

This report contains the collective views of an international group of experts on available literature. The generation of this report followed a clearly defined process: literature search>evaluation of data and writing of the draft by a specialist (s)>review of the draft by a group of scientists>peer-reviewed by an expert (s) in the said field > and finalisation.

Assessment of Toxicological Endpoints for the Registration, Evaluation and Authorisation of Chemicals, Regulation (EC) No. 1907/2006 (REACH)

MANGANESE AND ITS INORGANIC COMPOUNDS:

4. REPROTOXICITY ASPECTS

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In accordance with the requirements of Regulation (EC) No. 1907/2006 (REACH), a literature search is required in order to assess relevant information available to the registrant, in order to include where appropriate the information into the technical dossier required for registration. One of the endpoints requiring information is reproductive and developmental toxicity. This report is written with a view to assisting the registrant with this requirement in mind.

The literature search strategy is detailed towards the end of the report. Briefly, 2 searches were made:

Literature search using Datastar:

Medline (1966+), Embase (1974+) and Toxfile (1966+) in December 2001, with a further update search being performed in August 2002.

A second literature search using STN:

The databases Medline, Embase, Biosis, HCAPLUS and Toxcenter were searched from 1959 to May 2009

In addition various reference lists from publically available review documents were also assessed and additional literature deemed relevant was obtained for review.

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Harlan Project 3326/C64850

Inorganic manganese

Expert Literature Evaluation Report for Reproductive Toxicity

22 October 2009

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Introduction

Manganese (Mn) is a heavy metal with interesting intrinsic properties. It is mainly used in steel production where its affinity to sulphur leads to improved material characteristics. Furthermore, it is classically used in battery production, but to a lower extent.

Manganese is an essential trace element for mammals. It is the central atom in a number of enzymes. Most of the manganese in the body is stored in the bones.

Metals have properties which can complicate toxicity evaluation, which include different kinetic and toxicological characteristics of soluble versus insoluble forms (pure metal, salts, alloys). Among soluble forms, the oxidation state can definitively influence the toxicological properties as well. That this is true for manganese was demonstrated in comparative absorption studies with manganese compounds [Dorman *et al.*, 2001, Roels *et al.*, 1997]. The different forms of manganese, especially the well-soluble like manganese chloride should be treated separately from the less soluble form like pure metal and oxides when toxicological data are evaluated in a quantitative way.

The purpose of this document is to critically evaluate the data published in the scientific literature on the potential impacts of manganese and its inorganic salts on reproductive performance. As such, this literature review is restricted to manganese and its inorganic salts. Data on organic manganese -containing compounds like maneb or mancozeb are not part of this review. Manganese acetate was not evaluated due to the organic counter-ion. Data quality was assessed by the scoring system of Klimisch [Klimisch *et al.*, 1997]¹. In addition to this review, two further reviews on Absorption, distribution, Metabolism and Excretion (ADME) and Neurotoxicity have been written (Bounds, 2009 and Gut, 2009) and focus on those two important topics that are not within the main scope of this document.

The review has been separated into three aspects of the reproductive process.

- i) Fertility and effects on reproductive organs
- ii) Pregnancy and prenatal development
- iii) Neonatal and juvenile offspring development.

For each study reviewed, a general summary and results are followed by a detailed evaluation by the author of this document.

Literature search protocol: The literature from the IEH/IOM report (2002) was screened for relevant hits, as a fully documented literature search was conducted for this report. An additional literature search covering the timeframe from 1959 up to May 2009 was performed. 58 articles considered relevant by expert judgment were selected and evaluated. More details on restrictions, references not considered relevant etc. can be found in Appendix 1.

¹ 1=reliable without restrictions, 2= reliable with restrictions, 3= not reliable, 4=not assignable

General Considerations for manganese

Manganese exposure of the general public can occur either through dietary uptake (considered the main route of exposure) or via inhalation of air / aerosols of water in air. Dermal exposure is considered negligible for the general public.

Occupational exposure is likely to be restricted to inhalation exposure. Oral exposure is considered negligible during production work, and the skin is considered to be a relatively strong barrier towards metals and salts.

The route of exposure can heavily influence toxicity of manganese and its inorganic salts. While oral absorption is relatively low (in the range of approximately 3-4%), absorption after inhalation exposure is higher. This may be partly due to the fact that uptake is not only via the lung, but direct transport along the olfactory neurons into the brain is possible at least in rodents. However, the relevance of this olfactory neuronal route to humans remains unclear.

The manganese body load is subject to homeostatic regulation. Uptake and excretion are dependent on the nutritional status, age and form of manganese administered. Thus, blood- and urine-concentrations are not reliable markers for manganese exposure [Gennart *et al.*, 1992; Ellingsen *et al.*, 2003a and b].

High manganese exposure is considered to be the cause of manganism, a neuronal central nervous disorder that affects similar regions of the brain to those affected by Parkinson's disease, and thus leads to similar symptoms. However, manganism is not sensitive to dopamine-treatment as is the case in Parkinson's disease, but rather to chelation therapy during the early stages to remove the manganese.

Manganese ions cross the placental barrier to embryos, as the manganese content of embryonic tissues was elevated after intravenous administration of radiolabelled manganese chloride to pregnant maternal animals [Koshida *et al.*, 1965; Onoda *et al.*, 1978]. Placental transfer was also demonstrated after inhalation exposure of maternal animals [Dorman *et al.*, 2005]. However, the exposure of the embryo after inhalation exposure seems to be limited, as inhalation of manganese sulphate in pregnant rats resulted in a clear elevation in manganese levels in the maternal animal, but only a small increase in livers of the embryos [Dorman *et al.*, 2001]. Limited transfer was also supported by *in vitro* data on human placenta [Miller *et al.*, 1987]. There are hints that young animals may take up more manganese than adults, but the situation is not completely clear and may just reflect a need of the neonate for more manganese to build up enzymes [Fechter, 1999].

Overall Summary and Conclusion

The quality of the reviewed studies varies largely, triggering the necessity to take this into consideration when reviewing the results. 25 out of a total of 58 evaluated studies were considered reliable (Klimisch 1 or 2), while the other studies were in most of the cases reported without giving enough details to scientifically judge on the quality of the study (Klimisch 4), or had severe shortcomings in the experimental work (Klimisch 3).

Marked differences in reaction to treatment were generally seen when different manganese compounds were administered: while studies with manganese chloride demonstrated effects on fertility and juvenile development, no effects were observed with manganese sulfate administered at considerably higher doses.

Very few of the studies addressed the relevant route of occupational exposure, which is, in the case of manganese, inhalation. Some studies on intra-tracheal administration have been indentified, but cannot be used for quantitative risk assessment, as this local administration cannot be translated into inhalation exposure due to circumventing important clearing mechanisms and high local concentrations in the lung.

A risk for reduced **fertility** of manganese workers is considered rather unlikely based on the data available, as neurotoxicity seems to occur at lower doses, and the observed effects may be secondary to this primary toxic action². However, well-designed animal studies on the relevant route of exposure with adequate doses (specifically a guideline inhalation two-generation reproduction study with full functional observation battery, ideally on different manganese compounds) are lacking, and the conclusion can only be regarded as preliminary due to conflicting evidence.

With regards to **prenatal development**, no major malformations were observed upon treatment with manganese compounds. However, skeletal effects were observed *in vivo* and *in vitro*, for manganese chloride even below the threshold for maternal toxicity. It should be noted that no skeletal effects were observed with manganese sulphate. Based on these findings, manganese compounds are not considered teratogenic, but may have a potential of embryotoxicity, depending on the compound. The relevant route of exposure is not covered, and relevant data exists only on manganese chloride and sulphate.

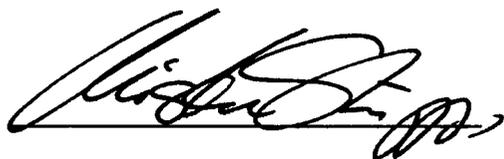
The effect of manganese exposure on **juvenile development** was investigated in several studies, however, only mechanistic studies have been conducted and no full developmental neurotoxicity study is available to judge on the endpoint. Furthermore, relevant human data are not available. A relationship between manganese chloride exposure and increased sex hormones has been established. However, no neurodegenerative changes or adverse effects on the development/learning capability of the animals have been reported in studies considered as reliable. Ideally, a developmental neurotoxicity study would give a solid database for judgement on juvenile development. However, the need of such a study based on the rather low indications for a relevant developmental effect maybe subject for discussion.

In conclusion, manganese exposure is considered rather unlikely to cause infertility at doses below the threshold for neurotoxicological symptoms. Observed effects seem to be secondary to primary neurotoxicity. Prenatal development may be affected by exposure resulting in embryotoxicity; however, the form of manganese and the route of exposure are

² for further detailed information on neurotoxicity of manganese and its compounds, please refer to the separate report on neurotoxicity of manganese by Prof. Dr. Gut

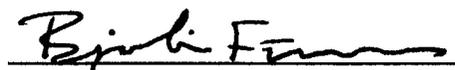
considered to have a significant impact on this endpoint. Effects on juvenile development are considered unlikely, while symptoms of neurotoxicity were observed as in adults. The study database is weak with respect to the relevant route of exposure and relevant manganese compounds found in the environment/occupational settings are missing for all endpoints.

Ittingen, Oct. 22, 09



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1. Fertility and Effects on Reproductive Organs

Several studies involving inorganic salts of manganese have been conducted in humans and animals. None of the studies involved manganese metal. Only 13 out of 28 studies could be evaluated as reliable, while the other studies in most of the cases did not provide enough details to judge on their quality.

Exposure in epidemiological studies **in humans** was in most cases through mining, where oxides are predominant. Conflicting evidence for impotence and reduced birth rates in manganese -exposed male workers was found: while one study [Lauwerys *et al.*, 1985] identified clearly reduced birth rates, another (later) study by the same working group [Gennart *et al.*, 1992] identified no relationship. In a later study by another workgroup, no effects on reproductive outcomes were identified [Jiang, 1996]. Impotence was commonly described, but in all cases as a symptom of chronic manganese poisoning/manganism, accompanied by strong neurotoxic signs [Rodier, 1955; Mena *et al.*, 1967; Penalver, 1955; Schuler, 1957; Emara *et al.*, 1971], or can be considered to be a symptom of manganism where there is lack of information on health status [Jiang, 1996]. Hypersexuality was also reported [Emara *et al.*, 1971]. Low semen quality was correlated with high blood manganese [Wirth *et al.*, 2007] or semen manganese [Wu, 1996], but no conclusion on exposure and causality can be drawn based on this study data due to blood concentration not being a reliable measure of exposure (homoeostatic regulation), and due to the presence of other heavy metals with known effects on fertility in semen of the same workers. Recent epidemiological investigations on infertility patients established correlation of blood Cd, Pb and Mo levels with infertility, but not with manganese although the level in blood was determined in the study [Meeker *et al.*, 2009 and in press]. A correlation between manganese exposure and inhibin B levels in serum was identified, while prolactin levels were not affected [Ellingsen *et al.*, 2007], or slightly increased [Mutti *et al.*, 1996]. However, the association appears rather weak. The effect of welding fumes, in which Mn is present, on male fertility and sperm quality has provided conflicting results: two studies demonstrated no effect [Bonde 1990a, Hjollund 1998] while two studies demonstrated a relatively weak effect [Bonde 1990b, Mortensen 1988]. Since Mn is one of many components of welding fumes, the relevance to Mn is not clear.

