973). Absence of teratogenicity of intrauterine copper wire in rats, hamsters and rabbits. Contraception, **7(5):** 413 - 434 (published). *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).*
232.2 Data protection	No. *(indicate if data protection is claimed)*
232.2.1 Data owner	*Give name of company* Public domain.
232.2.2 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.
232.2.3 Criteria for data protection	*Choose one of the following criteria (see also TNsG on Product Evaluation) and delete the others:* No data protection claimed.
	233 GUIDELINES AND QUALITY ASSURANCE
233.1 Guideline study	No. This was a non-regulatory study carried out to determine whether copper wire, placed within the uterus after implantation and kept *in situ* throughout pregnancy, produced any teratogenic effects on the embryo, or alters in any way development and subsequent growth of the offspring. *(If yes, give guidelines; if no, give justification, e.g. "no guidelines available" or "methods used comparable to guidelines xy")*
233.2 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)*
233.3 Deviations	Yes. Refer to section 4.3.4 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")*
	234 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.
234.1 Test material	Cu^{2+} as copper wire *Or give name used in study report*
234.1.1 Lot/Batch number	*List lot/batch number if available* Not available.
234.1.2 Specification	Deviating from specification given in section 2 as follows *(describe specification under separate subheadings, such as the*

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following; additional subheadings may be appropriate):

234.1.2.1 Description — *If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)*
Copper wire, 0.1 mm in diameter.

234.1.2.2 Purity — *Give purity in % active substance*
[redacted]

234.1.2.3 Stability — *Describe stability of test material*
Not applicable to inorganic substances.

234.2 Test Animals — Non-entry field

234.2.1 Species — Rat, hamster and rabbit.
if other, state reason for non standard species

234.2.2 Strain — **Rat:** Holtzman strain.
Hamster: Not stated.
Rabbit: New Zealand White.

234.2.3 Source — **Rat:** Not stated.
Hamster: Lakeview Hamster Colony, Newfield, New Jersey. **Rabbit:** Not stated.

234.2.4 Sex — Male and female.

234.2.5 Age/weight at study initiation — **Rat:** Weight 180 – 220 g; age not stated.
Hamster: Weight 100 – 120 g; age not stated.
Rabbit: Weight not stated; age 9 - 91/2 months.

234.2.6 Number of animals per group — *Give number*

Treatment Group (Parent Generation)	Species		
	Rat	Hamster	Rabbit
Copper	12	11	9
Control	7	6	5

should be enough to yield 20 pregnant females per group

234.2.7 Mating — The mating procedure and the symbols designated to descendants of either copper wire treated parent or control parent are shown in **Figure A6.8.2(05)-3**.

Rat P generation: 19 females were mated with untreated males. Of these females, copper wire was inserted into the endometrial cavities of both uterine horns of 12 animals. The remaining 7 animals were considered to be untreated controls.

Rat F_1 generation: When F_1 females reached the age of 90 days, each was cohabited with one fertile male for 10 days. When F_1 males reached the age of 120 days, each was cohabited with 2 virgin cycling females for 10 days.

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	Rat F_2 generation: F_2 animals were tested in the same manner as F_1 animals, giving rise to an F_3 generation. **Hamster P generation:** 17 females were mated with untreated males. Of these females, copper wire was inserted into the endometrial cavities of both uterine horns of 11 animals. The remaining 6 animals were considered to be untreated controls. **Hamster F_1 generation:** When F_1 females reached the age of 90 days, each was cohabited with one fertile male for 10 days. When F_1 males reached the age of 120 days, each was cohabited with 2 virgin cycling female for 10 days. **Hamster F_2 generation:** F_2 animals were tested in the same manner as F_1 animals, giving rise to an F_3 generation. **Rabbit P generation:** 14 females were mated with untreated males. Of these females, copper wire was inserted into the endometrial cavities of both uterine horns of 9 animals. The remaining 5 animals were considered to be untreated controls. **Rabbit F_1 generation:** When F_1 animals reached the age of 71/2 - 8 months, each was cohabited with normal animals. **Rabbit F_2 generation:** No F_2 generation was bred in the rabbit.	
234.2.8 Duration of mating	*2 weeks or other* 10 days for mating of F_1 and F_2 animals.	
234.2.9 Deviations from standard protocol	*i.e. second mating of parent or F1 generations, standardisation of litter size.* Refer to section 6.5.5.	X
234.2.10 Control animals	Yes	
234.3 Administration/ Exposure	Intrauterine *Fill in respective route in the following, delete other routes*	
234.3.1 Animal assignment to dosage groups	*See table below* Not applicable.	
234.3.2 Duration of exposure before mating	None *10 weeks or other (mice at least 56 days, rats 70 days)*	
234.3.3 Duration of exposure in general females only). P, F1, F2 males, females	**Rat:** From day 6 of pregnancy until sacrifice of parent (P generation **Hamster:** From day 6 of pregnancy until sacrifice of parent (P generation females only). **Rabbit:** From day 7 of pregnancy until sacrifice of parent (P generation females only). F_1 and F_2 generation animals were not exposed to copper wire.	
234.3.4 Method of exposure	**Intrauterine** **Rat and Hamster:** Copper wire was inserted in the endometrial cavities of both uterine horns of rats and hamsters on Day 6 of pregnancy. The wire was inserted by means of a half-circle suture needle through the antimesometrial surface about 5 mm below the uterotubal junction and led through the uterine lumen and brought out 2 – 3 mm below the original entry. The two ends were tied together, thus	

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	making a ring 5 – 7 mm in diameter. The surface area of copper wire within the uterine cavity was approximately 3 mm^2. **Rabbit:** Copper wire was inserted into the uterine horns on day 7 of pregnancy at a position approx. 2 cm below the utero-tubal junction and a ring of 1 – 1.5 cm in diameter was made. This provided a surface area of the copper wire within the uterus of approximately 6 mm^2.
234.3.5 Vehicle	None.
234.3.6 Copper dose received by test animals.	**Rat and Hamster:** It was estimated that the rate of dissolution of the wire was approximately 2.75 µg per day. **Rabbit:** It was estimated that the rate of dissolution of the wire was approximately 5.50 µg per day.
234.3.7 Controls	Untreated animals (no copper wire introduced).
234.4 Examinations	Non-entry field.
234.4.1 Clinical signs	Yes
234.4.2 Body weight	Yes
234.4.3 Food/water consumption	No
234.4.4 Oestrus cycle	No
234.4.5 Sperm parameters testis weight	
234.4.6 Offspring	number and sex of pups presence of gross anomalies weight gain physical or behavioural abnormalities Survival rate at time of weaning.
234.4.7 P, F_1 and F_2 organ weights	uterus ovaries testis ventral prostate (rat and hamster only) Epididymi (rabbit only) seminal vesicles adrenal glands
234.4.8 Histopathology P (females only), F_1 and F_2 (males and females).	uterus ovaries adrenal glands testis ventral prostate (rat and hamster only) Epididymi (rabbit only) seminal vesicle

