Manganese and its compounds

Evaluation of the effects on reproduction, recommendation for classification

Aan de Minister van Sociale Zaken en Werkgelegenheid

Onderwerp	:	Aanbieding advies over mangaan en mangaan verbindingen
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Mijnheer de Minister,

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, 2 of 3 wat betreft de effecten op de voortplanting en stoffen die schadelijk kunnen zijn voor het nageslacht via borstvoeding. 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 - een publicatie van de commissie aan over mangaan en mangaan-verbindingen. Deze publicatie heb ik heden aan de Staatssecretaris van Sociale Zaken en Werkgelegenheid aangeboden.

Hoogachtend,
w.g.
prof. dr JJ Sixma

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Committee for compounds toxic to reproduction
A Committee of the Health Council of the Netherlands

to

the Minister and State Secretary of Social Affairs and Employment

No. 2001/02OSH, The Hague, 30 March 2001

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is "to advise the government and Parliament on the current level of knowledge with respect to public health issues..." (Section 21, Health Act).

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

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

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Samenvatting

Op verzoek van de Minister van Sociale Zaken en Werkgelegenheid beoordeelt de Gezondsheidsraad de effecten op de reproductie van stoffen waaraan mensen tijdens de beroepsuitoefening kunnen worden blootgesteld. De Commissie Reproductie- toxische 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 mangaan en mangaanverbindingen onder de loep genomen.

De aanbevelingen van de commissie zijn:

- Voor effecten op de fertiliteit adviseert de commissie mangaan en mangaanverbindingen 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 ontwikkelingsstoornissen adviseert de commissie mangaan en mangaanverbindingen 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 mangaan en mangaanverbindingen niet te kenmerken wegens onvoldoende gegevens.

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 manganese and its compounds.

The committee's recommendations are

- For effects on fertility, the committee recommends to classify manganese and its compounds in category 3 (*substances which cause concern for human fertility*) and to label manganese and its compounds with R62 (*possible risk of impaired fertility*).
- For developmental toxicity, the committee recommends to classify manganese and its compounds in category 3 (*substances which cause concern for humans owing to possible developmental effects*) and to label manganese and its compounds with R63 (*possible risk of harm to the unborn child*).
- For effects during lactation, the committee is of the opinion that due to a lack of appropriate data manganese and its compounds should not be labelled.

Chapter 1

Scope

1.1 Background

As a result of the Dutch regulation on registration of compounds toxic to reproduction that came into force on 1 April 1995, the Minister of Social Affairs and Employment requested the Health Council of the Netherlands to classify compounds toxic to reproduction. The classification is performed by the Health Council's Committee for Compounds Toxic to Reproduction according to the guidelines of the European Union (Directive 93/21/EEC). The committee's advice on the classification will be applied by the Ministry of Social Affairs and Employment to extend the existing list of compounds classified as toxic to reproduction (class 1, 2 or 3) or labelled as may cause harm to breastfed babies (R64).

1.2 Committee and procedure

The present document contains the classification of manganese 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 DH Waalkens-Berendsen at the Department of Neurotoxicology and Reproduction Toxicology of the TNO Nutrition and Food Research Institute, Zeist, The Netherlands, by contract with the Ministry of Social Affairs and Employment. The classification is based on the evaluation of published human and animal studies concerning adver-

se 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 November 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, after prenatal exposure, small reversible changes in foetal growth and in skeletal development (e.g. wavy ribs, short rib XIII, incomplete ossification) in offspring occur in a higher incidence than in the control group in the absence of maternal effects, the substance will be classified in category 3 for developmental toxicity. If

these effects occur in the presence of maternal toxicity, they will be considered as a consequence of this and therefore the substance will not be classified for developmental toxicity (see Annex C, 4.2.3.3 developmental toxicity final paragraph).

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

1.4 Data

Literature searches were conducted in the on-line databases Toxline and Medline, starting from 1966 up 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 finalizing 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 summarized in Annex D.

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* Organisation for Economic Cooperation and Development

1.5 Presentation of conclusions

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

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

1.6 Final remark

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

* for definitions see Tox95

Chapter **2**

Manganese and its compounds

2.1 Introduction

Name	:	manganese
Use	:	in a variety of industrial processes including steelmaking and in the manufacture of dry-cell batteries, as a substitute for lead in petrol, as component of several fungicides, as a contrast agent in radiodiagnostic studies
Atom weight	:	54.938
Chem formula	:	Mn
CAS no	:	7439-96-5

Manganese (Mn) can exist in 11 oxidation states from -3 to +7, with the most common valences being +2, +4 and +7. The +2 valence is the predominant form in biological systems, the +4 occurs in MnO_2 and the +7 valence is found in permanganate. Mn is an essential trace element for men and animals.

2.2 Human studies

Fertility

Exposure to very high levels of Mn may lead to sexual impotence of men (Penalver 1955 cited in WHO 1981) (Pen55).

An epidemiological study was conducted among workers recruited from a Belgian factory producing Mn salts from concentrated ores. The airborne concentration of Mn (total dust) had a median value of 0.97 mg/m^3. Mean duration of exposure was 7.9 years. During the exposure period, the number of children born was significantly lower than the expected number (Lau85).

Another epidemiological study was conducted into the effects of Mn on Belgian workers, exposed to Mn as MnO_2 dust in a dry alkaline battery plant (median atmospheric concentration of total Mn: 0.71 mg/m^3; mean duration of exposure: 6.2 years). After standardization, their fertility rate was similar to that observed in unexposed workers (Gen92).