Focusing on the studies considered reliable, conflicting evidence of an association between manganese exposure and fertility remains [Gennart *et al.*, 1992; Lauwerys *et al.*, 1985], with the most recent study demonstrating no association and containing a statement that the positive study may not have been corrected for all confounders. The study on association between blood manganese and semen quality/hormone levels [Wirth *et al.*, 2007] is not considered to be appropriate for manganese, as blood is not a reliable marker of exposure to manganese. Observed effects on fertility of welders cannot be traced back to manganese-exposure, as exposure was to many heavy metals [Bonde *et al.*, 1990a/b, Hjollund *et al.*, 1998]. The slight increase in prolactin seen by Mutti *et al.* (1996) is probably not biologically significant. Furthermore, a later study [Ellingsen *et al.*, 2007] demonstrated no increase in prolactin. An association between Mn exposure and inhibin B elevation does also not appear to be biologically significant.

The **animal** experimental studies indicate toxicity to testes in rabbits [Chandra, 1973, Seth *et al.*, 1973] after intra-tracheal administration of manganese oxide, but no effects were observed in rats or mice after chronic administration of manganese sulphate in the diet for 2 years [NTP, 1993]. Oral administration of manganese chloride to monkeys was reported to produce histopathological testis damage to monkeys [Murthy *et al.*, 1980], but it was quite moderate at doses already producing neurotoxic symptoms. In a reduced two-generation study in rats, reduced fertility was found after dietary administration of trimanganese tetraoxide [Laskey *et al.*, 1982], but no effects were observed on testis and ovary weights, sperm counts or hormone levels. Subchronic (12-week) administration of manganese

chloride via drinking water to mice resulted in reduced male and female fertility, and effects on female reproductive organ weights [Elbetieha *et al.*, 2001]. Although a study with intraperitoneal injection of manganese chloride indicated effects in the rat, this study is not considered reliable due to the non-relevant route of exposure and important reporting deficiencies [Chandra, 1971]. Several studies were performed to investigate the mechanism of the observed effects. One study described markers of oxidative stress being present in the testes after *in vivo* treatment of rats with manganese chloride, but it remains unclear if this is a mechanism of toxicity or a sign of toxicity, as no full toxicity data from this study are available [Wu *et al.*, 2004]. Another study *in vitro* found an association between manganese chloride exposure of the hypothalamus and increased release of luteinizing hormone-releasing hormone and increased nitric oxide synthase [Prestfilippo *et al.*, 2007].

Focusing on the studies considered reliable, oral manganese chloride exposure seems to trigger reduced fertility in mice at very high doses [Elbetieha *et al.*, 2001]. However, neurological symptoms are considered to be present at this dose. Increased organ weights of female reproductive organs were found after administration of the chloride, but not the sulphate [NTP, 1993]. *In vitro* investigations indicate an influence of manganese on LH-RH in the hypothalamus at very high doses [Prestfilippo *et al.*, 2007].

Taken together, no clear picture of reduced male fertility (neither semen quality nor birth rates) can be derived from the existing studies in man. It seems unlikely that impotence occurs at exposure levels below the threshold of exposure causing manganism, and is likely to be a symptom of neurotoxicity rather than a specific effect on the reproductive organs. No epidemiological studies on female reproductive performance exist.

Animal data indicates toxic effects of different manganese species on fertility. However, it should be noted that the exposures used in those studies were very high compared to normal exposures, and it cannot be concluded that those effects are critical for risk assessment, as information on general toxicity and especially neurotoxicity is generally lacking in those studies. Under still very high, but more realistic dose levels (in a 2-year study in rats and mice), no pathological effects on reproductive organs were observed. Taking the mode-of-action investigations into account, at very high doses effects on reproductive organs and fertility seem to occur in experimental animals, but at more realistic exposure levels this was not demonstrated.

In conclusion, a primary risk for reduced fertility of manganese workers is considered rather unlikely based on the data available, as neurotoxicity seems to occur at lower doses, and the observed effects may be secondary to this primary toxic action. However, well-designed animal studies on the relevant route of exposure with adequate doses (specifically a guideline inhalation two-generation reproduction study with full functional observation battery, ideally on different manganese compounds) is lacking, and the conclusion can only be regarded as preliminary due to the described conflicting evidence.

1.1 Human Data

manganese compound	Reference	Data Quality acc. Klimisch
manganese oxides	Gennart <i>et al.</i> , 1992	2
	Emara <i>et al.</i> , 1971	3
	Mutti <i>et al.</i> , 1988	2
Mixed exposure: mining	Mena <i>et al.</i> , 1967	4
	Penalver, 1955	4
	Schuler <i>et al.</i> , 1957	4
	Rodier, 1955	4
	Jiang <i>et al.</i> , 1996	4
	Wu <i>et al.</i> , 1996	4
Mixed exposure: salts, oxides, carbonates	Lauwerys <i>et al.</i> , 1985	2
unknown	Wirth <i>et al.</i> , 2007	2
	Meeker <i>et al.</i> , in Press	2
	Meeker <i>et al.</i> , 2009	2
	Ellingsen <i>et al.</i> , 2007	2
	Alessio <i>et al.</i> , 1989	4
	Bonde, 1990a	2
	Bonde, 1990b	2
	Hjollund <i>et al.</i> , 1988	2
	Mortensen, 1988	4

Manganese oxides:

Study: Gennart *et al.*, 1992

Type of Study: epidemiological study, retrospective

Data Quality: Klimisch 2 (reliable with restrictions); well-documented study, non-audited, no history on wives

Number of Individuals in exposed/control group: 70/138

Sex: male

Manganese compound: manganese dioxide (dry alkaline battery plant (Belgian “blue-collar” workers))

Exposure conditions: mean duration 6.2 years, median atmosphere dust concentration of 0.71 mg/m³ airborne manganese dust

Correction for confounding factors: smoking, alcohol use (not for wives’ medical history or habits)

Results: no effect of manganese dioxide exposure towards male fertility

Executive summary: workers were occupationally exposed to manganese dioxide by inhalation at mean concentrations of 0.71 mg /m³ for a mean of 6.2 years. The birth rate was slightly reduced in the group of exposed individuals; however, this is due to the fact that the manganese -exposed group was younger in average compared to the control. After correcting for age, no effects on birth rate compared to control workers were identified.

Evaluation: state-of-the art epidemiological study, corrected for confounders, with exposure records

Study: Emara *et al.*, 1971

Type of Study: survey and reporting of old exposure data

Data Quality: Klimisch 3 (not reliable); no control group, only few details on subjects, exposure was determined 12 years before survey, duration of exposure not stated; only as support literature

Number of Individuals in exposed/control group: 36/no control group; 8 cases of chronic manganese poisoning

Sex: male

Manganese compound: dust containing 65-70% manganese oxide (not further defined)

Exposure conditions: 3/8 cases at approximately 7 mg/m³ airborne manganese dust, 5/8 at approximately 40 mg/m³ airborne manganese dust

Results: 3/8 cases of chronic manganese poisoning reported impotence, while 1 case reported hypersexuality

Executive summary: a survey on occupationally exposed workers was conducted. The scope was to determine common symptoms. Impotence and hypersexuality were reported among the cases considered to be chronically poisoned.

Evaluation: the number of cases is too small to conclude, the exposure data is too old to be correlated to the study (from 1950's, while the survey was done in the 70's; workplace protective measures may have improved and the people exposed in the 50's may have retired)

Study: Mena *et al.*, 1967

Type of Study: elimination study in man with radiolabelled manganese

Data Quality: Klimisch 4 (not assignable); level of details too low to allow conclusions

manganese compound: not specified ("manganese miners")°

Exposure conditions: not specified ("manganese miners")°

Executive summary: the main scope of the study is not reproductive toxicity; only the symptom "impotence" was extracted from the 13 cases of "chronic manganese poisoning" described. Among the 13 cases of chronic manganese poisoning, 8 out of 12 individuals described impotence among other symptoms indicative of neurotoxicity.

Evaluation: the number of cases is too small to conclude, exposure is not described. It remains unclear whether impotence is a symptom of manganism or a separate disorder.

° Typically mined ores contain mainly oxides

Review: Penalver, 1955

Scope : review on manganese exposure, symptomatic of poisoning and treatment opportunities

Data Quality: Klimisch 4 (not assignable); no raw data given

manganese compound: not specified (“manganese miners”)[°]

Exposure conditions: not specified (“manganese miners”)[°]

Executive summary: in this review, exposure conditions to manganese, symptoms of manganese poisoning and treatment opportunities are discussed. Impotency is cited as a common symptom of chronic manganese poisoning. Two opinions describe it as a symptom of manganism, one as unrelated to manganism.

Evaluation: the number of cases is too small to conclude, exposure is not described. It remains unclear whether impotence is a symptom of manganism or a separate disorder.

[°]Typically mined ores contain mainly oxides

Review: Rodier, 1955

Scope: review on manganese mining conditions in Morocco, exposure characterization in different mines, symptoms of poisoning, treatment opportunities, and protective measure to reduce exposure

Data Quality: Klimisch 4 (not assignable); no raw data given

manganese compound: not specified (“manganese miners”)[°]

Exposure conditions: not specified (“manganese miners”)[°]

Executive summary: In this review, exposure conditions to manganese in Moroccan mines are characterized, symptoms of manganese poisoning, treatment opportunities and protective measures are discussed. Impotence is cited as a common symptom of chronic manganese poisoning, occurring in 80% of the patients.

Evaluation: The number of cases is too small to conclude, exposure is not described. It remains unclear whether impotence is a symptom of manganism or a separate disorder.