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234.4.9 Histopathology P (females only), F_1 and F_2 (males and females) not selected for mating.	uterus ovaries adrenal glands testis ventral prostate (rat and hamster only) Epididymi (rabbit only) seminal vesicle
234.5 Further remarks	Gestation period
	RESULTS AND DISCUSSION *Describe findings. If appropriate, include table. Sample tables are given below.*
234.6 Effects	**Table A6.8.2(05)** has been amended to take account of available data. Copies of tables taken from the published report are appended, as follows: **Table A6.8.2(05)-1**: Effect of Copper Wire on Pregnancy and Parturition in Rats; **Table A6.8.2(05)-2**: Survival of F_1 Generation Rats at the Time of Weaning (25 days old); **Table A6.8.2(05)-3**: Organ Weights of F_1 Generation Rats; **Table A6.8.2(05)-4**: Organ Weights of F_2 Generation Rats; **Table A6.8.2(05)-5**: Effect of Copper Wire on Pregnancy and Parturition in Hamsters; **Table A6.8.2(05)-6**: Organ Weights of F_1 Generation Hamsters; **Table A6.8.2(05)-7**: Organ Weights of F_2 Generation Hamsters; **Table A6.8.2(05)-8**: Effect of Copper Wire on Pregnancy and Parturition in Rabbits; **Table A6.8.2(05)-9**: Organ Weights of F_1 Generation Rabbits. Growth rate data presented in graphical form are appended as follows: **Figure A6.8.2(05)-1**: Growth rates of F_1 Generation Female Rats; **Figure A6.8.2(05)-2**: Growth rates of F_1 Generation Male Rats.
234.6.1 Parent males	*No effects / describe significant effects referring to data in results table* **Rat:** Not applicable. Parental males were not treated. **Hamster:** Not applicable. Parental males were not treated. **Rabbit:** Not applicable. Parental males were not treated.
234.6.2 Parent females	*No effects / describe significant effects referring to data in results table* **Rat:** There was no difference in gestation periods between the mothers bearing the wire in the uterus and the controls (23 and 22.5 days,

respectively). All copper wire-treated and control mothers delivered normally. However, a comparison of the average number of pups delivered from treated mothers to those from untreated rats showed that

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~~the copper wire-treated females delivered 6.5 ± 0.7 pups, a number~~ significantly lower than that of the untreated controls (8.6 ± 0.6) at the 5% confidence level. Since the rat blastocysts are spaced along the longitudinal axis of the endometrial wall and may implant in the immediate vicinity of the utero-tubal junction, it seems likely that the incidence of fewer pups in the treated group was due to manipulation of the uterus and damage to the embryos at or near the site when the copper wire was inserted.

At autopsy, there were no gross anatomical deformities noted in female parents. Histological examination of the ovaries, uteri and adrenals did not show deviation from normal.

There was no evidence of teratogenic effects in pups of either sex. No abnormalities were observed at birth, at weaning or at fertility testing. There was no effect on survival rates of the F_1 generation animals at the time of weaning. Survival rates of the descendants of treated and untreated mothers indicate that lactation was not interrupted by the wire. Pups grew normally, as evidenced by the fact that the increase in body weight measured at 5-day intervals from 5 days up to 60 days of age in treated animals was similar to that in untreated animals.

Hamster: Copper wire had little effect on gestation or parturition when the wire was inserted into the uterine lumen after implantation. There was no difference in the average number of pups born between the group bearing copper wire and the control group (6.9 vs. 6.7). The gestation period for treated animals was not different from the controls (17 vs. 16.5 days). Lactation in treated mothers was considered to be normal, using as the criteria the average body weight of the pups and the percentage of loss of pups at weaning (25 days of age).

No teratogenic effects were observed in the F1 generation animals at birth and at weaning. Histological examination of the ovaries, uteri and adrenals of mothers with copper wire showed no deviation from normal.

Rabbit: At the time of insertion of the copper wire (Day 7 of pregnancy) there was no difference in the average number of implantation sites between the animals which were to be exposed to copper wire and the controls. However, at laparotomy on Day 15 of pregnancy (laparotomy was done only in animals bearing copper wire), the number of implantation sites was significantly less than that observed on Day 7 of pregnancy. The number of pups subsequently delivered from these animals was reduced as compared to that in the control animals (32.4% vs. 44.2%). This difference is thought to be due to manipulation of the uterus at the time of insertion of the copper wire.

There were no gross anatomical deformities noted in the F_1 generation at birth, at weaning or at autopsy.

The Parent females were autopsied after weaning. Histological examination of the ovaries, uteri and adrenals showed no deviations from normal.

234.6.3 F_1 males	*No effects / describe significant effects referring to data in results table*

Copper Oxide

Section A6.8.2 Fertility Study

Annex Point IIA6.8.2
IUCLID: 5.8.1(05)

Specify section no., heading, route and species as appropriate

A6.8.2(05), Toxicity to fertility

Rat: The fertility of the F_1 males was tested when the animals reached the age of 120 days. Each male was cohabited with two virgin cycling females for 10 days. Fourteen of 15 males (A') born to Parent mother bearing copper wire and 6 of 7 males (B') born to Parent untreated controls mated. The average number of pups born to group A' males was 9.3 ± 0.6 as compared to the average number of 8.8 ± 0.7 for group B' males.

The weights of the reproductive organs of the F_1 generation animals are shown in **Table A6.8.2(05)**. There were no significant differences in organ weights of male offspring of copper wire-treated and untreated mothers. Since 93% of the animals in group A' have normal fertility, it is not surprising that the weights of their reproductive organs are comparable to those of the animals in group B'.

No gross anatomical deformities were noted at autopsy in F_1 males. Histological examination of tissues showed no deviation from normal.

Hamster: There was no apparent effect on fertility of offspring of treated and untreated mothers in males of the F_1 generation. Seven of 10 males in group A' and 6 of 10 males in group B' were mated with normal females. The average number of pups was 8.0 ± 0.8 in group A' and 6.4 ± 1.2 in group B'. These differences are not significant.

F_1 generation males were autopsied at the age of 155-160 days. Weights of the reproductive organs are shown in **Table A6.8.2(05)**. There were no significant differences in organ weights of male offspring of copper wire treated and untreated mothers. Although s slight decrease was noted in the weight of the testes in these animals, fertility appeared not to be affected (although only 10 randomly selected animals from the total 32 were used). In addition, the weights of accessory sex organs, such as seminal vesicles and ventral prostates, increased in this group indicating that from a hormonal viewpoint, the function of the testes was not subnormal.

At autopsy, there were no demonstrable macroscopic anatomical deformities in F_1 males. Histological examination of the tissues of F_1 males showed no abnormalities.

Rabbit: The fertility of F_1 generation males was tested when the animals reached the age of 7V2 - 8 months by mating with normal animals. The average number of implantation sites in females mated with A' males was 7.2 (range 1 - 10). This represents a normal degree of fertility.

Some of the F_1 generation animals were autopsied at either the age of 3 or 6 months. No teratogenic effects were observed in these animals and their growth rate was normal. The reproductive organs were fixed for histological examination. The remaining animals were autopsied at the age of 8V2 months. There were no significant differences in body weight

and organ weights between the male F1 generation animals from copper treated and untreated mothers. Histological examination of the male reproductive tissues of F1 generation animals showed no deviations from normal.

Copper Oxide

Section A6.8.2
Annex Point IIA6.8.2
IUCLID: 5.8.1(05)
234.6.4 F1 females

Fertility Study

Specify section no., heading, route and species as appropriate

A6.8.2(05), Toxicity to fertility

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

Rat: The fertility of F1 females was tested when the animals reached the age of 90 days. Each female was housed with one fertile male for 10 days. Thirty-three of 38 females (A) born to Parent mother bearing copper wire, and 11 of 16 females (B) born to Parent untreated controls were mated successfully. The average number of pups was 10.1 ± 0.5 in group A as compared to the average of 8.5 ± 0.9 pups in group B. It was apparent that there were no significant differences in fertility of offspring of copper treated and untreated mothers of either sex in the F1 generation.

The weights of the reproductive organs of the F1 generation animals are shown in **Table A6.8.2(05)**. The females were autopsied after weaning their pups (F2 generation) at 125-130 days of age. There were no significant differences in organ weights of female offspring of copper wire treated and untreated mothers. Since 86% of the animals in group A have normal fertility, it is not surprising that the weights of their reproductive organs are comparable to that of the animals in group B.

