Copper sulphate

Evaluation of the effects on reproduction, recommendation for classification

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Committee for Compounds toxic to reproduction,
a committee of the Health Council of the Netherlands

to:

the Minister and State Secretary of Social Affairs and Employment

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Contents

Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondsheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductietoxische stoffen, een commissie van de Raad, adviseert een classificatie van reproductie toxische stoffen volgens Richtlijn 93/21/EEC van de Europese Unie. In het voorliggende rapport heeft de commissie kopersulfaat onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit meent de commissie dat er voldoende bewijs is om kopersulfaat niet te classificeren.
- Voor ontwikkelingsstoornissen, adviseert de commissie kopersulfaat in categorie 3 (*stoffen die in verband met hun mogelijke voor de ontwikkeling schadelijke effecten reden geven tot bezorgdheid voor de mens*) te classificeren en met R63 (*mogelijk gevaar voor beschadiging van het ongeboren kind*) te kenmerken.

Executive summary

On request of the Minister of Social Affairs and Employment, the Health Council of the Netherlands evaluates the effects on the reproduction of substances at the workplace. The Health Council's Committee for Compounds Toxic to Reproduction recommends to classify compounds toxic to reproduction according to the Directive 93/21/EEC of the European Union. In the present report the committee has reviewed copper sulphate.

The committee's recommendations are:

- For effects on fertility, the committee is of the opinion that sufficient data show that no classification of copper sulphate is indicated.
- For developmental toxicity, the committee recommends to classify copper sulphate in category 3 (*substances which cause concern for humans owing to possible developmental toxic effects*), and to label copper sulphate with R63 (*possible risk or harm to the unborn child*).

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed by the Health Council's Committee for Compounds Toxic to Reproduction according to the guidelines of the European Union (Directive 93/21/EEC). The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (class 1 and 2) of the European Union.

1.2 Committee and procedure

The present document contains the classification of copper sulphate by the Health Council's Committee for Compounds Toxic to Reproduction. The members of the committee are listed in Annex A. The first draft of this report was prepared by Mrs AE Smits-van Prooije and Mrs ir DH Waalkens-Berendsen at the Department of Neurotoxicology and Reproduction Toxicology of the TNO Nutrition and Food Research Institute, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development of the above mentioned compound.

Classification was performed according to the guidelines of the European Union listed in Annex C.

Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans (R61)
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic effects (R63)

No classification for effects on fertility or development

In November 1998, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

The classification of compounds toxic to reproduction on the basis of the Directive 93/21/EEC is ultimately dependent on an integrated assessment of the nature of all parental and developmental effects observed, their specificity and adversity, and the dosages at which the various effects occur. The directive necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the directive, the committee has agreed upon a number of additional considerations.

- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex C, 4.2.3.1 category 1).
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up to 1995. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft the committee performed an additional literature search in Medline and Toxline for the period 1995 to 1997. The results of this search were no reason for the committee to adjust the recommendations.

The committee chose to describe human studies in the text, starting with review articles. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

Animal data are described in the text and summarised in Annex D.

1.5 Presentation of conclusions

The classification is given with key effects, species and references specified. In case a substance is not classified as toxic to reproduction, one of two reasons is given:

- Lack of appropriate data preclude assessment of the compound for reproductive toxicity.
- Sufficient data show that no classification for toxic to reproduction is indicated.

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* Organisation for Economic Cooperation and Development

1.6 Final remark

The classification of compounds is based on hazard evaluation* only, which is one of a series of elements guiding the risk evaluation process. The committee emphasizes that for derivation of health based occupational exposure limits these classifications should be placed in a wider context. For a comprehensive risk evaluation, hazard evaluation should be combined with dose-response assessment, human risk characterization, human exposure assessment and recommendations of other organisations (GR89).

* for definitions see Tox95

Chapter 2

Copper sulphate

2.1 Introduction

Copper sulphate is a blue crystalline solid. It is an essential element.

Name	:	copper sulphate ($CuSO_4$)
CAS-no	:	7758-98-7
Use	:	as antifungal agent
Mol weight	:	159.6

In 1989, the Netherlands Food and Nutrition Council recommended a dietary copper intake (Cu^+ or Cu^{2+}) of 1.5-3.5 mg per day for male and female adults (Voe89). For pregnant women, the Council recommended a dietary intake of 2-3.5 mg per day.

2.2 Human studies

No data were found on the effects of copper sulphate on human fertility and development.