[°]Typically mined ores contain mainly oxides

Review: Schuler, 1957

Scope: description of exposure conditions, description of symptoms

Data Quality: Klimisch 4 (not assignable); only few details on the definition of the symptoms

manganese compound: not specified (“manganese miners”)[°]

Exposure conditions: between 0.5 (pure rock drilling) and 46 (manganese -bearing rock drilling) mg manganese /m³

Executive summary: in this review, exposure conditions to manganese in a Chilean mine was characterized, 15 workers were diagnosed with manganese poisoning and their symptoms were described. 3/15 workers described disturbances of libido, and 3/15 disturbances in ejaculation. No controls to compare are available.

Evaluation: the number of cases is too small to conclude, exposure is not described. It remains unclear whether impotence is a symptom of manganism or a separate disorder.

° Typically mined ores contain mainly oxides

Study: Mutti *et al.*, 1988

Type of Study: epidemiological study, prolactin level in workers exposed to Mn

Data Quality: Klimisch 2 (well documented study, includes clear exposure scenario and measurements of airborne Mn)

Number of Individuals in the study: 31 exposed/34 not exposed to Mn

Sex: male

manganese compound: 95% manganese dioxide and manganese tetroxide (Ferroalloy producing plant)

Exposure conditions: airborne Mn: furnace area: 460 µg/m³ (210-980), maintenance area: 200 µg/m³ (144-560), 4 days per week for an average of 14.5 years.

Correction for confounding factors: Age, smoking, alcohol, educational level

Results: levels of Mn in blood and urine of exposed workers were higher than in workers not exposed: blood 9.84 vs 6.78 µg/ L, urine 2.69 vs. 0.40 µg/ L. Exposed workers also had higher levels of serum prolactin (9.77 ng/ mL, geometric mean) compared to unexposed workers (4.65 ng/ mL)

Executive summary: the level of Mn (blood and urine) and prolactin (serum) was measured in workers in a ferroalloy plant. A total of 31 exposed workers were compared to 34 workers with no known Mn exposure. The Mn concentration in blood and urine of exposed workers was higher than in the control group. Exposed workers also had increased concentration of serum prolactin.

Study: Jiang *et al.*, 1996

This study was not available in English, only in Chinese. Therefore, it cannot be evaluated properly and the evaluation has to rely on the evaluation in the ATSDR-review of 2001 and the abstract/tables in the publication

Data Quality: Klimisch 4 (not assignable); article only available in Chinese

Summary of the ATSDR-evaluation:

A study conducted on 314 men (with up to 35 years of exposure) from a manganese plant, where airborne concentrations of 0.145 mg/m³ were reported, showed no evidence of adverse effects on reproductive outcome (children/couple). Impotence and lack of sexual desire were higher amongst exposed individuals.

Discussion: The observed impotence/ejaculation disturbances may well be symptoms of manganism rather than a specific effect upon reproductive organs.

Study: Wu *et al.*, 1996

This study was not available in English, only in Chinese. Therefore, it cannot be evaluated properly and the evaluation has to rely on the evaluation in the ATSDR-review of 2001 and the abstract/tables given in the publication

Data Quality: Klimisch 4 (not assignable); article only available in Chinese

Summary of the ATSDR-evaluation:

In a study amongst manganese miners, ore processors and welders with airborne manganese concentrations between 0.14 and 5.5 mg/m³, miners showed decreased sperm count and viability, with welders showing decreased sperm viability. It was noted however that other metal levels (copper, nickel, chromium) in seminal fluid were also present at elevated levels, and that due to those confounders (which were demonstrated to have impact on the endpoint in this study) no direct conclusions can be drawn on manganese .

Discussion: The view of ATSDR is shared here: in presence of the other metals, no conclusion can be drawn from the data.

Unknown

Study: Wirth *et al.*, 2007

Type of Study : epidemiological study on infertility patients, determination of semen quality and blood manganese levels in parallel

Data Quality: Klimisch 2 (reliable with restrictions); well-documented study, data and statistics are considered to be reliable; however, conclusions are not considered to be derivable from the data and confounding factors were not analysed

Number of Individuals in the study: 200

Sex: male

manganese compound: not determined

Exposure conditions: no information

Correction for confounding factors: smoking, age: no significant differences between the groups, no correction applied

Results: reduced number of children in the groups where male workers were exposed to manganese compared to control workers

Executive summary: 200 patients of an infertility clinic in Michigan, USA, gave blood and semen samples. Semen quality (concentration, motility and morphology) and blood manganese levels were determined. A correlation between high manganese blood levels and low semen quality was identified.

TABLE 2. Distribution of Low Semen Parameters and High Se Level by Blood Manganese Category

	High Manganese*	
	Yes (n = 70) No. (%)	No (n = 130) No. (%)
Sperm parameter		
Low motility	31 (44)	35 (27)
Low concentration	21 (30)	22 (17)
Low morphology	41 (59)	69 (53)
High selenium	25 (36)	25 (19)

*High manganese level was defined as at or greater than the 75th percentile of the manganese distribution for the study population.

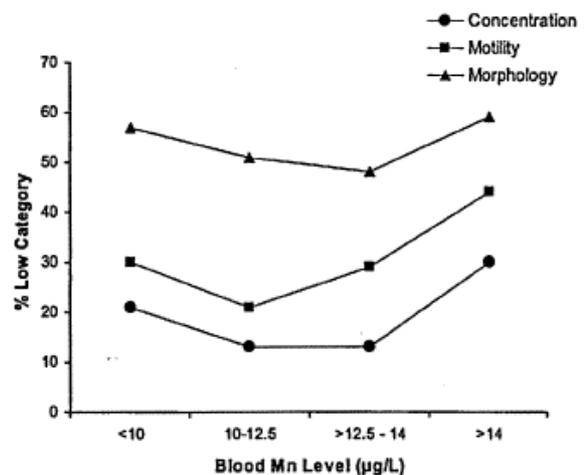


FIGURE 1. Dose-response relationships between quartiles of blood manganese levels and the sperm parameters.

Evaluation: the study is not considered to be relevant for a causal exposure-disease relationship due to the following reasons:

- blood manganese is not a reliable biomarker of exposure due to the homeostatic regulation
- other heavy metal levels (lead, mercury) were not determined and may well confound the study outcome

- elevated manganese levels in blood may well be a second symptom (together with low semen quality) of an unidentified underlying illness
- the response is U-shaped. This may be interpreted in a way that an optimum level of manganese in the blood would be ideal for reproduction, and may support the argument above

Study: Meeker *et al.*, 2009

Type of Study: epidemiological study on infertility patients, determination of blood metal levels and neuroendocrine/thyroid hormones (PART REPORT TO WIRTH *ET AL*, 2007)

Data Quality: Klimisch 2 (reliable with restrictions); well-documented study, data and statistics are considered to be reliable; however, conclusions are not considered to be derivable from the data and confounding factors were not analysed

Number of Individuals in the study: 219

Sex: male

manganese compound: not determined

Exposure conditions: no information

Correction for confounding factors: smoking, alcohol use (only described in main study publication by Wirth)

Results: no correlation between blood manganese concentration and hormone levels (prolactin, TSH)

Executive summary: workers were occupationally exposed to manganese dioxide by inhalation at mean concentrations of 0.71 mg /m³ for a mean of 6.2 years. Blood metal concentrations were determined and compared with blood hormonal levels (prolactin, TSH). While correlations with Cd, Pb, Mo were identified, no association with manganese was found.

Evaluation: state-of –the art epidemiological study. However, the study is not valid to establish a link between manganese exposure and disease, as blood manganese is not a reliable marker of exposure due to homoeostatic regulation. Several metals contribute to the potential actions, and thus conclusions are difficult to interpret. Furthermore, infertility patients form the study cohort, with obviously high percentage of fertility/sexual problems that are not associated with metal exposure.

Study: Ellingsen *et al.*, 2007

Type of Study : epidemiological study on prolactin and inhibin B levels in Mn welders

Data Quality: Klimisch 2 (reliable with restrictions); well-documented study, data and statistics are considered to be reliable;

Number of Individuals in the study: 42 exposed/42 control/23 patients diagnosed with neurotoxic symptoms

Sex: male

manganese compound: not determined; welding in a shipyard/plant producing heavy machines

Exposure conditions: mean: 121 $\mu\text{g Mn/m}^3$ (air sampling from breathing zone)

Correction for confounding factors: smoking, alcohol consumption

Results: elevated inhibin B levels in serum of exposed workers, but reduced in patients with neurotoxic symptoms

Executive summary: manganese-levels in blood and urine were determined in 96 Mn-exposed welders, 120 control subjects and 27 patients diagnose with neurotoxic symptoms who were former welders. In parallel, serum prolactin and inhibin B were determined. Exposure of the welders was estimated by air sampling parallel to the study. While prolactin levels were not correlated with Mn exposure, inhibin B levels were slightly elevated in exposed welders, but not in patients.

Evaluation: state-of-the art epidemiological study. However, the correlation observed between manganese exposure and inhibin B levels is biologically not convincing due to the large and overlapping range observed, although statistically it reaches significance.

Table II. Background characteristics, exposure and the serum concentrations of prolactin and inhibin B in 23 male patients (all former welders) who have received the diagnosis of manganism, 42 current welders (exposed) and 42 referents.

	Patients		Exposed		Referents		p ANOVA
	Mean	Range	Mean	Range	Mean	Range	
Age* (years)	50.2	41–58	46.8	41–65	48.4	41–66	0.10
Smokers (%)	47.8	–	59.5	–	52.4	–	–
No. of cigarettes per day	6.6	0–20	9.9	0–40	8.6	0–30	0.39
Alcohol consumption* (g per year)	1950	0–8112	10258	0–47320	6349	0–72800	0.01
Duration of welding (years)	22.7	15–30	21.4	1–40	–	–	–
Time since welding cessation (years)	5.7	4–7	–	–	–	–	–
B-Mn*** (nmol l ⁻¹)	152 ^d	94–345	152	84–391	127	68–258	0.07
B-Pb ^{a,****} (μmol l ⁻¹)	0.17 ^d	0.10–0.41	0.24	0.09–0.71	0.18	0.08–1.04	0.01
U-Mn ^{a,****} (nmol mmol cr ⁻¹)	0.13 ^d	0.06–0.26	0.25 ^d	0.05–2.2	0.38	0.04–21.0	0.001
Prolactin ^a (mIU l ⁻¹)	151	62–379	173	58–708	162	74–1082	0.59
Adjusted ^{a, b} (mIU l ⁻¹)	150	–	174	–	162	–	0.56
Inhibin B ^{*****} (ng l ⁻¹)	83	8–200	157	22–348	127	8–242	<0.001
Adjusted ^{c, *****} (ng l ⁻¹)	87	–	154	–	128	–	<0.001

*p <0.05 between patients and exposed; **p <0.05 between patients and referents; ***p <0.05 between exposed and referents; ^ageometric mean, otherwise arithmetic mean; ^badjusted for age and smoking status; ^cadjusted for alcohol consumption; ^done subject missing; cr, creatinine.

