Cadmium and its compounds

Evaluation of the effects on reproduction, recommendation for classification

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

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Mijnheer de Staatssecretaris,

Bij brief van 3 december 1993, nr. DGV/BMO-U-932542, verzocht de Staatssecretaris van Welzijn, Volksgezondheid en Cultuur namens de Minister van Sociale Zaken en Werkgelegenheid om naast het afleiden van gezondheidskundige advieswaarden ook te adviseren over andere onderwerpen ten behoeve van de bescherming van beroepsmatig aan stoffen blootgestelde personen. In 1995 heeft de Staatssecretaris van Sociale Zaken en Werkgelegenheid besloten tot het opstellen van een zogenaamde niet-limitatieve reprotox-lijst. Op deze lijst komen stoffen die volgens de richtlijnen van de Europese Unie ingedeeld moeten worden in categorie 1 of 2 wat betreft effecten op de voortplanting. De Gezondheidsraad is verzocht om voor stoffen een classificatie volgens de EU-criteria voor te stellen.

In 1996 heb ik hiervoor de Commissie Reproductietoxische stoffen ingesteld.

Hierbij bied ik u - gehoord de Beraadsgroep Gezondheid en Omgeving - de publikatie van de commissie aan over cadmium en cadmiumverbindingen. Deze publicatie heb ik heden ter kennisname aan de Minister van Volksgezondheid Welzijn en Sport en aan de Minister van Volkshuisvesting, Ruimtelijke Ordening en Milieubeheer gestuurd.

Hoogachtend,
w.g.
prof. dr JJ Sixma

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Evaluation of the effects on reproduction, recommendation for classification

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

to

the Minister and State Secretary of Social Affairs and Employment

No. 2000/04OSH, The Hague, 3 May 2000

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Samenvatting

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

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit adviseert de commissie cadmium en cadmiumverbindingen in categorie 3 (*stoffen die in verband met hun mogelijke voor de vruchtbaarheid van de mens schadelijke effecten reden geven tot bezorgdheid*) te classificeren en met R62 (*mogelijk gevaar voor verminderde vruchtbaarheid*) te kenmerken.
- Voor effecten op de ontwikkeling adviseert de commissie cadmium en cadmiumverbindingen in categorie 3 (*stoffen die in verband met hun mogelijke voor de ontwikkeling schadelijke effecten reden geven tot bezorgdheid voor de mens*) te classificeren en met R63 (*mogelijk gevaar voor beschadiging van het ongeboren kind*) te kenmerken.
- Voor effecten tijdens lactatie adviseert de commissie om cadmium en cadmiumverbindingen met R64 (*kan schadelijk zijn via de borstvoeding*) te kenmerken.

Executive summary

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

The committee's recommendations are:

- For effects on fertility, the committee recommends to classify cadmium and its compounds in category 3 (*substances which cause concern for human fertility*) and to label cadmium and its compounds with R62 (*possible risk for impaired fertility*).
- For developmental toxicity, the committee recommends to classify cadmium and its compounds in category 3 (*substances which cause concern for humans owing to possible developmental toxic effects*) and to label cadmium and its compounds with R63 (*possible risk of harm to the unborn child).*
- For effects during lactation, the committee recommends that cadmium and its compounds should be labelled with R64 (*may cause harm to breastfed babies*)

Annex **1**

Scope

1.1 Background

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

1.2 Committee and procedure

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

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

Classification for fertility and development:	
Category 1	Substances known to impair fertility in humans (R60)
	Substances known to cause developmental toxicity in humans (R61)
Category 2	Substances which should be regarded as if they impair fertility in humans (R60)
	Substances which should be regarded as if they cause developmental toxicity in humans (R61)
Category 3	Substances which cause concern for human fertility (R62)
	Substances which cause concern for humans owing to possible developmental toxic effects (R63)
No classification for effects on fertility or development	
Labelling for lactation:	
	May cause harm to breastfed babies (R64)
	No labelling for lactation

In December 1999, the President of the Health Council released a draft of the report for public review. The individuals and organisations that commented on the draft report are listed in Annex B. The committee has taken these comments into account in deciding on the final version of the report.

1.3 Additional considerations

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

- If there is sufficient evidence to establish a causal relationship between human exposure to the substance and impaired fertility or subsequent developmental toxic effects in the progeny, the compound will be classified in category 1, irrespective the general toxic effects (see Annex C, 4.2.3.1 category 1).
- Adverse effects in a reproductive or developmental study, in the absence of data on parental toxicity, occurring at dose levels which cause severe toxicity in other studies, need not necessarily lead to a category 2 classification.

- If, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).
- Clear adverse reproductive effects will not be disregarded on the basis of reversibility per se.
- Effects on sex organs in a general toxicity study (e.g. in a subchronic or chronic toxicity study) may warrant classification for fertility.
- The committee not only uses guideline studies (studies performed according to OECD standard protocols*) for the classification of compounds, but non-guideline studies are taken into consideration as well.

1.4 Labelling for lactation

The recommendation for labelling substances for effects during lactation is also based on Directive 93/21/EEC. The Directive defines that substances which are absorbed by women and may 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, should be labelled with R64. Unlike the classification of substances for fertility and developmental effects, which is based on a hazard identification only (largely independent of dosage), the labelling for effects during lactation is based on a risk characterisation and therefore also includes consideration of the level of exposure of the breastfed child.

Consequently, a substance should be labelled for effects during lactation when it is likely that the substance would be present in breast milk in potentially toxic levels. The committee considers a compound as potentially toxic to the breastfed child when exposure to this compound via the milk results in an intake exceeding an exposure limit for the general population, eg the acceptable daily intake (ADI).

1.5 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up 1995. Literature was selected primarily on the basis of the text of the abstracts. Publications cited in the selected articles, but not selected during the primary search, were reviewed if considered appropriate. In addition, handbooks and a collection

* Organisation for Economic Cooperation and Development

of most recent reviews were consulted. References are divided in literature cited and literature consulted but not cited. Before finalising the public draft the committee performed an additional literature search in Medline and Toxline for the period 1995 to 1999. The results of this search were no reason for the committee to adjust the recommendations.

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

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

1.6 Presentation of conclusions

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

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

1.7 Final remark

The classification of compounds is based on hazard evaluation* only, which is one of a series of elements guiding the risk evaluation process. The committee emphasises 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 characterisation, human exposure assessment and recommendations of other organisations.

* for definitions see Tox95

Chapter 2

Cadmium and its compounds

2.1 Introduction

Name	:	cadmium
Use	:	protective plating on steel, electrode material in batteries, pigment, plastic stabilizer, by-product of zinc production
Atom weight	:	112.40
Chem formula	:	Cd / Cd^{2+}
CAS number	:	7440-43-9

Cadmiumchloride, cadmiumoxide and cadmiumsulphate are considered to be (non-genotoxic) carcinogens.

2.2 Human studies

Man may be exposed to cadmium (Cd) either occupationally, via smoking of tobacco or via the diet and drinking water. Exposure via food is the main route for the non-smoking population.