2.3 Animal studies

Tables 1 and 2 (Annex D) summarize the fertility and developmental toxicity studies with copper sulphate in experimental animals.

Fertility

Lecyck (Lec80) exposed sexually mature male and female C57BL and DBA mice to $CuSO_4$ in the diet at levels of 0, 0.5, 1.0, 1.5, 2.0, 3.0 or 4.0 g/kg feed. After one month males and females were mated within the dose groups; the females were sacrificed on day 19 of gestation. No effect was observed on the number of successful matings and the number of litters.

Aulerich et al. (Aul82) exposed male and female mink to $CuSO_4$ in the diet (0, 25, 50, 100, 200 mg/kg feed) for 8 months before mating, and during mating, gestation and lactation. Neither reproductive nor general toxic effects were observed in the parental animals. As to the offspring, a dose-related increase in postnatal litter mortality and a decrease in litter weight were found.

Developmental toxicity

Male and female C57BL and DBA mice were exposed to $CuSO_4$ in the diet at levels of 0, 0.5, 1.0, 1.5, 2.0, 3.0 or 4.0 g/kg feed (Lec80). After one month, males and females were mated within the dose groups; the females were sacrificed on day 19 of gestation. An increase in foetal weight was observed in the lowest dose groups (0.5 and 1.0 g/kg feed), and an increase in foetal mortality in the higher dose groups (3.0 and 4.0 g/kg feed). A few congenital malformations, *eg.* fused vertebrae and ribs, hydrocephalus and encephalocele were found in the highest dose groups of both strains. No data were available on the maternal status.

Ferm and Hanlon (Fer74) injected $CuSO_4$ intravenously into pregnant hamsters (0, 2.13, 4.25, 7.50, 10.0 mg/kg bw) on day 8 of gestation. Upon sacrifice on day 12 or 13 of gestation, they found teratogenic effects (midline defects, microphthalmia, ectopia cordis) from the lowest dose level (based on Cu^{2+}) of 2.13 mg/kg bw, and upwards. The highest dose of 10.0 mg/kg was lethal for the mothers. However, no additional maternal toxicity data were available.

O'Shea (O'S79) injected 0.08 mg Cu^{2+} (as $CuSO_4$) intravenously in female CFLP mice on day 7, 8 or 9 of gestation. Upon sacrifice on day 10 of gestation, evidence of both embryolethality and teratogenicity was observed. Resorptions were always found, but the teratogenic effect was dependent on the day of dosing. Administration on day 7

caused abnormalities in the head region. Administration on day 8 resulted in the absence of 'turning' of the embryo and in abnormalities of the neural tube, whereas administration on day 9 caused abnormalities of the neural tube and heart. No data were available on the maternal status.

Giavini et al. (Gia80) injected a $CuSO_4$ dose of 7.5 mg/kg bw intraperitoneally in female Sprague Dawley rats on day 3 of gestation. Upon sacrifice on day 5 of gestation, morphological alterations and degeneration of the blastocysts were observed.

2.4 Conclusion

No data were available on the effects of exposure to $CuSO_4$ on human fertility or development.

In general, the impact of $CuSO_4$ in animal studies depends among others on the route of administration: oral administration has considerably less effect on the offspring than intravenous or intraperitoneal administration.

No effects of $CuSO_4$ were observed in animal studies on male or female fertility (Lec80; Aul82). Therefore, sufficient animal data show that no classification of $CuSO_4$ for fertility effects is indicated.

Exposure to $CuSO_4$ resulted in an increased body weight of the offspring (Lec80), whereas higher levels caused a reduction in foetal body weight or foetal mortality (Lec80; Aul82). The embryotoxic or teratogenic effects of $CuSO_4$ appeared to be dependent on the moment of pregnancy when $CuSO_4$ was administered; the effects ranged from degeneration of blastocysts and embryolethality, to midline defects, ectopia cordis, defects of ribs and vertebrae (Fer74; Gia80; O'S79). The committee is of the opinion that the pattern of malformations found in these animal studies were in resemblance with the pentalogy of Cantrell in humans (Can58, Car92). The committee concludes that the effects of $CuSO_4$, found in two independent studies, were reproducible and specific embryopathic effects. However, classification in category 2 is not appropriate in view of the insufficient data on the maternal toxicity and the less relevant route of exposure in several studies.

Therefore, in view of the animal data with respect to the effects on development, the committee recommends to classify $CuSO_4$ in category 3 ('substances which cause concern for humans owing to possible developmental toxic effects'). It should be labeled with R 63 (possible risk or harm to the unborn child).