Development

No human data concerning developmental effects of Mn were found.

Lactation

Casey (1977) reported a concentration of 20 µg Mn/l in breast milk of New Zealand women (Cas77).

Vaughan *et al.* (1979) studied the longitudinal changes in mineral content in human milk of Caucasian women in the United States and found concentrations ranging from 14 to 25 µg/l (Vau79).

Vuori *et al.* 1980 observed a Mn concentration of 4-4.5 µg/l in breast milk of Finnish women (Vuo80).

Dang *et al.* (1985) analysed the Mn content in human breast milk of economically poor Indian women and found concentrations of 20 µg/l in the colostrum of 10 tribal women and of 15 µg/l in breast milk of 19 urban women (Dan85). Mn-concentration increased with the post-partum period (1-6 months 13 µg/l, 6-12 months 23 µg/l and 1-2.5 years 36 µg/l) in undernourished city women.

Casey *et al.* (1989) reported declining Mn concentrations in breast milk of women living in Denver (United States) (3.7 µg/l at 1 months after start of lactation to 2 µg/l at 2 months and than remaining constant to 7 months, thereafter levels tended to rise) (Cas89).

Dorner *et al.* (1989) detected a mean Mn concentration in breast milk of 6.2 µg/l (depending on stage of lactation) of German women (Dor89).

Wilson *et al.* (1992) reported Mn levels of 4.1 µg/l (range 1.5-10.3) in human breast milk of mothers of preterm-babies in Northern Ireland (Wil92).

Arnaud and Favier (1995) determined Mn content in breast milk 2 days postpartum and 6 days postpartum in lactating women of France and found concentrations of 12 $\pm$ 6 µg/l and 3.4 $\pm$ 1.6 µg/l, respectively (Arn95).

No Acceptable Daily Intake (ADI) has been established in the Netherlands. Based on a TLV (threshold limit value) for Mn of 0.2 mg/m^3 (ACG99) a maximal acceptable level of about 167 µg/l breast milk might be calculated (see Annex E).

2.3 Animal studies

Tables 1 and 2 summarize the fertility and developmental studies with Mn in laboratory animals.

Fertility studies

Chandra *et al.* (1973) tested the effects of a single intraperitoneal injection of 250 mg MnO_2/kg body weight in male rabbits (Cha73). After 8 months fertility was evaluated; no pregnancies occurred when Mn-treated males were mated with untreated females. At autopsy after 4 months, testis showed a reduced size. At histopathological examination of the testis, slight oedema of interstitial tissue, degeneration and desquamation of the seminiferous tubular epithelium and reduction in number of spermatids were observed. At autopsy after 8 months testes were reduced in size. At histopathological examination, the tubular structure was disorganized, extensive desquamation and cytolysis of various elements of seminiferous epithelium, marked degeneration of spermatocytes and spermatids, and a reduced number of spermatids were observed. In the homogenate of the testis, biochemical alterations were detected. General toxicity was not described. The following remark was made by the authors:" testicular toxicity occurs much earlier than the neurological syndrome"

Singh *et al.* (1974) studied the effects of Mn after an intraperitoneal injection daily for 25 days (0 or 6 mg Mn/kg body weight per day as $MnSO_4$) in male Albino rats (Sin74). At autopsy, 48 hour after the last injection, in the testis a complete absence of spermatocytes in the degenerated seminiferous tubules was observed and in the testis homogenate decreased succinic dehydrogenase, lactic dehydrogenase, acid phosphatase and an increased ribonuclease adenosine triphosphatase was measured. Furthermore, the liver was necrotic and in the homogenates of liver and brain enzyme activity was altered (brain: decreased succinic dehydrogenase and lactic dehydrogenase and increased ribonuclease; liver: decreased succinic dehydrogenase and lactic dehydrogenase and increased ribonuclease).

Chandra *et al.* (1975) treated male hooded rats (intraperitoneally) with 0, 2 or 6 mg Mn/kg body weight per day (as $MnSO_4.4H_2O$) alone or 6 mg Mn/kg body weight per day in combination with 2 mg Zn/kg body weight per day (as $ZnSO_4.7H_2O$) during 30 days (Cha75a). At autopsy slight oedema of interstitial tissue of the testis was observed in the 2 mg/kg group. In the 6 mg Mn/kg bw group degenerative changes in the seminiferous epithelium of some tubules, depletion of spermatids, decreased number of spermatocytes were observed in the testis. In male rats treated with 6 mg Mn in combination with Zn, no pathological changes were observed in the testis. General toxicity was not described.

Murthy *et al.* (1980) treated male Macaca mulatta monkeys (by gavage) during 18 months with 0 or 25 mg $MnCl_2$/kg body weight per day (Mur80). No mortalities occurred. The monkeys developed neurological symptoms. At gross examination, the testes were swollen and a decreased testis weight was recorded. Pathological examination revealed interstitial oedema and mild degeneration of the seminiferous tubules of the testis and furthermore, reduced activities of succinic dehydrogenase, glucose-6-phosphate dehydrogenase and acid phosphatase were observed.

