Health Council of the Netherlands

Penicillamine

Evaluation of the effects on reproduction, recommendation for classification

2014/15

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Gezondheidsraad
Health Council of the Netherlands

Aan de minister van Sociale Zaken en Werkgelegenheid

Onderwerp : Aanbieding advies *Penicillamine*
Uw kenmerk : DGV/MBO/U-932542
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Geachte minister,

Graag bied ik u hierbij het advies aan over de effecten van penicillamine op de vruchtbaarheid en het nageslacht; het betreft ook effecten op de lactatie en via de moedermelk op de zuigeling.

Dit advies maakt deel uit van een uitgebreide reeks waarin voor de voortplanting giftige stoffen worden geclassificeerd volgens richtlijnen van de Europese Unie. Het gaat om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld.

Dit advies is opgesteld door een vaste commissie van de Gezondheidsraad, de Subcommissie Classificatie reproductietoxische stoffen. Het is vervolgens getoetst door de Beraadsgroep Gezondheid en omgeving van de Gezondheidsraad.

Ik heb dit advies vandaag ter kennisname toegezonden aan de staatssecretaris van Infrastructuur en Milieu en aan de minister van Volksgezondheid, Welzijn en Sport.

Met vriendelijke groet,

prof. dr. W.A. van Gool,
voorzitter

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Penicillamine

Evaluation of the effects on reproduction,
recommendation for classification

Subcommittee on the Classification of Reproduction Toxic Substances,
a Committee of the Health Council of the Netherlands

to:

the Minister of Social Affairs and Employment

No. 2014/15, The Hague, May 22, 2014

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues and health (services) research...” (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Infrastructure & the Environment, Social Affairs & Employment, Economic Affairs, and Education, Culture & Science. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

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Contents

Samenvatting

In het voorliggende advies heeft de Gezondheidsraad penicillamine onder de loep genomen. Penicillamine wordt gebruikt als chelator bij behandeling van de ziekte van Wilson, bij vergiftigingen met koper, kwik en lood en kan worden voorgeschreven bij cystinurie en reumatoïde artritis. Dit advies past in een reeks adviezen waarin de Gezondheidsraad op verzoek van de minister van Sociale Zaken en Werkgelegenheid de effecten van stoffen op de voortplanting beoordeelt. Het gaat vooral om stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Subcommissie Classificatie reproductietoxische stoffen van de Commissie Gezondheid en beroepsmatige blootstelling aan stoffen van de raad, hierna aangeduid als de commissie, kijkt zowel naar effecten op de vruchtbaarheid van mannen en vrouwen als naar effecten op de ontwikkeling van het nageslacht. Daarnaast worden effecten op de lactatie en via de moedermelk op de zuigeling beoordeeld.

Op basis van Verordening (EG) 1272/2008 van de Europese Unie doet de commissie een voorstel voor classificatie. Voor penicillamine komt de commissie tot de volgende aanbevelingen:

- voor effecten op de fertiliteit adviseert de commissie om penicillamine niet te classificeren wegens onvoldoende geschikte gegevens
- voor effecten op de ontwikkeling adviseert de commissie penicillamine te classificeren in categorie 1B (*stoffen waarvan verondersteld wordt dat zij*

toxisch zijn voor de menselijke voortplanting) en te kenmerken met H360D (*kan het ongeboren kind schaden*)

- voor effecten tijdens of via lactatie adviseert de commissie om penicillamine niet te kenmerken wegens onvoldoende geschikte gegevens.

Executive summary

In the present report the Health Council of the Netherlands reviewed penicillamine. Penicillamine is used as a chelator in Wilson's disease, in copper, mercury and lead intoxications and can be prescribed in case of cystinuria and rheumatoid arthritis. This report is part of a series, in which the Health Council evaluates the effects of substances on reproduction, at request of the Minister of Social Affairs and Employment. It mainly concerns substances to which man can be exposed occupationally. The Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety of the Health Council, hereafter called the Committee, evaluates the effects on male and female fertility and on the development of the progeny. Furthermore, the Committee considers the effects of a substance on lactation and on the progeny via lactation.

The Committee recommends classification according to Regulation (EC) 1272/2008 of the European Union. For penicillamine, these recommendations are:

- for effects on fertility, the Committee recommends not classifying penicillamine due to a lack of appropriate data
- for effects on development, the Committee recommends classifying penicillamine in category 1B (*presumed human reproductive toxicant*) and labelling with H360D (*may damage the unborn child*)
- for effects on or via lactation, the Committee recommends not labelling penicillamine due to a lack of appropriate data.

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. This classification is performed by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances of the Dutch Expert Committee on Occupational Safety (DECOS). The classification is performed according to European Union Regulation (EC) 1272/2008 on classification, labelling and packaging (CLP) of substances and mixtures. The CLP guideline is based on the Globally Harmonised System of Classification and Labelling of Chemicals (GHS). The Subcommittee'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 reproductive toxicant (category 1A and 1B and 2) or compound with effects on or via lactation.

1.2 Committee and procedure

This document contains the classification of penicillamine by the Health Council's Subcommittee on the Classification of Reproduction Toxic Substances, hereafter called the Committee. The members of the Committee are

listed in Annex A. The submission letter (in English) to the Minister can be found in Annex B.

In 2014, the President of the Health Council released a draft of the report for public review. The individuals and organizations that commented on the draft report are listed in Annex C. The Committee has taken these comments into account in deciding on the final version of the report. The comments received, and the replies by the Committee, can be found on the website of the Health Council.

The classification is based on the evaluation of published human and animal studies concerning adverse effects with respect to fertility and development as well as lactation of the above-mentioned compound.

Classification for reproduction (fertility (F) and development (D)):	
Category 1	Known or presumed human reproductive toxicant (H360(F/D))
Category 1A	Known human reproductive toxicant
Category 1B	Presumed human reproductive toxicant
Category 2	Suspected human reproductive toxicant (H361(f/d))
No classification for effects on fertility or development	
Classification for lactation:	
	Effects on or via lactation (H362)
	No labelling for lactation

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC) 1272/2008) presented in Annex D. The classification of compounds 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 guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations (see Annex E).

1.3 Effects on or via lactation

The recommendation for classifying substances for effects on or via lactation is also based on Regulation (EC) 1272/2008. The guideline defines that substances which are absorbed by women and have been shown to interfere with lactation or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified

and labelled. Unlike the classification of substances for fertility and developmental effects, which is based on hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on risk characterization and therefore, it also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects on or via lactation when it is likely that the substance would be present in breast milk at potentially toxic levels. The Committee considers a concentration of a compound as potentially toxic to the breastfed child when this concentration exceeds the exposure limit for the general population, e.g. the acceptable daily intake (ADI).

1.4 Data

Literature searches were conducted in the on-line databases XTOXLINE, MEDLINE and CAPLUS, up to June 2009 without a starting date; the final update was performed in TOXNET in October 2013. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not retrieved 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.

The Committee describes both the human and animal studies in the text. The animal data are described in more detail in Annex F as well. Of each study the quality of the study design (performed according to internationally acknowledged guidelines) and the quality of documentation are considered.

In the assessment of the potential reproduction toxic effects of penicillamine, the Committee also used data on adverse effects related to its application as a therapeutic agent.

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 precludes assessment of the compound for reproductive toxicity
- sufficient data show that no classification for toxic to reproduction is indicated.

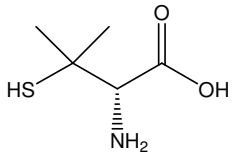
1.6 Final remark

The classification of compounds is based on hazard evaluation only (Niesink et al., 1995)[13], 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 organizations.

Chapter 2

Penicillamine

2.1 Introduction

name	:	penicillamine
CAS registry number	:	52-67-5
EC/EINECS number	:	200-148-8
CAS registry name	:	D-valine, 3-mercapto-
synonyms	:	D-penicillamine; (S)-penicillamine; D-mercaptovaline; β-mercaptovaline; 3-mercapto-D-valine; (S)-3,3-dimethylcysteine; α-amino-β-methyl-β-mercaptobutyric acid; β-thiovaline
colour and physical state	:	fine, (practically) white, crystalline powder
molecular weight	:	149.2
molecular formula	:	$C_5H_{11}NO_2S$
structural formula	:	
melting point	:	202-206 °C
vapour pressure	:	$8x10^{-5}$ Pa at 25 °C (estimated)
Log P octanol-water)	:	-1.8 (experimental)
solubility	:	freely soluble in water; slightly soluble in alcohol; insoluble in ether, chloroform.
use	:	as a chelator: in Wilson's disease (a genetic disorder characterized by high tissue copper); cystinuria; copper, mercury, and lead intoxications; rheumatoid arthritis.[2,3,10]

general toxicity	:	effects seen with penicillamine can be ascribed for a large part to its chelating properties; especially, copper deficiency can produce a variety of clinical effects in animals and humans due to its role in the structure and function of a number of enzymes including cytochrome c oxidase (electron transport), superoxide dismutase (free-radical detoxification), tyrosinase (melanin production), dopamine beta-hydroxylase (catecholamine production), lysyl oxidase (cross-linking of collagen and elastin), ceruloplasmin (ferroxidase, transport), and unidentified copper enzyme involved in cross-linking of disulphide bonds of keratin.[26]
kinetics	:	penicillamine is well absorbed from the gastrointestinal tract and metabolized for the largest part; half the oral dose is excreted in faeces, but metabolites have not been characterized yet; in urine, penicillamine is excreted as S-methyl-penicillamine, cysteine-penicillamine mixed sulphide, penicillamine disulphide and homocysteine-penicillamine mixed disulphide.[2,14]

Data from[20,21], unless otherwise noted.

2.2 Human studies

Fertility studies

No data were available regarding the effects of exposure to penicillamine on human fertility.

Developmental toxicity studies

Data on developmental effects in humans are available from reports on single cases or small case series of women with Wilson's disease, rheumatoid arthritis or cystinuria treated with penicillamine during pregnancy.