Fertility

Mason (1990) studied the effects of occupational exposure to cadmium (concentration unknown) on the pituitary-testicular endocrine axis of workers (n=77), as measured by

serum testosterone (T), luteinizing hormone (LH) and follicular-stimulating hormone (FSH) (Mas90). Cd exposure estimates were constructed for each subject from atmospheric measurements; these exposure estimates were validated by *in vivo* neutron activation analyses of liver Cd burdens. The lack of testicular endocrine effects was in contrast to significant dose-related changes in renal glomerular and tubular function demonstrated in the same population.

Gennart *et al*. (1992) studied the male reproductive function in Belgian melters (n=83) who were exposed to Cd (Gen92). The mean urinary Cd level was 6.9 µg per gram creatinine. Fertility in these workers was not affected when compared to an unexposed population.

Xu *et al*. (1993) determined the concentrations of Cd and other trace elements (Pb, Se and Zn) in blood and seminal plasma of men (n=221) undergoing initial screening for infertility (Xu93). The authors detected a relation between increased Cd concentrations in blood and decreased sperm density in oligozoospermic men but not in normospermic men. High Cd concentration in seminal plasma was associated with low semen volume.

Keck *et al*. (1995) did not find a correlation between Cd levels in seminal plasma and semen parameters (semen volume, count, motility and morphology) and fertility in 12 men with proven fertility and 44 normozoospermic patients as well as 118 unselected patients of an infertility clinic in Germany (Kec95).

Development

Huel *et al*. (1984) investigated the effects of maternal occupational Cd exposure on child development (Hue84). Despite statistically significant evidence of increased exposure to Cd, no adverse health effects were documented in the exposed new-borns included in this study. Six years later, each of these 26 children was given a psychometric test. Statistical analysis showed a significant inverse relationship between the degree of in utero exposure to Cd and Pb and the child's motor and perceptual abilities (Bon86).

Kuhnert *et al*. (1987) studied the effects of smoking during pregnancy on decreased infant weight (Kuh87). In smokers (n=77) maternal whole blood Cd, placental Cd and placental Zn levels were inversely related to birth weight.

Loiacono *et al*. (1992) studied the effects of Cd on birth weight of children of mothers who lived in the vicinity of a Pb smelter in Titova Mitrovica, Yugoslavia (Loi92). Control subjects were studied in Pristina, a non-exposed town located 25 miles to the south. Birth weights of children of a group of non-smoking women from Titova Mitrovica were compared to those of non-smoking women from Pristina. No association between placental Cd concentrations and birth weight was observed.

Marlowe *et al*. (1983) suggested a relation between pre- and postnatal Cd and Pb exposure and mental retardation in children (Mar83).

Lactation

Casey (1977) studied the levels of some trace elements in human breast milk in New Zealand and detected concentrations lower than 1 µg Cd/l (Cas77).

Larsson *et al*. (1981) reported levels of 0.1 µg Cd/l (median) in breast milk of Swedish women (Lar81). They did not find significant differences in Cd levels between 3- and 6-month samples. The authors calculated a median weekly intake of Cd by 3-months old infants of 0.1 µg/kg body weight.

Sharda *et al*. (1983) found no significant changes in Cd levels from colostrum (20 µg Cd/l) to mature milk (17 µg Cd/l) in Indian women (Sha83).

Vuori *et al*. (1983) studied Cd concentrations in human breast milk of Finnish women in the first, third and sixth months and detected levels of 2.0, 1.5 and 1.6 µg Cd/l (median) (Vuo83).

Kovar *et al*. (1984) collected 5 days postpartum breast milk from 28 British mothers from an urban population and detected mean concentrations Cd of 0.4 µg/l (Kov84).

Sternowsky and Wessolowski (1985) found Cd concentrations in colostrum and mature milk of non-smoking women living in an urban area in Germany of 23.2 and 15.1 µg Cd/l, respectively and in milk of women living in a rural area in Germany of 26.6 and 13.5 µg Cd/l, respectively (Ste85).

Radish *et al*. (1987) studied the concentrations of Cd in human breast milk in smokers and non-smokers in various districts of West-Berlin (Germany) and detected median levels of 0.16 and 0.07 µg Cd/l (Rad87).

Schramel *et al*. (1988) detected levels of 0.88 µg Cd/l in human breast milk in Germany (Sch88).

Zahradnicek *et al*. (1989) found levels of 0.31 µg Cd/l in Czechoslovakia in 1985-1986.

Ende and Hille (1992) reported Cd levels of lower than 0.4 (in 1987), 2 (in 1988), 2 (in 1989) and 0.4 µg Cd/l (in 1990) in human breast milk in Germany (End92).

Plöckinger *et al*. (1993) reported Cd levels in human milk below the detection limit in Austrian women (Plö93).

Hallén *et al*. (1995) determined Cd levels in breast milk obtained 6 weeks after delivery from women living in the vicinity of a copper and lead metal smelter and in a control area (Hal95). Milk Cd levels in women in the control area, 0.07 µg Cd/l, were somewhat higher than in women from the smelter area, 0.05 µg Cd/l.

From the ADI for Cd of 400-500 µg/person/day an acceptable level of about 5 µg/l breast milk can be calculated (see Annex E).

2.3 Animal studies

Tables 1 and 2 (annex D) summarise fertility and developmental studies with cadmium in experimental animals.

Fertility studies, oral exposure

In a multigeneration reproduction study with Charles River CD mice, Cd was offered continuously in the drinking water at a level of 10 mg/l. In this study the following effects were observed: arterial hypertension, a high incidence of preweaning mortality, small litters, a low mating index and an extended precoital time (Sch71). The strain could not be bred beyond the F2b-generation.

Chronic exposure of groups of random bred white male rats to 0.00005, 0.0005 or 0.005 mg/kg body weight in the drinking water (dose levels corresponding to 0.001, 0.01 and 0.1 mg Cd^{2+}/l) resulted in the high dose group in several alterations, among them loss of body weight and a reduced spermatogenesis, and an increase of number of tubules with cast-off epithelium and with 12^{th} stage meiosis (Kra76).

Dixon *et al*. (1976) studied the effects of (I) a single oral dose of 0, 6.25, 12.5 and 25 mg Cd/kg body weight or (II) of drinking water containing 0.001, 0.01 and 0.1 mg Cd/l for 90 days in rats (Dix76). After acute dosing serial matings were performed for 70 days and testes were examined microscopically on day 1 and 7 and for 6 succeeding 7-day intervals. No effect on fertility was observed after acute (I) or chronic (II) oral Cd administration .

Kotsonis and Klaassen (1978) studied the toxicity of Cd in male Sprague Dawley rats after oral administration of $CdCl_2$ in the drinking water (0, 10, 30 or 100 mg/l) for 24 weeks (Kot78). Fertility was tested after 3, 6, 12 and 24 weeks and appeared not affected. At necropsy after 24 weeks no effect on testis weight and histopathology was observed. At 30 and 100 mg/l, effects on the kidney were detected.

Groups of 9-10 male and female Sprague-Dawley rats were, starting from conception, chronically exposed to 0, 0.1, 1.0 or 5.0 mg/l $CdCl_2$ in the drinking water. They were sacrificed either on postnatal day (PN) 50 or around PN 130. Sacrifice on PN 50 revealed a decreased liver weight in the 5.0 mg/l group, and no effect on sperm count. Adult sacrifice showed a decreased liver weight in the 1.0 and 5.0 mg/l groups, and a decreased serum progesterone level and litter size, and an increased preimplantation loss in the 5.0 mg/l group (Las80).