Eight groups of pregnant Long-Evans rats were given from gestation day (GD) 2 onwards a either a low iron (Fe) (20 mg/kg) or a normal Fe (240 mg/kg) containing basal diet supplemented with 0 or 50 mg/kg Mn as $MnSO_4$, or the same diets supplemented with 0, 350, 1050 or 3500 mg/kg Mn as Mn_3O_4 (Las82). The offspring was kept on the same diets through postnatal day 130. Body weights were only statistically significantly reduced in the low Fe diets, even in the 0 mg Mn group; male body weights was reduced through day 100, female body weights through day 60. No significant differences were detected in ovary weights. Absolute testis weights were significantly reduced in the low Fe-high Mn groups; no effect on relative testis weights was observed. No treatment-related effects were found on serum LH, FSH or testosterone concentrations. Serum testosterone concentrations were dose (Mn)-related decreased without reaching the level of statistical significance. Epididymal sperm counts were statistically significantly depressed in the low Fe-high Mn groups, at day 100 only. At 90-100 days the male and female offspring were mated within the groups. The number of pregnancies was statistically significantly reduced in the 3500 mg/kg Mn groups; litter size, ovulations, resorptions, preimplantation loss and F_2 foetal weight were normal.

Scheufler and Schmidt (1983) performed a dominant lethal test and injected male AB Jena/Halle or DBA Halle mice intraperitoneally with Mn ($MnCl_2$) at level of ½ LD_{50} (LD_{50}=121 mg/kg bw) and mated the males with untreated females for 5-6 weeks (Sch83). No effects on reproduction and pre- and postimplantation loss were observed. General toxicity was not described.

Groups of 36 female and 12 male 3 month old albino rats were given a basal diet containing 50 mg Mn/kg feed, or the same diet supplemented with 500 or 1000 mg

Mn/kg feed (as $MnSO_4$) (Var84). At the age of 6 months, each male was paired within the dose groups with 3 females, 1 per 3 weeks. General health and development of the male rats, female rats and offspring was followed. Reproductive performance was statistically significantly reduced in the 500 and 1000 mg/kg diet groups, as did the litter size, growth and survival of the offspring. The effects increased with each litter due to the cumulative effect of Mn. Histological examination of the testis and ovaries showed reduction of spermatogenesis, changes in the tubular epithelium, atretic follicles and persistence of the corpora lutea, indicative of a disfunction of the sexual organs. General toxicity was not described.

Groups of Long-Evans rat pups were dosed orally from birth to postnatal day 21 with Mn_3O_4 to obtain a daily dose of 0, 71 or 214 µg Mn/g body weight (Las85). Only slight effects were observed in body and testis weights in 21 and 28 day old pups. No effects were observed in seminal vesicle weight or tissue concentrations of Mn either on unstimulated or stimulated FSH or LH serum-concentrations. There was a depression of sustained serum testosterone concentration, which should be the result of Mn induced damage in the testicular Leydig cell, since no indication of hypothalamic or pituitary malfunction was found.

Grant *et al.* (1997) studied the effects of mangafodipir trisodium (MnDPDP, a Mn chelate, used as a contrast agent) on male and female fertility after repeated intravenous injection in Sprague Dawley rats (Gra97). Male rats were dosed daily during the premating period of 70 days with 0, 10, 30 or 100 µmol MnDPDP/kg body weight per day (0, 0.55, 1.65 or 5.5 mg Mn/kg body weight/day) and until necropsy on day 84 or 85. Male rats were mated with untreated female rats. About 50% of the rats of the high dose group had yellow discoloration of the urine. There were no treatment-related effects on mortality, feed intake or other clinical signs. Body weights were reduced in a dose-related manner and reached statistical significance in the high dose group. There were no treatment-related effects on male fertility and fecundity indices. At autopsy of the female rats there were no treatment-related effects on the numbers of corpora lutea, implantations, resorptions, foetuses, foetal body weights and the incidences of external abnormalities.

Female rats were dosed daily (intravenously) during a premating period of 15 days and during gestation up to GD 7 with 0, 10, 30 or 100 µmol MnDPDP/kg body weight per day (0, 0.55, 1.65 or 5.5 mg Mn/kg body weight/day) and mated with untreated male rats (Gra97). During treatment of the female rats there were no adverse effects on clinical signs or body weight gains. There were no adverse effects on oestrus cycling, mating performance or fertility of the treated female rats. There were no treatment-related findings at autopsy (Gra97).

Developmental toxicity

The effects of prenatal and postnatal Mn deficiency (i.e. early neonatal death, skeletal abnormalities, congenital irreversible ataxia, increased brain convulsability) were summarized by Hurley (Hur81).

Groups of 13 pregnant mice (CD-1) were placed on a casein-based diet containing a basal level of 50 mg Mn/kg diet as $MnSO_4$ (Gra80). From day 15 of lactation one group received the same diet containing an additional 1050 mg Mn/kg diet as Mn_3O_4. At postnatal day 30 the male offspring was weaned and kept on the same diet. Their growth, general appearance and the presence of gross behavioural abnormalities were recorded until postnatal day 90 when the last animals were killed. The growth and general appearance of the Mn-treated males was normal throughout the experiment, but their activity levels were significantly reduced; the growth of their reproductive tissues (seminal vesicles, testes and preputials) was statistically significantly retarded. Maternal toxicity was not described.

