Mercury and its compounds

Evaluation of the effects on reproduction, recommendation for classification

Aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid

Onderwerp	:	aanbieding advies
Uw kenmerk	:	DGV/BMO-U-932542
Ons kenmerk	:	U 949/AB/jt/543-Z2
Bijlagen	:	1
Datum	:	3 mei 2000

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 repro-tox-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 kwik en kwikverbindingen. Deze publikatie 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

Mercury and its compounds

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. 2000/05OSH, The Hague, 3 May 2000

Preferred citation:
Health Council of the Netherlands: Committee for Compounds toxic to reproduction. Mercury and its compounds; Evaluation of the effects on reproduction, recommendation for classification. The Hague: Health Council of the Netherlands, 2000; publication no. 2000/05OSH.

ISBN: 90-5549-314-7

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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 kwik en kwikverbindingen onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Metallisch kwik
  - voor effecten op de fertiliteit adviseert de commissie om metallisch kwik niet te classificeren wegens onvoldoende gegevens
  - voor effecten op de ontwikkeling adviseert de commissie metallisch kwik in categorie 2 te classificeren (*stoffen die dienen te worden beschouwd alsof zij bij de mens ontwikkelingsstoornissen veroorzaken*) en met R61 (*kan het ongeboren kind schaden*) te kenmerken
  - voor effecten tijdens de lactatie is de commissie van mening dat er onvoldoende gegevens zijn om metallisch kwik te kenmerken.
- Methylkwik
  - voor effecten op de fertiliteit adviseert de commissie om methylkwik niet te classificeren wegens onvoldoende gegevens

- voor effecten op de ontwikkeling adviseert de commissie methylkwik in categorie 1 te classificeren (*stoffen waarvan bekend is dat zij bij de mens ontwikkelingsstoornissen veroorzaken*) en met R61 (*kan het ongeboren kind schaden*) te kenmerken
- voor effecten tijdens de lactatie adviseert de commissie om methylkwik met R64 (*kan schadelijk zijn via de borstvoeding*) te kenmerken.

- Fenylkwikacetaat
  - voor effecten op de fertiliteit adviseert de commissie om fenylkwikacetaat niet te classificeren wegens onvoldoende gegevens
  - voor effecten op de ontwikkeling adviseert de commissie om fenylkwikacetaat niet te classificeren wegens onvoldoende gegevens
  - voor effecten tijdens de lactatie is de commissie van mening dat er onvoldoende gegevens zijn om fenylkwikacetaat te kenmerken.
- Kwikchloride/kwiknitraat
  - voor effecten op de fertiliteit adviseert de commissie om kwikchloride/ kwiknitraat niet te classificeren wegens onvoldoende gegevens
  - voor effecten op de ontwikkeling adviseert de commissie om kwikchloride/kwiknitraat niet te classificeren wegens onvoldoende gegevens
  - voor effecten tijdens de lactatie is de commissie van mening dat er onvoldoende gegevens zijn om kwikchloride/kwiknitraat 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 mercury and its compounds.

The committee's recommendations are:

- Mercury (metallic)
  - for effects on fertility, the committee recommends no classification of metallic mercury, due to lack of data
  - for developmental toxicity, the committee recommends to classify metallic mercury in category 2 (*substances which should be regarded as if they impair fertility in humans*) and to label metallic mercury with R61
  - the committee is of the opinion that a lack of appropriate data precludes the labelling of metallic mercury for effects during lactation.
- methylmercury
  - for effects on fertility, the committee recommends no classification of methylmercury, due to lack of data
  - for developmental toxicity, the committee recommends to classify methylmercury in category 1 (*substances known to cause developmental toxicity in humans*) and labelled with R61

- for effects during lactation, the committee recommends that methylmercury should be labelled with R64 (*may cause harm to breastfed babies).*

- phenylmercury acetate
  - for effects on fertility, the committee recommends no classification of phenylmercury acetate, due to lack of data
  - for developmental toxicity, the committee recommends no classification of phenylmercury acetat, due to lack of data
  - the committee is of the opinion that a lack of appropriate data precludes the labelling of phenylmercury acetate for effects during lactation.
- mercuric chloride/mercuric nitrate
  - for effects on fertility, the committee recommends no classification of mercuric chloride/mercuric nitrate, due to lack of data
  - for developmental toxicity, the committee recommends no classification of mercuric chloride/mercuric nitrate, due to lack of data
  - the committee is of the opinion that a lack of appropriate data precludes the labelling of mercuric chloride and nitrate for effects during lactation.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed 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 mercury and its compounds by the Health Council's Committee for Compounds Toxic to Reproduction. The committee distinguishes four different groups of mercury compounds in this report: (I) Metallic mercury (chapter 2); (II) Methyl mercury (chapter 3); (III) phenylmercury acetate (chapter 4); (IV) Mercury chloride and mercury nitrate (chapter 5). In chapter 6, the effects of the different mercury compounds during lactation are described.

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 ef-

fects 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, e.g. the acceptable daily intake (ADI).

* Organisation for Economic Cooperation and Development

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 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 and animal 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.

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

Mercury (metallic)

2.1 Introduction

Name	:	mercury
CAS-no	:	7439-97-6
Use	:	as dental amalgam, in common consumer products such as light bulbs, barometers, thermometers and other medical equipment
Atom weight	:	200.59
Chem formula	:	Hg

2.2 Human studies

2.2.1 Fertility

Lauwerys *et al*. (1985) examined the fertility of Belgian male workers employed in a zinc-mercury factory, in a chloralkali plant or in plants manufacturing electrical equipment (103 workers in total, versus 101 controls) (Lau85). No adverse effects were detected on male fertility, expressed as the difference between expected and observed number of children.

Rowland *et al*. (1994) examined the reproductive effects in 296 female dental assistants (versus 111 controls) in California USA. after exposure to metallic mercury vapour (Row94). Preparation of a high number of amalgams per week and/or poor

hygienic working conditions were associated with effects on fertility. However, in case of a low number of amalgams and/or good hygienic circumstances, fertility was even better than in the controls, which was considered an unaccountable difference.

Alcer *et al*. (1989) examined the effects of long-term exposure to elemental mercury at an Energy plant in Michigan USA. in 241 male workers (versus 254 controls) (Alc89). No effects were detected on the total number of pregnancies, number of live born children, congenital abnormalities and illnesses; a difference was found in the number of miscarriages, which was already elevated in the "exposed" group, before actual exposure started.