No gross anatomical deformities were noted at autopsy in F1 females. Histological examination of tissues showed no deviation from normal.

Hamster: There was no apparent effect on fertility of offspring of treated and untreated mothers in females of the F1 generation. Twelve of the 16 females in group A and 7 of 7 females in group B were mated and delivered normally. The average number of pups was 7.9 ± 0.8 in group A and 7.8 ± 0.9 in group B. These differences are not significant.

F1 generation females were autopsied at the age of 145-150 days. Weights of the reproductive organs are shown in **Table A6.8.2(05)**. There were no significant differences in organ weights of female offspring of copper wire treated and untreated mothers.

At autopsy, there were no demonstrable macroscopic anatomical deformities in F1 females. Histological examination of the tissues of F1 females showed no abnormalities.

Rabbit: The fertility of F1 generation females was tested when the animals reached the age of 7V2 - 8 months by mating with normal animals. The average number of implantation sites in A females was 7.5 (range 5 - 10). This represents a normal degree of fertility.

Some of the F1 generation animals were autopsied at either the age of 3 or 6 months. No teratogenic effects were observed in these animals and their growth rate was normal. The reproductive organs were fixed for

X

histological examination. The remaining animals were autopsied at the age of 8V2 months. There were no significant differences in body weight and organ weights between the female F_1 generation animals from copper treated and untreated mothers. Histological examination of the female reproductive tissues of F_1 generation animals showed no deviations from normal.

234.6.5 F_2 males *No effects / describe significant effects referring to data in results table*

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Rat: The F_2 generation males were examined grossly at birth for possible malformations. The animals were weaned at 25 days of age and some were eliminated when they reached the age of 60 days. Some were autopsied and the reproductive organs preserved for histological study. The remaining animals were used for fertility testing at age 120 days. The mating procedures for F_2 generation animals were the same as those of the F_1 generation. Offspring of F_1A' and F_1B' males were designated A'A' and B'B' females and A'A' and B'B' males, respectively.

All F_2 generation animals used for assessment of fertility, were mated. The average number of implantation sites in each group was as follows: A'A' females, 10.0 ± 1.0; B'B' females, 11.5 ± 0.5; A'A' males, 10.5 ± 0,3; and B'B' males, 10.7 ± 0.4. These results show that there were no significant differences in fertility among F_2 generation descendants of copper wire treated and untreated animals.

The organ weights of F_2 generation males are shown in **Table A6.8.2(05)**. There were no significant differences in body weights or organ weights. The autopsy data obtained for F_2 male rats were in the normal range.

No gross anatomical deformities were noted at autopsy in F_2 males. Histological examination of tissues showed no deviation from normal.

Hamster: F_2 generation males were examined macroscopically at birth and at weaning for possible malformations. At weaning, some animals were eliminated and others were eliminated when they reached the age of 45-50 days. Some were autopsied and the reproductive organs were fixed for histological examination. Some of the remaining animals were used for fertility testing when the males reached 120 days of age. The mating procedures were the same as in the F_1 generation.

The results show that there were no differences in the fertility of AA or A'A' males. However, the average number of pups delivered in AA males (descendants of copper treated Parent) was significantly lower than that of normal animals (3.1 ± 0.6 vs. 7.9 ± 0.8). The cause for this difference in the F_2 generation is not known.

The body weight and organ weights of F_2 generation males are shown in **Table A6.8.2(05)**. There were no significant differences in the body weight and organ weights, with the exception of the adrenal weights. The adrenal weights of the A'A' males were significantly increased. At autopsy, there were no demonstrable macroscopic anatomical deformities in F_2 males. Histological examination of the tissues of F_2 males showed no abnormalities.

Rabbit: Not applicable

234.6.6 F_2 females

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

Rat: The F_2 generation females were examined grossly at birth for possible malformations. Animals were weaned at 25 days of age and some were eliminated when they reached the age of 60 days. Some were autopsied and the reproductive organs were preserved for histological study. The remaining animals were used for fertility testing

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at an age of 90 days. The mating procedures for the F2 generation animals were the same as those of the Fl generation. Offspring of F_1A and F_1B females were designated as AA and BB females, and AA and BB males, respectively.

All F_2 generation animals used for assessment of fertility, were mated. The average number of implantation sites in each group was as follows: AA females, 12.8 ± 0.7; BB females, 10.0 ± 1.1; AA males, 10.9 ± 0.5; BB males, 11.1 ± 0.5. These results show that there were no significant differences in fertility among F_2 generation descendants of copper wire treated and untreated animals.

The organ weights of F_2 generation females are shown in **Table A6.8.2(05)**. There were no significant differences in body weights or organ weights. The autopsy data obtained for F_2 female rats were in the normal range.

No gross anatomical deformities were noted at autopsy in F_2 females. Histological examination of tissues showed no deviation from normal.

Hamster: F_2 generation females were examined macroscopically at birth and at weaning for possible malformations. At weaning, some animals were eliminated and others were eliminated when they reached the age of 45-50 days. Some were autopsied and the reproductive organs were fixed for histological examination. Some of the remaining animals were used for fertility testing when the females reached 90 days of age. The mating procedures were the same as in the F_1 generation.

The results show that there were no differences in the fertility of AA or A'A' females. However, the average number of pups delivered in BB females (descendants of control Parent) was significantly lower than that of normal animals (2.0 ± 1.0 vs. 7.8 ± 0.9 for the female Parents). The cause for this difference in the F_2 generation is not known.

The body weight and organ weights of F_2 generation females are shown in **Table A6.8.2(05)**. There were no significant differences in the body weight and organ weights.

At autopsy, there were no demonstrable macroscopic anatomical deformities in F_2 females. Histological examination of the tissues of F_2 females showed no abnormalities.

Rabbit: Not applicable.

234.7 Other

Describe any other significant effects

None.

235 APPLICANT'S SUMMARY AND CONCLUSION

235.1 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 copper wire, placed within the uterus after implantation and kept in situ throughout pregnancy, produced any teratogenic effects on the embryo, or altered in any way the development and subsequent growth of the offspring of rats, hamsters and rabbits. The potential for adverse effects on the fertility of treated animals was also assessed.

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Nulliparous female rats of the Holtzman strain (bodyweights in the range 180-220 g); adult cycling female hamsters (bodyweights in the range 100 - 120 g); and adult albino New Zealand female rabbits (age 9 to 91/2 months old) were used. The animals were housed in temperature (24.5 – 26.7°C) and illumination (14 hr light and 10 hr dark) controlled rooms and maintained on standard laboratory chow specific to each species. Tap water was provided *ad libitum*.

In rats and hamsters, positive matings were verified by the presence of sperm in vaginal smears. The day of insemination was designated as Day 1 of pregnancy. In rabbits, visual observation only was used to confirm copulation, and that day was designated as Day 0 of pregnancy.

Copper wire (99.9% pure, 0.1 mm in diameter) was inserted into the endometrial cavities of both uterine horns of rats and hamsters on Day 6 of pregnancy. The wire was inserted by means of a half-circle suture needle through the antimesometrial surface about 5 mm below the uterotubal. junction and led through the uterine lumen and brought out 2-3 mm below the original entry. The two ends were tied together, thus making a ring 5-7 mm in diameter. The surface area of copper wire within the uterine cavity was approximately 3 mm^2. It was estimated that the rate of dissolution of the wire used in the cycling rat was approximately 2.75 µg per day.