Proposed classification for effects on fertility

Sufficient animal data show that no classification of $CuSO_4$ for toxic to fertility is indicated.

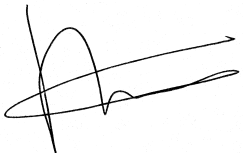
Proposed classification for effects on development

Category 3, R63

For the committee,
The Hague, 23 June 1999

dr ASAM van der Burght,
scientific secretary

dr BJ Blaauboer,



References

Aul82 Aulerich SJ, Ringer RK, Bleavins MR, Napolitano A. Effects of supplemental dietary copper on growth, reproductive performance and kit survival of standard dark mink and the acute toxicity of copper to mink. J. An. Sci. 1982; 55:337-343.

Can58 Cantrell JR, Haller JA, and Ravitz MM. A Syndrome of congenital defects involving the abdominal wall, sternum, diaphragm, pericardium and heart. Sur. Gynecol. Obstet. 1958; 107:602-614.

Car92 Carmi R, Boughman JA. Pentalogy of Cantrell and associated midline anomalies: a possible ventral midline developmental field. Am. J. Med. Genet. 1992; 1:42(1):90-95.

Fer74 Ferm VH, Hanlon DP. Toxicity of copper salts in hamster embryonic development. Biol. Reprod. 1974; 11:97-101.

Gia80 Giavini E, Prati M, Vismara C. Effects of cadmium, lead and copper on the rats preimplantation embryos. Bull. Environm. Contam. Toxicol. 1980; 25:702-705.

GR89 Gezondheidsraad. Koper, toetsing van een ontwerp basis-document. Den Haag: Gezondheidsraad, 1989. Publicatienummer 1989/09

Lec80 Lecyk M. Toxicity of $CuSO_4$ in mice embryonic development. Zool. Pol. 1980; 28:101-105.

O'S79 O'Shea KS, Kaufman MH. Influence of copper on the early post-implantation mouse embryo: an in vivo and in vitro study Roux's. Arch. Dev. Biol. 1979; 186:297-308.

Tox95 Niesink RJM, de Vries J, Hollinger MA, eds, Toxicology, Principles and Applications, Boca Raton: CRC Press, 1995:385

Voe89 Voedingsraad, Nederlandse voedingsnormen 1989, Advies opgesteld door de Commissie Voedingsnormen, Voorlichtingsbureau voor de Voeding, Den Haag, 1989

Literature consulted but not referred to in the text of this report

Bar82 Barlow SM, Sullivan FM (editors). Reproductive Hazards of Industrial Chemicals. An evaluation of animal and human data. 1982; 316-325 Academic Press, London - New York.

Cla83 Clarkson ThW, Norberg GF, Sager PR (editors). Reproductive and developmental toxicity of metals. V.H. Ferm and D.P. Hanlon: Metal-induced congenital malformations. 1983; 383-397 Plenum Press, New York and London.

Had91 Haddad DS, Kantarjian AH. The effects of copper loading on pregnant rats and their offspring. Funct. Dev. Morphol. 1991; 1(3):17-22.

Sch93 Schardein JL. Chemical induced birth defects, Seconds edition, revised and expanded Marcel Dekker, Inc, New York, Basel, Hong Kong, 1993; 374-375.

Annexes

Annex **A**

The committee

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The first draft of the present document was prepared by A.E. Smits-van Prooije and DH Waalkens-Berendsen, from the TNO Nutrition and Food Research Institute in Zeist, by contract with the Ministry of Social Affairs and Employment.

Secretarial assistance: E Vandenbussche-Parméus.
Lay-out: J van Kan.

Annex **B**

Comments on the public draft

- Dr D James
  Health and Safety Executive, United Kingdom

Annex **C**

Directive (93/21/EEC) of the European Community

4.2.3 Substances toxic to reproduction

4.2.3.1 For the purposes of classification and labelling and having regard to the present state of knowledge, such substances are divided into 3 categories:

Category 1:

Substances known to impair fertility in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility.

Substances known to cause developmental toxicity in humans

There is sufficient evidence to establish a causal relationship between human exposure to the substance and subsequent developmental toxic effects in the progeny.