Pregnant female AB Jena/Halle or DBA Halle mice received a single intraperitoneal injection with Mn at levels of $^1/_{30}$ to $^1/_2$ of the LD_{50} (121 mg/kg bw) on GD day 4 (Sch83). They were killed on GD 18. At all dose levels but the lowest, preimplantational death was observed. Daily injections from GD 1-5 showed that the no effect level was $^1/_{90}$ of LD_{50} (1.34 mg/kgbody weight/day). Injection on GD 12 of doses up to 4 mg/kg body weight per day ($^1/_{30}$ of LD_{50}) resulted in embryolethality; the no effect level was $^1/_{60}$ of LD_{50}; with daily injections from GD 1-14 the no effect level was again 1.34 mg/kg body weight/day. High doses of Mn (¼ - ½ LD_{50}) on GD 4 and 10 resulted in a low incidence of teratogenic effects (runts, cleft palate, fused ribs or vertebra) in the DBA-strain, but nothing was observed in the AB strain. No effects were described concerning maternal toxicity.

Groups of about 80 random bred female Swiss (ICR) mice were exposed to MnO_2 dust or filtered air from the age of 1 month for 16 weeks (7 h/d, 5 d/w; 12 w to 50 mg/m^3, then to 85 mg/m^3), whereafter the female mice were mated with untreated males (Low84). On day 1 of gestation, the groups were split and half was exposed to MnO_2, half to filtered air for 17 d, thus creating 4 groups. Upon birth, the groups were cross-fostered. Preconceptionally exposed dams produced significantly larger litters; prenatal exposure resulted in reduced neonatal activity scores (which were intensified when the offspring was also exposed via lactation) and growth retardation that persisted into adulthood. Offspring reared by preconceptionally exposed dams had significantly lower postnatal 7 body weights, and higher day 12 activity scores. Sexually mature offspring exposed *in utero* and during lactation had a depressed rearing frequency and exploratory behaviour. Mn exposure via the mother also resulted in lower cerebral mitochondrial Mn levels. Maternal toxicity was not described in this study.

Pregnant QS mice were given a single intraperitoneal injection of 0, 12.5, 25 or 50 Mn^{2+} mg/kg body weight on GD 8, 9 or 10 (Web87). The animals were killed on GD 18. Fifty mg appeared to be embryolethal on any day of injection; 25 mg was teratogenic (low incidence of exencephaly) when administered on GD 8. On GD 9, 25 mg/kg bw caused growth retardation and embryonic loss and on GD 10 it was embryolethal. 12.5 mg on GD 8 induced 2% exencephaly, slight growth retardation on GD 9 and severe retardation and embryonic death on GD 10. The maternal effects of the intraperitoneal injections consisted of severe physiological changes, including a dose response rise in blood glucose.

The LD_{50} of $MnCl_2.4H_2O$ (single dose) in Swiss mice was established as 320 mg/kg body weight. Five groups of 20 pregnant Swiss mice were subcutaneously injected with 0, 2, 4, 8 or 16 mg $MnCl_2.4H_2O$/kg body weight on GD 6-15 (San93). The dams were killed on GD 18. 32% of the 16 mg group females died during gestation. A significant reduction of maternal body weight and food consumption were observed in the 8 and 16 mg groups, resulting in a significant reduction of carcass weight and adjusted body weight gain in these groups. Adjusted body weight gain in the 4 mg group was slightly but not significantly reduced. Gravid uterine weights and relative liver weights were also decreased in these groups, whereas absolute and relative kidney weights were increased. No significant treatment related changes were found in number of implantation sites, early resorptions, dead foetuses or sex ratio. A significant increase in late resorptions was found in the 4, 8 and 16 mg groups; in the 16 mg group 7 dams with 100% resorptions were found. The average foetal body weight decreased in a dose-related way reaching the level of statistical significance in the 8 and 16 mg groups. Significant delay in ossification of the sternebrae was found in the 4, 8 and 16 mg groups; significant reduced ossification of the parietal and occipital bones was observed in the 8 and 16 mg groups

Treinen *et al.* (1995) studied the effects of MnDPDP in Sprague Dawley rats after intravenous injection during gestation (Tre95). Several dosing regimens of MnDPDP were studied: (I) 0, 2, 5 or 20 µmol/kg body weight per day (0, 0.11, 0.275 or 1.1 mg/kg body weight/day) during GD 6-17, (II) 0, 20, 40 or 80 µmol/kg body weight per day (0, 1.1, 2.2 or 4.4 mg/kg bw/day) during GD 6-8, 9-11, 12-14 or 15-17. Furthermore, in a third study (III) $MnCl_2$ was injected intravenously during GD 6-17 at dose levels of 0, 5, 20 or 40 µmol/kg body weight per day (0, 0.275, 1.1 or 2.2 mg/kg body weight/day). In the first study (I) no effects were observed on the number of foetuses, foetal viability, number of resorptions, number of implantations and pre-and post-implantation loss. However, at 20 µmol MnDPDP/kg body weight/day, foetal body weights were significantly decreased and a significant increased number of specific skeletal abnormalities (irregularly shaped clavicle, femur, fibula, humerus, ilium, radius, scapula, tibia and/or ulna) were observed. In the second study (II), skeletal malformations identical to those observed

in the previous study, were increased in a dose-dependent manner with the highest incidences occurring in foetuses from females dosed during GD 15-17. In the third study (III) identical skeletal malformations were induced by $MnCl_2$ from dose levels of 20 µmol/kg body weight/day onwards. No maternal toxicity was observed in study (I) and (II); the effect on maternal body weight gain in the highest dose groups was related to the lower litter weights. In the third study (III) flushing of ears, eyes and/or extremities and increased post-dose irritabilty up to 30 minutes were observed. The effect on maternal body weight gain in the highest dose groups was related to the lower litter weights.