Study: Alessio et al. 1989

Type of Study: epidemiological study, effect of Mn on LH, FSH, and cortisol levels

Data Quality: Klimisch 4 (relevance of endpoint cortisol, confounder variables not fully accounted for, analytical performance not indicated)

Number of Individuals in the study: 14 exposed/14 control

Sex: male

manganese compound: not stated (ferrous-manganese foundry)

Exposure conditions: exposure from inhalation: rotary mill area (0.04-1.1 mg/m³) and casting furnace area (0.05-0.9 mg/m³)

Correction for confounding factors: alcohol and smoking, age, length of employment, metallurgic work background.

Results: the exposed group had higher urine concentration of Mn. Serum concentration of prolactin and cortisol was also elevated.

Executive summary: serum concentrations of FSH, LH, prolactin, and cortisol were measured in 14 male workers in a ferrous-manganese foundry and compared with a control group. The control group was selected from the general population and was

selected according to similar socioeconomic conditions, age, and length of employment. The mean levels of Mn in urine were ~8 times higher than in the control group. The exposed group also had statistically significant increased levels of serum prolactin and cortisol.

Evaluation: the effects of Mn on prolactin and cortisol in this study are relatively minor and based on a limited statistical material. The authors also acknowledge significant differences in confounder variables such as smoking and drinking between the exposed group and the control group. As the levels of cortisol have been found to be affected by a number of stress-related events it is not unlikely that these are connected. The study does not demonstrate a relevant effect of Mn on reproductive parameters.

Study: Bonde, 1990a

Type of Study: epidemiological study, subfertility in metal welders

Data Quality: Klimisch 2 (well reported study, statistical approach seemingly valid and based on good number of subjects)

Number of Individuals in the study: 339 welders, 198 non-welding metalworkers

Sex: male

manganese compound: not defined

Exposure conditions: welding fumes, >1 year welding

Correction for confounding factors: yes, including disease (maternal and paternal), tobacco and alcohol consumption

Results: an increased risk of infertility (no conception after 2 years of unprotected intercourse) was observed in welders compared to the control group. However, when confounding factors were taken into consideration the risk was reduced to insignificant levels.

Executive summary: the occupational exposure, reproductive experience, and medical history were obtained from 339 welders by a self-administered questionnaire. The control group consisted of 198 non-welding metal workers and were given the same questionnaire. Infertility was defined as no conception after 2 years of unprotected intercourse. At the time of welding exposure, there was an increased risk of sub-fertility relative to age-matched controls. When taken into consideration confounding factors (smoking/drinking habits and maternal/paternal disease) this risk was insignificant.

Evaluation: the conclusions of the study are based on a relatively solid number of exposed individuals and controls. A number of important confounder variables have been taken into consideration.

Study: Bonde, 1990b

Type of Study: epidemiological study in welders, effect on welding fumes on sperm and sex hormones

Data Quality: Klimisch 2 (well documented study, exposure levels measured and taken into consideration)

Number of Individuals in the study: 35 stainless steel welders, 46 mild steel welders, 54 non-welding metal workers (control group)

Sex: male

manganese compound: not clearly defined

Exposure conditions: welding fumes. Exposure level was measured by personal sampler and divided up into two categories based on location and type of welding: The concentration of Mn for mild steel welding: high exposed 132.1 $\mu\text{g}/\text{m}^3$ and low exposed 64.8 $\mu\text{g}/\text{m}^3$. Stainless steel welding: 4.0 $\mu\text{g}/\text{m}^3$.

Correction for confounding factors: control group were from the same geographical, occupational and social setting. Age, alcohol and tobacco consumption, and frequency of sauna use were also taken into consideration.

Results: sperm count/ejaculate, sperm motility and linear penetration rate, and proportion of normal sperm forms were significantly decreased in mild steel welders. These effects were dose dependent. There was also a non-significant increase in FSH in the exposed group. The stainless steel welders also had deteriorations in some sperm parameters.

Executive summary: semen quality and hormone levels (FSH, LH, and testosterone) were determined in workers exposed to welding fumes. Workers were recruited from six workplaces in Denmark and asked to provide a semen sample for sperm quality and blood for monitoring sex hormones. Exposure (Fe, Mn, Cu, and Cr) in the workplace was determined by personal air monitoring. A total of 46 mild steel welders and 35 stainless steel welders fulfilled the criteria of the study. The control group consisted of 54 individuals. There was a significant decrease in sperm count per ejaculate, motility and linear penetration rate and proportion of sperm with normal morphology in mild steel welders. These effects were dose-dependent (low vs high exposed groups). There was also a weak increase in FSH in mild steel welders. In

stainless steel welders, some sperm parameters were changed: decreased semen volume, sperm count per ejaculate, motility, and increase in number of immature sperm forms.

Evaluation: this study investigated the impact on welding fumes on sperm quality and sex hormones. The study is well-designed and takes into account important confounding variables. Nevertheless, participation was relatively low (37.1% for welders, 36.7% for non-welders) and might have introduced a selection bias (as suggested by the author). The effects seen on various parameters used to measure sperm quality were relatively minor. Furthermore, these parameters were still significantly above the criteria set by the World Health Organisation for healthy sperm. Most importantly, Mn is one of many chemicals present in welding fumes and the effects seen in this study can not be directly attributed to Mn.

Study: Hjollund *et al*, 1998

Type of Study: epidemiological study on sperm quality in welders

Data Quality: Klimisch 2 (well-documented study, raw data included)

Number of Individuals in the study: exposed group: 130 welders Control: 68 non-welders (no welding activity for >3 months, internal control group), 200 nonmetal workers

Sex: male

manganese compound: not defined

Exposure conditions: welding fumes, unknown Mn level

Correction for confounding factors: yes, most important being self reported reproductive disease, smoking, drinking

Results: none of the parameters measured (semen quality, testosterone, FSH, LH) were affected by exposure to welding fumes.

Executive summary: semen quality and sex hormones were measured in 130 workers exposed to welding fumes for less than 1 hour/day (38%), 1-2 hours/day (27%) or 3+ hours/day (33%). The results were compared against an internal group consisting of metal workers with no welding experience for the last 3 months (internal reference) and a group of non-metal workers (external reference). There were no differences in the parameters used to study semen quality or sex hormones.

Evaluation: the study is well designed with inclusion of potential confounding effects and as well as a relatively solid number of study subjects. Furthermore, to reduce selection bias, the cohort was selected from people with no known reproductive or fertility problems. Since the study is on general welding fumes and the actual Mn levels in the fumes is not indicated, the relevance to Mn is not known.

Study: Mortensen, 1988

Type of Study: epidemiological study on welders and reproductive success

Data Quality: Klimisch 4 (data on assessment of sperm health not included, conclusions based on limited material)

Number of Individuals in the study: 27 welders/ 2517 total

Sex: male

manganese compound: not defined

Exposure conditions: welding, exact exposure scenario not described

Correction for confounding factors: yes, living quarters, age, smoking and drinking habits, medicine use, earlier diagnosis of mumps w/wo orchitis

Results: there was an increased risk for poor sperm quality among welders compared to men not exposed to agents thought to influence sperm quality.

Executive summary: the cohort was recruited from men who had delivered a semen sample to 4 local hospitals in connection with fertility problems. A total of 2517 men having submitted a semen sample responded to a questionnaire with questions on occupational exposure, alcohol/tobacco use, and health status in general. The men were placed in one of the following categories: 1) welders, 2) metal workers not exposed to welding, 3) other industrial workers and 4) unexposed workers. Semen quality was considered poor if one of more of the following criteria was fulfilled: 1) sperm concentration less than $20 \cdot 10^6/\text{ml}$, 2) less than 50% of sperm motile and 3) less than 50% of sperm with morphological appearance. Of the 2517 men, 27 were welders. In this group, there was an increased risk of poor sperm quality compared to the men not exposed to agents believed to affect sperm quality.

Evaluation: the cohort for this study was selected among men visiting the hospital because of fertility problems and a certain level of selection bias is expected. The conclusions of the study rely heavily on the definition of- and cut-off criteria for poor sperm quality. These criteria follow roughly the criteria from the World Health Organisation, with the exception that it does not take into consideration one of the

criteria for male fertility, ejaculate volume. The study does not present any data on sperm quality and the severity of “poor sperm quality” can not be properly assessed. Since the study does not include data on Mn levels in welding fumes, the relevance to Mn is uncertain.

1.2 Animal Data

manganese compound	Reference	Data Quality acc. Klimisch
MnCl ₂	Elbetieha <i>et al.</i> , 2001	2
	Prestfilippo <i>et al.</i> , 2007	2
	Chandra, 1971	4
	Wu <i>et al.</i> 2004	4
	Murthy <i>et al.</i> , 1980	4
MnO ₂	Chandra <i>et al.</i> , 1973	4
	Seth <i>et al.</i> , 1973	4
Mn ₃ O ₄	Laskey <i>et al.</i> , 1982	4
MnSO ₄	NTP, 1993	1

Manganese oxides:

Study: Chandra, 1973

Type of Study: intra-tracheal 8 month study on reproductive performance and testis histopathology/testes enzyme activity in rabbits

Data Quality: Klimisch 4 (not assignable); the test item and its purity are not characterized, and observations after treatment are not described

Test animals: rabbit, approximately 1.5 kg, from I.T.R.C. colony (not further specified)

Housing conditions: not reported

Sex: male

Test item: manganese dioxide (source and purity not defined)

Substance administration: single intra-tracheal at 250 mg/kg bw, formulated in normal saline at 1.5 ml/animal

Dose group size: 5 control and 10 treated animals at each sacrifice

Observations during treatment: none reported

Sacrifice: after 4 and 8 months

Investigations: fertility was assessed by repeated pairing with females of proven fertility after 8 months

Post-mortem studies: excision of testes, standard histopathological processing to eosin-haematoxylin slides and staining for calcium; assessment of acid phosphatase, adenosine triphosphatase and succinic dehydrogenase in testis homogenate

Results: after 4 months: oedema, degradation of seminiferous tubules/reduced number of spermatids with abnormal morphology; after 8 months: reduced testis size (macroscopy), significantly reduced enzymatic activity on all three enzymes investigated, no fertility upon pairing (while control rabbits produced offspring with a mean litter size of 4), disorganized tubular structures with extensive desquamation and spermatid degeneration, calcification of tubules

Executive summary: male rabbits were exposed to a single intra-tracheal dose of manganese oxide in normal saline, or the vehicle only as a control. One group of males was sacrificed after 4 months of treatment and processed for histopathology. The remaining animals were paired with females of proven fertility after 8 months, and thereafter sacrificed. Testes were processed for histopathological examination, and parts of testes homogenized for enzyme activity determination.