Endres summarized several case reports from the period 1967-1977 including a total of 87 exposed pregnancies (0.5-3.0 g/day penicillamine for the entire pregnancy or parts thereof) that resulted in two newborn infants with severe connective tissue defects.[4] Rosa described these two infants in more detail and added three later cases, all having cutis laxa and several other abnormalities, such as growth retardation, micrognatia, low set ears and inguinal hernia. He also mentioned four infants born without cutis laxa, but with a variety of other birth defects after maternal penicillamine treatment.[16] Gregory and Mansell on the other hand, reported on 46 pregnancies among patients with cystinuria treated with penicillamine in which four miscarriages but no congential malformations occurred.[5] In the absence of controlled studies, Sternlieb pooled data concerning

the results of pregnancy in women with Wilson's disease receiving penicillamine from the National Center for the Study of Wilson's Disease (n=71), from case reports in Medline (n=41), and from personal communications (n=41). Of these 153 reported pregnancies, two ended in induced abortions because of oesophageal and neural tube defects, one child was born with a cleft lip, one with mannosidosis, and two with transient cutis laxa.[19] In 2004, Pinter et al. also described an infant with cutis laxa, severe micrognathia and other defects born to a mother treated with penicillamine for Wilson's disease.[15] Albukerk[1] and Hanukogli et al.[6] reported on two miscarriages and two siblings born with goitrous hypothyroidism, respectively, in two women treated for Wilson's disease.

Lactation

Penicillamine was not detectable by HPLC in the breast milk of any of the milk samples from four patients with Wilson's disease receiving daily doses of 500-800 mg penicillamine.[22]

In three infants breastfed by mothers taking penicillamine, no adverse effects were observed that could be related to penicillamine.[22]

2.3 Animal studies

Fertility and developmental toxicity studies in laboratory animals are summarized in Annex F.

Fertility studies

Yamada et al. administered oral (gavage) doses of penicillamine of 0, 62.5, 125, 250 and 500 mg/kg bw/day to groups of 20 male and female SLC:Wistar rats. Male rats were treated for 60 days starting before mating (not further specified); female rats from 14 days before mating, throughout mating and until 7 days of gestation. All pregnant females were sacrificed on gestational day 20 and their foetuses were examined. Body weight and food consumption were decreased at 500 mg/kg bw/day in males before mating accompanied by soft faeces, lustress hair and inhibition of spontaneous activity. Treated females did not show clinical signs of toxicity or effects on body weight or oestrous cycle. Decreased mating rates and pregnancy rates and prolongation of the mating period were observed at 500 mg/kg bw/day. The number of corpora lutea and implantations were not affected (report in Japanese with summary and tables in English).[24]

Developmental toxicity studies

Diet

Female Sprague-Dawley rats (n=6-14) were given amounts of penicillamine in the diet of 0, 0.17, 0.83 or 1.66% throughout gestation (according to Keen et al. approximately 22-27, 108-137 and 166-274 mg/day; i.e. approximately 100, 490 and 880 mg/kg bw/day assuming an average body weight of 250 g). Addition of penicillamine did not affect the trace element composition of the diet. Dams were sacrificed on gestational day 21 and foetuses were removed by Caesarean section.

Dams appeared healthy and normal throughout gestation. Treatment did not affect food intake. Body weight gain was reduced at 490 and 880 mg/kg bw/day.

Litter size and placental weight were comparable between treated and control groups. No effect was observed on the number of implantations or the number of live foetuses. The number of dead foetuses was increased in all treated groups. Foetal weight was decreased at 490 and 880 mg/kg bw/day ($p<0.01$, both groups) and crown-rump length at 880 mg/kg bw/day ($p<0.001$). Compared to the control group, increases were seen in the percentage of resorbed foetuses (1, 8, 7, 10 at 0, 100, 490, 880 mg/kg bw/day, respectively), in the number of foetuses with malformations (0, 3, 39, 36), the percentage of malformed foetuses (0, 3, 30, 64%) and the percentage of total sites affected (1, 10, 35, 68%). On external examination, in decreasing order of incidence, flexed limbs, loose skin, abdominal herniation and spina bifida were noted at 490 and 880 mg/kg bw/day. At the highest dose, oedema and cleft palate were also observed. Internal examination showed small lungs and diaphragm lesion at 490 and 880 mg/kg bw/day. Subdermal haemorrhaging mostly on the back over the spinal column and on the head and jaw was seen in treated foetuses, particularly at 490 and 880 mg/kg bw/day. Skeletal variations such as abnormal ossification of the sternum, wavy and irregular ribs, and reduced ossification of the middle phalanges of the fore and hind limbs and tail vertebrae were noted in the groups receiving 490 and 880 mg/kg bw/day. No skeletal variations were seen in the control and 100 mg/kg bw/day groups.

Tissue concentrations of several trace elements were measured in dams and foetuses. Compared to controls, copper levels in plasma and kidney of dams of all dose groups and in liver and muscles of dams given 490 and 880 mg/kg bw/day were decreased. Copper contents of the uterus, whole foetus, foetal liver and placenta were dose dependently reduced at all dose levels. Zinc levels were only decreased at 880 mg/kg bw/day in maternal liver and plasma. Zinc levels

were slightly increased in the whole foetus at 490 and 880 mg/kg bw/day, and decreased in foetal liver at 880 mg/kg bw/day. Iron levels in dams were doubled at 490 and 880 mg/kg bw/day; no effects were seen on other tissues in dams or foetuses. Manganese and magnesium level measured in dams in liver, plasma and kidney and calcium in liver and plasma were not affected by penicillamine treatment. Levels of manganese, calcium and magnesium in uterus or placenta did not differ between groups. Manganese levels were increased at 490 and 880 mg/kg bw/day in the whole foetus, but not in the foetal liver. Magnesium levels were decreased at 880 mg/kg bw/day in foetal liver, but not in the whole foetus. Calcium levels were not affected in the foetal liver.

The types of malformations found after penicillamine administration are similar to those seen in copper deficiency suggesting this is at least one of the mechanisms for penicillamine-induced developmental toxicity.[9]

In an additional study, this group investigated the effect of a copper-supplemented diet on the effects induced by dietary penicillamine administration. Mated female Sprague-Dawley rats (n=10) were given 5 (control), 50 or 100 mg copper/kg food. Half of the animals in each group (n=5) received penicillamine added to the diet at a concentration of 0.83% (i.e. 880 mg/kg bw/day) (addition of penicillamine did not affect the trace element composition of the diet). Four or five females per group were confirmed to be pregnant. Dams were sacrificed on gestational day 21 and foetuses were removed by Caesarean section.

Food intake was similar among all groups. No effect was seen on maternal weight gain, litter size, or foetal or placental weight. Crown-rump length was reduced in the copper-control group receiving penicillamine. The frequency of resorptions in the copper control group receiving penicillamine was 23%, while that in the groups with supplemental copper and penicillamine (6 and 4% at 50 and 100 mg/kg, respectively) was only slightly higher than in the 50 and 100 mg/kg copper group without penicillamine (both 3%); no resorptions were observed in the control group. Malformations were seen in the groups given penicillamine only: 21, 2 and 4% at 5, 50 and 100 mg/kg copper. The total of implantation sites with either a dead or malformed foetus was affected in the penicillamine-treated groups: 39, 8 and 8% at 5, 50 and 100 mg/kg copper, respectively. The types of malformation seen in the unsupplemented penicillamine group were similar to those reported in the study above (Keen, 1983): loose skin, small lungs, and diaphragmatic lesions. Only one foetus in the 50 μg/g copper + penicillamine and 100 μg/g copper + penicillamine group each had small lungs and in the latter group, one foetus had an incomplete diaphragm.

Copper levels in the liver, plasma, kidney, muscle and intestine of dams were similar for groups only receiving copper in the diet. Copper levels in these tissues were comparable between penicillamine-treated groups, but lower than in the correlated copper-only group. This was most pronounced for the 5 mg/kg copper + penicillamine group, where the copper level was only about 20% of the control level. Copper content in placenta, whole foetus and foetal liver was comparable between the different copper levels in the diet. Addition of penicillamine in the diet reduced the copper concentration at all copper dose levels. The groups with supplemental copper had higher copper concentrations in placenta, whole foetus and foetal liver than the unsupplemented group. Zinc concentration in the liver, plasma, kidney, muscle or intestine of dams was not affected by penicillamine or copper-supplementation as was zinc concentration in placenta, whole foetus and foetal liver. The iron content was not significantly different between the penicillamine groups and the correlated only-copper groups in all dam tissues except the kidney. In the kidney, iron concentration at the 100 mg/kg copper + penicillamine was higher than the 100 mg/kg copper group. The pattern of higher iron content was seen in the copper-supplemented penicillamine groups in the liver, plasma and kidney and highest copper-supplemented group in muscle and intestine. Iron levels in placenta, whole foetus and foetal liver showed a similar pattern as in maternal tissues. Manganese concentrations were not affected in any of the maternal or foetal tissues tested.[11]

Irino et al. administered amounts of 0 or 4000 mg penicillamine/kg diet (i.e. 0, 480 mg/kg bw/day; assuming a daily food intake of 3 g and an average body weight of 25 g) in the diet of 18 or 28 three-month-old female DDD mice, respectively, for two months. Animals were mated 1:1 with untreated males, and females were treated during pregnancy and lactation. Nine of the penicillamine-treated dams were supplemented with copper immediately after delivery. Two to three pups per litter were sacrificed at 1, 7, 14 and 21 days of age for metal determinations in various organs. Three dams per group were used for the cytochrome c oxidase assay.

Data on maternal toxicity were not presented, but in an accompanying study in which male and female mice were fed diets containing 4000 mg penicillamine/kg diet from three to 15 weeks after birth, toxic signs such as dorsal hernia, priapism and paralysis of the digits were observed present in almost all mice after six weeks of treatment.

Body weights of the pups in the penicillamine-only group were decreased at postnatal day 7 ($p<0.01$), 14 (not statistically significant (n.s.) and 21 ($p<0.01$). In the copper-supplemented group, body weights were lower at postnatal day 7

($p<0.01$) but similar to those in controls at postnatal day 14 and 21. At postnatal day 10, hind limb paralysis, motor incoordination and tremors began to appear in pups. Aortic aneurysms were observed at various time points. Toxic signs and mortality were most prominent from postnatal days 14 to 21.

Absolute brain weights were decreased at postnatal days 1 ($p<0.01$) and 7 ($p<0.05$) and increased at postnatal days 14 ($p<0.01$). In the copper-supplemented group, brain weights were similar to those in the penicillamine-only group at postnatal day 7 and 14 but higher at postnatal day 21 ($p<0.05$).