Groups of 14 male or female Sprague-Dawley rats were administered 0, 0.1, 1.0 or 10 mg/kg body weight/day Cd (as $CdCl_2$) by gavage for 6 weeks prior to and during a 3 weeks mating period (with untreated females) (Sut80a and b). Body weights of males

treated with 1.0 and 10 mg/kg body weight/day were depressed. No effects were found on copulation ability, pregnancy or litter size. The treated males were also mated with the treated females whose body weight was only depressed in the 10 mg group. Adverse effects were observed in the 10 mg group on numbers of copulations, pregnancies, implantation sites and on foetal body weight and length. The females of the mid and high dose groups showed mild to overt signs of toxicity as concluded from body and organ weights.

Groups of male Sprague-Dawley rats were exposed to 0, 17.2, 34.4 or 68.8 mg/l Cd in the drinking water for 70 days (Zen82). Following mating with untreated females, no differences were observed in mating success, number of pregnancies, litter size and pup body weight. Histological examination of the testis revealed no differences with the controls.

Bomhard *et al*. (1987) studied the chronic effects of single and multiple oral and subcutaneous Cd administrations on the testis of Wistar rats (Bom87). Animals having received orally (gavage) 1 x 100 or 1 x 200 mg/kg body weight or subcutaneously 1 x 2 or 1 x 2.5 mg/kg body weight showed severe lesions of the whole testicular parenchyma with massive calcification of the necrotic tubules and pronounced fibrosis of the interstitium. All animals receiving 2.5 mg/kg body weight subcutaneously had a Leydig cell tumour in at least one testis. Rats treated with 2.5 mg/kg body weight did not differ in appearance, general behaviour or food and water consumption of the control animals; some transient inflammatory reactions were observed at the injection site.

Andersen *et al*. (1988) studied the effects of a single oral dose of $CdCl_2$ (0, 270, 530 and 790 µmol g/kg body weight 0, 30, 60 and 90 mg/kg) on mortality and tissue damage in CBA/Bom male mice (And88). In the 30 mg/kg bw group effects were observed on liver, stomach and duodenum. Effects on the testis were firstly observed in the 60 mg/kg group.

Male and female Sprague Dawley derived rats received $CdCl_2$ by gavage at doses of 25, 51, 107 and 225 mg/kg body weight/day for 1 or 10 consecutive days or in drinking water solutions at concentrations of 13-323 mg $CdCl_2$/l for 10 consecutive days (Bor89). After a single dose of $CdCl_2$, an apparent treatment-related but not statistically significant decrease in body weight was observed in the males. Furthermore, absolute and relative spleen weights were significantly lower and lung weights were significantly higher. No effects were observed in the females after a single dose. Dose-dependent mortality was observed in the 10 day gavage study. Body weight gain was reduced in a dose-dependent manner in both males and females. Absolute and/or relative brain, liver, spleen, lung, thymus, kidney and testis weights of treated males were reduced in a dose dependent manner. In females, absolute and/or relative liver, spleen, thymus and kidney weights were reduced in a dose dependent manner. Focal necrotic changes were observed in renal tubular epithelium and tubular degeneration was reported in males and females.

Testicular and hepatic changes were observed in males. In the drinking water study dose-dependent changes in body weight gain and absolute and/or organ weights were observed. However, the testis weights of the Cd dosed males were comparable to those in the controls.

Male Wistar rats, age 5 weeks, were given 0 or 10 mg Cd/l in drinking water for 52 weeks. Animals were sacrificed at the end of week 28, 40 and 56 and examined for histopathological examination of testis, kidney and liver (Say91). All Cd-treated male rats showed pathological testicular alterations, and liver and kidney damage after chronic exposure. Cd levels were found to be highest in the kidney. At the end of the 52-week period, reproductive capacity of the Cd-treated rats was investigated by mating the treated males with 2 untreated females. All control males and only 60% of the Cd-treated males were fertile.

Fertility studies, subcutaneous exposure

A single subcutaneous injection of $CdCl_2$ (10 mg/kg body weight) induced profound cellular and vascular changes in the ovaries of prepuberal rats (Kar59). At 96 hours after treatment some recovery of the ovary changes was observed; by 168 hours the process of recovery was complete.

A single subcutaneous injection of $CdCl_2$ in adult rats (40 µmol Cd/kg body weight, ~ 4.5 mg/kg bw) resulted in decreased weights of testis, seminal vesicles and prostate and severe histopathological changes of reproductive organs (Par60).

Male CBA-mice were exposed to Cd by subcutaneous injection of 2.2 µmol $CdCl_2$/kg body weight (~ 0.2 mg/kg bw) for 5 days/week for 6 months (Nor75). A decrease in normal (testosterone-dependent) proteinuria was shown and morphological examination of the seminal vesicles revealed a smaller weight and size as well as histological indications of lower secretory activity of the epithelium compared to controls.

Lohiya *et al*. (1976) injected male adult Langurs subcutaneously with a single dose of 0, 4 or 12 mg $CdCl_2$/kg body weight (Loh76). Animals dosed with 4 mg/kg were sacrificed after 30 days and animals dosed with 12 mg/kg were sacrificed after 60 days. No effects of $CdCl_2$ treatment on body weights were observed. Testes of the 12 mg/kg group were small and oedematous; the weight of the reproductive organs was reduced. In the 4 mg/kg group, no macroscopical effects on organ weights were observed. Histological examinations of the testis and epididymis of $CdCl_2$ treated animals showed severe lesions in the testis and epididymis which were more severe in the high dose group.

Groups of sexually immature and mature female Syrian hamsters (Cr:RGH), mice (BALB/cAnNCr, DBA/2NCr, C57BL/6NCr, NFS/NCr) and rats (F344/NCr, WF/NCr) received a single subcutaneous injection with 20 to 47.5 µmol $CdCl_2$/kg body weight (2.2 to 5.3 mg/kg body weight) in the dorsal thoracic midline (Reh88). They were killed

1 to 56 days after administration. Upon sacrifice ovarian necrosis was observed. The lowest adverse effect levels depended on species (hamster was the most susceptible), strain (BALB mice were most, DBA mice were least susceptible; no difference between the rat strains), and age (immature animals were more susceptible).

Wlodarczyk *et al*. (1995) studied the effect of Cd on the male reproductive system of Golden hamsters (Wlo95). Hamsters were subcutaneously injected with a single dose of 0 or 0.5 mg Cd/kg body weight. Five males of each dose group were sacrificed 1, 4 and 10 weeks after treatment. The testis, epididymis and accessory sex glands were weighed and histologically examined. Epididymal sperm was enumerated and sperm morphology was evaluated. In the Cd-treated animals decrease in organ weight and pathological changes of the reproductive organs were detected at all time intervals but intensified to the end of the experiment (10 weeks). Furthermore, sperm number in the epididymis was decreased.