In rabbits, the wire was inserted into the uterine horns on Day 7 of pregnancy at a position approximately 2 cm below the utero-tubal junction and a ring of 1-1.5 cm in diameter was made. This provided a surface area of the copper wire within the uterus of approximately 6 mm^2. The amount of copper released in 24 hrs from the wire was estimated to be approximately 5.50 µg on the assumption that the rate of dissolution of the wire used in the rabbit is similar to that in the rat.

The wire was left *in situ* during pregnancy and lactation, and the gestation period was recorded. The mothers were sacrificed at the time of weaning and the ovaries, uteri and adrenals were fixed with Bouin's solution for histological examination. The number and sex of the pups of rats and hamsters were recorded at birth and the offspring were observed for gross abnormalities. The body weight of F_1 generation rats was recorded at 5-day intervals from the age of 5 days through 60 days. The offspring of rats and hamsters were weaned at the age of 25 days and the number of surviving F_1 generation was recorded. In the meantime, the females were separated from the males and maintained in separate cages to raise F_2 and F_3 generations. In rabbits, laparotomy was done on Day 15 of pregnancy and the number of implantation sites was recorded. The offspring were weaned when 30-35 days of age. Some of the F_1 generation rabbits were sacrificed at the age of either 3 or 6 months.

When the F_1 generation rat and hamster females reached the age of 90 days and the males 120 days, each female was cohabited with one fertile male and each male with 2 virgin cycling females for 10 days. The fertility of the F_1 generation animals was evaluated by the following regimens: a) the ratio of the animals mated over the animals used and b) the number of implantation sites or the number of pups delivered. Some of the animals delivered by the F_1 generation were eliminated at the time of weaning and examined for gross malformations. The remaining

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animals were used for fertility testing when they reached maturity. The fertility of F_2 generation animals was tested in a manner similar to that described for the F_1 generation.

At autopsy, the body weight and the weights of the following organs were determined: ovaries, uteri and adrenals in the females; testes, seminal vesicles, epididymus (in the rabbit only), ventral prostate and adrenals in the males. All tissues were fixed in Bouin's solution. Histological sections were stained with haematoxylin and eosin. All the results obtained were analysed statistically using Student's t-test. A probability of less than 0.05 was considered as statistically significant.

235.2 Results and discussion

Summarize relevant results; discuss dose-response relationship.

Rats: There was no difference in gestation periods between the mothers bearing the wire in the uterus and controls. All copper wire treated and control mothers delivered normally. However, a comparison of the average number of pups delivered from treated mothers to those from untreated rats showed that the copper wire treated females delivered 6.5 ± 0.7 pups, a number significantly lower than that of the untreated controls (8.6 ± 0.6) at the 5% confidence level. Since rat blastocysts are spaced along the longitudinal axis of the endometrial wall and may implant in the immediate vicinity of the utero-tubal junction, it is considered likely that the incidence of fewer pups in the treated group was due to manipulation of the uterus and damage to the embryos at or near the site when the copper wire was inserted.

No teratogenic effects were evident in offspring. No abnormalities were observed at birth, at weaning or at the time of the fertility test. There was no effect of copper wire on survival rates of the F_1 generation animals at the time of weaning. Survival rates of the descendants of treated and untreated mothers indicates that lactation was not interrupted by the wire. F_1 generation animals of both sexes grew normally, as evidenced by the increases in body weight. There were no significant differences in fertility of offspring of copper treated and untreated mothers of either sex in the F_1 generation. There were no significant differences in organ weights of offspring of copper wire treated and untreated mothers in either sex of the F_1 generation.

There were no significant differences in fertility among F_2 generation descendants of copper wire treated and untreated animals. There were no significant differences in body weights or organ weights in either sex of the F_2 generation.

At autopsy, there were no gross anatomical deformities noted in Parent, F_1 or F_2 generations. Histological examination of the ovaries, uteri and adrenals of Parent females, and of female and male tissues of F_1 and F_2 generations did not show deviations from normal.

Hamsters: There was no difference in the average number of pups born between the group bearing copper wire and the control group. The gestation period for treated animals was not different from the controls. Lactation in treated mothers was considered to be normal, based on the average body weights of pups and the percentage lost at weaning. No teratogenic effects were observed in the F_1 generation animals at birth and at weaning. Histological examination of the ovaries, uteri and adrenals of mothers with copper wire showed no deviation from normal.

Section A6.8.2	**Fertility Study**	
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	There was no apparent effect on the fertility of offspring of treated and untreated mothers in either sex of the F_1 generation. There were no significant differences in organ weights of offspring of copper wire treated and untreated mothers in either sex of the F_1 generation.	
	There were no significant differences in fertility among F_2 generation descendants of copper wire treated and untreated animals. There were no significant differences in body weights or organ weights in either sex of the F_2 generation, other than an unexplained increase in the adrenal weights of control males.	X
	At autopsy, there were no gross anatomical deformities noted in Parent, F_1 or F_2 generations. Histological examination of the ovaries, uteri and adrenals of Parent females, and of female and male tissues of F_1 and F_2 generations did not show deviations from normal.	
	Rabbits: At the time of insertion of the copper wire (Day 7 of pregnancy), there was no difference in the average number of implantation sites between the animals which were to be exposed to copper wire and the controls. However, at laparotomy on Day 15 of pregnancy, the number of implantation sites was significantly less than that observed on Day 7 of pregnancy. The number of pups subsequently delivered from these animals was reduced as compared to that in the control animals. This difference was thought to be due to manipulation of the uterus at the time of insertion of the copper wire.	
	There were no gross anatomical deformities noted in F_1 generation at birth, at weaning or at autopsy. The fertility of F_1 generation was normal.	
	The Parent females were autopsied after weaning. Histological examination of the ovaries, uteri and adrenals showed no deviations from normal.	
	No teratogenic effects were observed in F_1 generation animals and their growth rate was normal. There were no significant differences in body weight and organ weights between the F_1 generation animals of either sex from copper treated and untreated mothers. Histological examination of the female and male reproductive tissues of F_1 generation animals showed no deviations from normal.	
235.3 Conclusion	The fertility of rats, hamsters and rabbits of the parent, F_1 and F_2 generations was unaffected by exposure of parent animals to copper from wire placed into the uterus after implantation of embryos. Similarly, no adverse effects (teratogenicity or growth and development) attributable to the exposure of parent females to copper were seen in F_1 or F_2 animals.	
235.3.1 LO(A)EL	*Give critical effect and dose/concentration*	
	Non-entry field	

Section A6.8.2		**Fertility Study**
Annex Point IIA6.8.2		*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1(05)		**A6.8.2(05), Toxicity to fertility**
235.3.1.1 males	Parent	*give critical effect and concentration* Not applicable.
235.3.1.2 females	Parent	*give critical effect and concentration* No dose-related effects were observed in any of the species tested..
235.3.1.3	F1 males	*give critical effect and concentration* No dose-related effects were observed in male offspring (F_1 generation) of dosed females in any of the species tested.
235.3.1.4	F1 females	*give critical effect and concentration* No dose-related effects were observed in female offspring (F_1 generation) of dosed females in any of the species tested.
235.3.1.5	F2 males	*give critical effect and concentration* No dose-related effects were observed in male descendents (F_2 generation) of dosed females in any of the species tested.
235.3.1.6	F2 females	*give critical effect and concentration* No dose-related effects were observed in female descendents (F_2 generation) of dosed females in any of the species tested.
235.3.2 NO(A)EL		Non-entry field
235.3.2.1 males	Parent	*give concentration* Not applicable.
235.3.2.2 females	Parent	*give concentration* Rat and Hamster : The NOAEL following intrauterine exposure is estimated to be ≥ 2.75 µg per day (see section 3.3.6). Rabbit : The NOAEL following intrauterine exposure is estimated to be ≥ 5.50 µg per day (see section 3.3.6).
235.3.2.3	F1 males	*give concentration* No dose-related effects were observed in male offspring (F_1 generation) of dosed females in any of the species tested.
235.3.2.4	F1 females	*give concentration* No dose-related effects were observed in female offspring (F_1 generation) of dosed females in any of the species tested.
235.3.2.5	F2 males	*give concentration* No dose-related effects were observed in male descendents (F_2 generation) of dosed females in any of the species tested.
235.3.2.6	F2 females	*give concentration* No dose-related effects were observed in female descendents (F_2 generation) of dosed females in any of the species tested.
235.3.3 Reliability		*Based on the assessment of materials and methods include appropriate reliability indicator 0, 1, 2, 3, or 4* 2
235.3.4 Deficiencies		Yes. This study was not conducted and/or reported in compliance with GLP. When compared with generally accepted principles to be applied to reproductive toxicity and teratogenicity studies, it is also apparent that there were a number of methodological deviations, including the