The effect of MnDPDP on developmental toxicity in New Zealand White rabbits was studied by Blazak *et al.* (1996) (Bla96). MnDPDP was intravenously injected during GD 6-18 at dose levels of 0, 5, 20, 40 and 60 µmol MnDPDP/kg body weight per day (0, 0.275, 1.1, 2.2 or 3.3 mg/kg body weight/day). Treatment with MnDPDP did not result in overt symptoms of maternal toxicity and there were no significant effects on maternal body weight gains or feed consumption. Treatment with 60 µmol MnDPDP/kg body weight per day resulted in a significant increase in post-implantation loss, but there was no significant increase in external, visceral or skeletal abnormalities at any dose.

Grant *et al.* (1997) studied the effects of MnDPDP on the developmental toxicity in Sprague Dawley rats after intravenous injections during GD 6-17 at dose levels of 0, 10, 20 or 40 µmol/kg body weight per day (0, 0.55, 1.1 or 2.2 mg/kg body weight/day) (Gra97). No treatment-related adverse effects on clinical signs or mortality were observed. Body weights were reduced in the 40 µmol/kg body weight group; this correlated with reduced foetal weights. At autopsy of the female rats, there were treatment-related effects on the incidences of post-implantation losses, total numbers of foetuses, viable and non-viable foetuses and foetal weights in the high dose group and foetal weights in the mid dose group. In the mid- and high-dose group the incidence of foetuses with skeletal abnormalities (distorted/misshapen humerus, radius, ulna, scapula, clavicle, femur, tibia and fibula) was increased. The incidence of foetuses with wavy ribs was increased in all treatment groups. Furthermore, in the rat the effects of 40 µmol MnDPDP/kg body weight per day (intravenously), 40 µmol DPDP/kg body weight per day (intravenously), 6 or 30 µmol $MnCl_2$/kg body weight per day (intravenously) (0.33 or 1.65 mg/kg body weight/day) and 400 µmol $MnCl_2$/kg body weight per day (22 mg/kg body weight/day) (orally) during GD 6-17 were studied (Gra97). There were no mortalities in any of the groups, nor treatment-related adverse effects on clinical signs, body weights, feed or water consumption. In the 6 µmol $MnCl_2$/kg body weight group foetal weight was increased and decreased in the 40 µmol MnDPDP/kg body weight and 30 µmol $MnCl_2$/kg body weight groups. Clear treatment-related skeletal abnormalities were seen in the foetuses of the dams injected with 40 µmol MnDPDP/kg body weight per

day and 30 µmol $MnCl_2$/kg body weight per day. No effect was observed in the foetuses of dams treated orally with 400 µmol $MnCl_2$/kg body weight.

Grant *et al.* (1997) also studied post-natal development after intravenous injections at dose levels of 0, 10, 20 or 40 µmol MnDPDP/kg body weight per day in Sprague Dawley rats during GD 6-17(Gra97). There were treatment-related reductions in total numbers of viable neonates at delivery in the 40 µmol MnDPDP/kg body weight group. Pup body weights and pup survival were decreased in this high dose group. Post-natal examination indicated that there were no treatment-related adverse effects on physical development. However, there were apparent adverse effects on functional development (shortened grasp/holding time, increased recovery times for surface righting ability and negative geotaxis) in the mid-and high-dose group. There were no effects on the auditory startle test or on pupil constriction.

Grant *et al.* (1997) studied the development effects of MnDPDP in New Zealand White rabbits after intravenous injections during GD 6-18 at dose levels of 0, 10, 20 or 40 µmol/kg body weight per day (0, 0.55, 1.1 or 2.2 mg/kg body weight/day) (Gra97). No treatment-related adverse effects on clinical signs or mortality or abortions were observed. At autopsy of the female rabbits, there were no treatment-related effects on the incidences of post-implantation losses, total numbers of foetuses, viable and non-viable foetuses, foetal weights and incidence of foetuses with abnormalities. An increased incidence of foetuses with incomplete ossification of the sternebrae was observed in the high-dose group.

Lactation

No animal studies concerning Mn in milk of lactating animals were available.

2.4 Conclusion

The data concerning the effects of manganese on fertility in man are ambiguous. In an epidemiological study, Lauwerys *et al.* 1985 found a decreased number of birth among workers exposed to manganese. However, Gennart *et al.* 1992 observed an unaffected fertility rate among workers exposed to manganese.

In animal studies effects on fertility were observed. Vargas *et al* (1984) found effects in rats after exposure to manganese via the diet on reproductive performance and changes in the male and female reproductive organs (Var84). Maternal toxicity was not described in this publication but not expected at the used concentrations in the diet. Murthy *et al.* (1980) dosed male Macaca mulatta monkeys by gavage and found effects on the testis in the presence of neurological effects (Mur80). Laskey *et al.* (1982) exposed pregnant rats and their offspring via the diet to manganese (Las82). Body

weights and testis weight of the male offspring were reduced. The reproductive performance of the manganese exposed F1-animals was reduced in the high dose group. Other studies via the less relevant intraperitoneal route (Cha73: rabbits, Sin 74: rats and Cha 75: rats) showed also effects on reproductive performance and or reproductive organs. Grant *et al.* 1997 found no effect on fertility after intravenous injection during a premating period of 70 days at levels which cause effects on body weights and clinical signs in male rats. The same dose levels were tested in female animals and no effects on general toxicity and fertility were detected.

In view of the animal data concerning fertility, the committee recommends to classify manganese and its compounds in category 3 (substances which may cause concern for human fertility) and to label it with R 62 (possible risk of impaired fertility).

No publications concerning developmental effects in man were found.