Developmental toxicity, oral exposure

A multigeneration reproduction study with CD mice in which Cd was offered continuously in the drinking water at a level of 10 mg/l, resulted in maternal arterial hypertension and a high incidence of runts and bent tails (Sch71).

Groups of 14 female and male Sprague-Dawley rats were administered 0, 0.1, 1.0 or 10 mg Cd/kg food/day for 6 weeks by gavage, after which they were mated; exposure continued during mating and gestation up to GD 20, when the females were sacrificed (Sut80a and b). Adverse effects were observed in the 10 mg/kg group on foetal body weight and length; the foetuses were anaemic and malnutritioned, but the placenta weight was increased. Numbers of implantation sites and live foetuses decreased, and number of resorptions increased significantly in the 10 mg group, and slightly in the 1.0 mg group. No malformations were observed. The females of the mid and high dose groups showed mild to overt signs of toxicity. The treated males also mated with untreated females which showed no effects upon sacrifice.

Untreated female Sprague-Dawley rats were mated to males, previously exposed to 0, 17.2, 34.4 or 68.8 mg Cd/l in drinking water (Zen82). Part of the females was sacrificed on GD 20. No adverse effects were observed. The other females were allowed to litter; the resulting offspring was followed for general and behaviourial changes, which were not detected.

Female CF1 mice were bred for 6 consecutive generations (Whe88). Levels of 0.25, 5.0 and 50 mg/kg food Cd ($CdCl_2$) were added to the diet. The highest dose had no effect on fertility or pup survival during lactation, but caused a 15% decrease in litter size at birth and a 25% decrease in pup growth.

Andersson *et al*. (1997) gave Cd in the drinking water (5 mg/l) to female Sprague Dawley rats during the lactation period, from parturition to postnatal day 17, and/or to the offspring until postnatal day 42 (And97). Mean body weights of the dams and offspring, food and water intake were comparable to controls. Relative weights of liver, kidney and brain were comparable as well. Plasma urea nitrogen levels in rats exposed to Cd postweaning were significantly higher than in the control group, whereas exposure during lactation and postweaning did not induce differences. No obvious neuropathology was observed after Cd exposure. Exposure to Cd during lactation as low as 5 mg/l in drinking water lead to neurochemical disturbances of the serotonergic system in offspring.

Three consecutive generations of Wistar rats were orally (gavage) treated with 0, 3.5, 7.0 and 14.0 mg Cd/kg body weight as $CdCl_2$ over the period of gestation, lactation and 8 weeks after weaning (Nag97). Behavioural tests were performed in male rats from each generation at the age of 12 weeks. Main results of the behavioural tests were changed in vertical exploration activity (rearing) and increased exploration of an open field centre. The spontaneous and evoked electrophysiological variables showed dose-and generation dependent changes. In most tests the 3.5 mg/kg group was not affected. Treatment with the mid- and high- dose resulted in significantly lower body weights. Pup body weights were comparable to those in the control groups and there were no visible malformations. In the F3-generation reduced relative kidney and spleen weights were observed in the high-dose group.

Antonio *et al*. (1998) reported altered neurotransmitter levels in brains of new-born Wistar rats after exposure to cadmiumacetate (10 mg/l) via the drinking water from the start of pregnancy to parturition or until postnatal day 5 (Ant98).

Corpas and Antonio (1998) exposed Wistar rats to Cd (10 mg/l) via drinking water during gestation or during early lactation (from delivery until PN 5) (Cor98). Pups exposed in utero were sacrificed on PN 0. Pups exposed during early lactation were sacrificed on postnatal day 5. Pup weight, testicular and ovary weight were recorded at necropsy. Furthermore, seminiferous tubule diameter and number of prospermatogonia in the testis of the pups were measured. On postnatal day 5 ovary and testis weights were reduced in the Cd-treated pups; in addition seminiferous tubule diameter and number of prospermatogonia were reduced in Cd-treated pups on PN 0 and 5. On PN 0 and 5 a reduced DNA/RNA ratio was observed in ovary and testis of the Cd-treated pups.

Female Wistar rats were given orally (gavage) 0, 3.5, 7.0 or 14 mg Cd/kg body weight as $CdCl_2$ in 3 different treatment regimens: (I) GD 5-15, (II) GD 5-15 and 4 weeks postnatally or (III) GD 5-15, 4 weeks postnatally followed by the same treatment of male rats of the F1-generation for 8 weeks (Dés98). Behavioural tests were performed in F1-males at the age of 12 weeks. The number of pups/litter was slightly lower and the pup weights were slightly (not significantly) decreased in the high dose group. Body

weight gain of the male offspring was comparable to the control group. At necropsy kidney weights of all treatment regimes of the high dose group were decreased. Behavioural changes were observed in the 7 and 14 mg/kg group in the groups exposed during gestation and lactation. Some changes in the behavioural tests were only seen when F1-pups were also treated with Cd during the postweaning period.

Developmental toxicity, other exposure routes

Parízek studied the effects in rats of Cd on the placenta after injection of 0.02 mmol Cd salt/kg body weight (~ 2.2 mg/kg) between gestation day (GD) 17 and 21 (Par64). Subcutaneous injection of Cd was followed in all cases by rapidly progressive placental changes, chiefly in the pars foetalis, which was completely transformed within 24 hours into an extensive blood clot with little remaining necrotic tissue. In most cases the placental changes were accompanied by haemorrhages into the uterine cavity and were found within 6 hour after Cd injection. Complete destruction of the pars foetalis resulted in interruption of pregnancy, with either delivery of the dead conceptus or resorption.

Groups of 4 to 8 mated female Wistar rats [Crl:(WI)BR] received a subcutaneous injection with 0, 40 or 50 µmol $CdCl_2$/kg (~ 4.5 or 5.6 mg/kg) on GD 12 or 18 (Sal89). They were sacrificed on GD 19 or 20. A reduced blood flow in the chorionallantoic placenta was measured after 12 days of exposure, both in the 40 and in the 50 µmol groups. No foetal changes were inflicted in the 40 µmol Cd/kg group on GD12. On GD 18, 40 µmol/kg resulted in resorptions; the 50 µmol/kg group showed on GD 12 foetal lethality, and on GD 18 both foetal lethality and a decreased foetal body weight. Malformations were not observed, apart from 1 case of cleft palate in the 50 µmol/kg on GD 12 group.

Pelletier and Satinder (1991) studied the effects of daily subcutaneous injection of 0.075 or 0.225 mg $CdCl_2$/kg body weight during gestation in 3 genetic lines of rats: Roman HighAvoidance (RHA), Roman Low Avoidance (RLA) and Satinder's Heterogeneous stock (SHS) (Pel91). Cd-exposed progeny from the RHA line weighed significantly less than control RHA progeny (PN 41-44); however, SHS progeny of the low-dose group weighed significantly more than progeny from any other group (PN 14-44). Unconditioned response level (UER) was determined on PN 39 and acquisition of conditioned avoidance responses was tested from PN 41-44. Significant effects on behaviour were observed in the high dose group. Maternal body weights, food consumption, length of gestation, litter size at birth and weaning, foetal mortality and physical development were not affected by Cd treatment.