Category 2:

Substances which should be regarded as if they impair fertility in humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in impaired fertility on the basis of:

- Clear evidence in animal studies of impaired fertility in the absence of toxic effects, or, evidence of impaired fertility occurring at around the same dose levels as other toxic effects but which is not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Substances which should be regarded if they cause developmental toxicity to humans:

There is sufficient evidence to provide a strong presumption that human exposure to the substance may result in developmental toxicity, generally on the basis of:

- Clear results in appropriate animal studies where effects have been observed in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects.
- Other relevant information.

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of impaired fertility in the absence of toxic effects, or evidence of impaired fertility occurring at around the same dose levels as other toxic effects, but which is not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.
- Other relevant information.

Substances which cause concern for humans owing to possible developmental toxic effects:

Generally on the basis of:

- Results in appropriate animal studies which provide sufficient evidence to cause a strong suspicion of developmental toxicity in the absence of signs of marked maternal toxicity, or at around the same dose levels as other toxic effects but which are not a secondary non-specific consequence of the other toxic effects, but where the evidence is insufficient to place the substance in Category 2.

- Other relevant information.

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:
T; R60: May impair fertility

For substances that cause developmental toxicity:
T; R61: May cause harm to the unborn child

Category 2:

For substances that should be regarded as if they impair fertility in humans:
T; R60: May impair fertility

For substances that should be regarded as if they cause developmental toxicity in humans:
T; R61: May cause harm to the unborn child.

Category 3:

For substances which cause concern for human fertility:
Xn; R62: Possible risk of impaired fertility

For substances which cause concern for humans owing to possible developmental toxic effects:
Xn; R63: Possible risk of harm to the unborn child.

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

Reproductive toxicity includes impairment of male and female reproductive functions or capacity and the induction of non-inheritable harmful effects on the progeny. This may be classified under two main headings of 1) Effects on male or female fertility, 2) Developmental toxicity.

1 *Effects on male or female fertility*, includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would interfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.
2 *Developmental toxicity*, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well

as those manifested postnatally. This includes embrytoxic/fetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peripostnatal defects, and impaired postnatalmental or physical development up to and including normal pubertal development.

Classification of chemicals as toxic to reproduction is intended to be used for chemicals which have an intrinsic or specific property to produce such toxic effects. Chemicals should not be classified as toxic to reproduction where such effects are solely produced as a non-specific secondary consequence of other toxic effects. Chemicals of most concern are those which are toxic to reproduction at exposure levels which do not produce other signs of toxicity.

The placing of a compound in Category 1 for effects on Fertility and/or Developmental Toxicity is done on the basis of epidemiological data. Placing into Categories 2 or 3 is done primarily on the basis of animal data. Data from *in vitro* studies, or studies on avian eggs, are regarded as 'supportive evidence' and would only exceptionally lead to classification in the absence of *in vivo* data.

In common with most other types of toxic effect, substances demonstrating reproductive toxicity will be expected to have a threshold below which adverse effects would not be demonstrated. Even when clear effects have been demonstrated in animal studies the relevance for humans may be doubtful because of the doses administrated, for example, where effects have been demonstrated only at high doses, or where marked toxicokinetic differences exist, or the route of administration is inappropriate. For these or similar reasons it may be that classification in Category 3, or even no classification, will be warranted.

Annex V of the Directive specifies a limit test in the case of substances of low toxicity. If a dose level of at least 1000 mg/kg orally produces no evidence of effects toxic to reproduction, studies at other dose levels may not be considered necessary. If data are available from studies carried out with doses higher than the above limit dose, this data must be evaluated together with other relevant data. Under normal circumstances it is considered that effects seen only at doses in excess of the limit dose would not necessarily lead to classification as Toxic to Reproduction.

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known antifertility agents or other information from humans which would lead to the conclusion that effects would be likely to be seen in humans. Where there are studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

Since impaired fertility may occur as a non-specific accompaniment to severe generalised toxicity or where there is severe inanition, classification into Category 2 should only be made where there is evidence that there is some degree of specificity of toxicity for the reproductive system. If it was demonstrated that impaired fertility in animal studies was due to failure to mate, then for classification into Category 2, it would normally be necessary to have evidence on the mechanism of action in order to interpret whether any adverse effect such as alteration in pattern of hormonal release would be likely to occur in humans.

Developmental toxicity

For classification into Category 2 there should be clear evidence of adverse effects in well conducted studies in one or more species. Since adverse effects in pregnancy or postnatally may result as a secondary consequence of maternal toxicity, reduced food or water intake, maternal stress, lack of maternal care, specific dietary deficiencies, poor animal husbandry, intercurrent infections, and so on, it is important that the effects observed should occur in well conducted studies and at dose levels which are not associated with marked maternal toxicity. The route of exposue is also important. In particular, the injection of irritant material intraperitoneally may result in local damage to the uterus and its contents, and the results of such studies must be interpreted with caution and on their own would not normally lead to classification.