The rate of spontaneous abortions among wives of 152 workers in a chloralkali plant occupationally exposed to mercury vapour was compared with the rate among the wives of 374 controls in the same plant (Cor91). As an increased number of abortions was observed in the exposed group in the presence of confounding factors such as smoking and alcohol consumption, the committee concluded that this study did not provide information on causality.

2.2.2 *Developmental studies*

In a preliminary study, Sikorski *et al*. (1987) examined the reproductive effects of exposure to metallic mercury in Polish female dentists and dental assistants (81 females, versus 34 controls) (Sik87). An increase in spontaneous abortions, stillbirths and congenital malformations (a.o. 5 babies with spina bifida) was observed as well as an increase in menstrual disorders, which significantly correlated with the number of years active in the dental profession. Larsson (1995) discussed the validity of the original publication of Sikorski and commented or cited the following comments made by others: "The base population from which the 81 study subjects, or 34 controls, were selected, was not defined. Exposed and control subjects were not matched according to age and medical history" (Lar95). Exposure to mercury was measured in scalp or pubic hair; this determination was performed at examination in 1985 and 1986 and not at the time of pregnancy (children with spina bifida were born in 1968, 1972, 1977, 1980 and 1982). In the opinion of Larsson (Lar95), the study of Sikorski contained an erroneous interpretation of results and distortion of conclusions. The committee supports these conclusions of Larsson.

Alcer *et al*. (1989) examined the effects of long-term exposure to elemental mercury at an Energy plant in Michigan USA., in 241 male workers (versus 254 controls) (Alc89). No effects were detected on the total number of pregnancies, number of live born children and congenital abnormalities.

Ericson and Källén (1989) examined the reproductive effects of exposure to metallic mercury in Swedish female dentists, dental assistants and dental technicians (Eri89). No

adverse effects were found as to spontaneous abortions, birth weight, congenital malformations or infant survival. No information was presented about the actual exposure level.

2.2.3 *Lactation*

See chapter 6.

2.3 Animal studies

2.3.1 *Fertility*

Baranski and Szymczyk (1973 cited in: Barlow and Sullivan 1982) exposed groups of 24 female rats to mercury vapour (0 and 2.5 mg/m^3 for 6h/day for 21 days) (Bar 73). A significant increase in the length of the oestrous cycle in mercury-exposed rats, from a mean of 4.3 days before exposure to a mean of 6.7 days at the end of exposure was found. Oestrous cycle duration in control animals increased from 4.5 to 5.1 days during the same period. The authors also exposed female rats to 0 or 2.5 mg/m^3 metallic mercury vapour, 6h/day for 6-8 weeks before mating. All 18 treated and 23 control females that did mate became pregnant. The total numbers of treated and untreated females was not reported. The occurrence of slight general toxicity was presented as a non significant reduction in mean body weight.

2.3.2 *Developmental toxicity*

Baranski and Szymczyk (1973 cited in: Barlow and Sullivan 1982) exposed female rats to 0 or 2.5 mg/m^3 metallic mercury vapour for 6h/day for 6-8 weeks before mating, but not during pregnancy (Bar73). A non significant reduction in mean maternal body weight was recorded. There were no effects on the mean number of pups/litter or on the number of live pups/litter at birth. However, postnatal mortality was significantly increased, particularly during the first 4 days of life when 26% of the pups from the mercury-exposed group died compared with 1% in the control group. At 2 months of age, offspring body and organ weights were determined: male offspring weights were unaffected, but female offspring from the mercury-exposed group had significantly lower kidney and liver weights and significantly higher ovary weights than controls.

They also exposed groups of 12 female rats to 0 or 2.5 mg/m^3 metallic mercury vapour by inhalation for 6h/day for 3 weeks before pregnancy and again on days 7-20 of pregnancy, and allowed them to litter. Maternal weight gain was reduced, but not significantly, in the mercury-exposed group. There were no significant effects on pregnancy ra-

te, number of litters born, or on total litter size (i.e. live and dead pups) at birth. However, mean live litter size at birth was reduced significantly to 7.6 pups in the mercury-exposed group compared with 9.6 pups in controls. In the first 4 days of life 96% of pups from the exposed group died; none survived to weaning (Bar73).

In a further experiment, groups of 8 female rats were similarly exposed, and killed on gestation day 20. A significant reduction in live litter size was confirmed, and found to be due to a decrease in the number of implantations, reflecting a decrease in ovulation and/or increase in pre-implantation losses.

Berlin *et al*. (1992) exposed 10 pregnant squirrel monkeys to mercury vapour at a concentration of 1 mg/m^3, beginning at week 3 to week 7 of gestation and continuing to the termination of pregnancy (approximately week 22) (Ber92). One monkey was exposed to 24 h/day, 5 days/week, 4 monkeys were exposed for 7h/day, 5 days/week and 5 monkeys were exposed for 4 h/day, 5 days/week. Ten unexposed monkeys were assigned as controls in addition to historical controls. There was a 60% incidence of abnormal pregnancies in the exposed monkeys compared to 5% in the breeding colony. The incidence of abortion and neonatal mortality showed a dose-related increase. A decrease in birth weight was also observed. Histopathological examination of the brain of the offspring showed a number of changes. Maternal toxicity was not reported.

The effect of neonatal exposure of rats to mercury vapour at a concentration 0.05 mg/m^3, 1 h or 4 h from postnatal days 11-17, on the behaviour in adulthood were studied by Fredriksson *et al*. (1992) (Fre92). Tests for spontaneous motor activity were performed at the ages of 2 and 4 months. Rats exposed for 4 h/day showed a marked increase in locomotion and total activity but a decrease for rearing when tested at 2 months of age. At 4 months of age these rats showed marked hypoactivity with respect to all three variables. Rats exposed for 1h/day showed no significant differences at 2 months compared to controls. However, at the age of 4 months the same pattern (increase in variables locomotion and total activity but a decrease for rearing) already noticed in the 4h/day group at 2 months was observed in this group. In the spatial learning tasks applied, neonatally exposed pups showed a retarded acquisition to the radial maze, while there was no difference compared to controls in the circular swim maze.

The behaviour of offspring of squirrel monkeys exposed to 0.5 or 1 mg/m^3 of mercury vapour during the last $^2/_3$ part or more of gestation were studied by Newland *et al* 1996 (New96). Persistent behavioural effects of prenatal mercury vapour exposure included instability in lever-press durations and steady-state performance under concurrent schedules of reinforcement as well as aberrant transitions.

2.3.3 Lactation

See chapter 6.