Soukupova and Dostal (1991a) administered a single dose, subcutaneously, of 0, 2, 4 or 6 mg $CdCl_2$/kg body weight on GD 8, 9, 10, 11, 12, 13 or 14 to random bred ICR mice (Sou91a). The embryolethal effect was highest after treatment with 6.0 mg/kg on GD 12 and 13 (50.0 and 61.3%). Among survivors foetuses with haemorrhagic bullae,

limb malformations, exencephaly, cleft palate, open eyelids and tail deformities occurred. Mainly right-sided limbs were malformed. Administration of 2 and 4 mg/kg body weight induced fore limb polydactylies, whereas with 6 mg also oligodactylies were induced. Foetal weight was only reduced in the 6 mg group dosed on GD 12. Reduction of the foetal thymus weight was observed in the 6 mg groups dosed from GD 9-14.

Soukupova *et al*. (1991b) exposed mated random bred ICR mice, subcutaneously, to 2.5 and 5 mg $CdCl_2$/kg body weight on GD 16 and detected that the immune response of their offspring was altered (Sou91b).

Ferm *et al*. (1968) studied the effects of intravenous injection of 2 or 4 mg $3CdSO_4.8H_2O$/kg body weight to pregnant golden hamsters on GD 7 (Fer68). Animals were killed on GD 11 or 14. The number of embryonic resorptions and malformed foetuses was increased in the Cd-treated groups. Maternal animals injected with 10 mg $3CdSO_4.8H_2O$/kg body weight or more, all died shortly after injection. Cd levels from 5 - 10 mg induced foetal resorption rates of almost 100% without noticeable effect on the mothers.

Groups of 15 pregnant Wistar-Porton rats received a single intravenous injection of 1.1 or 1.25 mg Cd/kg body weight between GD 9 and 15 (Sam79). They were sacrificed on GD 20. No effects were observed after administration of the low dose; the effects of the high dose depended on, amongst others, the moment of injection: the later exposed, the stronger the increase of resorptions and the larger the decrease in number of abnormal foetuses. The observed malformations included hydrocephalus, an- and microphthalmia, gastroschisis and umbilical hernia. A reduction in maternal blood flow was observed.

Hartsfield *et al*. (1992) injected Syrian golden hamsters intravenously with 0, 2 and 3 mg $CdCl_2$ on GD 7; females were sacrificed on GD 14 and foetuses were weighed and examined for viability and external malformations (Har92). Resorptions were noted. Maternal body weight of the Cd treated groups was reduced. The number of non-viable foetuses and foetuses with malformations were increased in the 2 and 3 mg Cd group and foetal weight and length were decreased in these groups.

De *et al*. (1993) studied the effects of Cd exposure during the preimplantation period of pregnancy on the subsequent development and implantation of mouse embryos. Injection with 38 µmol Cd/kg body weight (~4.3 mg) on GD 1 had little effect on the initiation and maintenance of pregnancy when examined on GD 7 (De93). At examination of a similar treated group on GD 4, sites were absent in 62 % of the females. Thus, Cd treatment on GD 1 delayed temporarily, but did not prevent implantation and was not embryolethal. The same dose administered on GD 3 caused pregnancy failure in all mice examined on GD 7; no implantation sites were detected on GD 4.

Lactation

Lucis and Shaikh (1972) injected ^{109}Cd subcutaneously to rats during pregnancy or during the lactation period (Luc72). In both cases ^{109}Cd was present in a low concentrations in the milk recovered from the stomach of the pups.

Houpert *et al*. (1997) studied the amount of Cd eliminated through milk in ewes (Hou97). After Cd was orally or intravenously administered, the levels in milk increased rapidly and could be detected 6 hours after administration. Levels reached a maximum value on the 2nd or 3rd day after administration. These values then decreased, first quickly and later slowly.

2.4 Overall conclusion

Only a few publications concerning the effects of cadmium and its compounds on human fertility were found. Mason (Mas90), Gennart *et al*. (Gen92) and Keck *et al*. (Kec95) did not report an effect on fertility related to Cd exposure. Xu *et al*. (Xu93) detected a relation between increased Cd concentrations in blood and decreased sperm density in oligozoospermic men but not in normospermic men. Furthermore, in the publication of Mason (Mas90) it was reported that renal effects were found in the absence of testicular endocrine effects.

In animal studies, effects of Cd treatment on male and female reproductive organs were observed after oral or subcutaneous administration (gavage, drinking water or diet) in rats, mice, hamsters and monkeys (Kar59, Par60, Sch71, Nor75, Kra76, Loh76, Las80, Sut80a, 80b, Bom87, And88, Reh88, Bor89, Say91, Wlo95). Laskey *et al*. (Las80), Sutou *et al*. (Sut80a, 80b), Andersen (And88), Borzelleca *et al*. (Bor89) and Saygi *et al*. (Say91) detected effects on the reproductive organs or fertility at dose levels which caused general toxicity (effects on kidney, liver or body weight). In other studies general toxicity was not always described but may be present in view of the levels used (Kar59, Par60, Sch71, Nor75, Kar76, Loh76, Bom87, Reh88, Wlo95). Dixon *et al*. (Dix76), Kotsonis *et al*. (Kot78) and Zenick *et al*. (Zen82) did not find any effects on fertility.

In conclusion, the committee recommends to classify cadmium and its compounds for fertility in category 3 (substances which cause concern for human fertility) and to label the compounds with R 62 (possible risk of impaired fertility).

In human studies involving Cd, effects were observed on birth weight (Kuh87, Loi92), motor and perceptual abilities (Bon86) and mental retardation of offspring (Mar83). However, it is not clear from these studies whether the effects described were due to exposure to Cd or to other substances (i.e Pb exposure).

In animal studies, effects of Cd on development (resorptions, foetal death, malformations, foetal/pup weight, behavioural performance, neurochemical disturbances of the serotonergic system and altered immune response) were observed after subcutaneous or oral administration (gavage, drinking water or diet) in rats, mice, hamsters (Par64, Fer68, Sch71, Sam79, Sut80a and b, Whe88, Sal89, Pel91, Sou91a and b, Har92, De93, And97, Nag97, Ant98, Dés98). Furthermore, *in utero* Cd exposure affected the development of the reproductive organs in the offspring (Cor98). In the animal studies maternal toxicity was often not described in detail; however, general toxicity may be assumed due to the levels used.

In conclusion, based on the effects found in animal studies, which predominantly occurred at levels of maternal toxicity, the committee recommends to classify cadmium and its compounds in category 3 (substances which cause concern for humans owing to possible developmental toxic effects) and label to compounds with R 63 (possible risk of harm to the unborn child).

In most publications cited (Cas77, Lar81, Vuo83, Kov84, Rad87, Sch88, Zah89 End92 Plö93, Hal95), Cd levels in human breast milk were below the calculated safe level of about 5 µg/l. However, in 2 publications from India and Germany Cd levels were detected in human breast milk in concentrations of about 20 µg/l (Sha83, Ste85).

Cd was detected in milk of animals (Luc72, Hou97).

In conclusion, the committee recommends to label Cd with R64 (May cause harm for breastfed babies).

Proposed classification for fertility

Category 3, R 62.

Proposed classification for developmental toxicity

Category 3, R 63.