While copper levels in serum, brain, kidney and skin of pups started to deviate from controls from postnatal day 7 onwards, copper levels in spinal cord, liver and milk were statistically significantly lower from postnatal day 1 onwards. Copper levels in the aorta were statistically significantly decreased on postnatal day 7 and 14. While copper levels in the brain and spinal cord increased with time in the control group, copper levels in the penicillamine-only group were constant. Copper supplementation increased copper levels close to control levels or to a higher level compared to those in the penicillamine-only group and completely prevented neurological disorders. Zinc and iron levels in the brain of pups were similar to those of controls. Zinc and iron levels in the liver were lower and higher than controls at postnatal day 1 and 14, respectively. Brain cytochrome c oxidase activity was statistically significantly lower in the penicillamine-only group compared to the control and copper-supplemented group. Histological investigation of hepatocytes and ganglion cells showed less granules present in pups from penicillamine-only-treated mice.[8]

Irino et al. investigated the effects on offspring from female mice treated with penicillamine during gestation and/or lactation. Females (n=4-6) were given one of the following diet regimes (during gestation + lactation): control diet only; control diet + 4000 mg/kg diet penicillamine (c+480pa)*; 4000 mg/kg diet penicillamine + control diet (480pa+c); 2000 mg/kg diet penicillamine + 2000 mg/kg diet penicillamine (240pa+240pa); 4,000 mg/kg diet penicillamine + 4000 mg/kg diet penicillamine (480pa+480pa); or 4000 mg/kg diet penicillamine + 4000 mg/kg diet penicillamine + $5x10^{-3}$ M copper sulphate in drinking water (480pa+480pa+Cu). In pups, abnormal signs and gross lesions in the offspring (n=11-21/sex/dose) were recorded up to postnatal day 35; at postnatal day 1, 7, 14 and 21, selected numbers of pups were killed for histological examinations.

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* assuming a food intake of 3 g/day and an average body weight of 25 g, 2000 or 4000 mg penicillamine/kg diet is equivalent to 240 or 480 mg/kg bw/day.

No abnormal clinical signs or lesions were seen in the treated dams. Treatment did not affect litter size and no external malformations were observed at birth. No mortality was seen in the control group and in group 240pa+240pa and 480pa+480pa+Cu. In group 480pa+480pa, pup survival decreased from postnatal day 14 onwards, only 1/39 (3 %) pups remaining alive at postnatal day 35. In group 480pa+c, 20% of the pups died within 14 days after birth, the remaining surviving until postnatal day 35. In group c+480pa, pup mortality occurred after 21 days reaching 50% on postnatal day 35. No sex differences were noted. In the pups of the control group and group 480pa+480pa+c, no abnormal clinical signs or lesions were noted. In group 480pa+c, only digit paralysis was seen in some survivors at postnatal 35 while in group 240pa+240pa, digit paralysis and scrotal subcutaneous haemorrhages were observed. In group c+480pa, this paralysis and haemorrhages appeared already at postnatal day 28 and priapism at postnatal day 35. Group 480pa+480pa showed already gangrenous digits two days after birth. Within fourteen days after birth, motor incoordination, tremor, hyperexcitability on stimulation, and hind limb paralysis appeared. Ptosis and ruptured aortic aneurysm occurred within 21 days, and digit paralysis and scrotal subcutaneous haemorrhage within 28 days after birth. No particular pathological findings were noted in the skin, lung, liver and kidney of offspring. The aortas in the 480pa+480pa group showed dissecting aneurysms and focal breaks in the elastic lamellae; neuronal degeneration was noted in the cerebral cortex, thalamic nuclei and spinal ganglion on postnatal day 14 and 21 but not on postnatal day 1 and 7. In the c+480pa group, changes indicative of minimal neurodegeneration were seen on postnatal day 21. No neuronal degeneration was noted in the remaining groups.[7]

Gavage

Yamada et al. administered oral (gavage) doses of penicillamine of 0, 62.5, 125, 250 and 500 mg/kg bw/day to groups of 20 male and female SLC:Wistar rats. Male rats were treated for 60 days before mating; female rats from 14 days before mating, throughout mating and until gestational day 7. All pregnant females were sacrificed on gestational day 20 and their foetuses were examined.

Treated females did not show clinical signs of toxicity or effects on body weight. No effects on the number of live foetuses, mortality, sex ratio and foetal body weight and no increases in the incidences of external, visceral and skeletal abnormalities were observed.[24]

Pregnant Sprague-Dawley rats (n=3-10) were given doses of penicillamine of 360, 375 or 400 mg/day penicillamine (i.e. 1440, 1500, 1600 mg/kg bw/day; assuming an average body weight of 250 g) during gestational days 10 to 15 and of 200 or 400 mg/day ((i.e. 800, 1600 mg/kg bw/day) during gestational days 10 to 17. Dams were sacrificed one day before expected delivery and foetuses were examined.

Apart from mortality at doses of 1600 mg/kg bw/day, no maternal effects were reported. Of the four pregnant rats given 1600 mg/kg bw/day on gestational days 10-17, two died; the number of resorptions was 16/18 (89%) and the incidence of cleft palate 2/2 (100%). Of the ten rats given the same dose on gestational days 10-15, one died. The number of resorptions was 7/82 (8%) and the incidence of cleft palate 60/75 (80%). At 1440 and 1500 mg/kg bw/day given on gestational days 10-15, no mortality occurred. The number of resorptions was 0 and 1/44 (2%), respectively; the incidence of cleft palate 17/43 (40%) and 16/37 (43%), respectively. No mortality, cleft palate or resorptions were noted at 800 mg/kg bw/day given during gestational days 10-17.[18]

Rousseaux and MacNabb administered daily doses of penicillamine of 0, 250, 500, 1000 or 0 and 2000 mg/kg bw to CD-1 mice (n=7-10/group) during gestational days 1 to 12. On gestational day 18, foetuses were removed per Caesarean section. Two-thirds were examined for skeletal abnormalities and one-third for soft tissue alterations.

Food consumption did not differ between groups between gestational days 0 to 12 and 13 to 18. Body weight (gain) of all treated dams was reduced by 3-4% from gestational day 13 to 18 ($p=0.004$); relative liver weights of the treated dams were not affected. No effect was observed on the number of implantations, live foetuses, dead foetuses, early resorptions, embryonic losses, foetal losses and sex ratio. At 2000 mg/kg bw, there were increases in the number of non-viable foetuses and decreases in foetal weight and crown-rump length (all $p<0.001$). No external malformations were noted in penicillamine-treated animals.[17]

Female DDI mice (n=20-35) were treated with 3200 or 4000 mg penicillamine/ kg bw penicillamine for five consecutive days starting on gestational day 10 or for four consecutive days starting on gestational day 11. A vehicle (distilled water) and an untreated control group were included. Dams were sacrificed on gestational day 17. Foetuses were examined with emphasis on cleft palate. No statistical analysis was performed.

In the groups given 3200 or 4000 mg/kg bw/day for five days and 4000 mg/kg for four days, 12 to 17 percent of the animals had aborted, while no abortions occurred in the animals given 3200 mg/kg bw/day for four days and in the animals of the control groups. The total percentage of foetal deaths and resorptions was increased in both groups given 4000 mg/kg bw/day compared to both control groups. Average foetal body weights were decreased in all treated groups compared to both control groups. The percentages of litters with cleft palate were increased in all treated groups (77%, 9% at 3200 mg/kg bw for five and four days, respectively; 79%, 52% at 4000 mg/kg bw for five and four days, respectively; 0% in controls).[12]

Pregnant Syrian golden hamsters (number per group not reported) were given doses of penicillamine of 3200, 4000 or 4800 mg/kg bw on gestational day 7. A vehicle and a control group were included. Dams were sacrificed on gestational day 14 and foetuses were examined.

The numbers of litters, number of implantations and number of live foetuses were not affected compared to the control groups. Foetal mortality expressed as percentage of resorptions was 11, 28 and 15% at 3200, 4000 and 4800 mg/kg bw, respectively (vs. 10 and 1% in untreated and vehicle controls, respectively). At 4000 and 4800 mg/kg bw, mean foetal weights were decreased ($p<0.05$). The major malformation observed was encephalocele (1, 8 and 13%, respectively, vs. 0% in the control groups). Other defects not dose-relatedly observed at low frequency (1-9%) were exencephaly and slightly malpositioned limbs. Cleft palate was seen in three litter mates at 4000 mg/kg bw. No visceral abnormalities were found.[23]

Drinking water

Yurdakök et al. studied the cerebellar maturation of the offspring of female albino rats (n=3-4/group) given 0, 300 or 400 mg penicillamine (i.e. 0, 1000, 1333 mg/kg bw/day; assuming an average body weight of 300 g) in their drinking water during the last six days of gestation. On gestational day 20-21, two rats per group were delivered by Caesarean section. The remaining two treated dams receiving 1333 mg/kg bw/day during gestation and together with the two controls were kept with their offspring for three weeks after delivery, when their offspring was sacrificed. The following observations were performed in six to ten offspring per group. Birth weight was decreased in the penicillamine groups compared to control. The hair of the pups was short, sparse, easily pulled out and of coarse texture compared to control. Cerebellum weight was decreased

at the highest dose. Histological examination revealed a significant decrease in myelination, reduction in the thickness of the molecular layer and the number of Purkinje cells relative to controls. These effects were absent in the offspring sacrificed three weeks after delivery, indicating a reversal of degeneration of the cerebellum.[26]

Yurdakök et al. investigated the effect of penicillamine on the development of lung and kidney tissue in offspring from albino rats (n=5/group) given 0 or 300 mg of penicillamine (0 or 1000 mg/kg bw/day; assuming an average body weight of 300 g) in their drinking water during the last six days of gestation. Treated dams had lower serum copper levels than those in the control group. Pups from treated dams (n=21) had decreased body and kidney weights compared to controls (n=17). Lung weights were comparable. Histological examination of the lung and kidneys of the pups revealed pulmonary dysplasia with bronchomegaly, cystic alveoli, interstitial emphysema, atelectasis, perivascular loose of connective tissue and vascular aneurysm in the lung and caliectasis in the kidneys in the treated group.[25]

Lactation

In one of the mouse studies by Irino et al., one group received penicillamine during lactation only. Pup mortality was observed from postnatal day 21 onward and amounted to 50% by postnatal day 35. The offspring showed digit paralysis and scrotal subcutaneous haemorrhages at postnatal day 28 and priapism at postnatal day 35; changes indicative of minimal neurodegeneration were observed at post-mortem examination on postnatal day 21.[8]

In another study by Irino et al., stomach milk copper levels in sucklings of mothers treated with 0.4% penicillamine in the diet during pregnancy were decreased compared to controls at 1, 7 and 14 days of age. These suckling mice showed signs of neurological dysfunction which disappeared after copper-supplementation in their drinking water three weeks after birth.[7]

2.4 Conclusion

Fertility

No human studies on fertility effects of penicillamine were available.

One animal study of which only the summary and tables were available showed decreased mating and pregnancy rates and prolongation of the mating

period at the same dose levels as decreased body weight and food consumption in males.[24]

Therefore, the Committee proposes not to classify penicillamine for effects on fertility due to a lack of appropriate human and animal data.