2.4 Overall conclusions

Male fertility man was not affected in any of the human studies (Lau85, Alc89). The effects described by Cordier *et al*. 1991 on spontaneous abortion after paternal exposure did not provide evidence of a causal relationship. The data on human female fertility and reproduction were ambiguous: Sikorski *et al*. 1987 and Rowland *et al*. 1994 reported effects on fertility (disturbance of the menstrual cycle) and reproduction (spontaneous abortion, perinatal death), whereas Ericson and Kallèn 1989 did not observe effects in their Swedish epidemiological study among dental workers. The committee is of the opinion that the differences between these studies might be caused by a lack of control for age, social status, diet, smoking, selection bias and quality of the working conditions which influence the actual exposure to metallic mercury vapour.

The effects found on fertility in rats (Baranski and Szymczyk 1973) were found at high concentrations at which general toxicity may be assumed.

In conclusion, the committee recommends not to classify metallic mercury with respect to effects on fertility because of a lack of appropriate data.

Data from human studies for developmental effects provided no basis for classification of metallic mercury.

Exposure of rats to metallic vapour before mating and during gestation resulted in an increased pre-implantation loss, reduced number of live pups at birth and in postnatal mortality in the absence of clear maternal toxicity (Bar73). In squirrel monkeys (Ber92) abnormal pregnancies, increased number of abortions, and neonatal mortality were found after exposure to high concentrations of mercury vapour at which general toxicity may be assumed. Neonatal exposure of rats (Fre92) and prenatal exposure in squirrel monkeys (New96) resulted in persistent behavioural changes.

In conclusion, with respect to effects on development the committee recommends to classify metallic mercury in category 2 (substances that should be regarded as if they cause developmental toxicity in humans) and to label the compound with R61 (may cause harm to the unborn child).

Proposed classification for fertility

Lack of appropriate data precludes assessment of the metallic mercury for fertility.

Proposed classification for developmental toxicity

Category 2, R 61.

Proposed labelling for effects during lactation

See chapter 6.

Chapter 3

Methylmercury

3.1 Introduction

Name	:	methylmercury
CAS-no	:	22967-92-6
Use	:	fungicide
Mol weight	:	215.59
Chem formula	:	CH_3Hg^+

3.2 Human studies

3.2.1 Fertility

No publications were found concerning effects of methylmercury on human fertility.

3.2.2 Developmental studies

Methylmercury has caused major epidemics of poisoning in the general population. The two epidemics of methylmercury poisoning in Japan, in Minamata Bay and in Niigata, were caused by accumulation of industrially released mercury in edible fish. In Iraq, poisoning was caused by ingestion of bread contaminated with methylmercury fungicides (WHO76).

The reports on the Minamata outbreak described only slight symptoms in the mothers whose children, which had been exposed in utero, had cerebral palsy and/or microcephaly. In this study, it was concluded that the foetus is more sensitive to the effects of methylmercury than adults (WHO76).

Surveys conducted on a selected Iraqi population to determine the extent of exposure to methylmercury revealed cerebral palsy and psychomotor retardation in children which had been exposed in utero (Ami74, Ami79). The number of births registered in 1973, the year following the methylmercury poisoning epidemic, was decreased by 2000, whereas increases in number of births of 10000 and 8000 had occurred in the 2 years prior to the epidemic (registered births in Iraq: 1970, 48055; 1971, 58837; 1972, 66549; 1973 64582). However, no data were reported for the years after 1973 (Gre85).

Further analysis of the Japanese and Iraqi data revealed additional information relating to the effects of prenatal methylmercury exposure: methylmercury inhibits the growth of the foetal brain and the migration of neurones from the embryological generation layer to the final destination in the cortex. This inhibition in foetal brain development results in the behavioural changes and reduced cognitive and motor ability found in clinical cases. Cytological studies demonstrated that methylmercury interferes with microtubule formation, cell division, and neuronal protein synthesis (WHO 1990).

Grandjean *et al*. (1997) studied the neurodevelopmental effects in children (age 7 years) in the Faroe islands (Gra97). The median hair concentration was 4.5 ppm (mg/kg hair) and in 13% of the children more than 10 ppm (mg/kg hair). In the cohort with maternal hair mercury concentrations from 3-10 ppm neuropsychological changes were observed.

Davidson *et al*. (1998) studied the neurodevelopmental consequences of exposure to methylmercury from fish eating in Seychelles children at the age of 66 months (Dav98). Mean maternal hair mercury concentration were 6.8 ppm (range 3-26.7 ppm). In this study no adverse effects of prenatal or postnatal methylmercury exposure were found.

Mahaffey (1998) suggested that the discrepancy in outcome between the studies of Grandjean *et al*. 1997 and Davidson *et al*. 1998 might be explained by the use of tests with a lower detection limit for subtle cognitive and neuromotor disturbances in the latter study (Mah98). The committee supports the suggestion of Mahaffey.

3.2.3 Lactation

See chapter 6.

3.3 Animal studies

3.3.1 Fertility

Lee and Dixon (1975) reported effects on spermatogenesis, testis histology and fertility in CDF1 mice after intraperitoneal exposure to a dose of methylmercury equivalent to 1 mg mercury/kg; no data on general toxicity were presented in this study (Lee75). Fertility as studied from serial matings up to 70 days after exposure indicated a significant decrease in fertility, compared with controls. The dose levels used in this study were close to doses at which general toxicity was observed in other studies.

Mohamed *et al*. (1987) studied the effects of oral treatment with methylmercury of adult male monkeys (Macaca fascicularis) on sperm production, motility and morphology and serum testosterone levels (Moh87). Sperm parameters were affected; these effects were not accompanied by histological abnormalities at the end of the treatment period.

Burbacher *et al*. (1984) did not observe an effect on menstrual cycle or menses length after oral treatment of 0, 50 or 90 µg/kg bw/day methyl mercury hydroxide to monkeys (macaca fascicularis) (Bur84). Pregnancy and abortion rates were not statistically significantly changed. The offspring of the methyl mercury-treated females tended to be smaller (birth weight and crown-rump length). Treatment with 90 µg/kg bw per day produced signs of toxicity in all 4 female monkeys.

Mitsumori *et al*. (1990) reported tubular atrophy of the testes in B6C3F1 mice after 2-year feeding of 0.4, 2 and 10 ppm (mg/kg feed) methylmercury at the highest dose-level (Mit90). At 2 ppm (mg/kg feed) effects on the kidneys and at 10 ppm on the peripheral nervous system, cerebrum and cerebellum, kidneys and stomach were observed.