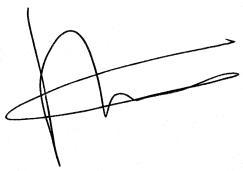
Proposed labelling for effects during lactation for Cd

R64.

For the committee,
The Hague, 3 May 2000

dr ASAM van der Burght,
scientific secretary

dr BJ Blaauboer,
chairman



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A The committee

B Comments on the public draft

C Directive (93/21/EEG) of the European Community

D Fertility and developmental toxicity studies

E Calculation safe levels of Cd in (human) breast milk

F Abbreviations

Annexes

Annex **A**

The committee

- BJ Blaauboer, *chairman*
  Toxicologist; Research Institute of Toxicology, Utrecht
- JN van den Anker
  Professor of pediatrics and Neonatology; Erasmus University, Rotterdam
- AM Bongers, *advisor*
  Ministry of Social Affairs and Employment, The Hague
- HFP Joosten
  Toxicologist; NV Organon, Department of Toxicology and Drug Disposition, Oss
- D Lindhout
  Professor of Clinical Genetics/Teratology; Erasmus University, Rotterdam
- JHJ Copius Peereboom-Stegeman
  Toxicologist; Catholic University Nijmegen, Nijmegen
- AH Piersma
  Reproductive toxicologist; National Institute of Public Health and the Environment, Bilthoven
- A Stijkel
  Toxicologist; Environmental Awareness Foundation, ‘s-Graveland
- PJJM Weterings
  Toxicologist; Weterings Consultancy BV, Rosmalen
- ASAM van der Burght, *scientific secretary*
  Health Council of the Netherlands, The Hague

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

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

Annex **B**

Comments on the public draft

- A draft of this report was released in 1999 for public review. No persons or organisations have commented on the draft document.

Annex **C**

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

4.2.3 Substances toxic to reproduction

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

Category 1:

Substances known to impair fertility in humans

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

Substances known to cause developmental toxicity in humans

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

Category 2:

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

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

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

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

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

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

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

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

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

Generally on the basis of:

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

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

For substances that should be regarded as if they impair fertility in humans:

T; R60: May impair fertility

For substances that should be regarded as if they cause developmental toxicity in humans:

T; R61: May cause harm to the unborn child.

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

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

Xn; R63: Possible risk of harm to the unborn child.

4.2.3.3 *Comments regarding the categorisation of substances toxic to reproduction*

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

1 *Effects on male or female fertility*, includes adverse effects on libido, sexual behaviour, any aspect of spermatogenesis or oogenesis, or on hormonal activity or physiological response which would in-

terfere with the capacity to fertilise, fertilisation itself or the development of the fertilised ovum up to and including implantation.

2 *Developmental toxicity*, is taken in its widest sense to include any effect interfering with normal development, both before and after birth. It includes effects induced or manifested prenatally as well as those manifested postnatally. This includes embrytoxic/fetotoxic effects such as reduced body weight, growth and developmental retardation, organ toxicity, death, abortion, structural defects (teratogenic effects), functional defects, peri-postnatal defects, and impaired postnatal mental or physical development up to and including normal pubertal development.

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

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

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

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

Effects on fertility

For the classification of a substance into Category 2 for impaired fertility, there should normally be clear evidence in one animal species, with supporting evidence on mechanism of action or site of action, or chemical relationship to other known antifertility agents or other information from humans which would

lead to the conclusion that effects would be likely to be seen in humans. Where there are studies in only one species without other relevant supporting evidence then classification in Category 3 may be appropriate.

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

Developmental toxicity

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

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

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

Effects during Lactation

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

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

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

R64 would normally be assigned on the basis of:

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

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

c on the basis of evidence in humans indicating a risk to babies during the lactational period. Substances which are known to accumulate in the body and which subsequently may be released into milk during lactation may be labelled with R33 and R64.

Annex **D**

Fertility and developmental toxicity studies

Table 1.1 Fertility studies in animals with Cd.

authors	species	route	experimental period	dose	findings	remarks
Kar *et al*. (1959)	Colony bred albino rat (n=?)	s.c. injection	administration: a single dose sacrifice: 0,6,24,48,96,168,360 h after injection	0 or 10 mg $CdCl_2$/ kg bw	Cellular and vascular changes in the ovary; partly recovered after 96 h, completely recovered after 168 h	
Parízek (1960)	Rat (n=?)	s.c. injection	administration: a single dose sacrifice: up to 4 months after injection	0 or 40 µmol $CdCl_2$/kg bw (4.5 mg/kg bw)	Decreased weights of testis, seminal vesicles and prostate. Severe histopathological changes of reproductive organs	Experimental procedures poorly described.
Schroeder and Mitchener (1971)	Charles River DC mouse (males & females) (:n=5)	drinking water	multigeneration study administration: continuously	0 or 10 mg Cd/l	arterial hypertension, low mating index, small litters, high pup mortality, strain could not be bred beyond F2b-generation	
Nordberg (1975)	Male CBA-mice (n=8)	s.c. injection	administration: 5 d/w for 6 m.	0 or 2.2 µmol $CdCl_2$/kg bw (0.2 mg/kg bw)	Decreased proteinuria and a decreased weight and size of the seminal vesicles. Indications of a lower secretory capacity of seminal vesicles.	
Dixon *et al*. (1976)	Sprague-Dawley rats (n=10)	gavage (1) or drinking water (2)	1) acute, serial breeding 2) subchronic administration: 1) single dose to males 2) 30, 60, 90d. sacrifice: 1) up to 6 w after injection 2) 30, 60, 90 d	1) 0, 6.25, 12.5 or 25 mg $CdCl_2$ 2) 0, 0.001, 0.01 or 0.1 mg $CdCl_2$/l	1) no effects on male fertility were observed 2) no effects on body weight and weights of testis, prostate and seminal vesicles. No effects on clinical parameters and serum hormone levels. No effect on testis histopathology.	
Krasovskii *et al*. (1976)	random bred white rat (males) (n=?)	drinking water	administration: continuously	0, 0.00005, 0.0005 or 0.005 mg Cd/kg bw	0.005 mg: decreased body weight, reduced spermatogenesis	

bw = body weight; w=week; d = day; s.c. = subcutaneous; PN = postnatal

Table 1.2 Fertility studies in animals with Cd.