After 4 months, degenerative changes in testis histopathology were observed. After 8 months, animals were found to be infertile in the pairing experiment. Testes appeared macroscopically smaller than those of control animals, and a higher degree of degenerative changes were observed histopathologically. The number and morphology of spermatides was affected, and enzymatic activity was significantly reduced compared to control.

Evaluation: the description of material and methods applied in this study is very short, making evaluation difficult. Other measures of toxicity are not given; it cannot be excluded that toxicity was present in the animals leading to a secondary effect on testes. Intra-tracheal administration is covering the relevant route of exposure (inhalation), but high local concentrations in the lower lung parts are reached which is physiologically not ideal. It is unclear which inhalation exposure would relate to this

manganese oxide lung burden. Only one dose group was used, no NOAEL was identified. Thus, the study is of use for mode of action investigations, but not considered adequate for quantitative risk assessment purposes.

Study: Seth *et al.*, 1973

Type of Study : intra-tracheal 2-8 month study on reproductive performance and testis histopathology/testes enzyme activity in rabbits

Data Quality: Klimisch 4 (not assignable); the test item and its purity are not characterized, procedures are not described in sufficient details

Test animals: rabbit, approximately 1.5 kg, from I.T.R.C. colony (not further specified)

Housing conditions: diet ad lib., no further details reported

Sex: male

Test item: manganese dioxide, particulate, particle size below 5 µm (source and purity not defined)

Substance administration: single intra-tracheal at 250 mg/kg bw, formulated in normal saline at 1.5 ml/animal; administration by exposing the trachea under anaesthesia and with ligature

Dose group size: 12 control and 6 treated animals at each sacrifice

Observations during treatment: general condition reported to be normal up to 6 months

Sacrifice: after 2, 4, 6 and 8 months

Post-mortem studies: excision of testes, one for standard histopathological processing to eosin-haematoxylin slides and staining for calcium; one homogenized for assessment of succinate oxidoreductase and ATPase in testis homogenate

Results: after 6/8 months: reduced testis size; after 2 months: mild oedema and 10-20% degenerative changes in seminiferous tubules; after 4 months: 30% degenerative changes in seminiferous tubules, reduction in number of spermatids; after 6 months: 50% degenerative changes in seminiferous tubules, calcification of tubules; after 8 months: disorganized tubular pattern, few entangled spermatids, patchy calcification; enzyme activity was reduced, and strength of the effect increased over time

Executive summary: male rabbits were exposed to a single intra-tracheal dose of manganese oxide in normal saline, or the vehicle only as a control. One group of males was sacrificed after 2, 4, 6 and 8 months of treatment and processed for histopathology. Testes were processed for histopathological examination, and parts of testes homogenized for enzyme activity determination. Histopathology identified increasing severity of tubular degeneration in the seminiferous tubules, and terminally reduced spermatid counts and patch calcification of the tubules. Enzymatic activity of ATPase and SDH was reduced increasingly with time, with a maximum decrease of 40% for ATPase and 78% for SDH.

Evaluation: the description of material and methods applied in this study is very short, making evaluation difficult. The test item is not defined, and the surgical exposure of the trachea for intra-tracheal administration is considered rather uncommon. Other measures of toxicity are not given; it cannot be excluded that toxicity was present in the animals leading to a secondary effect on testes. Intra-tracheal administration is covering the relevant route of exposure (inhalation), but high local concentrations in the lower lung parts are reached which is physiologically not ideal. It is unclear which inhalation exposure would relate to this manganese oxide lung burden. Only one dose group was used, no NOAEL was identified. Thus, the study is of use for mode of action investigations, but not considered adequate for quantitative risk assessment purposes.

Study: Murthy *et al.*, 1980

Type of Study : oral 18 month study on testis histopathology/testes enzyme activity in monkeys

Data Quality: Klimisch 4 (not assignable); the test item and its purity are not characterized, animal origin and housing as well as all procedures are not described in sufficient details

Test animals: *macaca mullata*, approximately 5 kg (not further specified)

Housing conditions: individual, monkey pellets and 2 bananas per day, water ad lib., no further details reported

Sex: male

Test item: manganese chloride (source and purity not defined)

Substance administration: daily 25 mg/kg bw in 1 ml physiological saline, peroral

Dose group size: 4 control and 4 treated animals

Observations during treatment: general condition

Sacrifice: after 18 months

Post-mortem studies: excision of testes, pieces for histopathological processing to eosin-haematoxylin slides and staining for calcium; air dried sections for determination of SDH, G-6-PD, NADH-diaphorase

Results: neurological symptoms at the end of treatment. Degenerative changes in seminiferous tubules were reported, affecting approximately 40% of the tissue. Reduced enzymatic activities in the sections.

Executive summary: male monkeys were exposed to a daily oral dose of 25mg/kg bw of manganese chloride in physiological saline, or the vehicle only as a control. Towards end of the study, the animals were found to have neurological symptoms. The animals were sacrificed after 18 months. Testes of treated animals appeared swollen and increased in weight compared to control. Histopathology and enzymatic activity were investigated on testis slices. Relatively mild degenerative changes were found, and enzymatic activity seemed to be decreased.

Evaluation: the description of material and methods applied in this study is very short, making evaluation difficult. The test item is not defined, and the number of animals used does not deliver a reliable database to conclude on differences. Body weight was not taken into account when discussing testis weight. Neurological symptoms were observed in the animals in parallel to relatively low testis damage, demonstrating that neurotoxicity occurs in monkeys at lower levels. Only one dose group was used, no NOAEL was identified. Thus, the study is of use for mode of action investigations, but not considered adequate for quantitative risk assessment purposes.

Study: Laskey *et al.*, 1982

Type of Study : reduced two-generation study in rats

Data Quality: Klimisch 4 (not assignable); the group size is not defined, and thus the study and its statistical basis cannot be evaluated

Test animals: pregnant Long-Evans rats from Blue Spruce Farms, (age not specified)

Housing conditions: water and diet ad lib., 12:12 h light dark

Sex: male and female

Test item: trimanganese tetroxide (Alfa product, purity 98%)

Study design: pregnant females were exposed to trimanganese tetroxide in diet from day 2 of pregnancy until weaning of the offspring. Offspring was further exposed and mated at day 90-100. Litters were culled to 3 animals/sex.

Substance administration: in diet, during pregnancy initial mother animals, continuously for the F1 generation until termination of the pregnant F1 females; at 0, 350, 1050, 3500 ppm (resulting in an estimated exposure of 30, 100 and 300 mg/kg bw/d*)

Dose group size: group size not defined

Observations during treatment: body weights were monitored at the sacrifice time points

Sacrifice: after 24, 40, 60, 100 and 224 days of age (5-8 non-littermates) for testis weight, sperm count and/or blood analysis. F1 females used for mating were sacrificed for caesarean section at the end of pregnancy for fertility parameters.

Results: no effects on body weight were observed during treatment. Reduced numbers of pregnant animals were observed in the high-dose group, while other reproductive indices/litter parameters were not affected. Testis weights seemed reduced in the high dose only, but after correction for body weight no difference to control remained. Ovary weights, testosterone, LH, FSH and epididymal sperm count remained unaffected.

Executive summary: pregnant females were exposed to trimanganese tetraoxide in diet from day 2 of pregnancy until weaning of the offspring. Offspring were further exposed and mated at day 90-100. Litters were culled to 3 animals/sex. Animals were sacrificed after 24, 40, 60, 100 and 224 days of age for testis weight, sperm count and/or blood hormone analysis. F1 females used for mating were sacrificed at the end of pregnancy for fertility and litter parameters. No effects on body weight were observed during treatment. Reduced numbers of pregnant animals were observed in the high-dose group, while other reproductive indices/litter parameters were not affected. Testis weights were reduced in the high dose only, but after correction for body weight no difference to control remained. Ovary weights, testosterone, LH, FSH and epididymal sperm count remained unaffected.

Evaluation: the study cannot be evaluated as no information on group size is given, and thus validity of the statistics cannot be guaranteed.

Parameter	Dietary Mn as Mn ₂ O ₄ (ppm)			
	0	350	1050	3500
Percent pregnant	{43} ^a 84	{45} 84	{47} 79	{24} 63 ^b
Litter size (average per litter)	{20} ^c 10.5	{19} 10.4	{20} 9.8	{10} 10.6
Ovulations (average per dam)	{20} ^c 15.6	{19} 15.2	{20} 14.5	{10} 15.0
Resorptions (average per litter)	{20} ^c 2.2	{19} 1.6	{20} 2.6	{10} 1.6
Preimplantation deaths (average per litter)	{20} ^c 2.9	{19} 3.2	{20} 2.1	{10} 2.8
Mean F ₂ fetal weights (g)	{20} ^c	{19}	{20}	{10}
Males	5.7	6.0	6.0	6.1
Females	5.5	5.6	5.6	5.8

^aNumber of females bred.

^bSignificantly different ($p < 0.05$) from control (chi-square analysis).

^cNumber of litters.

TABLE 5. Testes Weights during Chronic Exposure to Mn₂O₄

Dietary Mn as Mn ₂ O ₄ (ppm)	Weight (g) at age				
	24 d	40 d	60 d	100 d	224 d
Normal Fe diet (240 ppm)					
0 (control)	{6} ^a 0.36 ± 0.02 ^b	{6} 1.70 ± 0.14	{6} 3.15 ± 0.71	{6} 3.32 ± 0.30	{8} 3.91 ± 0.90
350	{6} 0.33 ± 0.05	{6} 1.67 ± 0.24	{6} 3.14 ± 0.40	{6} 3.12 ± 0.46	{7} 3.96 ± 0.89
1050	{6} 0.36 ± 0.05	{6} 1.54 ± 0.17	{6} 2.85 ± 0.25	{6} 3.40 ± 0.26	{8} 3.26 ± 0.82
3500	{6} 0.37 ± 0.04	{5} 1.67 ± 0.15	{6} 2.97 ± 0.62	{6} 3.56 ± 0.34	{8} 3.06 ± 0.95

* roughly estimated during summary

Manganese salts:

Study: Chandra, 1971

Type of Study: intra-peritoneal 6 month study on testis histopathology with monthly sacrifices

Data Quality: Klimisch 4 (not assignable); documentation on the materials and methods section is not sufficient to judge on the quality of the study (test item purity, vehicle, test animals not defined)

Results: reduced number of spermatids in the tubules, degeneration of the seminiferous tubules, degeneration of spermatocytes

Executive summary: rats were exposed to manganese chloride by daily intra-peritoneal injection for periods between 30 and 180 days. At monthly intervals, control rats and exposed rats were sacrificed and histopathological investigation of the testes was conducted. No differences to control were noted up to 3 months of exposure. Thereafter, reduced number of spermatids in the tubules, degeneration of the seminiferous tubules, degeneration of spermatocytes was observed, with severity increasing from the 120- to the 180-day sacrifice time point.