Vachhrajani *et al*. (1992) studied the cellular distribution pattern of methylmercury at different stages of the seminiferous epithelium in the testis of rats intraperitoneally injected with 5 or 10 µg/kg body weight per day during 15, 30, 60 or 90 days. As profound cell death occurred between zygotene to pachytene stages and dividing spermatocytes to step 1 spermatids, the number of spermatids was conspicuously decreased at later times. General toxicity was not described in this study. The committee considers the exposure route and duration of exposure not relevant for classification for fertility.

3.3.2 Developmental toxicity

In 1990 the WHO concluded that the nervous system is a target of methylmercury, and that foetuses appear to be more sensitive than adults. Methylmercury is foetotoxic in

mice (Verschaeve and Leonard 1984 in: WHO 1990), teratogenic in rats (e.g. Kutscher *et al*. 1985 in: WHO 1990), and adversely affects the behaviour of rat (Elsner *et al*. 1988) and monkey offspring (e.g. Gunderson *et al*. 1986 in: WHO 1990).

3.3.3 Lactation

See chapter 6.

3.4 Overall conclusion

No reports were found concerning effects of methylmercury on human fertility. The effects of methylmercury found in animal studies in which fertility was studied were insukficient to classify methylmercury for effects on fertility.

In conclusion, the committee recommends not to classify methyl mercury with respect to effects on fertility because of a lack of appropriate data.

Methylmercury affects the development of the central nervous system both in humans and animals: it seriously affects the development of the brain, resulting in behavioural disorders (Ami74, Ami79; WHO90).

In view of human and animal data with respect to developmental toxicity, the committee recommends to classify methylmercury in category 1 (substances known to cause developmental toxicity in humans) and to label the compound with R 61 (may cause harm to the unborn child).

Proposed classification for fertility

Lack of appropriate data precludes assessment of the methylmercury for fertility.

Proposed classification for developmental toxicity

Cat 1, R 61.

Proposed labelling for effects during lactation

See chapter 6.

Chapter 4

Phenylmercury acetate

4.1 Introduction

Name	:	phenylmercury acetate
CAS-no	:	62-38-4
Use	:	fungicide
Mol weight	:	336.75
Chem formula	:	C_6H-Hg-$OOCCH_3$

4.2 Human studies

4.2.1 Fertility

No publications were found concerning effects of phenylmercury on human fertility.

4.2.2 Developmental toxicity

With respect to human data, Schardein (1993) reported that exposure to phenylmercury acetate was found to have no relation with congenital malformations among 889 pregnancies (Sch93). No details were reported.

4.2.3 *Lactation*

See chapter 6.

4.3 Animal studies

4.3.1 *Fertility*

Five groups of rats, 24 animals per group, received Fungitox OR, which contains phenylmercury acetate, in their food over a 6-months period. The fungicide was given in doses of 0, 1, 2, 4 and 8 ppm mercury equivalents (mg/kg feed). Gain in body weight was not affected by the fungicide. The mercury compound accumulated in all the organs studied but particularly in the kidneys. After 6 months, 6 males and 6 females were mated within the groups. Rats receiving 8 ppm mercury equivalents (mg/kg feed) had a 44% drop in progeny compared to the controls (Pie68). The study was poorly reported and exposure to other compounds in Fungitox OR was not described.

4.3.2 *Developmental toxicity*

Dzierzawski (1979) reported that phenylmercury acetate caused defects of the central nervous system in mice and hamsters, and multiple defects in rabbits (cited in Schardein 1993) (Dzi79). Teratogenic effects of the compound have not been demonstrated in the rat.

Murakami *et al*. (1956) studied the effects of phenylmercury acetate after vaginal application and subcutaneous injection in albino mice and observed an increased number of resorptions and fetuses with abnormalities (Mur56). In maternal animals exposure to phenyl mercury acetate resulted in effects on parenchymal cells of liver and kidney.

Gale and Ferm (1971) administered phenylmercury acetate intravenously to pregnant golden hamsters and induced resorptions and fetuses with abnormalities (Gal71). General toxicity is not described.

4.3.3 *Lactation*

See chapter 6.

4.4 Overall conclusion

No data were found with respect to effects on human fertility. The committee concluded that the animal data presented by Piechocka (1968) were insufficient for classification for fertility as well, because the study was poorly reported and the exposure to other compounds was clear.

In conclusion, the committee recommends not to classify phenylmercury acetate with respect to effects on fertility because of a lack of appropriate data.

No data were found with respect to effects on development in man.

Congenital malformations, predominantly of the central nervous system, were reported in animals (Mur56, Gal71, Dzi79). However, general toxicity was either not described in the publications, or the effects were observed at levels causing general toxicity.

In conclusion, the committee recommends not to classify phenylmercury acetate with respect to developmental effects because of a lack of appropriate data.

Proposed classification for fertility

Lack of appropriate data precludes assessment of phenylmercury acetate for fertility.

Proposed classification for developmental toxicity

Lack of appropriate data precludes assessment of phenylmercury acetate for developmental effects.

Proposed labelling for effects during lactation

See chapter 6.

Chapter **5**

Mercuric chloride and Mercuric nitrate

5.1 Introduction

Name	:	mercuric chloride
CAS-no	:	7487-94-7
Use	:	preservation wood; disinfectant; etching steel and iron; tanning leather; electroplating aluminium; treatment seed potatoes
Mol weight	:	271.52
Chem formula	:	$HgCl_2$

Name	:	mercuric nitrate
CAS no: 10045-94-0	:	10045-94-0
Use	:	manufacture of felt; mercury fulminate; fungicide (destroying phylloxera)
Mol weight	:	324.66
Chem formula	:	$Hg(NO_3)_2$

Mercuric chloride and mercuric nitrate are both inorganic mercury salts which were assumed to show the same kinetics in the human body; both will form a Hg^+ ion.

5.2 Human studies

5.2.1 Fertility

Schardein (1993) reported that mercuric chloride has been related to spontaneous abortion in two studies (1950 and 1960). No details were reported.

5.2.2 Developmental toxicity

No publications were found concerning effects of mercuric chloride and mercuric nitrate on developmental toxicity.

5.2.3 Lactation

See chapter 6.