authors	species	route	experimental period	dose	findings	remarks
Lohiya *et al*. (1976)	Langurs (male adult) (n=3)	s.c. injection	administration: single dose sacrifice: 1) 30 d after injection; 2) 60 d after injection	1) 0 or 4 mg $CdCl_2$/ kg bw 2) 0 or 12 mg $CdCl_2$/ kg bw	2) testis small and oedematous; weight reproductive organs reduced. 1) + 2) dose depended histological lesions in testis and epididymis.	
Kotsonis and Klaassen (1978)	Sprague-Dawley rats (males) (n=6)	drinking water	administration: continuously for 24 w sacrifice: 3,6,12,24 w	0, 10, 30 or 100 mg/l $CdCl_2$	At 30 and 100 mg/l tubular necrosis in kidney. No effect on fertility; no effects on histopathology and weight of testis	
Laskey *et al*. (1980)	Sprague-Dawley rat (males & females) (n=9-10)	drinking water	administration: continuously sacrifice: PN 50, 130	0, 0.1, 1.0 or 5.0 mg $CdCl_2$/l	1.0 mg: PN 130 depressed liver weight 5.0 mg: PN 50+130 depressed liver weight; preimplantation loss, reduced litter size	
Sutou *et al*. (1980a,b)	Sprague-Dawley rat (males & females) (n=13-14)	gavage	administration: 6 w prior to mating, mating, gestation	0, 0,1, 1.0 or 10 mg $CdCl_2$ /kg bw	untreated females: - treated females, mid & high dose: reduced body and organ weight, high dose: reduced copulation, no. of implants, no. live foetuses, foetal body weight, length	NB treated males were mated with treated and untreated females
Zenick *et al*. (1982)	Sprague-Dawley rat (males) (n=5)	drinking water	administration: 70 d	0, 17.2, 34.4 or 68.8 mg Cd/l (0, 1 2 and 4 mg/kg bw)	no effect on reproductive system no effect on reproductive parameters	

bw = body weight; w=week; d = day; s.c. = subcutaneous; PN = postnatal

Table 1.3 Fertility studies in animals with Cd.

authors	species	route	experimental period	dose	findings	remarks
Bomhard *et al*. (1987)	Wistar rats male n=30/ Cd group n=10/control group	1) gavage	1) single dose	1) 0 or 50 mg $CdCl_2$/ kg bw	1) effects comparable with controls	
		2) s.c. injection	2) single dose	2) 0 or 2.5 mg $CdCl_2$/ kg bw	2) transient inflammatory reaction at injection site, severe lesions testes and malignant Leydig cell tumours in all animals	
		3) gavage	3) 10 weekly doses	3) 0 or 5 mg $CdCl_2$/kg bw	3) effects comparable with control	
		4) s.c. injection	4) 10 weekly doses Studies 1-4: necropsy after 12, 18 and 30 months	4) 0 or 0.25 mg $CdCl_2$ /kg bw	4) effects comparable with controls	
	Supplementary studies 5 and 6 25 males/group		*Supplementary studies:*	*Supplementary studies:*		
		5) gavage	5) single dose	5) 0 or 200 mg $CdCl_2$ /kg bw	5) most animals (28 of 35) died within 3 days after administration; other animals severe lesions testis	
		6) s.c. injection	6) single dose	6) 0 or 2 mg $CdCl_2$ /kg bw	6) transient inflammatory reaction at injection site and transient decrease body weight; severe lesions testis	
	study 7 35 males/group	7) gavage	7) single dose Studies 5-7: necropsy after 6 months	7) 0 or 100 mg $CdCl_2$ /kg bw	7) most animals died (24 of 25); 1 animal severe lesions testis	

bw = body weight, s.c. = subcutaneous

Table 1.4 Fertility studies in animals with Cd.

authors	species	route	experimental period	dose	findings	remarks
Andersen *et al.* (1988)	CBA/Bom male mice (n=7-15)	oral (stomach tube)	administration: single dose sacrifice: 10 d after dosing	0, 270, 530, 790 µmol/kg bw (0,30,60,90 mg/kg bw)	Histological effects on liver, stomach and duodenum were firstly observed in the 30 mg/kg group Histological effects on testis were firstly observed in the 60 mg/kg group	
Rehm and Waalkes (1988)	hamster (1 strain) (n=6-48) mouse (3 strains) (n=5-12) rat (2 strains) (n=4-35) (females)	s.c. injection	administration: a single dose at 21-24 d, or 8 w old sacrifice: 1,2,3,4,7,14,28,56 d after administration	0 or 20-47.5 µmol/kg bw (2.2 -5.3 mg/ kg bw)	40 µmol/kg group: species, age and strain dependent severe toxicity to lethality due to liver necrosis all hamsters, most groups mice and high dose groups rats: haemorrhagic necrosis of the ovaries	
Borzella *et al.* (1989)	Sprague-Dawley rats (male and female) (n=10)	1) gavage or 2) drinking water	administration: 1) 1 or 10 consecutive days, 2) 10 d necropsy: 24 h after last dosing	1) 0, 25, 51, 107 or 225 mg $CdCl_2$/ kg bw, 2) 0 or 13-323 mg $CdCl_2$/l	1d gavage: not statistically significant effects on body weights, statistically significant effects on spleen and lung weights of males 10 d gavage: dose-dependent effects on mortality, body weights, organ weights (inclusive testis) of male and females. Histopathological changes in kidney, testis and liver Drinking water: dose-dependent effects on body weights and organ weights (not testis)	

bw = body weight; d = day; s.c. = subcutaneous; w = week

Table 1.5 Fertility studies in animals with Cd.

authors	apecies	route	experimental period	dose	findings	remarks
Saygi *et al.* (1991)	Wistar rats (control n=8; experimental group n=20)	drinking water	administration: continuously for 52 w to males only necropsy: 28, 40, 56 w	0 or 10 mg $CdCl_2$/l	Histopathological effects on testis, liver and kidney. Reduced fertility after 52 weeks Cd exposure (100% control *v.s.* 60% Cd-treated males)	
Wlodarczyk *et al.* 1995	Golden hamsters (males) (n=15)	s.c injection	administration: single dose necropsy: 1,4,10 w after treatment	0 or 0.5 mg $CdCl_2$/kg bw	Decreased weights and histopathological changes of testis, epididymis and accessory sex organs. Decreased number of sperm cells in epididymis after 4 weeks of treatment.	

bw = body weight; s.c. = subcutaneous; w = week

Table 2.1 Developmental toxicity studies in animals with Cd.

authors	species	route	experimental period	dose	findings	remarks
Pa ízek (1964)	Wistar rat (control n=12; Cd group n=70)	s.c. injection	Administration: single dose between GD 17-21	0 or 0.02 mmol $CdCl_2$/kg bw (2.2 mg/kg)	rapid progressive placental changes mainly of pars foetalis resulting in a extensive blood clot within 24 h and interruption of pregnancy.	
Ferm *et al*. (1968)	Golden hamsters (n=14-20)	i.v. injection	Administration: single dose of on GD 7. Sacrifice: GD 11 or 14	0, 2 or 4 mg $3CdSO_4.8H_2O$/ kg bw	Increased number of embryonic resorptions and malformed foetuses.	Maternal animals receiving more than 10 mg/kg all died. Cd levels of 5-10 mg/kg induced 100% fetal resorptions without effects on mothers.
Schroeder & Mitchener (1971)	Charles River CD mouse (n=5)	drinking water	multigeneration study administration: continuously	0 or 10 mg $CdCl_2$/l	maternal arterial hypertension, runts, bent tails	
Samarawickrama and Webb (1979)	Wistar-Porton rat (n=15)	i.v. injection	administration: 1x, on GD 10,11,12,13,14 or 15 sacrifice: GD 20	0, 1.1 or 1.25 mg Cd/kg bw	1.1 mg: no effects 1.25 mg: reduced maternal blood flow, time of administration-dependent decrease litter size, increase resorptions, incidence of hydrocephalus, an-&microphthalmia, gastroschisis, umbilical hernia	
Sutou *et al*. (1980a,b)	Sprague-Dawley rat (n=13-14)	gavage	administration: 6 w prior to mating, mating, gestation sacrifice: GD 20	0, 0.1, 1.0, 10 mg $CdCl_2$ /kg bw	1-10 mg: reduced maternal body and organ weight 10 mg: increased placenta weight, no. resorptions; reduced foetal bw & length, no. implantation sites, live foetuses	