Evaluation: very little is reported on material and methods applied in this study, making evaluation difficult. Other measures of toxicity are not given. Although liver congestion is mentioned, it cannot be excluded that toxicity was present in the animals leading to a secondary effect on testes. Intra-peritoneal administration is not a relevant route of exposure, and bioavailability can only be guessed (especially as the vehicle is unknown). Only one dose group was used, no NOAEL was identified. Thus, the study is of limited use for mode of action investigations, and not usable for quantitative risk assessment purposes.

Study: Wu *et al.*, 2004

Type of Study : oral gavage, 4-week study in rats, determination of enzyme activity in testes at termination

Data Quality: Klimisch 4 (not assignable); article only available in Chinese, only abstract is translated

Test animals: male Wistar rats, healthy and mature

Housing conditions: not given in the abstract

Sex: male

Test item: manganese chloride (purity 99.9%)

Substance administration: daily gavage for 30 days

Dose group size: 10 animals/dose group

Observations during treatment: non mentioned in abstract

Sacrifice: after 30 days

Post-mortem studies: enzymatic activity in serum and testes

Results: increased MDA and ROS in treated animals compared to control, reduced SOD, GSH-Px, LDHx, G-6-PD compared to control.

Executive summary: rats were daily exposed to manganese chloride by oral gavage for 30 days. Animals were sacrificed and enzymatic activity/markers of oxidative stress in testis-homogenate and serum determined, and compared to vehicle-treated animals. A statistically significant relationship between manganese chloride exposure and oxidative stress was found.

Evaluation: the study cannot be used for evaluation without full translation. However, a causal relationship between oxidative stress and a disease picture (especially claiming oxidative stress being the reason for the disease state) is difficult based on the huge number of triggers for oxidative stress. Without a complete view on the health state/general toxicity in response to treatment of the animals and data on what is happening in other tissues at those exposure levels, this study is difficult to interpret.

Study: NTP, 1993

Type of Study : dietary carcinogenicity studies in rats and mice

Data Quality: Klimisch 1 (reliable without restrictions); well-documented guideline study under GLP

Test animals: 1. Rat, F344/N, from Frederik Cancer research Facility, approximately 41 days old at start of the study

2. Mouse, B6C3F1, from Frederik Cancer research Facility, approximately 41 days old at start of the study

Housing conditions: 5/cage, water and diet ad lib.,. 23.3 ±2°C/40-80% relative humidity, 12 air changes/h, 12/12h light-dark cycle

Sex: male and female

Test item: manganese sulfate (JT Baker, purity > 97%)

Substance administration: continuously in diet for 103 weeks; rats: 0, 60, 200, 615 mg/kg bw/d in males and 0, 70, 230, 715 mg/kg bw/d in females; mice: 0, 160, 540, 1600 mg/kg bw/d in males, and 200, 700 and 2250 mg/kg bw /d in females

Dose group size: 70 animals/sex/dose group (10 animals were sacrificed after 9 months, 10 after 15 months and the remaining 50 at termination)

Observations during treatment: weekly (first 13 weeks) clinical investigations, thereafter monthly; no FOB, no bleedings

Sacrifice: after 9, 15 and 24 months

Post-mortem studies: full histopathological evaluation

Results: no distinct histopathological findings in the reproductive organs after chronic exposure to manganese sulfate in diet.

Executive summary: rats and mice were exposed to manganese sulphate in the diet for 2 years. Animals were sacrificed after 9, 15 and 24 months for full histopathological evaluation.

No hints of carcinogenicity were observed, and no changes in the reproductive organs were identified.

Evaluation: the study is a fully reliable guideline compliant GLP study on chronic toxicity. A full histopathological evaluation of the animals was conducted. As no findings on the reproductive organs were observed, it can be regarded as highly likely that none were present.

Study: Prestfilippo *et al.*, 2007

Type of Study : *in vitro* mode of action study in isolated rat hypothalamus

Data Quality: Klimisch 2 (reliable with restrictions); relatively well-documented study

Test system: freshly isolated hypothalamus from male Sprague-Dawley rat, incubated in Krebs-Ringer buffer on a shaker under cell culture conditions (37°C, 5% CO₂); pooled from 7-8 rats per condition

Test item: manganese chloride (Anedra, purity not stated)

Substance incubation: 30 min. (in case of use of an inhibitor, this inhibitor was pre-incubated for 15 min. before substance addition); 50, 250 and 500 µM

Repeats: not stated

Determined endpoints: luteinizing hormone releasing hormone (LHRH) in the culture medium by radio immunoassay, Nitric Oxide Synthase and cGMP-content in hypothalamus tissue by enzymatic activity measurement

Results: at high dose only (500 µM), increased LHRH-release, NOS-activity and cGMP-content were observed. The effects were blocked by specific inhibitors

Executive summary: freshly isolated rat hypothalamus samples were pooled and incubated either with manganese chloride alone, or with manganese chloride after pre-incubation with a specific inhibitor of cGMP or nitric oxide synthase. After 30 min., luteinizing hormone releasing hormone (LHRH) in the culture medium and Nitric Oxide Synthase / cGMP-content in hypothalamus tissue were determined.

Evaluation: the study is considered reliable despite some missing critical information (number of experimental repeats, purity/impurities in test item). However, it is difficult to judge on the relevance of the observed effect without knowing about osmolarity of

the incubation solutions. In concentration ranges of 0.5 mM, this maybe critical and may well trigger the observed effects alone without a systemic action of manganese.

Study: Elbetieha *et al.*, 2001

Type of Study : 12 weeks fertility study in mice

Data Quality: Klimisch 2 (reliable with restrictions); well-documented study with a clearly defined scope and sufficiently detailed material-methods description; no statement on impurity profile of the test item, no description of observations during treatment/symptomology

Test animals: Swiss mice from Jordan University, approximately 28 g and approximately 50 days old

Housing conditions: water and diet ad lib., 21 ±1°C, 12/12h light-dark cycle, humidity and air changes not stated

Sex: male and female

Test item: manganese chloride (Riedel de Haen, purity not stated)

Substance administration: via drinking water, continuously for 12 weeks at 1000, 2000, 4000 and 8000 mg/L (=108, 172, 352, 707 mg/kg bw in males or 100, 188, 359, 635 mg/kg bw in females)

Dose group size: 14 males/dose group, 15 females/dose group (males were paired with two females each after treatment, females with a buck of proven fertility)

Observations during treatment: none described

Sacrifice: after the 12 weeks of treatment, animals were paired for 10 days and thereafter sacrifices

Post-mortem studies: body weight and weight of paired ovaries and uterus in female mice at termination

Results: significantly reduced fertility in males at high dose only, no significant effects on fertility in females

Executive summary: male and female mice were exposed to manganese chloride in the drinking water for 12 weeks. Animals were paired thereafter with non-treated females/males, respectively. Fertility was determined by the number of pregnant females. Organ weights of female reproductive organs were recorded in a second group without pairing.

Fertility of males was significantly reduced in the high dose group. Uterine and ovary weights were increased in females treated for 12 weeks.

Evaluation: the study is reliable with regards to documentation and investigated endpoints. However, doses were very high. Body weight development indicates no significant toxicity in females, but is a very crude measure without additional information on behaviour etc. The doses chosen were very high, and it remains unclear whether males were so severely intoxicated that successful pairing was not possible anymore.

Effect of long-term exposure to manganese chloride (MnCl₂) via drinking water on fertility of male mice

Treatments	No. of males	No. of females	No. of pregnant females (%)	No. of implantations ^a	No. of viable fetuses ^a	Total no. of resorptions
Control	14	28	26/28 (92)	9.00 ± 2.22	8.76 ± 3.35	7
Manganese chloride (1000 mg/l)	14	28	25/28 (89)	8.73 ± 1.68	8.50 ± 1.74	13
Manganese chloride (2000 mg/l)	14	28	22/28 (78)	8.86 ± 1.75	8.40 ± 2.23	10
Manganese chloride (4000 mg/l)	14	28	20/28 (71)	8.15 ± 1.81	7.60 ± 1.87	11
Manganese chloride (8000 mg/l)	14	28	17/28 (66)*	8.00 ± 1.96	7.70 ± 1.89	6

^a Results are expressed as mean ± S.D.

* $P < 0.05$, significantly different compared to control value (Fisher's exact test (two-tail)).

Effect of long-term exposure to manganese chloride (MnCl₂) via drinking water on fertility of female mice

Treatments	No. of females	No. of pregnant females (%)	Number of implantations ^a	Number of viable fetuses ^a	No. of mice with resorptions (%)	Total no. of resorptions
Control	15	13/15 (86)	9.41 ± 1.68	9.41 ± 1.68	0/13 (0.0)	0
Manganese chloride (1000 mg/l)	15	13/15 (86)	9.08 ± 1.62	9.00 ± 1.68	3/13 (23)	3
Manganese chloride (2000 mg/l)	15	13/15 (86)	8.42 ± 1.92	8.25 ± 2.05	2/13 (15)	2
Manganese chloride (4000 mg/l)	15	9/15 (60)	8.43 ± 2.38	8.28 ± 2.22	1/9 (11)	1
Manganese chloride (8000 mg/l)	15	10/15 (66)	7.80 ± 1.55*	7.60 ± 1.58*	2/10 (20)	2

^a Results are expressed as mean ± S.D.

* $P < 0.025$ significantly different from control value (Student's *t*-test).

Body and organ weights of female mice exposed to manganese chloride (MnCl₂) for 12 weeks via drinking water^a

Details	Treatments				
	Control	1000 mg/l	2000 mg/l	4000 mg/l	8000 mg/l
No. of animals	8	12	12	12	12
Body weight (g)	34.6 ± 6.08	32.84 ± 5.12	33.31 ± 3.40	33.21 ± 3.02	33.33 ± 4.53
Ovarian weights (mg/10 gm b.wt) ^b	2.12 ± 0.83	2.5 ± 0.74	2.4 ± 0.79	3.50 ± 1.06*	4.70 ± 2.3*
Uterine weights (mg/10 gm b.wt) ^b	23.60 ± 8.51	31.80 ± 1.28*	34.50 ± 8.12*	35.50 ± 6.9**	33.7 ± 9.7*

^a Results are expressed as mean ± S.D.