5.3 Animal studies

5.3.1 Fertility

Lach and Srebro (1972) exposed groups of 10 female mice with normal cycles to mercuric nitrate by subcutaneous injection, at doses of 0.1 mg/day for 8 days, or 0.2 mg/day for 12 days (≈ 4 or 8 mg/kg bw/day, respectively). In the 0.1 mg/day group 3/10 showed no oestrous period at all and none had more than one oestrous period, during the 8 days of exposure (Lac72). Cycles returned to normal in the 12 days after the end of exposure. In de 0.2 mg/day group, 4/10 showed no oestrous period and 3/10 only one oestrous period during the 12 days of treatment. Duration of di-oestrous was significantly increased from 30% of the cycle length before treatment to 70% during treatment. In the 15 days after the end of exposure, 4/10 failed to show any oestrous period, only one of these 4 having an anoestrous during exposure. Total days in di-oestrus in the 15-day post-exposure were 45%. No data on general toxicity were presented in this study. However, the dose levels used in this study were close to doses at which general toxicity was observed in other studies.

Lee and Dixon (1975) reported effects on spermatogenesis, testis histology and fertility of methyl mercury in mice after intraperitoneal (i.p.) exposure to a dose of mercuric chloride equivalent to 1 mg mercury/kg bw (Lee75). Effects on fertility were studied from serial matings up to 70 days after exposure. A significant decrease was indicated in fertility, compared with controls. No data on general toxicity were presented in this stu-

dy. However, the dose levels used in this study were close to doses at which general toxicity was observed in other studies.

Histochemical investigations of 3 beta-hydroxy-delta 5-steroid dehydrogenase (delta 5-3 beta OHD) activity in the testicular tissue of rats administered mercuric chloride i.p. at dosages of 0.05 mg/kg bw and 0.1 mg/kg bw daily for 90 days revealed a graded inhibition of delta 5-3 beta OHD (last step of the testosterone synthesis) activity which was positively related to dosage and to the duration of treatment with this compound (Cho85).

Testicular changes following i.p. administration of mercuric chloride (1, 2 and 5 mg/kg bw per day) over one month were studied in rats, mice, guinea pigs and hamsters (Cho82). Mercuric chloride (5 mg/kg bw) caused testicular degeneration and cellular deformation, observed in both the seminiferous tubules and the Leydig cells in all species: a significant decrease of testicular weight was also observed. There was no cellular deformation at the dose of 2 mg/kg bw: only spermatogenic inhibition and Leydig cell atrophy were observed in the animals. At the dose of 1 mg/kg, testicular degeneration was observed only in the hamster, only partial degeneration was recorded in the rat and the mouse and no change was noted in the guinea pig. Effects of general toxicity were not described in this study. For the different mammalian species the LD_{50} ranged from 14-25 mg/kg bw.

5.3.2 Developmental toxicity

Holt and Webb (1986) exposed pregnant Wistar rats intravenously to mercuric chloride during different periods of gestation (Hol86). During the mid-gestation the minimum effective teratogenic dose of mercury (0.79 mg/kg bw) was high in relation to the maternal LD_{50} (c. 1 mg/kg bw) and the incidence of foetal malformations, mainly brain defects, was 23% in all live foetuses. In rats of different gestational ages, foetal Hg^{2+} decreased sharply between day 12 and day 13. The teratogenic effects on the foetus and damage to the maternal kidneys, however, were essentially the same in animals dosed with Hg^{2+} either immediately before or immediately after these gestational ages.

5.3.3 Lactation

See chapter 6.

5.4 Overall conclusion

With respect to human data, there are indications that mercuric chloride may cause spontaneous abortion. However, no information on this study were reported (Schardein 1993).

In animals, the effects on male fertility (Lee75, Cho82) were found at dosages at which toxicity may be expected. However, general toxicity is not described in these studies.

In conclusion, the committee recommends not to classify both mercury salts with respect to effects on fertility because of a lack of appropriate data.

No publications were found concerning effects of mercuric chloride and mercuric nitrate on developmental toxicity in man.

Mercuric chloride induces developmental effects in rats at a dose level which also may cause maternal toxicity (Holt and Webb 1986).

In conclusion, the committee recommends not to classify both mercury salts with respect to developmental effects because of a lack of appropriate data.

Proposed classification for fertility

Lack of appropriate data precludes assessment of the mercury salts for fertility.

Proposed classification for developmental toxicity

Lack of appropriate data precludes assessment of the mercury salts for developmental effects.

Proposed labelling for effects during lactation

See chapter 6.

Chapter 6

Lactation (organic and inorganic mercury)

6.1 Human studies

In 1968, a total mercury concentration of 63 µg/l (mean) and 50 µg/l (mean) was detected in breast milk of healthy women from the polluted Minamata district and from an agricultural area in Japan, respectively (Fuj77).

Pitkin *et al*. (1976) found detectable mercury levels in 14 of 32 samples of non-exposed women with an established lactation of 56 or more days (Pit76). The total mercury concentration was 0.9 µg/l.

Fujita and Takabatake (1977) detected total mercury levels of 3.6 $\pm$ 2.2 µg/l in breast milk of mothers of the Tokyo Metropolitan area (Fuj77). No correlation between mercury levels in blood and breast milk was detected.

Wilson *et al*. (1980) estimated that the concentration of methylmercury in human breast milk was around 29 µg/l in Iraqi women after the poisoning with methylmercury (Wil80).

Skerfving (1988) studied the total mercury content and the methylmercury content in breast milk (Ske88). The total mercury concentration in milk averaged 3.1 (range 0.2-6.3) µg/l in 15 mothers and the mean methylmercury concentration in milk was 0.6 (range 0.2-1.2) µg/l in 9 mothers.

Klemann *et al*. (1990) studied the relationship between dental amalgam fillings and mercury content in human breast milk and amniotic fluid (Kle90). The concentration of total mercury in the breast milk of 86 women, at five to ten days after delivery, was 1.9 $\pm$ 1.6 µg/l. No significant correlation to maternal amalgam surface area was found.

Oskarson *et al*. (1996) collected breast milk, blood and hair samples 6 weeks after delivery from 30 women who lived in the north of Sweden (Osk96). Total mercury concentration in breast milk was 0.6 ± 0.4 µg/kg. The level of mercury in milk reflected the plasma level. Methyl mercury is bound predominantly to erythrocytes, whereas inorganic mercury is distributed almost equally between erythrocytes and plasma; the latter distribution would favour the lactational transfer of inorganic mercury from blood. In addition, an efficient transfer of inorganic mercury from plasma into milk has been shown in animals (Sun91). Approximately half of the total mercury content in milk was in the form of inorganic mercury. Total and inorganic mercury in milk were correlated with the number of amalgam fillings. There was no significant correlation between milk levels of mercury in any chemical form and the estimated methylmercury intake (Osk96).

Vimy *et al*. (1997) detected that women with amalgam fillings had significantly higher mercury levels in breast milk (0.236 ± 0.034 µg/l) than women without amalgam fillings (0.146 ±0.025 µg/l) (Vim97).