Section A6.8.2	**Fertility Study**
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following:

- The toxicity of copper to reproduction / teratogenicity was assessed only after implantation of embryos in the Parent females. No copper was administered to males.
- Ony a single 'dose level' was used. The dose received by parent females was estimated, not measured.
- F_1 and F_2 generations were not exposed to copper during their growth, mating and reproduction.
- Test and control groups generally contain fewer animals than recommended.
- Effects on the oestrus cycle were not assessed.
- Sperm parameters were not assessed.

Reporting deficiencies included the following:

- Information on the numbers of animals mated is deficient in some cases.
- Detailed information on survival of pups at birth and at weaning (hamsters and rabbits).

These deficiencies do not, however, 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 and that the results obtained are consistent with work published by other researchers. Furthermore, this research (including its methodology) was published in a peer-reviewed publication, and has therefore been subject to the prior scrutiny of experts in the field. The study has also been referenced in a number of reviews of the toxicity of copper to reproduction.

No internationally accepted guidelines are available that specifically address the objective of the research presented in this summary.

Overall, this is a well-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 the embryotoxic / teratogenic potential of copper. 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 [redacted]

Section A6.8.2	**Fertility Study**
Annex Point IIA6.8.2	*Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1(05)	**A6.8.2(05), Toxicity to fertility**
Materials and Methods	[redacted]
Results and discussion	[redacted]
Conclusion	[redacted]
Reliability	[redacted]
Acceptability	[redacted]
Remarks	[redacted]

	COMMENTS FROM ...
Date	*Give date of comments submitted*
Materials and Methods	*Discuss additional relevant discrepancies referring to the (sub)heading numbers and to applicant's summary and conclusion.* *Discuss if deviating from view of rapporteur member state*
Results and discussion	*Discuss if deviating from view of rapporteur member state*
Conclusion	*Discuss if deviating from view of rapporteur member state*
Reliability	*Discuss if deviating from view of rapporteur member state*
Acceptability	*Discuss if deviating from view of rapporteur member state*
Remarks	

Section A6.8.2 **Fertility Study**
Annex Point IIA6.8.2 *Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1(05) **A6.8.2(05), Toxicity to fertility**

Table A6.8.2(05). Table for reproductive toxicity study (modify if appropriate)

If effects are found in one generation, the figures for the other generation(s) should be given as well (as shown as an example for mortality). Give only information on endpoints with effects, delete other endpoints.

Parameter		Genera-tion	control		Rat		Hamster		Rabbit	
			m	f	m	f	m	f	m	f
Mortality	incidence	P	0	0	0	0	0	0	0	0
		F_1	0	0	0	0	0	0	0	0
		F_2	0	0	0	0	0	0	0	0
		F_3	0	0	0	0	0	0	0	0
Food consumption	% of control	P	--*	--	--	--	--	--	--	--
		F		--	--	--	--	--	--	--
		F		--	--	--	--	--	--	--
Body weight gain	% of control	P	--	--	--	--	--	--	--	--
		F_1	100	100	98.0	98.1	99.6	98.4	100.6	110.0
		F_2	100	100	95.9	98.0	96.9	110.1	--	--
Clinical Observations *specify effects*	Incidence	P	0	0	0	0	0	0	0	0
		F_1	0	0	0	0	0	0	0	0
		F_2	0	0	0	0	0	0	0	0
		F_3	0	0	0	0	0	0	0	0
Organ weights	% of control									
OVARIES		P - -		--	--	--	--	--	--	--
		F_1	--	100	--	94.9	--	102.5	--	153.6
		F_2	--	100	--	94.7	--	98.3	--	--
Uteri		P	--	--	--	--	--	--	--	--
		F_1	--	100	--	88.6	--	85.3	--	144.6
		F_2	--	100	--	101.1	--	127.9	--	--

Section A6.8.2 **Fertility Study**
Annex Point IIA6.8.2 *Specify section no., heading, route and species as appropriate*
IUCLID: 5.8.1(05) **A6.8.2(05), Toxicity to fertility**

Adrenals		**P**	--	--	--	--	--	--	--	--
		F_1	100	100	98.4	92.5	113.4	90.0	100.2	100.2
		F_2	100	100	95.9	104.7	122.1	101.5	--	--
Testes		**P**	--	--	--	--	--	--	--	--
		F_1	100	--	100.8	--	96.5	--	120.6	--
		F_2	100	--	101.2	--	113.2	--	--	--
Epididymi		**P**	--	--	--	--	--	--	--	--
		F_1	100	--	--	--	--	--	120.6	--
		F_2 --		--	--	--	--	--	--	--
Seminal vescicle		**P**	--	--	--	--	--	--	--	--
		F_1	100	--	89.6	--	122.0	--	117.3	--
		F_2	100	--	104.4	--	104.9	--	--	--
Ventral prostate		**P**	--	--	--	--	--	--	--	--
		F_1	100	--	86.7	--	123.1	--	--	--
		F_2	100	--	102.7	--	98.4	--	--	--
Pathology										
Histopathologic examination *specify effects*	Incidence									
		P	0	0	0	0	0	0	0	0
		F_1	0	0	0	0	0	0	0	0
		F_2	0	0	0	0	0	0	--	--

Copper Oxide

Reproductive Performance	Generation	Species					
		Rat		Hamster		Rabbit	
		Control	Test Animals	Control	Test Animals	Control	Test Animals
Female fertility index (No. pregnant animals/ No. females mated) x 100.	P	--	--	--	--	--	--
	F_1	68.75	86.8	100	75.0	60.0	66.67
	F_2	--	--	--	--	--	--
Male fertility index (No. males that became sires / No. males placed with females) x 100.	P	--	--	--	--	--	--
	F_1	85.7	93.3	60.0	70.0	--	--
	F_2	--	--	--	--	--	--
Mean number of implantation sites (range)	P	--	--	--	--	8.6	7.9
	F_1	--	--	--	--	--	7.5 (5-10)
	F_2 offspring of F_1 females	(BB ♀) 10.0 ± 1.1	(AA ♀) 12.8 ± 0.7	--	--	--	--
		(BB ♂) 11.1 ± 0.5	(AA ♂) 10.9 ± 0.5	--	--	--	--
	F_2 offspring of F_1 males	(B'B'♀) 11.5 ± 0.5	(A'A'♀) 10.0 ± 1.0	--	--	--	--
		(B'B' ♂) 10.7 ± 0.4	(A'A' ♂) 10.5 ± 0.3	--	--	--	--
Mean number of lost implantations (No. implantation sites – No. pups born alive).	P	--	--	--	--	24	48
	F_1	--	--	--	--	--	--
	F_2	--	--	--	--	--	--
Post-implantation loss ((No. implantation sites – No. pups born alive)/ No. implantation sites) x 100.	P	--	--	--	--	55.81	67.6
	F_1	--	--	--	--	--	--
	F_2	--	--	--	--	--	--
Duration of pregnancy (mean days)	P	22.5	23.0	16.5	17.0	33.1	31.7
	F_1	--	--	--	--	--	--
		F_2 - -	--	--	--	--	--
Live birth index (No. pups born alive / No. pups born) x 100.	P	100	100	100	100	100	100
	F_1	--	--	--	--	--	--
	F_2	--	--	--	--	--	--