^b Relative weights.

* $P < 0.05$, significantly different compared to control value (Student's *t*-test).

** $P < 0.005$, significantly different compared to control value (Student's *t*-test).

2. Pregnancy and Prenatal Development

One study on humans is available, and several studies have been conducted in animals. None of the studies deal with manganese metal, all have been conducted with manganese compounds. Only 3 out of 8 studies could be evaluated as reliable, while the other studies in most of the cases did not provide enough details to judge on their quality.

A single **human** epidemiological study exists on the effect of manganese on pregnancy in humans [Kilburn, 1987]. The retrospective analysis was done on an isolated population living in an area rich in natural manganese and corresponding manganese mining. The exposure scenarios are not outlined but it can be assumed that it is significant since the population live in close proximity to nature and in many cases are dependent on what the land provides. There was an increase in the number of stillbirths in the population relative to a similar population living in an area where the exposure to manganese was assumed to be lower. However, a number of confounding factors are not taken into consideration and the conclusions of this study are not reliable.

The majority of the available literature on the effect of manganese on pregnancy and prenatal development of **animals** is based on manganese salts. None of the studies have been done with manganese metal. These studies were performed by dosing either manganese chloride or manganese sulfate at various points of gestation and corresponding analysis of the effect on fetuses. The most consistent observation was embryo/feto-toxicity and an increase in skeletal effects. These effects are dependent on dose and time of dosage. When dosed very early in pregnancy (day 1-3) with 50 mg/kg (subcutaneous) of manganese chloride no pregnancies were observed while a reduced number of pregnancies were observed in animals dosed on day 2-4 [Colomina *et al.* 1996] However, a 100% resorption rate was observed in the latter group. In a second study [Bataineh *et al.*, 2007], a 50 mg/kg dose (oral gavage) of manganese sulfate was administered later during gestation (day 8, 9, 10, 11) and there was significant post-implantation loss with corresponding fewer surviving pups per litter. Webster and Valois (1987) observed embryoletality when 50 mg/kg of manganese sulfate was delivered intra-peritoneally on day 8, 9, or 10. An increased incidence of resorptions was observed at lower doses. It should be noted that there is a lack of observations of maternal effects during exposure in many of these studies and no conclusions can be made regarding maternal toxicity. An increased incidence of skeletal effects was observed in three of the studies [Sanchez *et al.*, 1993; Colomina *et al.*, 1996; Grant *et al.*, 1998] and an *in vitro* study supported the findings of an effect on bone formation [Doyle, 2001]. Grant *et al.* (1988) observed skeletal abnormalities in almost half of the fetuses analyzed following intravenous administration of 30 $\mu\text{mol/kg}$ (3.8 mg/kg) while no effects were seen after 400 $\mu\text{mol/kg}$ (53 mg/kg) by oral gavage. This difference is believed to reflect bioavailability of manganese following oral administration.

Focusing on the studies considered reliable [Doyle, Sanchez, Jahrwinen], conflicting evidence of embryotoxicity (i.e. skeletal findings) was found. It is noted that one study with

manganese chloride in mice was positive for those effects, while the other study with manganese sulphate in rats was negative. Different bioavailability from the individual compounds may be the cause, or different species susceptibility, but the latter is considered less convincing when comparing the studies in mice on the fertility endpoint [Elbethieha and NTP]. The *in vitro* investigations in the micromass assay support the occurrence of skeletal findings, but have been conducted with chloride as well.

In conclusion, in the only epidemiological study of human manganese exposure, there is no proof of an effect on pregnancies and prenatal development. Animal studies indicate an effect on skeletal development, which is not considered teratogenic but embryotoxic. However, no study on the relevant route of exposure is available, and the bioavailability from different manganese compounds may affect the outcome significantly.

2.1 Human Data

manganese compound	Reference	Data Quality acc. Klimisch
Mixed exposure: mining	Kilburn, 1987	4

Study: Kilburn, 1987

Type of Study: retrospective epidemiological study and summary of neurological studies

Data Quality: Klimisch 4 (No raw data available, limited dataset and numbers, no information on statistical analysis, significant confounding factors not taken into consideration)

Number of Individuals in exposed/control group: population study (frequencies)

Sex: male and female

Manganese compound: manganese ore (primarily oxides)

Exposure conditions: relatively high concentrations found in the environment: chronic exposure via water, food, and activities

Correction for confounding factors: none

Results: no clear effect of environmental manganese on rate of stillbirths and malformations

Executive summary: this study attempts to delineate a causal effect between environmental manganese exposure and unusual neurological disorders, stillbirths, and congenital malformations in an island Aboriginal population. The area is rich in manganese deposits and manganese mining and it is speculated that the symptoms seen in the population are due to manganese exposure. The frequency of stillbirths in the Aboriginal population on Groote Eylandt was 42/1000 compared to 29.5/1000 in a similar mainland Aboriginal population (Northern Territory). The infant mortality rate was 40/1000 compared to 45.8/1000 in the mainland population. Over a 10 year period 293 children were born in the community with 8 born with congenital malformations. It was concluded that the rate of stillbirths were unusually high but a definite link to manganese exposure could not be made. The sample size was not large enough establish a link between manganese and malformations.

Evaluation: the publication is primarily focused on neurological status in subjects exposed to environmental manganese but includes some data on stillbirths and rate of malformations. Although there is a significantly increased number of stillbirths in the island population studied compared to a mainland Aboriginal population, it can not be established that manganese is involved. Furthermore, no information on access to healthcare, general health status, genetics, or other environmental/lifestyle exposures is included. The study is of very limited value in determining the impact of manganese exposure on pregnancies or prenatal development.

2.2 Animal Data

manganese compound	Reference	Data Quality acc. Klimisch
MnCl ₂	Doyle et al., 2002	2
	Colomina <i>et al.</i> , 1996	4
	Grant <i>et al.</i> , 1998	4
	Sanchez <i>et al.</i> , 1993	2
MnSO ₄	Bataineh, 2007	4
	Webster <i>et al.</i> , 1987	4
	Jahrvinen <i>et al.</i> , 1975	2

Manganese salts

Study: Colomina *et al*, 1996

Type of Study: subcutaneous administration of manganese chloride to pregnant Swiss mice on day 9, 10, 11, and 12 of gestation to determine eventual embryotoxicity and fetotoxicity

Data Quality: Klimisch 4 (lack of observations of maternal toxicity)

Test animals: adult Swiss Albino mice 28-31 g

Housing conditions: animals were kept at 22± 2°C, 40-60% humidity, 12 hour light cycle, food and water *ad libitum*

Sex: female

Test item: manganese chloride (Analytical grade, Source: Merck, Germany) in 0.9% saline (0.1 ml for 25 g animal)

Substance administration: pregnant mice were given a subcutaneous injection of 50 mg/ kg bodyweight of manganese chloride on day 9, 10, 11, or 12 of gestation

Dose group size: only number of pregnant animals at day of termination indicated (no treatment-related deaths): Control: 22, Day 9: 17, Day 10: 21, Day 11: 20, Day 12: 15

Observations during treatment: none reported

Sacrifice: Animals sacrificed on gestational day 18

Post-mortem studies: uterus was examined: number of implantation sites, resorptions and live/dead fetuses were recorded. Live fetuses were subject to weighing and examination for malformations (Stained with Bouin's for visceral examination and Alizarin for skeletal examination),

Results: no maternal deaths were observed. In animals administered manganese chloride on gestational day 9, 10, and 11, there was a significant decrease in live fetuses per litter. The bodyweight for these fetuses were slightly lower in animals dosed with manganese on day 9 and 10. There were no significant differences in the number of total implants, early resorptions, or sex ratios. No external or visceral malformations were observed. In animals exposed at all days of exposure there was a statistically significant increase in skeletal malformations.

Executive summary: to investigate the effect of manganese on rodent embryos and fetuses, pregnant mice were administered 50 mg/kg bw of manganese chloride on day 9, 10, 11, or 12 of gestation. The animals were killed on day 18 of gestation and

the contents of the uterus examined. The most significant observation was that the number of live fetuses/litter was significantly down in animals dosed on day 9, 10, or 11. Of the fetuses that were alive, there was a reduced bodyweight in animals from the day 9 and 10 dose group. The number of skeletal effects relative to control group was significantly up for day 9, 10, 11, and 12. No visceral or external effects of manganese exposure were observed.

Evaluation: the material and methods section is relatively comprehensive, including information on test animals and test substance. Since there were no reported observations of dams during pregnancy, it can not be excluded that there was maternal toxicity. Only one dose concentration was used and this prevents determining a NOAEL for the endpoints studied.

Study: Bataineh HN, 2007

Type of Study: oral gavage study to determine the impact of manganese sulfate exposure during pregnancy

Data Quality: Klimisch 4 (Limited information on substance and animals, no observations during exposure)

Test animals: Sprague-Dawley rats weighing 240-250 g

Housing conditions: animals were housed under “controlled conditions”. Individual housing during exposure and until termination

Sex: female (virgin)

Test item: manganese sulfate (analytical grade, source not stated)

Substance administration: Female pregnant rats were gavaged with 50 mg/ kg bodyweight (in 0.2 mL physiological saline) of manganese sulfate daily during day 1-3 or 2-4 of pregnancy.

Dose group size: 10 pregnant females received test substance, 10 pregnant animals received physiological saline. Similar exposures to other metals were done in parallel during the study

Observations during treatment: none reported

Sacrifice: animals sacrificed on gestational day 20 (cervical dislocation)

Post-mortem studies: implantation sites, number of live fetuses, and number of resorptions were recorded following sacrifice

Results: in animals exposed to manganese sulfate during gestational day 1-3: No pregnancies were recorded. In animals exposed to manganese sulfate during gestational day 2-4: Significantly reduced number of pregnancies (30% of control), no viable fetuses, and 100% resorption.

Executive summary: female rats were housed with a male rat of proven fertility for mating purposes. Pregnant animals were administered 50 mg/kg bodyweight manganese sulfate in 0.2 ml physiological saline by oral gavage during day 1-3 or 2-4 of pregnancy. On day 20 of pregnancy, all animals were sacrificed by cervical dislocation and the number of implantation sites, live fetuses and resorptions were recorded. In the animals dosed on day 1-3, no pregnancies were recorded and in animals dosed on day 2-4, a significantly reduced number of pregnancies were recorded with a 100% resorption rate.