Developmental toxicity

A number of case reports involving women who received penicillamine treatment during pregnancy described infants born with a variety of developmental defects, often including cutis laxa.[4,15,16,19] These findings consistently indicate that penicillamine may have human developmental toxic effects, mainly of presumed mesodermal origin. However, the lack of well conducted epidemiological studies, which include the use of appropriate controls, adequate exposure assessment and due consideration of bias or confounding, precludes final conclusions.

In developmental toxicity studies in rats and mice effects were observed in offspring at dose levels at which no effects were seen in dams.[8,18] The Committee noted the low number of animals used in these studies. In the study reported by Keen et al.[9], a variety of mesodermal malformations were seen at a dose level at which only slightly decreased body weights in dams were observed. Dams fed penicillamine had increased incidences of resorptions and malformations in their offspring, in the absence of effects on food intake or body weight.[11] In other studies[7,12,23,26], effects such as cleft palate, brain tissue morphological changes, hind limb paralysis, neurological effects and aortic aneurysms were observed. Although the absence or presence of maternal toxicity was not always reported in these studies, the Committee considers that the nature and severity of the effects observed indicate that they occurred independently from maternal toxicity.

Overall, based on the prenatal and postnatal effects found in animals (i.e. rats, mice, and hamsters), the Committee proposes to classify penicillamine for effects on development in category 1B (*presumed human reproductive toxicant*).

Lactation

In one study, penicillamine could not be detected in breast milk samples obtained from women receiving daily doses of penicillamine of 500-800 mg. No adverse effects that were likely to have been related to the administration of penicillamine were observed in three infants breastfed by mothers taking penicillamine as a drug.[22]

One mouse study showed mortality and serious defects after receiving penicillamine during lactation only.[8]

However, in the absence of data on concentrations of penicillamine in breast milk and on the safe/acceptable daily intake of penicillamine, the Committee is not able to calculate a safe level for penicillamine in human breast milk. Therefore, the Committee proposes not labelling penicillamine for effects on or via lactation due to a lack of appropriate human and animal data.

Proposed classification for fertility

Lack of appropriate data precludes the assessment of penicillamine for effects on fertility.

Proposed classification for developmental toxicity

Category 1B, H360D.

Proposed labelling for effect during lactation

Lack of appropriate data precludes the assessment of penicillamine for effects on or via lactation.

References

1 Albukerk JN. Wilson's disease and pregnancy. A case report. Fertil Steril. 1973;24:494-7.

2 Byrns MC, Penning T. Environmental toxicology: carcinogens and heavy metal. In: Brunton LL, Blumenthal DK, Murri N, Hilal-Dandan R, editors. Goodman & Gilman's the pharmacological basis of therapeutics. New York, USA: the McGraw-Hill Companies, Inc.; 2012. [cited 2013 November]. Available from: http://accessmedicine.com/resourceTOC.aspx?resourceID=651.

3 College ter Beoordeling van Geneesmiddelen (Medicines Evaluation Board) (CBG-MEB). [cited 2013 March]. Availbale from: http://www.cbg-meb.nl/CBG/nl/humane-geneesmiddelen/geneesmiddeleninformatiebank/default.htm.

4 Endres W. D-Penicillamine in pregnancy - to ban or not to ban? Klin Wochenschr. 1981;59:535-7.

5 Gregory MC, Mansell MA. Pregnancy and cystinuria. Lancet. 1983;322:1158-60.

6 Hanukoglu A, Curiel B, Berkowitz D, Levine A, Sack J, Lorberboym M. Hypothyroidism and dyshormonogenesis induced by D-penicillamine in children with Wilson's disease and healthy infants born to a mother with Wilson's disease. J Pediatr. 2008;153:864-6.

7 Irino M, Sanada H, Tashiro S, Yasuhira K, Takeda T. D-penicillamine toxicity in mice. III. Pathological study of offspring of penicillamine-fed pregnant and lactating mice. Toxicol Appl Pharmacol. 1982;65:273-85.

8 Irino M, Yasuhira K, Takeda T. D-penicillamine toxicity in mice. II. Concentrations of copper, zinc and iron related to development of toxicity. Toxicol Appl Pharmacol. 1982;63:1-12.

9 Keen CL, Mark-Savage P, Lonnerdal B, Hurley LS. Teratogenic effects of D-penicillamine in rats: relation to copper deficiency. Drug Nutr Interact. 1983;2:17-34.

10 Koninklijke Nederlandse Maatschappij ter bevordering der Pharmacie (KNMP), editor. Medicijn: penicillamine. [cited 2013 November]. Available from: http://www.apotheek.nl/Medische_informatie/Medicijnen/Medicijnen/penicillamine.as.

11 Mark-Savage P, Keen CL, Hurley LS. Reduction by copper supplementation of teratogenic effects of D-penicillamine. J Nutr. 1983;113:501-10.

12 Myint BA. D-penicillamine-induced cleft palate in mice. Teratology. 1984;30:333-40.

13 Niesink RJM, de Vries J, Hoolinger MA, editors. Toxicology, principles and applications. Boca Raton FL, USA: CRC Press; 1995.

14 Perrett D. The metabolism and pharmacology of D-penicillamine in man. J Rheumatol Suppl. 1981;7:41-50.

15 Pinter R, Hogge WA, McPherson E. Infant with severe penicillamine embryopathy born to a woman with Wilson disease. Am J Med Genet. 2004;128A:294-8.

16 Rosa FW. Teratogen update: penicillamine. Teratology. 1986;33:127-31.

17 Rousseaux CG, MacNabb LG. Oral administration of D-penicillamine causes neonatal mortality without morphological defects in CD-1 mice. J Appl Toxicol. 1992;12:35-8.

18 Steffek AJ, Verrusio AC, Watkins CA. Cleft palate in rodents after maternal treatment with various lathyrogenic agents. Teratology. 1972;5:33-40.

19 Sternlieb I. Wilson's disease and pregnancy. Hepatology. 2000;31:531-2.

20 US National Library of Medicine (NLM), editor. (D-)Penicillamine. CASNR: 52-67-5. In: Hazardous Substances Data Bank (HSDB). [cited 2013 March] . Available from:

21 US National Library of Medicine (NLM), editor. Penicillamine [USAN:USP;INN;BAN;JAN] RN: 52-67-5. In: ChemIDplus Lite. [cited 2013 July] . Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?CHEM

22 US National Library of Medicine (NLM), editor. Penicillamine. CASNR: 52-67-5. In: Drugs and lactation database (LactMed). [cited 2013 june]. Available from: http://toxnet.nlm.nih.gov/cgi-bin/sis/search/f?./temp/~MmgShM:1

23 Wiley MJ, Jonega MG. Neural tube lesions in the offspring of hamsters given single oral doses of lathyrogens early in gestation. Acta Anat. 1978;100:347-53.

24 Yamada T, Otomo S, Tanaka Y, Sasajima M, Ohzeki M. Reproduction studies of D-penicillamine in rats (1). Fertility study. Pharmacometrics. 1979;18:553-60.

25 Yurdakok M, Haziroglu R, Topaloglu H, Tinaztepe K. Pulmonary dysplasia with renal malformations: a common connective tissue disorder? Turk J Pediatr. 1993;35:111-4.

26 Yurdakok M, Topaloglu H, Haziroglu R. The effect of D-penicillamine in the developing rat cerebellum. Turk J Pediatr. 1989;31:137-43.

Literature consulted but not cited

- Beckman DA, Brent RL. The effects of maternal drugs on the developing fetus. In: Avery GB, Fletcher MA, MacDonald MG, editors. Neonatology: pathophysiology and management of the newborn. 5th ed. Philadelphia PA, USA: Lippincott;1999.p 209-36.
- Daston GP, Baines D, Elmore E, Fitzgerald MP, Sharma S. Evaluation of chick embryo neural retina cell culture as a screen for developmental toxicants. Fundam Appl Toxicol. 1995;26:203-10.
- Grasedyck K. D-penicillamin - Nebenwirkungen, Pathogenese und Risikominderung. Z Rheumatol. 1988;47 Suppl 1:17-9.
- Jelinek R, Peterka M, Rychter Z. Chick embryotoxicity screening test - 130 substances tested. Indian J Exp Biol. 1985;23:588-95.
- Johnson EM, Newman LM, Gabel BG, Boerner TF, Dansky LA. An analysis of the hydra assay's applicability and reliability as a developmental toxicity prescreen. Int J Toxicol. 1988;7:111-26.
- Keen CL, Lonnerdal B, Hurley LS. Drug-induced copper deficiency: a model for copper deficiency teratogenicity. Teratology. 1983;28:155-6.
- Khera KS, Whalen C. Detection of neuroteratogens with an in vitro cytotoxicity assay using primary monolayers cultured from dissociated fetal rat brains. Toxicol in Vitro. 1988;2:257-73.
- Kilburn KH, Hess RA. Neonatal deaths and pulmonary dysplasia due to D-penicillamine in the rat. Teratology. 1982;26:1-9.
- Schardein JL. Current status of drugs as teratogens in man. Prog Clin Biol Res. 1985;163C:181-90.
- Vogel HG. Collagen and mechanical strength in various organs of rats treated with D-penicillamine or amino-acetonitrile. Connect Tissue Res. 1975;3:237-44.

A	The Committee
B	The submission letter (in English)
C	Comments on the public draft
D	Regulation (EC) 1272/2008 of the European Community
E	Additional considerations to Regulation (EC) 1272/2008
F	Fertility and developmental toxicity studies

Annexes

Annex **A**

The Committee

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  Professor of Medical Genetics, Paediatrician (not practising), Clinical Geneticist, University Medical Centre, Utrecht
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- A.S.A.M. van der Burght, *Scientific Secretary*
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- J.T.J. Stouten, *Scientific Secretary*
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The first draft of the present document was prepared by Dr. H.M. Barentsen, from the Regulatory Affairs Department of WIL Research Europe B.V. (Den Bosch, the Netherlands), by contract with the Ministry of Social Affairs and Employment.

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the chairperson and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the inaugural meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

Annex **B**

The submission letter (in English)

Subject : Submission of the advisory report *Penicillamine*
Your reference : DGV/MBO/U-932542
Our reference : U-8136/HS/cn/543/U14
Enclosure(s) : 1
Date : May 22, 2014

Dear Minister,

I hereby submit the advisory report on the effects of penicillamine on fertility and on the development of the progeny; it also concerns effects on lactation and on the progeny via lactation.