Classification into Category 3 is based on similar criteria as for Category 2 but may be used where the experimental design has deficiencies which make the conclusions less convincing, or where the possibility that the effects may have been due to non-specific influences such as generalised toxicity cannot be excluded.

In general, classification in category 3 or no category would be assigned on an ad hoc basis where the only effects recorded are small changes in the incidences of spontaneous defects, small changes in the proportions of common variants such as are observed in skeletal examinations, or small differences in postnatal developmental assessments.

Effects during Lactation

Substances which are classified as toxic to reproduction and which also cause concern due to their effects on lactation should in addition be labelled with R64 (see criteria in section 3.2.8).

For the purpose of classification, toxic effects on offspring resulting *only* from exposure via the breast milk, or toxic effects resulting from *direct* exposure of children will not be regarded as ‘Toxic to Reproduction’, unless such effects result in impaired development of the offspring.

Substances which are not classified as toxic to reproduction but which cause concern due to toxicity when transferred to the baby during the period of lactation should be labelled with R64 (see criteria in section 3.2.8). This R-phrase may also be appropriate for substances which affect the quantity or quality of the milk.

R64 would normally be assigned on the basis of:

a toxicokinetic studies that would indicate the likelihood that the substance would be present in potentially toxic levels in breast milk, and/or

b on the basis of results of one or two generation studies in animals which in- dicate the presence of adverse effects on the offspring due to transfer in the milk, and/or

c on the basis of evidence in humans indicating a risk to babies during the lactational period.

Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **D**

Fertility and Developmental toxicity studies

Table 1 Fertility studies with copper sulphate.

authors	species	route	experimental period	dose	findings	Remarks
Lecyk (1980)	mouse (males and females)	diet	1 month + gestation up to d 19	0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 g CuSO4 /kg feed	no effect on number of successful matings and number of litters	
Aulerich et al. (1982)	mink (males and females)	diet	8 months + gestation, lactation	0, 25, 50, 100, 200 mg/kg feed CuSO4	no effects on male and female reproductive performance	

d = day

Table 2 Developmental toxicity studies with copper sulphate

authors	species	route	experimental period	dose	findings	remarks
Ferm & Hanlon (1974)	hamster (pregnant females)	i.v. injection	administration: gestation d 8 sacrifice: gestation d 12-13	0, 2.13, 4.25, 7.50, 10.0 mg Cu2+/kg bw	10 mg/kg bw: maternal lethality; all lower dose groups: midline defects, microphthalmia, ectopia cordis	no further data available on maternal toxicity
O'Shea & Kaufman (1979)	mouse (pregnant females)	i.v. injection	administration: gestation d 7, 8 or 9 sacrifice: gestation d 10	0.08 mg Cu2+/animal	embryolethality and teratogenicity (depending on day of dosing: abnormalities head region, no longitudinal rotation and abnormalities of neural tube, abnormalities of neural tube and heart, respectively)	no data available on maternal toxicity
Giavini et al. (1980)	rat (pregnant females)	i.p. injection	administration: gestation d 3 sacrifice: gestation d 5	7.5 mg CuSO4/kg bw	degeneration and morphological alterations blastocysts	no data available on maternal toxicity
Lecyk (1980)	mouse (males and females)	diet	1 month + gestation up to d19	0, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0 g CuSO4 /kg feed	0.5 and 1.0 g/kg feed: increased foetal weight 3.0 and 4.0 g/kg feed: decreased foetal weight A few cases of fused vertebrae and ribs, hydrocephalus, encephalocele	no data available on maternal toxicity
Aulerich et al. (1982)	mink (males and females)	diet	8 months + gestation, lactation	0, 25, 50, 100, 200 mg/kg feed CuSO4	dose-related increase in litter mortality, decrease in litter weight	

d = day; i.p. = intraperitoneal; i.v. = intravenous
To convert mg/kg feed to mg/kg bw a factor 20 is used in rats.

Annex **E**

Abbreviations

Abbreviations used:

bw	=	body weight
d	=	day
F	=	female(s)
i.p.	=	intraperitoneal
i.v.	=	intravenous
M	=	male(s)
n	=	number
NOAEL	=	no adverse effect level
OECD	=	Organisation for Economic Cooperation and Development
PN	=	postnatal