Table 2.2 Developmental toxicity studies in animals with Cd.

authors	species	route	experimental period	dose	findings	remarks
Zenick *et al*. (1982)	Sprague-Dawley rat (n=5 males and 15 females)	drinking water	administration males: 70 d sacrifice females: GD 20 or females were allowed to litter	0, 17.2, 34.4, 68.8 mg Cd/l (0, 1, 2 and 4 mg/kg bw)	GD 20 sacrifice: no foetal effects, postnatal behavioural assessment: no effects	NB: the females were *not* exposed
Whelton *et al*. (1988)	CF1 mice (n=60-77)	diet	administration: continuously for 6 generations	0, 0.25, 5 or 50 mg $CdCl_2$/kg	50 mg: no effect on fertility and survival, decreased litter size (15%) and decreased pup growth (25%)	
Saltzman *et al*. (1989)	Wistar rat (n=4-8)	s.c. injection	administration: GD 12 or 18 sacrifice: GD 20 or 19	0, 40 or 50 µmol $CdCl_2$/kg (4.5 or 5.6 mg/kg)	reduced maternal blood flow from uterus to chorioallantoic placenta causing time- and dose-dependent foetolethality	
Pelletier and Satinder (1991)	3 genetic lines of rats (n=9)	s.c. injection	administration: daily during gestation	0, 0.075 or 0.225 mg $CdCl_2$/kg bw	Different effects of Cd on progeny weights. Significant effects on behaviour high-dose group No effects on physical development, body weight, food consume, length of gestation, litter size, foetal mortality	
Suokupova *et al*. 1991a	ICR mice (n=?)	s.c. injection	administration: single dose on GD 8, 9, 10, 11, 12, 13, 14 sacrifice: GD18	0, 2 or 6 mg $CdCl_2$ kg/bw	Embryolethlity statistically sifnificantly correlated to day of treatment (highest on GD12 and 13.6 mg/kg). Haemorrhagic bullae, (right-sided) limb malformations, exencephaly, cleft palate, open eyelids and tail deformities were obeserved. Reduced foetal thymus weight GD9-14 in higher dose groups	

Table 2.3 Developmental toxicity studies in animals with Cd.

authors	species	route	experimental period	dose	findings	remarks
Suokupova *et al*. 1991b	ICR mice (n=4)	s.c. injection	administration: single dose on GD 16	0, 2.5 or 5.0 mg $CdCl_2$ kg/bw	Deviations in several immune functions of offspring	
Hartsfield *et al*. (1992)	Syrian golden hamsters (n=10)	i.v. injection	administration: single dose on GD 7 sacrifice: GD 14	0, 2 or 3 mg $CdCl_2$ /kg bw	Reduction maternal body weight increased number of non-viable foetuses and foetuses with malformations; fetal weight and length were decreased	
De *et al*. (1993)	CD-1 mice (n=3-15)	s.c. injection	administration: single injection on GD 1 or 3 sacrifice: GD 4 or 7	0, 25 or 38 µmol $CdCl_2$ /kg bw (2.9 or 4.3 mg/kg bw)	Cd on GD 1: Cd delayed implantation, not embryolethal at GD 7 Cd on GD 3: no implantation sited at GD 4, pregnancy failures at GD 7	
Andersson *et al*. (1997)	Sprague-Dawley rats (n=4)	drinking water	administration: dams from parturition to PN 17. Offspring: PN day 17-42 Sacrifice: 1 w after treatment	0 or 5 mg $CdCl_2$ /l	No effects on body weights, food and water intake of dams and offspring. No effect on weights of liver, kidney and brain of pups. Increased levels of plasma nitrogen only in pups exposed postweaning. No obvious neuropathological effects. Cortical serotonin levels were reduced in pups of all Cd groups	

w = body weight; s.c. = subcutaneous; i.v. = intravenous; GD = gestation day

Table 2.4 Developmental toxicity studies in animals with Cd.

authors	species	route	experimental period	dose	findings	remarks
Nagy-majtényi *et al*. (1997)	Wistar rats (n=5-10)	gavage	administration: 5 d/w during pregnancy, lactation and 8 weeks after weaning for 3 generations (females 7 d/w from start mating until weaning)	0, 3.5, 7.0 or 14.0 mg $CdCl_2$/kg bw	7 and 14 mg/kg reduced BW of parents 7 and 14 mg/kg affected most behavioural and electrophysiological parameters of male rats investigated at the age of 12 weeks No effects on pup body weights, no visible malformations and no clinical signs In F3-gen. kidney and spleen weights were reduced in highest dose group	
Antonio *et al*. (1998)	Wistar rat (n=4)	drinking water	administration: from initiation of pregnancy to parturition or to PN 5	0 or 10 mg Cd-acetate /l	Altered neurotransmitter levels in several regions of the brain	
Corpas *et al*. (1998)	Wistar rat (n=4)	drinking water	administration: 1) during gestation 2) during gestation and up to PN 5 sacrifice of pups: 1) PN 0; 2) PN 5	0 or 10 mg Cd-acetate /l	On PN 0 and 5 diameter of seminiferous tubule, number of prospermatogo nia and DNA, RNA and protein content of testis and ovary were reduced. On PN 5 weight of testis and ovary were reduced.	
Dési *et al*. (1998)	Wistar rat (n=?)	gavage	administration: 1) GD 5-15, 2) GD 5-15 + 4 w postnatal 3) GD 5-15 + 4 w postnatal + F1-males 8 w	0, 3.5, 7.0 or 14.0 mg $CdCl_2$ /kg bw	Pups, litter, pup weight and kidney weight were decreased in high dose group. Behavioural and electrophysiological tests of F1 males of 12 w old were influenced by Cd	

w = body weight; d = day; w = week; GD = gestation day; PN = postnatal

Annex **E**

Calculation safe levels of Cd in (human) breast milk

Assumptions:

- Body weight woman: 60 kg
- Body weight infant: 4.5 kg (4-5 kg)
- Intake breast milk: 900 ml (800-1000ml)
- An infant is as sensitive for the effects of Cd as an adult.

The Dutch Health Council (1988) proposed a safe intake level of 400-500 µg/person/week.

This results in a calculated intake of 57-71 µg/person/day and 0.95-1.18 µg/kg body weight/day. Safe intake level per infant is 4.28-5.31 µg/infant/day. Safe level of Cd in breast milk is 4.76-5.9 µg/l.

In conclusion, the committee considers 5 µg Cd/l breast milk as a safe level.

Annex **F**

Abbreviations

Abbreviations used:

bw	body weight
d	day
F	female(s)
GD	Gestation day
i.p.	intraperitoneal
i.v.	intravenous
M	male(s)
n	number of animals
no	number
ns	not significant
NOAEL	no adverse effect level
OECD	Organisation for Economic Cooperation and Development
PN	postnatal
w	week