This advisory report is part of an extensive series in which reproduction toxic substances are classified in accordance with European guidelines. This involves substances to which people may be exposed occupationally.

The advisory report was prepared by a permanent committee of the Health Council of the Netherlands, the Subcommittee on the Classification of Reproduction Toxic Substances. The advisory report was consequently reviewed by the Health Council's Standing Committee on Health and the Environment.

Today I sent copies of this advisory report to the State Secretary of Infrastructure and the Environment and to the Minister of Health, Welfare and Sport, for their information.

Yours sincerely,

(signed)
Prof. dr. W.A. van Gool,
President

Annex C

Comments on the public draft

A draft of the present report was released in 2014 for public review. The following organisation/person has commented on the draft document:

- T.J. Lentz. National Institute for Occupational Safety and Health, Cincinnati OH, USA.

The comment received, and the reply by the Committee can be found on the website of the Health Council.

Annex **D**

Regulation (EC) 1272/2008 of the European Community

3.7 Reproductive toxicity

3.7.1 Definitions and general considerations

3.7.1.1 Reproductive toxicity includes adverse effects on sexual function and fertility in adult males and females, as well as developmental toxicity in the offspring. The definitions presented below are adapted from those agreed as working definitions in IPCS/EHC Document No 225, Principles for Evaluating Health Risks to Reproduction Associated with Exposure to Chemicals. For classification purposes, the known induction of genetically based heritable effects in the offspring is addressed in Germ Cell Mutagenicity (section 3.5), since in the present classification system it is considered more appropriate to address such effects under the separate hazard class of germ cell mutagenicity.

In this classification system, reproductive toxicity is subdivided under two main headings:

(a) adverse effects on sexual function and fertility;

(b) adverse effects on development of the offspring.

Some reproductive toxic effects cannot be clearly assigned to either impairment of sexual function and fertility or to developmental toxicity. Nonetheless, substances with these effects, or mixtures containing them, shall be classified as reproductive toxicants.

3.7.1.2 For the purpose of classification the hazard class Reproductive Toxicity is differentiated into:

- adverse effects
  - on sexual function and fertility, or
  - on development;
- effects on or via lactation.

3.7.1.3 Adverse effects on sexual function and fertility

Any effect of substances that has the potential to interfere with sexual function and fertility. This includes, but is not limited to, alterations to the female and male reproductive system, adverse effects on onset of puberty, gamete production and transport, reproductive cycle normality, sexual behaviour, fertility, parturition, pregnancy outcomes, premature reproductive senescence, or modifications in other functions that are dependent on the integrity of the reproductive systems.

3.7.1.4 Adverse effects on development of the offspring

Developmental toxicity includes, in its widest sense, any effect which interferes with normal development of the conceptus, either before or after birth, and resulting from exposure of either parent prior to conception, or exposure of the developing offspring during prenatal development, or postnatally, to the time of sexual maturation. However, it is considered that classification under the heading of developmental toxicity is primarily intended to provide a hazard warning for pregnant women, and for men and women of reproductive capacity. Therefore, for pragmatic purposes of classification, developmental toxicity essentially means adverse effects induced during pregnancy, or as a result of parental exposure. These effects can be manifested at any point in the life span of the organism. The major manifestations of developmental toxicity include (1) death of the developing organism, (2) structural abnormality, (3) altered growth, and (4) functional deficiency.

3.7.1.5 Adverse effects on or via lactation are also included in reproductive toxicity, but for classification purposes, such effects are treated separately (see Table 3.7.1 (b)). This is because it is desirable to be able to classify substances specifically for an adverse effect on lactation so that a specific hazard warning about this effect can be provided for lactating mothers.

3.7.2 Classification criteria for substances

3.7.2.1 Hazard categories

3.7.2.1.1 For the purpose of classification for reproductive toxicity, substances are allocated to one of two categories. Within each category, effects on sexual function and fertility, and on development, are considered separately. In addition, effects on lactation are allocated to a separate hazard category.

Table 3.7.1(a) Hazard categories for reproductive toxicants.

Categories		Criteria
CATEGORY 1		Known or presumed human reproductive toxicant Substances are classified in Category 1 for reproductive toxicity when they are known to have produced an adverse effect on sexual function and fertility, or on development in humans or when there is evidence from animal studies, possibly supplemented with other information, to provide a strong presumption that the substance has the capacity to interfere with reproduction in humans. The classification of a substance is further distinguished on the basis of whether the evidence for classification is primarily from human data (Category 1A) or from animal data (Category 1B).
	Category 1A	Known human reproductive toxicant The classification of a substance in Category 1A is largely based on evidence from humans.
	Category 1B	Presumed human reproductive toxicant The classification of a substance in Category 1B is largely based on data from animal studies. Such data shall provide clear evidence of an adverse effect on sexual function and fertility or on development in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of other toxic effects. However, when there is mechanistic information that raises doubt about the relevance of the effect for humans, classification in Category 2 may be more appropriate.
CATEGORY 2		Suspected human reproductive toxicant Substances are classified in Category 2 for reproductive toxicity when there is some evidence from humans or experimental animals, possibly supplemented with other information, of an adverse effect on sexual function and fertility, or on development, and where the evidence is not sufficiently convincing to place the substance in Category 1. If deficiencies in the study make the quality of evidence less convincing, Category 2 could be the more appropriate classification. Such effects shall have been observed in the absence of other toxic effects, or if occurring together with other toxic effects the adverse effect on reproduction is considered not to be a secondary non-specific consequence of the other toxic effects.

Table 3.7.1(b) Hazard category for lactation effects.

EFFECTS ON OR VIA LACTATION
Effects on or via lactation are allocated to a separate single category. It is recognised that for many substances there is no information on the potential to cause adverse effects on the offspring via lactation. However, substances which are absorbed by women and have been shown to interfere with lactation, or which may be present (including metabolites) in breast milk in amounts sufficient to cause concern for the health of a breastfed child, shall be classified and labelled to indicate this property hazardous to breastfed babies. This classification can be assigned on the: (a) human evidence indicating a hazard to babies during the lactation period; and/or (b) results of one or two generation studies in animals which provide clear evidence of adverse effect in the offspring due to transfer in the milk or adverse effect on the quality of the milk; and/or (c) absorption, metabolism, distribution and excretion studies that indicate the likelihood that the substance is present in potentially toxic levels in breast milk.

3.7.2.2 Basis of classification

3.7.2.2.1 Classification is made on the basis of the appropriate criteria, outlined above, and an assessment of the total weight of evidence (see 1.1.1). Classification as a reproductive toxicant is intended to be used for substances which have an intrinsic, specific property to produce an adverse effect on reproduction and substances shall not be so classified if such an effect is produced solely as a non-specific secondary consequence of other toxic effects.

The classification of a substance is derived from the hazard categories in the following order of precedence: Category 1A, Category 1B, Category 2 and the additional Category for effects on or via lactation. If a substance meets the criteria for classification into both of the main categories (for example Category 1B for effects on sexual function and fertility and also Category 2 for development) then both hazard differentiations shall be communicated by the respective hazard statements. Classification in the additional category for effects on or via lactation will be considered irrespective of a classification into Category 1A, Category 1B or Category 2.

3.7.2.2.2 In the evaluation of toxic effects on the developing offspring, it is important to consider the possible influence of maternal toxicity (see section 3.7.2.4).

3.7.2.2.3 For human evidence to provide the primary basis for a Category 1A classification there must be reliable evidence of an adverse effect on reproduction in humans. Evidence used for classification shall ideally be from well conducted epidemiological studies which include the use of appropriate controls, balanced assessment, and due consideration of bias or confounding factors. Less rigorous data from studies in humans shall be supplemented with adequate data from studies in experimental animals and classification in Category 1B shall be considered.

3.7.2.3 Weight of evidence

3.7.2.3.1 Classification as a reproductive toxicant is made on the basis of an assessment of the total weight of evidence, see section 1.1.1. This means that all available information that bears on the determination of reproductive toxicity is considered together, such as epidemiological studies and case reports in humans and specific reproduction studies along with sub-chronic, chronic and special study results in animals that provide relevant information regarding toxicity to reproductive and related endocrine organs. Evaluation of substances chemically related to the substance under study may also be included, particularly when information on the substance is scarce. The weight given to the available evidence will be influenced by factors such as the quality of the studies, consistency of results, nature and severity of effects, the presence of maternal toxicity in experimental animal studies, level of statistical significance for inter-group differences, number of endpoints affected, relevance of route of administration to humans and freedom from bias. Both positive and negative results are assembled together into a weight of evidence determination. A single, positive study performed according to good scientific principles and with statistically or biologically significant positive results may justify classification (see also 3.7.2.2.3).

3.7.2.3.2 Toxicokinetic studies in animals and humans, site of action and mechanism or mode of action study results may provide relevant information which reduces or increases concerns about the hazard to human health. If it is conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals should not be classified.

3.7.2.3.3 If, in some reproductive toxicity studies in experimental animals the only effects recorded are considered to be of low or minimal toxicological significance, classification may not necessarily be the outcome. These effects include small changes in semen parameters or in the incidence of spontaneous defects in the foetus, small changes in the proportions of common foetal variants such as are observed in skeletal examinations, or in foetal weights, or small differences in postnatal developmental assessments.

3.7.2.3.4 Data from animal studies ideally shall provide clear evidence of specific reproductive toxicity in the absence of other systemic toxic effects. However, if developmental toxicity occurs together with other toxic effects in the dam, the potential influence of the generalised adverse effects shall be assessed to the extent possible. The preferred approach is to consider adverse effects in the embryo/foetus first, and then evaluate maternal toxicity, along with any other factors which are likely to have influenced these effects, as part of the weight of evidence. In general, developmental effects that are observed at maternally toxic doses shall not be automatically discounted. Discounting devel-

opmental effects that are observed at maternally toxic doses can only be done on a case-by-case basis when a causal relationship is established or refuted.

3.7.2.3.5 If appropriate information is available it is important to try to determine whether developmental toxicity is due to a specific maternally mediated mechanism or to a non-specific secondary mechanism, like maternal stress and the disruption of homeostasis. Generally, the presence of maternal toxicity shall not be used to negate findings of embryo/foetal effects, unless it can be clearly demonstrated that the effects are secondary non-specific effects. This is especially the case when the effects in the offspring are significant, e.g. irreversible effects such as structural malformations. In some situations it can be assumed that reproductive toxicity is due to a secondary consequence of maternal toxicity and discount the effects, if the substance is so toxic that dams fail to thrive and there is severe inanition, they are incapable of nursing pups; or they are prostrate or dying.