Drexler and Schaller (1998) studied the mercury concentration in breast milk resulting from amalgam fillings and dietary habits and found that the concentration of mercury in the breast milk collected immediately after delivery showed a significant association with the number of amalgam fillings as well as with the frequency of meals (Dre98). After 2 months of lactation, the concentrations of total mercury in breast milk were lower (mean <0.25 µg/l; range <0.25-11.7 µg/l) compared with the first sample (mean 0.90 µg/l, range <0.25-20.3 µg/l) and were positively associated with fish consumption and not longer with the number of amalgam fillings. The discrepancy between the studies of Kle90 and Fuj77, as regards the correlation between the number of amalgam fillings and mercury content of breast milk is probably related to the time of sampling (shortly after birth in Dre98). The authors concluded that mercury concentrations in breast milk were mainly the result of dietary habits. The influence of amalgam fillings, at least at the beginning of breast feeding, on the mercury concentration in breast milk appeared relatively small.

No publications were found concerning the excretion of phenylmercury acetate and mercury salts in human breast milk

6.2 Animal studies

Vimy *et al*. (1990) studied in 5 ewes whether mercury from amalgam fillings enters into breast milk in detectable amounts (Vim90). On day 112 of gestation, 12 occlusal amalgam fillings with radioactive mercury (^{203}Hg) were inserted. Radioactive mercury was detected in the milk. 2 Days after birth levels of 60 µg Hg/l were detected.

Yoshida *et al*. (1992) exposed guinea pigs to mercury vapour for 120 minutes within 12 hours after parturition at mean concentration of 6-10 mg/m^3 (Yos92). Mercury concentrations in milk were slightly lower than in plasma mercury concentrations of the maternal guinea pigs on PN (postnatal) days 3, 5 and 10. However, the decrease in mercury concentration in breast milk with time was slower than that in maternal plasma. The mercury concentrations detected in milk amounted to 9-14 µg/l.

A single oral doses of methyl ^{203}Hg (0.5, 3.3, 7.7 or 9.4 mg mercury/kg body weight) were given to rats on day 9 of lactation and the transfer of mercury in milk was investigated (Sun91). A linear, dose-dependent transfer of mercury from plasma to milk was found. After 24 hour the concentrations in milk were 6, 52, 210 or 240 µg/l; after 72 hours 6, 64, 160 or 170 µg/l. Transfer of mercury into milk is dependent on the exposure situation and preferentially inorganic mercury is transported into milk.

Nordenhäll *et al*. (1994) studied the effects of lactational exposure to ^{203}Hg -labelled methylmercury 1 day after parturition (Nor94). Milk was collected twice during the first week. The fraction of inorganic mercury in milk and kidneys of the pups was determined following separation of inorganic mercury and methylmercury by ion exchange chromatography. The concentration of ^{203}Hg in milk on the 1st day after methylmercury administration was 0.12 nmol/g (26 µg/l). ^{203}Hg was mainly excreted as methylmercury during the first 6 days of lactation. The excretion of methylmercury in milk corresponded to at least 5% of the dose administered to the dam.

Yoshida *et al*. (1994) injected maternal guinea pigs intraperitoneally with methylmercury and mercuric chloride (1mg/kg body weight) 12 hours after parturition (Yos94). In the offspring, the highest total methylmercury concentrations were found in the kidney, followed by the liver and the brain. Brain mercury concentrations were significantly higher in the offspring of methylmercury-treated dams than in those treated with mercuric chloride. Methylmercury levels in milk decreased on PN day 10 to 3 µg/l and were relatively constant on PN day 3 and 5 (13 and 11 µg/l, respectively).

After exposure to mercuric chloride, total mercury levels in milk were highest on PN day 3, (76 µg/l) and declined to 19 µg/l on PN day 10.

From the proposed ADI of 0.08 µg/kg body weight/day (RIVM: Jan94), a acceptable level of about 0.4 µg/l breast milk can be calculated for methyl mercury (see Annex E).

From the proposed ADI of 4 µg/kg body weight/day (RIVM: Jan94), a acceptable level of about 20 µg/l breast milk can be calculated for inorganic mercury (see Annex E).

6.3 Overall conclusion

From the above mentioned human and animal studies, it can be concluded that the concentrations of mercury due to the consumption of food contaminated with methylmercury (Fuj77, Wil80, Ske88) caused methylmercury concentrations in human breast milk which exceeded the calculated acceptable levels of 0.4 µg/l breast milk. Therefore, the committee recommends to label methylmercury with R64 (May cause harm to breastfed babies).

No appropriate data were available concerning the excretion of metallic mercury, phenyl mercury acetate and mercury salts in human or animal milk. Therefore, a lack of appropriate data precludes assessment of metallic mercury, phenyl mercury acetate and mercury salts for labelling for effects during lactation.

Proposed labelling for effects during lactation for metallic mercury

Lack of appropriate data precludes assessment of metallic mercury for labelling for effects during lactation.

Proposed labelling for effects during lactation for methyl mercury

R64.

Proposed labelling for effects during lactation for phenyl mercury acetate

Lack of appropriate data precludes assessment of phenyl mercury acetate for labelling for effects during lactation.

Proposed labelling for effects during lactation for mercury salts

Lack of appropriate data precludes assessment of mercury salts for labelling for effects during lactation.

For the committee,
The Hague, 3 May 2000

dr ASAM van der Burght, scientific secretary	dr BJ Blaauboer, chairman

References

Alc89 Alcer KH, Brix KA, Fine LJ, Kallenbach LR, Wolfe RA. Occupational mercury exposure and male reproductive health. Amer J Ind Med 1989; 15: 517-529.

Ami74 Amin-Zaki L, Elhassani SB, Majeed MA, Clarkson TW, Doherty RA, Greenwood MR. Intra-uterine methylmercury poisoning in Iraq. Pediatr 1974; 54: 587-595.

Ami79 Amin-Zaki L, Majeed MA, Elhassani SB, Clarkson TW, Greenwood MR, Doherty RA. Prenatal mercury poisoning, clinical observations over five years. AMA Dis Child 1979; 133: 172-177.

Bar73 Baranski B, Szymczyk I. Effects of mercury vapours upon reproductive function on white female rats. Medycyna Pracy 1973; 24 (3): 249-261 (see Barlow and Sullivan 1982).

Bar82 Barlow SM, Sullivan FM(editors). Reproductive Hazards of Industrial Chemicals. An evaluation of animal and human data, 1982; 386-406. Academic Press, London - New York.