Manganese induced developmental effects in various species by different exposure routes. In rats, skeletal abnormalities, decreased foetal weight, increased postimplantation loss and reduced postnatal activity was found in the absence of maternal toxicity after intravenous injections with manganese (Tre95, Gra97). In mice, an increased number of resorptions, reduced foetal weights and reduced and increased activity were observed (Gra80 (maternal toxicity not clear; diet), Low84 (maternal toxicity unknown; inhalatory), Web87 (maternal toxicity; intraperitoneally), San93 (maternal toxicity; subcutaneously). Blazak *et al* observed after intravenous injections an increase in postimplantation loss in New Zealand White rabbits in the absence of maternal toxicity (Bla96).

In conclusion, specific developmental effects were observed by Treinen *et al*, Blazak *et al* and Grant *et al* at dose levels at which no maternal toxicity was observed (Tre95, Bla96 and Gra97). However, these effects were found after a less relevant (intravenous) route of exposure. Other studies (Gra80 (diet), Low84 (inhalation), Web87 (intraperitoneally) and San93 (subcutaneously)) sustain the developmental toxicity of manganese however these effects were only observed in the presence of maternal toxicity or the maternal toxicity was not clear.

For that reason the committee recommends to classify manganese in category 3 ('substances which cause concern for humans owing possible developmental effects that should be regarded as if they cause developmental toxicity in humans') and labelled with R63 (Possible risk of harm to the unborn child).

Assuming ingestion of normal amounts of breast milk, the concentrations of manganese in human breast milk, as detected during the last decades, were between 2 and 25 µg/l. However, no acceptable daily intake (ADI) was available*. In addition, no animal studies concerning the concentration of Mn in milk were available.

In conclusion, the committee recommends not to label manganese and its compounds for effects during lactation due to a lack of sufficient data.

Proposed classification for fertility

Cat 3, R 62

Proposed classification for developmental toxicity

Cat 3, R 63

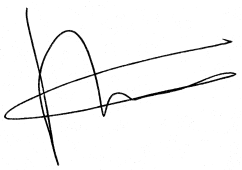
Proposed labelling for effect during lactation

No labelling.

* Based on a TLV (ACGIH) of 0.2 mg/m^3, the committee calculated a maximal acceptable intake level of 167 µg/l (breast milk).

For the committee,
The Hague, 30 March 2001

dr ASAM van der Burght,
scientific secretary



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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 Mn in (human) breast milk

F Abbreviations

Annexes

Annex **A**

The committee

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Secretarial assistance: M Javanmardi.
Lay-out: J van Kan.

Annex **B**

Comments on the public draft

A draft of the present report was released in 1999 for public review. The following persons and organisations have commented on the draft document:

- W ten Berge
  DSM, Heerlen
- EJ Kinghorn
  The Ferroalloys Association (TFA)
- P Jorgensen
  Joint European Manganese Industry Group (JEMIG)

Annex **C**

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

4.2.3 Substances toxic to reproduction

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

Category 1:

Substances known to impair fertility in humans

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

Substances known to cause developmental toxicity in humans

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

Category 2:

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

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

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

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

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

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

Category 3:

Substances which cause concern for human fertility:

Generally on the basis of:

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

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

Generally on the basis of:

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

4.2.3.2 The following symbols and specific risk phrases apply:

Category 1:

For substances that impair fertility in humans:

T; R60: May impair fertility

For substances that cause developmental toxicity:

T; R61: May cause harm to the unborn child

Category 2:

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

T; R60: May impair fertility

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

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

Category 3:

For substances which cause concern for human fertility:

Xn; R62: Possible risk of impaired fertility

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

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

4.2.3.3 Comments regarding the categorisation of substances toxic to reproduction

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

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

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

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

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

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

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

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

Effects on fertility

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

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

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

Developmental toxicity

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

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

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

Effects during Lactation

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

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

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

R64 would normally be assigned on the basis of:

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

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

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

Annex **D**

Fertility and developmental toxicity studies

Table 1.1 Fertility studies in animals with Mn .

authors	species	route	experimental period	dose	findings	remarks
Cha73	Rabbits males 20 in the Mn group and 10 controls	ip	single injection fertility testing after 8 months autopsy: after 4 and 8 months	0 or 250 mg Mn/kg bw (as MnO_2)	*After 4 months*: testis: reduced size at autopsy, slight oedema of interstitial tissue, degeneration and desquamation of the seminiferous tubules, reduction number of spermatids. *After 8 months*: no pregnancies after mating testis: reduced size. Tubular structure disorganized, extensive desquamation and cytolysis of various elements of seminiferous epithelium. Marked degeneration of spermatocytes and spermatids, reduced number of spermatids. Biochemical alterations	General toxicity not described. The following remark was made by the auteurs:" testicular toxicity occurs much earlier than neurological syndrome"
Sin74	Albino rats males (n=15/group)	ip	during 25 d autopsy 48 h after the last injection	0 or 6 mg Mn/kg bw/d (as $MnSO_4$)	complete absence of spermatocytes in degenerated seminiferous tubules; decreased succinic dehydrogenase, lactic dehydrogenase, acid phosphatase and increased ribonuclease adenosine triphosphatase Liver necrosis. Altered enzyme activity in liver and brain	

Table 1.1 Continued.