Copper Oxide

Gestation index (No. females with live pups/ No. females pregnant) x 100.	P	100	100	100	100	100	100
	F_1	--	--	--	--	--	--
	F_2	--	--	--	--	--	--
Mean litter size (range)	P	8.6 ± 0.6	6.5 ± 0.7	6.7 (3-11)	6.9 (2-10)	6.0 (5-7)	3.8 (1-5)
	F_1	(B' ♂) 8.8 ± 0.7	(A' ♂) 9.3 ± 0.6	(B' ♂) 6.4 ± 1.2	(A' ♂) 8.0 ± 0.8	--	--
		(B ♀) 8.5 ± 0.9	(A ♀) 10.1± 0.5	(B ♀) 7.8 ± 0.9	(A ♀) 7.9 ± 0.8	--	--
	F_2 offspring of F_1 females	--	--	(BB ♀) 2.0 ± 1.0	(AA ♀) 7.8 ± 0.9	--	--
		--	--	(BB ♂) 7.9 ± 0.8	(AA ♂) 3.1 ± 0.6	--	--
	F_2 offspring of F_1 males	--	--	--	--	--	--
		--	--	--	--	--	--
Sex ratio (male/female)	F_1	28/32	40/38	19/21	42/34	--	--
		F_2 - -	--	--	--	--	--
Survival rate at birth (%)	F_1	100	100	100	100	--	--
	F_2	--	--	--	--	--	--
Survival rate at weaning (25 days old) (%)	F_1	93.3	98.7	--	--	84.2	82.4
	F_2	--	--	--	--	--	--

* No data

Table A6.8.2(05)-1

Table I. Effect of Copper Wire on Pregnancy and Parturition in Rats

Treatment group	No. of Animals	No. of litters born	No. of pups born ♀	No. of pups born ♂	Av. No. of pups ± S.E.	Gestation period (days)
Copper	12	12	38	40	6.5 ± 0.7*	23.0
Control	7	7	32	28	8.6 ± 0.6	22.5

* $p < 0.05$

1

Table A6.8.2(05)-2

Table II. Survival of F_1 Generation Rats at the Time of Weaning (25 days old)

Treatment group	Number of pups At birth ♀	♂	total	At weaning ♀	♂	total	Survival Rate (%)
Copper	38	40	78	38	39	77	98.7
Control	32	28	60	28	28	56	93.3

Table A6.8.2(05)-3

Table III. Organ Weights of F_1 Generation Rats

Treatment group	Sex[1]	No. of animals	B.W. gm ±S.E.	Organ weights, mg ± S.E. Ovaries	Uteri	Adrenals	Testes	Seminal vesicles	Ventral prostate
A (Copper)	♀	32	250.8 ±2.5	87.5 ±2.5	416.1 ±22.1	70.6 ±1.8			
B (Control)	♀	14	255.6 ±3.7	92.2 ±3.9	469.8 ±40.8	76.3 ±2.1			
A' (Copper)	♂	38	404.4 ±5.1			49.9 ±0.9	3486.1 ±40.4	762.1 ±19.2	592.9 ±23.4
B' (Control)	♂	20	412.7 ±9.7			50.7 ±1.6	3459.8 ±50.4	850.6 ±24.8	684.1 ±27.2

[1]Female rats were autopsied at the age of 125-130 days; male rats were autopsied at the age of 145-150 days.

Table A6.8.2(05)-4

Table IV. Organ Weights of F_2 Generation Rats

Treatment group*	Sex[1]	No. of Animals	B.W. gm ±S.E.	Organ weights, mg ± S.E. Ovaries	Uteri	Adrenals	Testes	Seminal Vesicles	Ventral Prostate
AA	♀	10	248.5 ±4.6	86.5 ±1.5	456.3 ±32.4	74.8 ±1.6			
BB	♀	6	251.8 ±6.7	88.7 ±7.1	411.6 ±30.9	71.6 ±2.1			
A'A'	♀	8	261.1 ±3.7	84.4 ±5.3	393.3 ±28.1	76.0 ±1.8			
B'B'	♀	6	268.0 ±8.1	91.8 ±4.5	429.1 ±28.8	72.4 ±2.5			
AA	♂	10	384.1 ±11.8			48.6 ±2.5	3477.1 ±39.1	727.8 ±52.2	550.1 ±27.0
BB	♂	15	391.6 ± 9.0			50.6 ±2.1	3403.8 ±61.0	735.9 ±33.9	526.5 ±31.8
A'A'	♂	8	376.5 ±16.7			49.2 ±1.1	3481.0 ±41.4	820.7 ±21.5	590.8 ±31.3
B'B'	♂	8	401.2 ±6.5			51.4 ±2.8	3468.9 ±66.3	747.6 ±44.1	584.9 ±30.2

NOTE: Legend for table given on following page.

Legend for Table IV.

* AA -- offspring born to F_1A (Copper)

BB -- offspring born to F_1B (Control)

A'A' -- offspring born to F_1A' (Copper)

B'B' -- offspring born to F_1B' (Control)

AA -- offspring born to F_1A (Copper)

BB -- offspring born to F_1B (Control)

A'A' -- offspring born to F_1A (Copper)

B'B' -- offspring born to F_1B' (Control)

[1] Female rats were autopsied at the age of 125-130 days; male rats were autopsied at the age of 145-150 days.

Table A6.8.2(05)-5

Table V. Effect of Copper Wire on Pregnancy and Parturition in Hamsters

Treatment group	No. of animals	No. of litters born	No. of pups born ♀	No. of pups born ♂	Av. No. of pups (range)	Gestation period(days)
Copper	11	11	34	42	6.9 (2-10)	17.0
Control	6	6	21	19	6.7 (3-11)	16.5

Table A6.8.2(05)-6

Treatment group	Sex[1]	No. of animals	B. W. gm ± S.E.	Organ Weights, mg ± S.E. Ovaries	Uteri	Adrenals	Testes	Seminal Vesicles	Ventral prostate
A (Copper)	♀	23	129.3 ±3.5	32.8 ±1.6	361.5 ±38.9	15.3 ±0.8			
B (Control)	♀	11	131.4 ±7.0	32.0 ±1.5	423.8 ±41.7	17.0 ±0.6			
A' (Copper)	♂	32	129.5 ±3.1			27.9 ±0.9	3192.0 ±27.1	493.5 ±25.4	98.7 ±8.9
B' (Control)	♂	14	130.0 ±2.9			24.6 ±2.9	3307.7 ±107.6	404.5 ±17.4	80.2 ±7.7

[1] Female hamsters were autopsied at the age of 145-150 days; male hamsters were autopsied at the age of 155-160 days.