Ber92 Berlin M, Hua J, Lögdberg B, Warfvinge K. Prenatal exposure to mercury vapour: effects on brain development. Fund Appl Toxicol 1992; 19: 324-326.

Bur84 Burbacher TM, Monnett C, Grant KS, Mottet NK. Methylmercury exposure and reproductive dysfunction in the nonhuman primate. Toxicol Appl Pharmacol 1984; 75: 18-24.

Cho82 Chowdhury AR, Arora U. Toxic effect of mercury on testes in different animal species. Indian J Physiol Pharmacol 1982; 26: 246-249.

Cho85 Chowdhury AR, Vachhrajani KD, Chatterjee I ydroxy-delta 5-steroid dehydrogenase in rat testicular tissue by mercuric chlorid : 45-49.

Cor91 Cordier S, Deplan F, Mandereau L, Hemon D rcury and spontaneous abortions. Br J Ind Med 1991; 48: 375-381.

Dav98 Davidson PW, Myers GJ, Cox C *et al*. Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment. JAMA 1998; 280: 701-707.

Dre98 Drexler H, Schaller K-H. The mercury concentration in breast milk resulting from amalgam fillings and dietary habits. Environ Res 1998; Section A77: 124-129.

Dzi79 Dzierzawski, A. Embryotoxic and teratogenic effects of phenylmercuric acetate and methyl mercuric chloride in hamsters rats and rabbits. Pol Arch Weter 1979; 22: 263-287.

Els88 Elsner J, Hodel B, Suter K *et al*. Detection limits of different approaches in behavioral teratology, and correlation of effects with neurochemical parameters. Neurotox Teratol 1988; 10:155-167.

Eri89 Ericson A, Källén B. Pregnancy outcome in women working as dentists, dental assistants or dental technicians. Int Arch Occ Environ Health 1989; 61: 329-333.

Fre92 Fredriksson A, Dahlgren L, Danielsson B, Eriksson P, Dencker L, Archer T. Behaviorial effects of neonatal metallic mercury exposure in rats. Toxicology 1992; 74: 151-160.

Fuj77 Fujita M, Takabatake E. Mercury levels in human maternal and neonatal blood, hair and milk. Bull Environ Contam Toxicol 1977; 18(2): 205-209.

Gal71 Gale TF, Ferm VH. Embryopathic effects of mercury salts. Life Sci 1971; 10: 1341-1347.

Gra97 Grandjean P, Weihe P, White RF. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 1997; 19: 417-428.

Gre85 Greenwood MR. Methylmercury poisoning in Iraq. An epidemiological study of the 1971-1972 outbreak. J Applied Tox 1985; 5: 148-159.

Ino89a Inouye M. Teratology of heavy metals: mercury and other contaminants. Cong Anom 1989a; 29: 333-344.

Ino89b Inouye M. Teratology of heavy metals: mercury and other contaminants. Teratology 1989b; 40 (6): 652-653.

Jan94 Janus JA, Hesse JM, Rikken MGS (eds). Aandachtstoffen in het nederlandse milieubeleid - Overzicht 1994. RIVM rapport nr 601014 006 november 1994.

Lac72 Lach H, Srebro Z. The oestrous cycle of mice during lead and mercury poisoning. Act Biol Cracoviensia Series Zoologia 1972; 15: 121-30 (see Barlow and Sullivan 1982).

Lar95 Larsson KS. The dissemination of false data through inadequate citation. J Int Med 1995; 238: 445-450.

Lau85 Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A, De Cooman S. Fertility of male workers exposed to mercury vapor or to manganese dust: a questionniare study. Amer J Ind Med 1985; 7: 171-176.

Lee75 Lee IP, Dixon RL. Effects of mercury on spermatogenesis studied by velocity sedimentation cell separation and serial mating. J Pharmacol Exp Ther 1975; 104: 171-181.

Leo83 Leonard A, Jacquet P, Lauwerys R. Mutagenicity and teratogenicity of mercury compounds. Mut Res 1983; 114: 1-18.

Mah98 Mahaffey KR. Methylmercury exposure and neurotoxicity. JAMA 1998; 280: 737-738.

Mit90 Mitsumori K, Hirano M, Ueda H, Maita K, Shirasu Y. Chronic toxicity and carcinogenciity of methyl mercury chloride in B6C3F1 mice. Fund Appl Toxicol 1990; 14: 179-190.

Moh87 Mohamed MK, Burbacher TM, Mottet NK. Effects of methyl mercury on testicular functions in Macaca fascicularis monkeys. Pharmacol Toxicol 1987; 60: 29-36.

Mur56 Murakami U, Kameyama Y, Kato T. Effects of a vaginal applied contraceptive with phenyl mercuric acetate upon developing embryos and their mother animals. Annu Rep Res Inst Environ Med Nagoya Univ 1956; 88-99.

New96 Newland MC, Warfvinge K, Berlin M. Behavioral consequences of *in utero* exposure to mercury vapor: alterations in lever-press durations and learning in squirrel monkeys. Toxicol Appl Pharmacol 1996; 139: 374-386.

Nor95 Nordenhäll K, Dock L, Vahter M. Lactational exposure to methylmercury in the hamster. Arch Toxicol 1995; 69: 235-241.

Osk96 Oskarsson A, Schütz A, Skerfving S, Palminger Hallén I, Ohlin B, Lagerkvist BJ. Total and inorganic mercury in breast milk and blood in relation to fish consumption and amalgam fillings in lactating women. Arch Environ Health 1996; 51(3): 234-241.

Pie68 Piechocka J. Chemical and toxicological studies of the fungicide phenylmercury acetate. I. Studies of the effect of food contamination with Fungitox OR on rats. Roczn Panstw Zakl Hig 1968; 39: 385-388 (Dutch translation and English abstract from Polish paper).

Pit76 Pitkin RM, Bahns JA, Filer LJ, Reynolds WA. Mercury in human maternal and cord blood, placenta and milk. Proc Soc Exp Biol Med 1976; 151: 565-567.

Row94 Rowland AS, Baird DD, Weinberg CR, Shore DL, Shy CM, Wilcox AJ. The effect of occupational exposure to mercury vapour on the fertility of female dental assistants. Occ Environ Med 1994; 51: 28-34.

Sch93 Schardein JL. Chemical Induced Birth Defects, Second Edition, Revised and Expanded. Marcel Dekker, Inc. New York, Basel, Hong Kong 1993.

She89 Shepard TH. Catalog of Teratogenic agents. John Hopkins University Press, Baltimore-London 1989; 397-400.