3.7.2.4 Maternal toxicity

3.7.2.4.1 Development of the offspring throughout gestation and during the early postnatal stages can be influenced by toxic effects in the mother either through non-specific mechanisms related to stress and the disruption of maternal homeostasis, or by specific maternally-mediated mechanisms. In the interpretation of the developmental outcome to decide classification for developmental effects it is important to consider the possible influence of maternal toxicity. This is a complex issue because of uncertainties surrounding the relationship between maternal toxicity and developmental outcome. Expert judgement and a weight of evidence approach, using all available studies, shall be used to determine the degree of influence that shall be attributed to maternal toxicity when interpreting the criteria for classification for developmental effects. The adverse effects in the embryo/foetus shall be first considered, and then maternal toxicity, along with any other factors which are likely to have influenced these effects, as weight of evidence, to help reach a conclusion about classification.

3.7.2.4.2 Based on pragmatic observation, maternal toxicity may, depending on severity, influence development via non-specific secondary mechanisms, producing effects such as depressed foetal weight, retarded ossification, and possibly resorptions and certain malformations in some strains of certain species. However, the limited number of studies which have investigated the relationship between developmental effects and general maternal toxicity have failed to demonstrate a consistent, reproducible relationship across species. Developmental effects which occur even in the presence of maternal toxicity are considered to be evidence of developmental toxicity, unless it can be unequivocally demonstrated on a case-by-case basis that the developmental effects are secondary to maternal toxicity. Moreover, classification shall be considered where there is a significant toxic effect in the offspring, e.g. irreversible effects such as structural malformations, embryo/foetal lethality, significant post-natal functional deficiencies.

3.7.2.4.3 Classification shall not automatically be discounted for substances that produce developmental toxicity only in association with maternal toxicity, even if a specific maternally-mediated mechanism has been demonstrated. In such a case, classification in Category 2 may be considered more appropriate than Category 1. However, when a substance is so toxic that maternal death or severe inanition results, or the dams are prostrate and incapable of nursing the pups, it is reasonable to assume that developmental toxicity is produced solely as a secondary consequence of maternal toxicity and discount the developmental effects. Classification is not necessarily the outcome in the case of minor developmental changes, when there is only a small reduction in foetal/pup body weight or retardation of ossification when seen in association with maternal toxicity.

3.7.2.4.4 Some of the end points used to assess maternal effects are provided below. Data on these end points, if available, need to be evaluated in light of their statistical or biological significance and dose response relationship.

Maternal mortality:

an increased incidence of mortality among the treated dams over the controls shall be considered evidence of maternal toxicity if the increase occurs in a dose-related manner and can be attributed to the systemic toxicity of the test material. Maternal mortality greater than 10 % is considered excessive and the data for that dose level shall not normally be considered for further evaluation.

Mating index

(no. animals with seminal plugs or sperm/no. mated × 100) (*)

Fertility index

(no. animals with implants/no. of matings × 100)

Gestation length

(if allowed to deliver)

Body weight and body weight change:

Consideration of the maternal body weight change and/or adjusted (corrected) maternal body weight shall be included in the evaluation of maternal toxicity whenever such data are available. The calcula-

* () It is recognised that the Mating index and the Fertility index can also be affected by the male.

tion of an adjusted (corrected) mean maternal body weight change, which is the difference between the initial and terminal body weight minus the gravid uterine weight (or alternatively, the sum of the weights of the foetuses), may indicate whether the effect is maternal or intrauterine. In rabbits, the body weight gain may not be useful indicators of maternal toxicity because of normal fluctuations in body weight during pregnancy.

Food and water consumption (if relevant):

The observation of a significant decrease in the average food or water consumption in treated dams compared to the control group is useful in evaluating maternal toxicity, particularly when the test material is administered in the diet or drinking water. Changes in food or water consumption need to be evaluated in conjunction with maternal body weights when determining if the effects noted are reflective of maternal toxicity or more simply, unpalatability of the test material in feed or water.

Clinical evaluations (including clinical signs, markers, haematology and clinical chemistry studies):

The observation of increased incidence of significant clinical signs of toxicity in treated dams relative to the control group is useful in evaluating maternal toxicity. If this is to be used as the basis for the assessment of maternal toxicity, the types, incidence, degree and duration of clinical signs shall be reported in the study. Clinical signs of maternal intoxication include: coma, prostration, hyperactivity, loss of righting reflex, ataxia, or laboured breathing.

Post-mortem data:

Increased incidence and/or severity of post-mortem findings may be indicative of maternal toxicity. This can include gross or microscopic pathological findings or organ weight data, including absolute organ weight, organ-to-body weight ratio, or organ-to-brain weight ratio. When supported by findings of adverse histopathological effects in the affected organ(s), the observation of a significant change in the average weight of suspected target organ(s) of treated dams, compared to those in the control group, may be considered evidence of maternal toxicity.

3.7.2.5 Animal and experimental data

3.7.2.5.1 A number of internationally accepted test methods are available; these include methods for developmental toxicity testing (e.g. OECD Test Guideline 414), and methods for one or two-generation toxicity testing (e.g. OECD Test Guidelines 415, 416).

3.7.2.5.2 Results obtained from Screening Tests (e.g. OECD Guidelines 421 — Reproduction/ Developmental Toxicity Screening Test, and 422 — Combined Repeated Dose Toxicity Study with

Reproduction/Development Toxicity Screening Test) can also be used to justify classification, although it is recognised that the quality of this evidence is less reliable than that obtained through full studies.

3.7.2.5.3 Adverse effects or changes, seen in short- or long-term repeated dose toxicity studies, which are judged likely to impair reproductive function and which occur in the absence of significant generalised toxicity, may be used as a basis for classification, e.g. histopathological changes in the gonads.

3.7.2.5.4 Evidence from in vitro assays, or non-mammalian tests, and from analogous substances using structure-activity relationship (SAR), can contribute to the procedure for classification. In all cases of this nature, expert judgement must be used to assess the adequacy of the data. Inadequate data shall not be used as a primary support for classification.

3.7.2.5.5 It is preferable that animal studies are conducted using appropriate routes of administration which relate to the potential route of human exposure. However, in practice, reproductive toxicity studies are commonly conducted using the oral route, and such studies will normally be suitable for evaluating the hazardous properties of the substance with respect to reproductive toxicity. However, if it can be conclusively demonstrated that the clearly identified mechanism or mode of action has no relevance for humans or when the toxicokinetic differences are so marked that it is certain that the hazardous property will not be expressed in humans then a substance which produces an adverse effect on reproduction in experimental animals shall not be classified.

3.7.2.5.6 Studies involving routes of administration such as intravenous or intraperitoneal injection, which result in exposure of the reproductive organs to unrealistically high levels of the test substance, or elicit local damage to the reproductive organs, including irritation, must be interpreted with extreme caution and on their own are not normally the basis for classification.

3.7.2.5.7 There is general agreement about the concept of a limit dose, above which the production of an adverse effect is considered to be outside the criteria which lead to classification, but not regarding the inclusion within the criteria of a specific dose as a limit dose. However, some guidelines for test methods, specify a limit dose, others qualify the limit dose with a statement that higher doses may be necessary if anticipated human exposure is sufficiently high that an adequate margin of exposure is not achieved. Also, due to species differences in toxicokinetics, establishing a specific limit dose may not be adequate for situations where humans are more sensitive than the animal model.

3.7.2.5.8 In principle, adverse effects on reproduction seen only at very high dose levels in animal studies (for example doses that induce prostration, severe inappetence, excessive mortality) would

not normally lead to classification, unless other information is available, e.g. toxicokinetics information indicating that humans may be more susceptible than animals, to suggest that classification is appropriate. Please also refer to the section on maternal toxicity (3.7.2.4) for further guidance in this area.

3.7.2.5.9 However, specification of the actual 'limit dose' will depend upon the test method that has been employed to provide the test results, e.g. in the OECD Test Guideline for repeated dose toxicity studies by the oral route, an upper dose of 1 000 mg/kg has been recommended as a limit dose, unless expected human response indicates the need for a higher dose level.

3.7.3 Classification criteria for mixtures

3.7.3.1 Classification of mixtures when data are available for all ingredients or only for some ingredients of the mixture

3.7.3.1.1 The mixture shall be classified as a reproductive toxicant when at least one ingredient has been classified as a Category 1A, Category 1B or Category 2 reproductive toxicant and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for Category 1A, Category 1B and Category 2 respectively.

3.7.3.1.2 The mixture shall be classified for effects on or via lactation when at least one ingredient has been classified for effects on or via lactation and is present at or above the appropriate generic concentration limit as shown in Table 3.7.2 for the additional category for effects on or via lactation.

Table 3.7.2 Generic concentration limits of ingredients of a mixture classified as reproduction toxicants or foreffects on or via lactation that trigger classification of the mixture.

Ingredient classified as:	Generic concentration limits triggering classification of a mixture as:			
	Category 1A reproductive toxicant	Category 1B reproductive toxicant	Category 2 reproductive toxicant	Additional category for effects on or via l actation
Category 1A reproductive toxicant	≥ 0,3 % [Note 1]			
Category 1B reproductive toxicant		≥ 0,3 % [Note 1]		
Category 2 reproductive toxicant			≥ 3,0 % [Note 1]	
Additional category for effects on or via lactation				≥ 0,3 % [Note 1]

Note The concentration limits in the table above apply to solids and liquids (w/w units) as well as gases (v/v units).
Note 1 If a Category 1 or Category 2 reproductive toxicant or a substance classified for effects on or via lactation is present in the mixture as an ingredient at a concentration above 0,1 %, a SDS shall be available for the mixture upon request.

3.7.3.2 Classification of mixtures when data are available for the complete mixture

3.7.3.2.1 Classification of mixtures will be based on the available test data for the individual ingredients of the mixture using concentration limits for the ingredients of the mixture. On a case-by-case basis, test data on mixtures may be used for classification when demonstrating effects that have not been established from the evaluation based on the individual components. In such cases, the test results for the mixture as a whole must be shown to be conclusive taking into account dose and other factors such as duration, observations, sensitivity and statistical analysis of reproduction test systems. Adequate documentation supporting the classification shall be retained and made available for review upon request.

3.7.3.3 Classification of mixtures when data are not available for the complete mixture: bridging principles

3.7.3.3.1 Subject to paragraph 3.7.3.2.1, where the mixture itself has not been tested to determine its reproductive toxicity, but there are sufficient data on the individual ingredients and similar tested mixtures to adequately characterise the hazards of the mixture, these data shall be used in accordance with the applicable bridging rules set out in section 1.1.3.