Table A6.8.2(05)-7

Table VII. Organ Weights of F_2 Generation Hamsters

Treatment group*	Sex[1]	No. of animals	B. W. gm ± S.E.	Organ weights, mg ± S.E.					
				Ovaries	Uteri	Adrenals	Testes	Seminal vesicles	Ventral prostate
AA	♀	15	129.4 ±8.5	35.1 ±1.6	421.9 ±29.7	16.7 ±0.8			
BB	♀	14	116.1 ±6.2	32.9 ±1.4	339.3 ±28.5	16.0 ±0.9			
A'A'	♀	15	130.8 ±6.6	33.8 ±2.0	308.7 ±40.4	16.9 ±0.5			
B'B'	♀	12	120.2 ±10.3	37.2 ±2.6	231.8 ±55.7	17.1 ±0.9			
AA	♂	17	118.7 ±3.3			26.1 ±0.9	3219.2 ±97.1	485.9 ±34.4	247.4 ±64.9
BB	♂	11	111.1 ±4.0			22.8 ±0.7	3064.7 ±199.7	397.0 ±26.0	229.1 ±9.8
A'A'	♂	20	131.8 ±2.4			30.9 ±1.1	3675.1 ±45.1	533.5 ±43.0	169.0 ±10.1
B'B'	♂	7	147.4 ±5.9			23.9 ±1.5	3025.5 ±91.3	575.2 ±55.8	194.0 ±11.1

NOTE: Legend for table given on following page.

Legend for Table VII.

* AA -- offspring born to F_1A (Copper)

BB -- offspring born to F_1B (Control)

A'A' -- offspring born to F_1A' (Copper)

B'B' -- offspring born to F_1B' (Control)

AA -- offspring born to F_1A (Copper)

BB -- offspring born to F_1B (Control)

A'A' -- offspring born to F_1A' (Copper)

B'B' -- offspring born to F_1B' (Control)

[1] Female hamsters were autopsied at the age of 145-150 days; male hamsters were autopsied at the age of 155-160 days.

Table A6.8.2(05)-8

Table VIII. Effect of Copper Wire on Pregnancy and Parturition in Rabbits

Treatment group	No. animals	No. implantation sites (Lap. D_7)	No. litters born	No. pups born (%)	No. pups alive at weaning	Av. No. pups (range)	Gestation period (days)
Copper	9	71	6	23 (32.4)	19 (82.4)	3.8 (1-5)	31.7
Control	5	43	3	19 (44.2)	16 (84.2)	6.0 (5-7)	33.1

Table A6.8.2(05)-9

Table IX. Organ Weights of F_1 Generation Rabbits

Treatment group	Sex[1]	No. of animals	B.W. gm ± S.E.	Organ weights, mg ± S.E. Ovaries	Uteri	Adrenals	Testes	Epididymi	Seminal Vesicle
A* (Cu)	♀	5	3810.0 ±285.6	794.4 ±107.9	5844.7 ±269.2	371.5 ±17.1			
B**(Control)	♀	6	3462.5 ±78.8	517.3 ±70.1	3904.8 ±223.9	370.9 ±43.8			
A***(Cu)	♂	9	3422.7 ±113.5			368.3 ±32.8	5469.0 ±314.3	1867.3 ±93.9	996.4 ±101.1
B****(Control)	♂	4	3406.3 ±73.9			367.7 ±54.3	4566.4 ±494.1	1547.8 ±227.8	849.1 ±89.3

* Two female rabbits were autopsied at the age of 6 months and were not included here.

** Three female rabbits were autopsied at the age of 3 months and were not included here.

*** One male rabbit was autopsied at the age of 6 months and was not included here.

**** Three male rabbits were autopsied at the age of 3 months and were not included here.

[1] Both female and male rabbits were autopsied at the age of 8 1/2 months.

Figure A6.8.2(05)-1

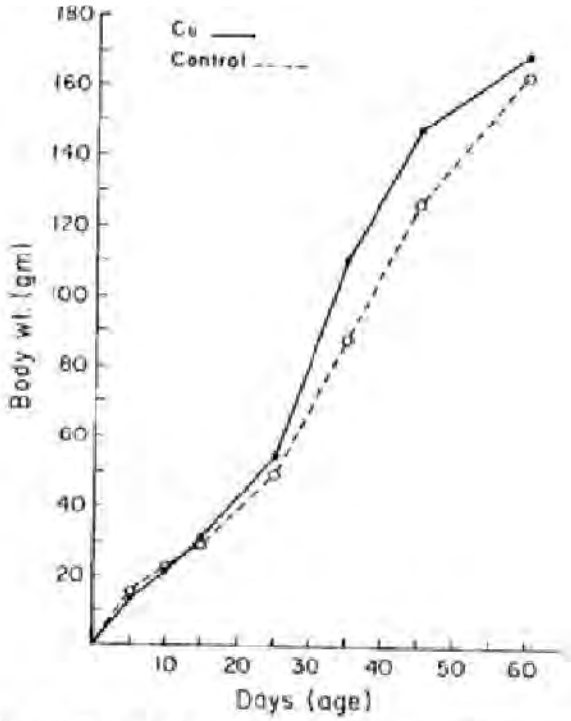


Fig. 1. Growth Rates of F_1 Generation Female Rats

Figure A6.8.2(05)-2

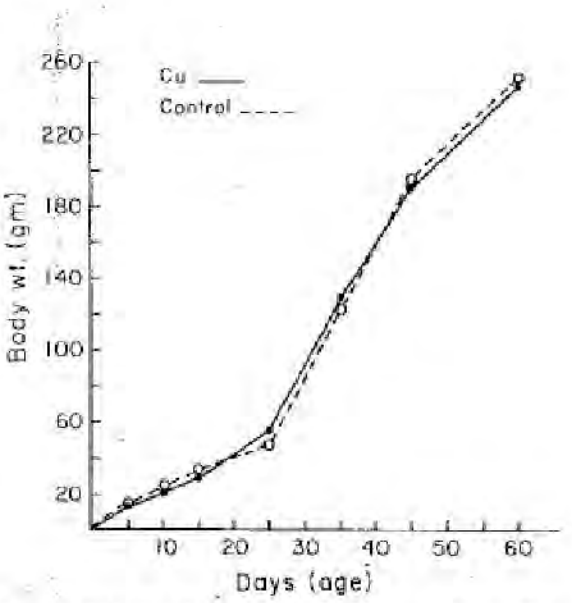
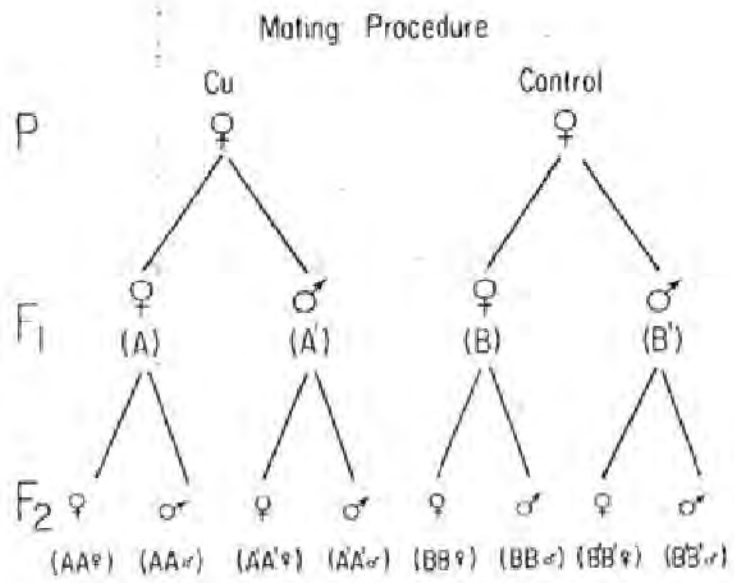


Fig. 2. Growth Rates of F_1 Generation Male Rats

Figure A6.8.2(05)-3



236 REFERENCE

Official use only

236.1 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] (2005). Copper Sulfate Pentahydrate: Multigeneration Reproduction Study in Rats. DuPont Haskell Laboratory for Health and Environmental Sciences. Laboratory Project ID: DuPont-14226. (unpublished)

236.2 Data protection

Yes

(indicate if data protection is claimed)

236.2.1 Data owner

Give name of company

European Copper Institute (ECI).