authors	species	route	experimental period	dose	findings	remarks
Cha75a	hooded rats males n=10/group	ip	during 30 d autopsy after 30 d administration	0, 2 or 6 mg Mn/kg bw/d and 6 mg Mn/kg bw/d in combination with 2 mg Zn/kg bw/d (as $MnSO_4$ and $ZnSO_4$)	*2 mg Mn*: testis, slight oedema of interstitial tissue *6 mg Mn*: testis, degenerative changes in the seminiferous epithelium of some tubules, depletion of spermatids, decreased number of spermatocytes *6 mg Mn in combination with Zn*: testis no pathological changes	General toxicity not described
Mur80	monkeys Macaca mulatta males n=4/group	gavage	during 18 months autopsy after 18 months	0 or 25 mg $MnCl_2$/kg bw/d	No mortalities. Development of neurological syndrome. Gross examination of the testis: swollen, decreased testis weight. testis: interstitial oedema and mild degeneration of the seminiferous tubules; inhibited activities of succinic dehydrogenase, glucose-6-phosphate dehydrogenase and acid phosphatase	
Las82	Long-Evans rats (females + offspring) n=6-7/group	diet	administration: GD 2 through PN d 130. Mating F1: PN 90-100	0, 350, 1050 or 3500 mg Mn_3O_4/ kg feed in either a low or normal Fe-diet	no general effects in normal Fe-diet *Low-Fe*: all groups incl. control: stat. sign. reduction bw offspring. 3500 mg Mn: stat. sign. reduction testis weight, epididymal sperm count. *Mating results both Fe-groups*: 3500 mg Mn stat. sign. reduction no. pregnancies; other parameters normal	the basic diets (20 mg/kg and 240 mg/kg Fe) contained 50 mg Mn/kg
Sch83	AB Jena/Halle, DBA Halle mice, males n=10/group untreated females	ip	single injection Mating with 3 untreated females/ male for 5-6 w autopsy females: GD 18	0 or ½ of LD_{50} $MnCl_2$ (121 mg/kg bw)	No effects on pre- or postimplantation loss; no dominant lethal effects.	no effects described general toxicity
Var84	rats (12 males and 36 females/group)	diet	administration: 3 month to end of study mating: at the age of 6 months	0, 500 or 1000 mg $MnSO_4$ /kg feed	both dose groups: stat. sign. decrease in reproduction, litter size, growth and survival offspring; cumulative effect exposure. Histological alternations of testes and ovaria.	the basic diet contained 50 mg/kg Mn

Table 1.1 Continued.

authors	species	route	experimental period	dose	findings	remarks
Las85	Long-Evans rats (male and female pups n= 11-49/group)	gavage	administration: birth to PN d 21. Autopsy: PN d 21 or 28	0, 71 or 214 µg Mn_3O_4 /g bw/d	no stat. sign. effects on body, testis or seminal vesicle weights; reduced testosterone level blood	
Gra97	Sprague Dawley rats males n=30/group mating with untreated females	iv	premating 70 d until autopsy on day 84 or 85	0, 10, 30 or 100 µmol MnDPDP/kg bw/d	10 and 30 µmol: no effect on general toxicity, no effect male fertility and litter parameters 100 µmol: 50% of the animals yellow staining of urine, decreased body weight, no effect male fertility and litter parameters	
Gra97	Sprague Dawley rats females n=25/group mating with untreated males	iv	premating 15 d and up to GD 7 autopsy GD20	0, 10, 30 or 100 µmol MnDPDP/kg bw/d	10, 30 or 100 µmol : no effect on general toxicity and female fertility and litter parameters	

n= number of animals; ip= intraperitoneal; n= number of animals; iv intravenous bw = body weight; d = day; w = week; GD = gestation day, PN = postnatal; stat. sign = statistically significant; ip = intraperitonal; no = number

Table 2.1 Developmental toxicity studies in animals with Mn.

authors	species	route	experimental period	dose	findings	remarks
Gra80	CD-1 mice (females + male offspring) n=13/group	diet	administration: PN 15 through PN 90 autopsy: PN 90	0 or 1050 mg Mn/ kg feed as Mn_3O_4	offspring: normal growth and appearance, stat.sign. reduced activity levels, retarded growth of reproductive tissues	the basis diet contained 50 mg/kg Mn
Sch83	AB Jena/Halle, DBA Halle mice females n=?/group	ip	administration: once, on GD 4 or 12, or repeated on GD 1-5 or 1-14 autopsy: GD 18	$^1/_{90}$ to ½ of LD_{50} $MnCl_2$	Single injections: preimplantation death or embryolethality, resp. No effect level: one-thirtieth (GD 4) or one-sixtieth (GD 12) of LD50 Repeated injections: idem single injections. No-effect level: 1.34 mg/kg/d (one ninetieth of LD50) ½-¼ LD50 on GD 4+10 resulted in a low incidence of teratogenic effects (runts, cleft palate, fused ribs and vertebra)	no effects described on maternal toxicity
Low84	Swiss ICR mice (females and offspring n=?/ group	inh	administration depending on group: exposure pre- or postconception, during gestation or not; offspring cross-fostered upon birth	MnO_2 dust 16 w 7h/d, 5d/w; 12 w: ± 50 mg/m^3. 4 w: ± 85 mg/m^3	preconceptional exposure resulted in sign. larger litters with sign. lower body weights and higher PN 12 activity scores; lactational and/or prenatal exposure in reduced neonatal activity scores and persisting growth retardation. Adults exposed in utero and via lactation had a depressed rearing frequency and exploratory behaviour	no effects described on maternal toxicity
Web87	QS mice females n=5-10 /group	ip	administration: once, on GD 8, 9 or 10 autopsy: GD 18	0, 12.5, 25 or 50 mg $MnSO_4$ /kg bw	maternal effects all dose groups: severe physiological changes included rise in blood glucose *50 mg*: embryolethal on every GD of injection *25 mg*: low teratogenicity after GD 8, growth retardation and embryo loss after GD 9 and embryolethal after GD 10 *12.5 mg*: slight teratogenicity after GD 8, slight growth retardation after GD 9, severe retardation and embryonic death after GD 10	