Sik87 Sikorski R, Juszkiewicz T, Paszkowski T, Szprengier-Juszkiewicz T. Women in dental surgeries: reproductive hazards in occupational exposure to metallic mercury. Int Arch Occ Env Health 1987; 59: 551-557.

Ske88 Skerving S. Mercury in women exposed to methylmercury thtough fish consumption, and their newborn babies and breast milk. Bull Environ Contam Toxicol 1988; 41: 475-482.

Sun91 Sundberg J, Oskarsson A, Albanus L. Methylmercury exposure during lactation: Milk concentration and tissue uptake of mercury in the neonatal rat. Bull Environ Contam Toxicol 1991; 46: 255-262.

Tho92 Thorp JM, Boyette DD, Watson WJ, Cefalo RC. Elemental mercury exposure in early pregnancy. Obstetr Gynecol 1992; 79: 874-876.

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

Vac92 Vachhrajani KD, Chowdbury AR, Dutta KK. Testicular toxicity of methyl mercury: analysis of cellular distribution pattern at different stages of seminiferous epithelium. Reprod Toxicol 1992; 6: 355-361.

Vim90 Vimy MJ, Takahashi Y, Lorschneider FL. Maternal-fetal distribution of mercury (^{203}Hg) released from dental amalgam fillings. Am J Physiol 1990; 258, R939-R945.

Vim97 Vimy JM, Hooper DE, King WW, Lorschneider FL. Mercury from maternal “Silver” tooth fillings in sheep and human breast milk. A source of neonatal exposure. Biol Trace Element Research 1997; 56: 143-152.

WHO76 WHO (1976). Environmental Health Criteria 1: Mercury. Geneva, World Health Organization.

WHO90 WHO (1990). Environmental Health Criteria 101: Methylmercury. Geneva, World Health Organization.

WHO91 WHO (1991). Environmental Health Criteria 118: Inorganic Mercury. Geneva, World Health Organization.

Wil80 Wilson JT, Brown RD, Cherek DR, Dailey JW, Hilman B, *et al*. Drug excretion in human breast milk. Clin Phamacokinet 1980; 5:1-66.

Yos92 Yoshida M, Satoh H, Kishimoto T, Yamamura Y. Exposure ot mercury via breast milk in suckling offspring of maternal guinea pigs exposedto mercury vapor after parturition. J Toxicol Environ Health 1992; 35: 135-139.

Yos94 Yoshida M, Watanabe C, Satoh H, Kishimoto T, Yamamura Y. Milk transfer and tissue uptake of mercury in suckling offspring after exposure of lactating meternal guinea pigs to inorganic or methylmercury. Arch Toxicol 1994; 68: 174-178.

Literature consulted but not cited

Bak73 Bakir F, Damluji SF, Amin-Zaki L. *et al*. Methyl mercury poisoning in Iraq. Science 1973; 181(96): 230-241.

Cla92 Clarkson T. Overview of mercury vapor toxicity, toxicokinetics, and critical target organs. Fund Appl Toxicol 1992; 19: 320-321.

Ern91 Ernst E, Lauritsen G. Effects of organic and inorganic mercury on human sperm motility. Pharmacol Toxicol 1991; 69: 440-444.

Fu93 Fu WZ. Effects of mercury exposure on reproduction in female workers. Chung Hua Yu Fang I Hsueh Tsa Chih 1993; 27: 347-349. (English abstract from Chinese paper)

Gel89 Gelbier S, Ingram J. Possible foetotoxic effects of mercury vapour: a case report. Public Health 1989; 103: 35-40.

Gon71 Goncharuk GA. Effect of chronic mercury poisoning on the immunological reactivity of offspring. Gigiena Truda 1971; 7: 73-75 (Dutch translation and see Barlow and Sullivan 1982).

Ign80 Ignatyev VM. Gonadotoxic and embryotoxic effects of metallic mercury. Gigiena Sanit 1980; 3: 72-73 (Dutch translation and see Shepard 1989).

Kuh81 Kuhnert PM, Kuhnert BR, Erhard P. Comparison of mercury levels in maternal blood, fetal cord blood, and placenta tissues. Amer J Obst Gynaecol 1981; 139: 209-213.

Lor92 Lorscheider A, Summers O, Magner PO, Boyd ND, Vimy MJ. Mercury from amalgan tooth fillings: its tissue distribution and effects on cell function. Fund Appl Toxicol 1992; 19: 321-324.

Mah98 Mahaffey KR. Methylmercury exposure and neurotoxicity. JAMA 1998; 280: 737-738.

Mot85 Mottet NK, Shaw C-M, Burbacher TM. Health risks from increase in methyl mercury exposure. Environ Health Perspect 1985; 63: 133-140.

Osk98 Oskarson A, Hallén, Sundberg J, Petersson Grawé K. Risk assessment in relation to neonatal metal exposure. Analyst 1998; 123: 19-23.

Rat96 Ratcliffe HE, Swanson GM, Fisher LJ. Human exposure to mercury: a critical assessment of evidence of adverse health effects. J Toxicol Environ Health 1996; 49: 221-270.

Row92 Rowland AS. Reproductive effects of mercury vapor. Fund Appl Toxicol 1992; 19: 326-329.

Wei84 Weigert P, Müller J, Klein H, Zufelde KP, Hillebrand J. Arsen, Blei, Cadmium un Quecksilber in und auf Lebensmitteln. ZEBS-Hefte: 1/1984, Seite 56.

Wol83 Wolff MS. Occupationally derived chemicals in breast milk. Am J Ind Med 1983; 4: 259-281.

Annexes

Annex **A**

The committee

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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**

Calculation safe levels of mercury in (human) breast milk

Assumptions:

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

Calculation safe levels of inorganic mercury in (human) breast milk

The RIVM (Jan94) proposed an ADI for inorganic mercury of 4 µg/kg body weight/day.

- Safe intake level per infant is 18 µg/infant/day.
- Safe level of mercury in breast milk is 20 µg/l.

In conclusion, the committee considers 20 µg inorganic mercury/l breast milk as a safe level.

Calculation safe levels of methylmercury in (human) breast milk

The RIVM (Jan94) proposed an ADI for methylmercury of 0.08 µg/kg body weight/day.

- Safe intake level per infant is 0.36 µg/infant/day.
- Safe level of methyl mercury in breast milk is 0.4 µg/l.

In conclusion, the committee considers 0.4 µg methylmercury/l breast milk as a safe level.

Annex **E**

Abbreviations

Abbreviations used:

ADI	Acceptable daily intake
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