236.2.2 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)

Wood Preservatives Copper Taskforce.

236.2.3 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. for the purpose of its entry into Annex I/IA.

237 GUIDELINES AND QUALITY ASSURANCE

237.1 Guideline study

Yes. The study report claims compliance with the following test guidelines:

- United States (U.S.) Environmental Protection Agency (EPA), Office of Prevention, Pesticides, and Toxic Substances (OPPTS) Health Effects Test Guidelines, OPPTS 870.3800 Reproduction and Fertility Effects. (August 1998).
- Organisation for Economic Cooperation and Development (OECD/OCDE). Guidelines for Testing of Chemicals, 416. (22nd January 2001).

Compliance with OECD test guideline 416 (adopted 22nd January 2001) is confirmed with the exception of deviations listed under point 2.3.

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

237.2 GLP

Yes.

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

237.3 Deviations

The following minor deviations occurred from the requirements of OECD guideline No. 416 (adopted 22nd

January 2001): X

- Animal rooms were maintained at a temperature of 18-26°C instead of the test guideline recommended 19-25%.
- The guideline indicates that "Twice daily, during the weekend once daily when appropriate, all animals should be observed for morbundity and mortality. However it is not clear from the report if these intervals were respected. The report indicates that "Cage-site examinations were conducted at least once daily throughout the study".
- Testicular histopathological examinations are not fully described.

These deviations are not considered to have affected the scientific integrity, or outcome of this study.

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

238 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.

238.1 Test material Copper sulphate pentahydrate

Or give name used in study report

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

Aldrich Lot 17919TA

238.1.2 Specification Deviating from specification given in section 2 as follows.

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

238.1.2.1	Description	*If appropriate, give e.g. colour, physical form (e.g. powder, grain size, particle size/distribution)* Colour: blue Physical form: crystal
238.1.2.2	Purity	*Give purity in % active substance* [redacted]
238.1.2.3	Stability	*Describe stability of test material* ***The stability of the test substance over the course of the study was confirmed by purity analyses conducted near the beginning and the end of the study. Analyses were conducted at:*** Exygen Research 3058 Research Drive State College, Pennsylvania 16801 U.S.A.
238.2 Test Animals		Non-entry field
238.2.1 Species		Rat. *if other, state reason for non standard species*
238.2.2 Strain		Crl:CD®(SD)IGS BR
238.2.3 Source		Charles River Laboratories, Inc., Raleigh, North Carolina, US.
238.2.4 Sex		Male and female.
238.2.5 Age/weight at study initiation		The P1 generation animals were approximately 8 weeks old at the start of treatment, and in the body weight ranges of approximately 262-332 g (males) and approximately 166-231g (females).
238.2.6 Number of animals per group		*Give number* *should be enough to yield 20 pregnant females per group* 30 rats/sex/concentration. See Table A.6.8.2-1.
238.2.7 Mating		*Start of Cohabitation* Animals were cohoused after approximately 10 weeks of exposure to the test substance. The day animals were first cohoused was designated as day 1 of cohabitation. *Duration of Cohabitation Period* Animals were cohoused until evidence of copulation was observed or until 2 weeks had elapsed. The cohabitation period ended in the morning of day 15 of cohabitation. *Evidence of Copulation*

	Once daily, each female was examined for the presence of an intravaginal copulation plug or sperm in the vaginal lavage sample, either of which was considered evidence of copulation. The presence of an intravaginal plug and/or sperm was recorded. The day evidence of copulation was observed was designated as day 0 of gestation. *Cohousing* Each female was continually housed on a 1:1 basis with a randomly selected, nonsibling male of the same dietary concentration level, in the male's cage. On the day copulation was confirmed, the female was transferred back to individual cage housing.
238.2.8 Duration of mating	*2 weeks or other* 2 weeks (see 3.2.7)
238.2.9 Deviations from standard protocol	*I.e. second mating of parent or F1 generations, standardisation of litter size* None.
238.2.10 Control animals	Yes. See Table A.6.8.2-1.
238.3 Administration/ Exposure	Oral *Fill in respective route in the following, delete other routes*
238.3.1 Animal assignment to dosage groups	*See table below* See to Table A6.8.2-1.
238.3.2 Duration of exposure before mating	*10 weeks or other (mice at least 56 days, rats 70 days)* At least 70 days for both P1 and F1 animals.

238.3.3 Duration of exposure in general P, F1, F2 males, females

From beginning of the study until sacrifice of parent, F1, F2-generation or other

Treatment Schedule

Gen.	Approximate Age at Start of Dosing (days)	Approximate No. of Study Days Before Mating	Duration of Dosing
P1	56	70 (at least)	Until sacrifice
F1	21	70 (at least)	Until sacrifice

Sacrifice Schedule

Animals	Gen.	Schedule
Adult Males	P1 F1	Test days 109-113 Test day 119
Pregnant Females	P1, F1	On day of weaning litters (Day 21 Postpartum)
Nonpregnant Females	P1, F1	Approximately Day 28 after the end of cohabitation
Culled Pups	F1, F2	Day 4 Postpartum
Weanlings	F1, F2	On day of weaning (except F1 rats selected as parental rats)

Oral

238.3.4 Type

Via the diet.

238.3.5 Concentration

0, 100, 500, 1000, 1500 ppm in the diet.

Diet Preparation Analysis

Diet preparation analysis demonstrated that the test substance was stable in the diet under study conditions. The homogeneity data support that the mixing procedure was adequate for all dietary levels. The concentration verification data indicate that the test substance was present at the targeted levels during the study.

238.3.6 Vehicle	*Moistened with water, aqueous solution, corn oil or other* No vehicle was used to mix the substance in the diet.
238.3.7 Concentration in vehicle	Not applicable.
238.3.8 Total volume applied	Not applicable.
238.3.9 Controls	*Vehicle, plain diet or other* Plain diet.
238.4 Examinations	*Non-entry field*
238.4.1 Clinical signs	Cage-site examinations were conducted at least once daily throughout the study. Moribund rats were sacrificed. At least once weekly throughout the premating feeding, gestation, and lactation periods, each of the P1 and F1 parental rats was individually handled and carefully examined for abnormal behavior and/or appearance.
238.4.2 Body weight	*Premating Period* All P1 and F1 rats were weighed once a week. All rats evaluated for developmental landmarks (vaginal patency, preputial separation) were weighed on the day of achievement. *Gestation and Lactation Periods* P1 and F1 dams were weighed on days 0, 7, 14, and 21 of each period. Females without evidence of copulation, those that copulated and did not deliver a litter, and males were weighed on a weekly schedule.
238.4.3 Food/water consumption	<u>Food consumption</u> *Premating and Cohabitation Periods* Individual food consumption was determined weekly for all P1 and F1 rats throughout the period, ending on test day 70. Food consumption was not measured during cohabitation for males and females or after cohabitation for males. *Gestation and Lactation Periods* Individual food consumption of pregnant P1 and F1 females was recorded on gestation days 0, 7, 14, and 21 and on lactation days 0, 7, and 14. Food consumption was not measured for males or females without evidence of copulation. From these determinations and body weight data, individual daily food consumption, food efficiency, and mean daily intake of the test substance were calculated. <u>Water consumption</u> Tap water was provided *ad libitum*. Water consumption was not measured.