Table 2.1 Continued.

authors	species	route	experimental period	dose	findings	remarks
Sán93	Swiss mice female n= 13-19/group	ip	administration: GD 6-15 autopsy: GD 18	0, 2, 4, 8, 16 mg $MnCl_2$ /kg bw/d	*16 mg*: 32% maternal death, *16+ 8 mg:* sign. reduction food consumption, body and carcass weight, gravid uterine and relative liver weight; increase relative and absolute kidney weight; decrease foetal body weight, delay in ossification. *4 mg*: slight effect on adjusted maternal bw gain; sign. increase late resorptions	
Tre95	Sprague Dawley rats females n= 25/group	iv	1) GD 6-17, autopsy GD 20	1) 0, 2, 5 or 20 µmol MnDPDP/ kg bw/d	1) *20 µmol*: decreased foetal body weights and increased no. of skeletal abnormalities (see text)	
			2) GD 6-8, 9-11, 12-14 or 15-17, autopsy GD 20	2) 0, 20, 40 or 80 µmol MnDPDP/ kg bw/d	2) *40 µmol*: GD 15-17 increased no. of skeletal abnormalities *80 µmol*: GD 12-14 and 15-17 increased no. of skeletal abnormalities	
			3) GD 6-17, autopsy GD 20	3) 0, 5, 20 or 40 $MgCl_2$ /kg bw/d	3) *20 and 40 µmol*: GD 6-17 increased no. of skeletal abnormalities in the presence of clinical signs	
Bla96	New Zealand White rabbits females n= 22/group	iv	GD 6-18, autopsy GD 29	0, 5, 20, 40 and 60 µmol MnDPDP/ kg bw/d	*5, 20 and 40 µmol:* no effects *60 µmol:* no maternal toxicity, increased postimplantation loss, no effect on skeletal abnormalities	
Gra97	Sprague Dawley rats females n= 24/group	iv	GD 6-17, autopsy GD 20	0, 10, 20 and 40 µmol MnDPDP/ kg bw/d	In all dose groups no effect on maternal toxicity *10 µmol*: increased no. of wavy ribs *20 µmol*: decreased foetal weight, increased no. of skeletal abnormalities, increased no. of wavy ribs *40 µmol:* increased postimplantation loss, decreased no. of foetuses, decreased foetal weight, increased no. of skeletal abnormalities, increased no. of wavy ribs	
Gra97	Sprague Dawley rats females n= 24/group	1) iv	GD 6-17, autopsy GD 20	1) 0 or 40 µmol MnDPDP/kg bw/d, or 40 µmol DPDP/ kg bw/d, or 6 or 30 µmol $MnCl_2$/ kg bw/d	1) *40 µmol MnDPDP*: decreased foetal body weights and increased no. of skeletal abnormalities (see text) *40 µmol DPDP*: no effect *6 µmol $MnCl_2$*: increased foetal weight *30 µmol $MnCl_2$*: decreased foetal body weights and increased no. of skeletal abnormalities	
		2) oral		2) 400 µmol $MgCl_2$ /kg bw/d	2) *400 µmol $MnCl_2$*: no effects.	

Table 2.1 Continued.

authors	species	route	experimental period	dose	findings	remarks
Gra97	New Zealand White rabbits females n= 22/group	iv	GD 6-18, autopsy GD 29	0, 10, 20 or 40 µmol MnDPDP/ kg bw/d	10, 20 and 40 µmol: no effect on maternal toxicity 40 µmol: increased number of foetuses with incomplete ossification of the sternebrae	
Gra97	Sprague Dawley rats females dams n= 12/group; 4 F1-pups/sex/litter for functional tests	iv	GD 6-17, autopsy PN 21	0, 10, 20 and 40 µmol MnDPDP/ kg bw/d	In all dose groups no effect on maternal toxicity. *40 µmol*: decreased no. of viable pups and pup survival, shortened grasp/holding time, increased recovery times for surface righting ability and negative geotaxis. No effect on physical development and auditory startle test and pupil constriction	

n= number of animals; bw = body weight; ip = intraperitoneal; inh = inhalation; PN = postnatal day; GD = gestation day, h= hour; d= day; w= week

Annex **E**

Calculation safe levels of Mn 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)
- Breath volume 10 m^3/day (8 hour) for an adult
- An infant is as sensitive for the effects of Mn as an adult.

The ACGIH (1997) proposed a TLV of 0.2 mg/m^3 (critical effect on central nervous system).

This results in a calculated intake of 2 mg/person/day or 33.3 µg/kg body weight/day. The maximal acceptable intake level per infant is 150 µg/infant/day. The maximal acceptable level of Mn in breast milk is 167 µg/l.

In conclusion, the committee considers 167 µg Mn/l breast milk as the maximal acceptable level.

Annex **F**

Abbreviations

Abbreviations used:

- body weight
- day
- female(s)
- intraperitoneal
- intravenous
- male(s)
- number
- no adverse effect level
- Organisation for Economic Cooperation and Development
- postnatal