3.7.4 Hazard Communication

3.7.4.1 Label elements shall be used for substances or mixtures meeting the criteria for classification in this hazard class in accordance with Table 3.7.3

Table 3.7.3 Label elements for reproductive toxicity.

Classification	Category 1A or Category 1B	Category 2	Additional category for effects on or via lactation
GHS Pictograms			No pictogram
Signal Word	Danger	Warning	No signal word
Hazard Statement	H360: May damage fertility or the unborn child (state specific effect if known)(state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H361: Suspected of damaging fertility or the unborn child (state specific effect if known) (state route of exposure if it is conclusively proven that no other routes of exposure cause the hazard)	H362: May cause harm to breast-fed children.
Precautionary Statement Prevention	P201 P202 P281	P201 P202 P281	P201 P260 P263 P264 P270
Precautionary Statement Response	P308 + P313	P308 + P313	P308 + P313
Precautionary Statement Storage	P405	P405	
Precautionary Statement Disposal	P501	P501	

Annex **E**

Additional considerations to Regulation (EC) 1272/2008

The classification and labelling of substances is performed according to the guidelines of the European Union (Regulation (EC)1272/2008) presented in Annex D. The classification of compounds 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 guideline necessarily leaves room for interpretation, dependent on the specific data set under consideration. In the process of using the regulation, the Committee has agreed upon a number of additional considerations:

- 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 offspring, the compound will be classified in category 1A, irrespective of the general toxic effects (see Annex D, 3.7.2.2.1.)
- adverse effects in a reproductive study, occurring without reporting the parental or maternal toxicity, may lead to a classification other than category 1B, when the effects occur at dose levels which cause severe toxicity in *general* toxicity studies
- clear adverse reproductive effects will not be disregarded on the basis of reversibility per se

- the Committee does not only use guideline studies (studies performed according to OECD* standard protocols) for the classification of compounds, but non-guideline studies are taken into consideration as well.

* Organisation for Economic Cooperation and Development.

Annex **F**

Fertility and developmental toxicity studies

Table 1 Fertility studies with penicillamine in laboratory animals.

authors	species	experimental period/ design	dose/route	general toxicity	effects on reproductive organs/effects on reproduction
Yamada et al. (1979)	SLC:Wistar rats (n=20/ sex/group)	males: 60 d before mating and throughout mating; females: 14 d before mating, throughout mating till gd 7; sacrifice: gd 20	0, 62.5, 125, 250, 500 mg/kg bw/d; oral (gavage)	decreased bw and food consumption in males at 500 mg/kg bw/d	mating rate: 19/20 (95%), 19/20 (95%), 20/20 (100%), 19/20 (95%), 10/20 (50%)*, resp.; pregnancy rate: 17/19 (90%), 16/19 (84%), 19/20 (95%), 17/19 (90%), 3/10 (30%)*; mating period (d): 18.1±11.6, 18.7±10.9, 18.7±9.2, 18.7±9.8, 33.4±11.7* no effect on oestrus cycle; on number of live foetuses, mortality, sex ratio, foetal weight, external, visceral and skeletal anomalies

bw=body weight; d=day(s); gd=gestational day(s)
* $p<0.01$

Table 2 Developmental toxicity studies with penicillamine in laboratory animals: diet studies.

authors	species	experimental period/design	dose/route	general toxicity	developmental toxicity
Keen et al. (1983)	female Sprague-Dawley rats (n=6-14/group)	throughout gestation; sacrifice: gd 21	0, 1700, 8300, 16,600 mg/kg diet (ca. 22-27, 108-137, 166-274 mg/d; equivalent to 0, 100, 490, 880 mg/kg bw/d assuming a bw of 250 g)	healthy and normal appearance; food intake not affected; bw gain during gestation (g): 116.7±5.9, 112.7±4.6, 104.9±6.2, 95.0±8.8, resp; Cu levels: decreased in liver, muscle at 490, 880 mg/kg bw decreased in plasma, kidney, uterus at all doses; Zn levels: decreased in liver, plasma at 880 mg/kg bw; Fe levels: increased at 490, 880 mg/kg bw; no effect on Mn, Ca, Mg levels	no effect on litter size and placental weight; foetal weight (mean of means of litters; g): 5.17±0.08, 4.91±0.13, 4.72±0.14**, 4.67±0.10***, resp.; crown-rump length (cm): 3.93±0.34, 3.63±0.05, 3.64±0.05, 3.17±0.07***; number of resorbed foetuses (dead/total sites): 2/139 (1%), 9/115 (8%), 9/139 (7%), 6/62 (10%); number of malformed foetuses (total malformed/total live): 0/137, 3/106 (3%), 39/130 (30%), 36/56 (64%); number of sites affected (malformed+dead/total sites): 2/139 (1%), 12/115 (10%), 48/139 (35%), 42/62 (68%) type and frequency of malformations: flexed limbs: 0/137, 2/106 (2%), 25/130 (19%), 27/56 (48%); loose skin: 0/137, 0/106, 11/130 (8%), 16/56 (29%); abdominal herniation: 0/137, 0/106, 6/130 (5%), 12/56 (21%); spina bifida: 0/137, 0/106, 2/130 (2%), 11/56 (20%); cleft palate: 0/137, 0/106, 0/130, 10/56 (18%); small lungs: 0/33, 0/29, 10/40 (25%), 7/16 (44%); diaphragm lesions: 0/33, 2/29 (7%), 5/40 (13%), 6/16 (38%); oedema: 0/137, 0/106, 0/130, 19/56 (34%); no tail/anus: 0/137, 0/106, 0/130, 1/56 (2%); club foot: 0/137, 0/106, 0/130, 1/56 (2%) subdermal haemorrhaging: at 490 and 880 mg/kg bw; abnormal ossification of sternum, wavy and irregular ribs, reduced ossification of middle phalanges of fore and hind limbs and tail vertebrae: at 490, 880 mg/kg bw Cu levels: decreased in whole foetus, foetal liver, placenta: at 100, 490, 880 mg/kg bw; Zn levels: increased in whole foetus: at 490, 880 mg/kg bw; decreased in foetal liver: at 880 mg/kg bw; Mn levels: increased in whole foetus:

					at 490, 880 mg/kg bw; Mg levels: decreased in foetal liver: at 880 mg/kg bw; Fe or Ca levels: no effects
Mark-Savage et al. (1983)	female Sprague-Dawley rats (n=5/group)	throughout gestation; sacrifice: gd 21	5 (control), 50 or 100 µg/g Cu with or without 8300 mg pa/kg diet (equivalent to 880 mg/kg bw/d)	no effect on food intake, bw gain; Cu levels: similar between Cu-only or Cu+pa groups; Cu+pa groups lower than Cu-only, especially at 5Cu+pa; no effect on Zn or Mg levels; Fe levels increased in 50 or 100 Cu+pa groups compared to Cu-only groups	no effect on litter size, foetal or placental weight; at 5Cu, 5Cu+pa, 50Cu, 50Cu+pa, 100Cu, 100Cu+pa: crown-rump length (cm): 4.12±0.02, 3.84±0.05*, 3.99±0.03, 4.03±0.04, 4.01±0.04, 4.06±0.03, resp.; number of resorbed foetuses (dead/total sites): 0/48, 14/62 (23%), 1/42 (2%), 3/51 (6%), 1/37 (3%), 2/55 (4%); number of malformed foetuses (total malformed/total live): 0/48, 10/48 (21%), 0/41, 1/48 (2%), 0/36, 2/53 (4%); number of sites affected (malformed+dead/total sites): 0/48, 24/62 (39%), 1/42 (2%), 4/51 (8%), 1/37 (3%), 4/55 (7%) type and frequency of malformations: loose skin: 0/48, 4/48 (8.3%),0/41, 0/48, 0/36, 0/53; diaphragm lesions: 0/15, 3/20 (15%), 0/17, 0/17, 0/12, 1/19 (5%); small lungs: 0/15, 7/20 (35%), 0/17, 1/17 (6%), 0/12, 1/19 (5.2%); misplaced gonads: 0/15, 1/20 (5%), 0/17, 0/17, 0/12, 0/19 Cu levels: similar between Cu-only groups; in Cu+pa groups lower than Cu-only group, especially at 5Cu+pa; Fe levels: increased in 50Cu+pa and 100Cu+pa groups compared to Cu-only group Zn or Mg levels: no effects
Irino et al. (1982)	female DDD mice (n=18-28/group) (3 mo old)	for 2 mo including gestation and lactation; mated with untreated males; interim sacrifice of pups at pnd 1, 7, 14, 21	0, 4000 mg/kg diet (equivalent to 480 mg/kg bw/d, assuming bw of 25 g and food intake of 3 g/d) (9 treated mice received $5x10^{-3}$ M $CuSO_4$ after delivery)	not reported (in accompanying study in which male and female mice were fed diets containing 0.4% penicillamine from 3-15 wk after birth, toxic signs such as dorsal hernia, priapism and paralysis of the digits were	at 0, 480 mg/kg bw, Cu supplementation: pup weight (g) at pnd 1: 1.71±0.03, 1.68±0.03, -, resp.; at pnd 7: 5.49±0.27, 4.17±0.03**, 4.64±0.21; at pnd 14: 6.41±0.57, 5.30±0.30, 6.77±0.94†; at pnd 21: 10.68±0.51, 5.94±0.70**, 11.73±0.29††; brain weight (g) at pnd 1: 0.092±0.002, 0.082±0.002**, - ; at pnd 7: 0.314±0.013, 0.279±0.003*, 0.277±0.007*; at pnd 14: 0.360±0.007, 0.392±0.009**, 0.384±0.005*; at pnd 21:

				observed present in almost all mice after 6 wk of treatment)	0.361±0.005, 0.378±0.012, 0.405±0.006**† Cu levels: decreased in serum, brain, kidney and skin of pups from pnd 7 onwards; in spinal cord, liver and milk statistically significantly from pnd 1 onwards; in aorta decreased on pnd 7 and 14; Cu levels in brain and spinal cord increased with time in control group, but constant in pa group; clinical signs: at pnd 10 hind limb paralysis, motor incoordination, tremors; from pnd14 to 21 toxic signs and mortality most prominent; aortic aneurysms observed at various time points Cu supplementation increased Cu levels close to control levels or to a higher level than that in the pa group and completely prevented neurological disorders; no effect on Zn and Fe levels in brain, in liver lower and higher than controls at pnd 1 and 14, resp.; brain cytochrome c oxidase: statistically significantly lower in pa group compared to control and Cu-supplemented group; histology: number of granules in hepatocytes and ganglion cells reduced
Irino et al. (1982)	female DDD mice (n=4-6/ group) (2-3 mo old)	during gestation and/or lactation; observations till pnd 35	0, 2000, 4000 mg/kg diet (equivalent to 0, 240, 480 mg/kg bw/d; assuming bw of 25 g and food intake of 3 g/d); groups (gestation + lactation): c(ontrol)+c(ontrol); c+480pa; 480pa+c; 240pa+240pa; 480pa+480pa; 480pa+480pa+Cu ($5x10^{-3}$M $CuSO_4$	no abnormal clinical signs or lesions seen	no effect on litter size; pup survival (%; estimated from a figure): at pnd 7: 100, 100, 98, 100, 94, 100; at pnd 14: 100, 93, 80, 100, 64, 100; at pnd 21: 100, 76, 80, 100, 36, 100; pnd 28: 100, 70, 80, 100, 16, 100; at pnd 35: 100, 57, 80, 100, 3, 100; no sex difference incidences of clinical signs and gross lesions; at birth: no external malformations; at pnd 1-7: none, none, none, none, [gangrene of digits: 3/39, aortic aneurysm rupture: 1/39, death; 1/39], none; at pnd 8-14: none, none, none, none, [motor incoordination: 10/38, hind limb paralysis: 9/38, tremor: 8/38, hyperexcitability: 8/38, death: 9/39], none; at pnd 15-21: none, none, none, none, [hind limb paralysis: 14/29, inactivity: 14/29, motor

	incoordination: 13/29, tremor: 10/29, ptosis: 8/29, hyperexcitability: 8/29, aortic aneurysm rupture: 5/29, death: 15/29], none; at pnd 22-28: none, [digit paralysis: 11/26, scrotal subcutaneous haemorrhage: 3/26], none, none, [inactivity: 9/14, digit paralysis: 5/14, hind limb paralysis: 5/14, motor incoordination: 4/14, tremor: 3/14, ptosis: 3/14, scrotal subcutaneous haemorrhage: 3/14, aortic aneurysm rupture: 1/14, death: 9/14], none; at pnd 29-35: none, [digit paralysis: 17/24, scrotal subcutaneous haemorrhage: 4/24; priapism: 4/10], [digit paralysis: 13/23], [digit paralysis: 16/23, scrotal subcutaneous haemorrhage: 7/23], [inactivity: 4/5, aortic aneurysm rupture: 2/5, digit paralysis: 1/5, hind limb paralysis: 1/5, ptosis: 1/5, death: 4/5], none histological examinations: 480pa+480pa group: dissecting aortas and breaks in elastic lamellae; neuronal degeneration in cerebral cortex, thalamic nuclei, spinal ganglion at pnd 14, 21 (not at pnd 1, 7); c+480pa: few abnormal neurons in cerebral cortex at pnd 21; no neuronal degenerations in other groups. no findings in skin, lung, liver, kidney

bw=body weight; d=day(s); g=gramme(s); gd=gestational day(s); mo=month(s); ns=not statistically significant; pa=penicillamine; pnd=postnatal day(s); wk=week(s)
*p<0.05; **P<0.01; ***p<0.001 for controls vs. penicillamine groups; † p<0.05; †† p<0.01 for copper supplemented vs. penicillamine groups

Table 3 Developmental toxicity studies with penicillamine in laboratory animals: gavage studies

authors	species	experimental period/design	dose/route	general toxicity	developmental toxicity
Yamada et al. (1979)	SLC:Wistar rats (n=20/sex/group)	males: 60 d before mating and throughout mating; females: 14 d before mating, throughout mating till gd 7; sacrifice: gd 20	0, 62.5, 125, 250, 500 mg/kg bw/d	decreased bw and food consumption in males at 500 mg/kg bw/d	no effect number of live foetuses, mortality, sex ratio, foetal weight, external, visceral and skeletal anomalies
Steffek et al. (1972)	female Sprague-Dawley rats (n=3-10/group)	gd 10-15 or 10-17	gd 10-15: 360, 375, 400 mg/d (ca. 1440, 1500, 1600 mg/kg bw/d, assuming a bw of 250 g); gd 10-17: 200, 400 mg/d (ca.800, 1600 mg/kg bw/d	at 1600 mg/kg bw: mortality in 1/10 (gd 10-15) and 2/4 (gd 10-17)	gd 10-17: 1600 mg/kg bw: number of resorptions: 16/18 (89%); incidence of cleft palate 2/2 (100%); 800 mg/kg bw: no resorptions, cleft palate: 0/27 gd 10-15: 1600 mg/kg bw: resorptions: 7/82 (8%); cleft palate 60/75 (80%); 1500 mg/kg bw: resorptions: 1/44 (2%); cleft palate: 17/43 (40%); 1440 mg/kg bw: no resorptions; cleft palate: 16/37 (43%)
Rousseau/ MacNabb (1992)	female CD-1 mice (n=7-10/group)	gd 1-12; sacrifice: gd 18	0, 250, 500, 1000 or 0, 2000 mg/kg bw/d	decreased bw gain at gd 13-18 in all treated groups (by 3-4%; p=0.004); no effect on food consumption and relative liver weight	2000 mg/kg bw: increased number of non-viable foetuses; decreased foetal weights and crown-rump lengths (all p<0.001) no effect on site and number of implantations, live foetuses, dead foetuses, early resorptions, embryonic loss, foetal loss and sex ratio; visceral and skeletal abnormalities comparable to control
Myint (1984)	female DDI mice (n=20-35/group)	gd 10-14 or gd 11-14; sacrifice: gd 17 (emphasis on cleft palate)	3200, 4000 mg/kg bw/d; vehicle (distilled water) control gd 10-14, untreated control groups included	not reported	gd 10-14: abortion (%): 12, 17; foetal death and resorption (%): 5, 15; litters with cleft palate (%): 77, 79; average foetal weight (g): 0.68, 0.67, at 3200 and 4000 mg/kg bw, resp. gd 11-14: abortion (%): 0, 13; foetal death and resorption (%): 3, 8; litters with cleft palate (%): 9, 52; average foetal weight (g): 0.71, 0.67, at 3200 and 4000 mg/kg bw, resp. controls: foetal death and resorption (%): 1, 3; average foetal weight (g): 0.78, 0.77, in vehicle and untreated controls, resp.; no abortions; no cleft palates

Wiley/Joneja (1978)	female Syrian golden hamsters (n=not reported)	gd 7; sacrifice: gd 14	3200, 4000, 4800 mg/kg bw; vehicle (distilled water), untreated control groups included	not reported	number of litters: 10, 6, 8, 9, 9 at 0 (untreated), 0 (vehicle only), 3200, 4000, 4800 mg/kg bw, vehicle controls, untreated controls, resp.; number of implants: 122, 79, 94, 107, 108, resp.; number of live foetuses: 110, 78, 84, 77, 82, resp.; mortality (including dead or resorbed foetuses, expressed as % implantations): 10, 1, 11, 28, 15, resp.; mean foetal weight (g): 2.09±0.29, 1.95±0.29, 1.99±0.40, 1.85±0.33 ($p<0.05$), 1.71±0.34 ($p<0.05$), resp.; % of liver foetuses weighing <60% of mean weight of untreated controls: 0, 3, 6, 7, 9, resp.; % of live foetuses with exencephaly: 0, 0, 5, 0, 1, resp.; with encephalocele: 0, 0, 1, 8, 13, resp.; with fore limb abnormalities: 0, 0, 2, 1, 1, resp.; with hind limb abnormalities: 0, 0, 2, 9, 4, resp.; fused ribs (expressed as % of skeletal stained foetuses),: 0, 0, 3, 0, 0, resp.; no visceral abnormalities observed

bw=body weight; d=day(s); gd=gestational day(s)

Table 4 Developmental toxicity studies with penicillamine in laboratory animals: drinking water studies.

authors	species	experimental period/design	dose/route	general toxicity	developmental toxicity
Yurdakök et al. (1989)	female albino rats (n=2-4/group)	last 6 d of gestation; sacrifice: gd 20-21 n=2/dose; sacrifice of remaining 2 at 0 and 400 mg at postnatal wk 3; pup cerebellum histologically examined and scored	0, 300, 400 mg (0, 1000, 1333 mg/kg bw/d; assuming a bw of 300 g)	not reported	pups had short, sparse, easily pulled out, coarsely textured hair at birth: bw (mg): 9537±390, 8378±483**, 4897±579**; cerebellar wt (as cerebellar wt/bw x 100; %): 0.36±0.04, 0.35±0.05*, 0.27±0.03**; score for cerebellar degenerative changes: 0, 0.4±0.1*, 2.5±0.3** [score based on thickness of granular layer, number and disarrangement of Purkinje cells and the heterotopic granular cells in the molecular layer; given 'from no change to severe pathological changes' as 0, +, ++, +++; maximum total score: 9] at postnatal wk 3: decreased bw and relative cerebellar wt (both $p<0.05$); no cerebellar degenerative changes

Yurdakök et al. (1993)	female albino rats (n=5/group)	last 6 d of gestation; Caesarean section: lungs, kidneys of offspring histologically examined	0, 300 mg (0, 1000 mg/kg bw/d)	lower serum copper	bw (mg): 9537±390, 8378±483**; left kidney wt (mg): 85±9.6, 60.5±8.2**; left kidney wt/bw (%): 0.89±0.08, 0.72±0.09**; lung wt (mg): 258.3±64.4, 233.3±55.6; lung wt/bw (%): 2.69±0.57, 2.78±0.63; histological examination: lung: cystic alveoli (in 17/21 (81%) of offspring), interstitial emphysema, atelectasis, perivascular loose of connective tissue and vascular aneurysm; kidney: caliectasis (in 12/21 (57%) of offspring)

bw=body weight; d=day(s); gd=gestational day(s); wk=week(s); wt=weight
*p<0.05; **p<0.001

Health Council of the Netherlands

Advisory Reports

The Health Council's task is to advise ministers and parliament on issues in the field of public health. Most of the advisory reports that the Council produces every year are prepared at the request of one of the ministers.

In addition, the Health Council issues unsolicited advice that has an 'alerting' function. In some cases, such an alerting report leads to a minister requesting further advice on the subject.

Areas of activity

Optimum healthcare
What is the optimum result of cure and care in view of the risks and opportunities?

Prevention
Which forms of prevention can help realise significant health benefits?

Healthy nutrition
Which foods promote good health and which carry certain health risks?

Environmental health
Which environmental influences could have a positive or negative effect on health?

Healthy working conditions
How can employees be protected against working conditions that could harm their health?

Innovation and the knowledge infrastructure
Before we can harvest knowledge in the field of healthcare, we first need to ensure that the right seeds are sown.

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