Evaluation: the study is relatively well reported. Only one dose group was used and this prevents the identification of a NOAEL and limits the statistical power of the study. No data were reported on potential maternal toxicity and it can not be excluded that the effects seen were secondary to that. The route of exposure is relevant for the general public.

Study: Doyle D, 2001

Type of Study: *in vitro* cell assay to determine if manganese chloride interferes with chondrogenesis

Data Quality: Klimisch 2

Test item: manganese chloride, (source or purity not stated)

Substance administration: manganese chloride was added to tissue culture at concentrations of 0.1, 1.0, 10, 100, 1000 or 10000 μM

Results: exposure to manganese chloride reduces cell viability and differentiation of limb bud cells from 11 day mouse embryos

Executive summary: an *in vitro* tissue culture assay was used as an approach to study the impact of manganese on the developing skeleton. The *in vitro* assay is based on rodent limb bud cells differentiating into chondrocytic cells. Chemicals inhibiting this differentiation are considered teratogenic. Isolated limb bud cells from 11 day embryos were isolated and incubated in the presence of 0, 1, 10, 100, 1000, or 10000 μM manganese chloride for 5 days. After 5 days exposure the cells were fixed and stained with Alcian blue. A dose-dependent reduction in cell viability (>1 mM) and differentiation (>10 μM) was observed.

Evaluation: the study is sufficient to demonstrate an *in vitro* effect of manganese chloride on embryonic cells involved with chondrogenesis. It is noted that doses applied are very high (up to 10 mM). However, the relevance to human health is uncertain. This study is a supplementary mode-of-action study.

Study: Grant D *et al.*, 1998

Type of Study: intravenous injection or by oral gavage of manganese chloride daily from day 6-17 of gestation

Data Quality: Klimisch 4 (abstract, limited information on background and results)

Test animals: CrI:CD (SD) BR VAF/Plus rats

Housing conditions: not stated

Sex: Female

Test item: manganese chloride (purity or source not stated)

Substance administration: intravenous injection via caudal vein of 6 or 30 µmol /kg or oral gavage of 400 µmol /kg of manganese chloride. The control group was injected with physiological saline.

Dose group size: 24 rats per dose group

Observations during treatment: None stated

Sacrifice: Day 20 of gestation

Post-mortem studies: fetuses were fixed and stained with alizarin and analyzed for skeletal malformations. Whole body autoradiogram of fetuses administered a single dose of 10 µmol /kg (54) manganese chloride on day 17.

Results: no effects seen in fetuses injected with 6 µmol/kg manganese chloride or in the animals receiving 400 µmol/kg manganese chloride by oral gavage. Skeletal abnormalities were observed with a high incidence (47.1%) in animals receiving 30 µmol/kg per i.v. These effects included distortions/misshaping of humerus, radius, ulna, scapula, clavicle, femur, tibia, fibula. Fetal weight was also reduced in the 30 µmol/kg dose group but there was no impact on viability. The whole body autoradiograms revealed uptake of manganese into bones and liver of fetuses.

Executive summary: female rats were administered manganese chloride daily from day 6-17 of gestation as either oral gavage (400 µmol /kg) or intravenous injection (6 or 30 µmol /kg). Animals were sacrificed on day 20 of gestation and the fetuses were

stained and analyzed for skeletal malformations. An increased incidence of skeletal malformations was observed in animals given 30 $\mu\text{mol /kg}$ per i.v. Whole body autoradiography demonstrated uptake of manganese into bones and liver of fetuses.

Evaluation: this study is presented as an abstract and very limited information on animals, treatment, and results, is available. As such, the evaluation of the validity of the study is difficult. Nevertheless, there seems to be a relatively clear trend of skeletal abnormalities at the highest intravenous dose studied.

Study: Webster WS, 1987

Type of Study: 1) Intra-peritoneal administration of manganese sulfate to pregnant mice on gestational day 8, 9, or 10 to determine whether manganese sulfate induces structural malformations, 2) Expose rat embryos *in vitro* to various concentrations of manganese sulfate to determine the highest maternal serum concentration compatible with normal development.

Data Quality: Klimisch 4 (Limited information on test animals, substance and observations during treatment)

Test animals: QS (Quackenbush special) mice, no further information stated. Sprague-Dawley rats, no further information stated

Housing conditions: No information

Sex: Female

Test item: manganese sulfate, (source or purity not stated)

Substance administration: 1) manganese sulfate was administered to pregnant mice as a single intra-peritoneal dose (12.5, 25, or 50 mg/kg) on day 8, 9 or 10 of gestation. Control animals received sodium sulphate. 2) Rat embryos incubated in homologous serum were exposed to 25, 50, 100, or 200 ng Mg^{2+} *in vitro*.

Dose group size: 50 mg/kg dose: 5 animals per day of injection, 25 and 12.5 mg/kg dose: 10 animals per day of injection, control group: 50 animals received sodium sulphate on day 8.

Observations during treatment: no information stated

Sacrifice: animals were sacrificed on gestational day 18

Post-mortem studies: reproductive parameters observed included number of litters/live fetuses/implantations resorbed. Live fetuses were weighed and examined for malformations,

Results: 1) Manganese sulphate was embryolethal at the highest dose studied (50 mg/kg). There was an increase in abnormal fetuses with exencephaly from animals exposed to 25 mg/kg only on day 8. The same dose was embryolethal on day 10 and reduced bodyweight and embryonic loss was seen following treatment on day 9. A slightly increased incidence of exencephaly was observed in the lowest dose group treated on day 8. After treatment on day 9 and 10 growth retardation was evident in the same dose group. 2) Embryos exposed to the highest concentration of manganese sulfate *in vitro* (200 ng/ ml) had dysmorphic growth and 50% had open neural tube. A reduction in embryonic growth was observed in both the 200 and the 100 ng/ml dose groups.

Executive summary: the effect of manganese sulfate on embryonic growth and organogenesis was investigated using a double approach. Pregnant mice were injected intraperitoneally with 12.5, 25, or 50 mg/kg of manganese sulfate on day 8, 9, and 10 of gestation. On day 18 the animals were sacrificed and the fetuses examined. In the second approach, rat embryos were removed from the dam at day 9 of gestation and exposed *in vitro* to manganese ²⁺ at 25, 50, 100, or 200 ng/ml. In embryos exposed to manganese sulfate *in utero*, embryolethality was observed at the highest dose. Exencephaly was observed in the middle dose on day 8 while the same dose caused growth retardation on day 9 and was embryolethal on day 10. Dysmorphic growth and open neural tube was common in embryos exposed to the 200 ng/ml manganese ²⁺ *in vitro*.

Evaluation: a limited experimental section, particularly on animal background and observations reduces the strength of the study. There were also no reported observations of possible maternal effects during exposure. Intraperitoneal administration is not relevant for human exposure, and the high local concentrations applied in the *in vitro* model are not representative for human exposure conditions. The study can be used as supplementary information, but not for quantitative risk assessment purposes.

Study: Jahrvinen *et al.*, 1975

Type of Study: oral administration of manganese sulfate to pregnant rats to determine the effect on embryofetal development and on tissue levels of manganese, Fe, Cu, and Zn in pregnant rats and their fetuses.

Data Quality: Klimisch 2 (test item purity and source not indicated)

Test animals: Sprague-Dawley

Housing conditions: individual wire bottom stainless steel cages, 20-24°C, 12 hour light cycle, food and water *ad libitum*

Sex: Female

Test item: manganese sulfate (purity and source not indicated)

Substance administration: manganese sulfate was added to diet to a final concentration of manganese of 24, 54, 154, 504, or 1004 mg/kg dry diet (estimated mg/kg of bodyweight: 2, 5, 13, 42, 84 mg/kg bw)*, and administered for eight weeks prior to mating and during pregnancy

Dose group size: 17 animals in each dose group

Observations during treatment: animals were weighed at the start of the study, week 8 and on gestational day 0 (day of fertilization) and 21, blood samples taken for haematological examination on day 21.

Sacrifice: Animals were sacrificed on gestational day 21

Post-mortem studies: fetuses: weighed, examination for gross malformations, skeletal staining, determination of Mn, Fe, Cu, and Zn concentration in body. Maternal: number of implantation sites, resorptions and fetuses were recorded, liver isolated and determination of concentration of Mn, Fe, Cu and Zn was done.

Results: manganese did not cause gross malformations or bone structure anomalies at the concentrations used in this study. Fetal weight was also not affected.

Executive summary: manganese sulfate was administered to female rats in the diet at concentration of 24, 54, 154, 504, or 1004 mg manganese /kg dry diet for 8 weeks. After 8 weeks the animals were allowed to mate. On gestational day 21 the animals were sacrificed, the uterus removed and the number of fetuses, implantation sites and resorptions were determined. The fetuses were subject to skeletal staining and the concentrations of manganese, Fe, Cu and Zn were determined in both whole fetus and maternal liver. There was no impact on fetuses at any of the doses studied. There was also no impact on reproductive parameters at any dose level. It was also observed that increasing dietary concentrations of manganese caused a decrease in iron concentration in the fetus and in the maternal liver. The effect of manganese in diet on concentration of Cu and Zn in fetus and maternal liver was relatively modest.

Evaluation: a relatively comprehensive study summary including detailed information on manganese content in animal diet. Since only the level of manganese in diet is indicated, the actual dose levels can not be established. As a rough indication of

exposure, the level of manganese in food can be divided by a factor of 10-12 to obtain an estimated dose in mg/kg bodyweight.

* roughly estimated during summary

Study: Sanchez *et al*, 1993

Type of Study: subcutaneous administration of manganese chloride from gestation day 6 to 15

Data Quality: Klimisch 2

Test animals: Swiss albino mice 28-32 g

Housing conditions: controlled conditions, 22±2°C, 50±10% humidity, 12 hour light cycle, food and water *ad libitum*

Sex: male and female

Test item: manganese chloride·4H₂O (analytical grade, Merck)

Substance administration: manganese chloride at dose level of 2, 4, 8, or 16 mg/kg/day was administered subcutaneously in 0.9% saline (0.1 ml/ mouse). Control animals received only saline

Dose group size: 20

Observations during treatment: body weight, food consumption, and general appearance

Sacrifice: day 18 of gestation

Post-mortem studies: implantation sites, resorptions, dead/live fetuses, live fetuses were weighed and checked for external abnormalities. Animals were either fixed in Bouin's for visceral anomalies or stained with Alizarin for skeletal defects

Results: dams: 32% of dams died in the highest dose group, 16 mg/kg. In the next two dose groups there was a decrease in both body weight and food consumption at different time points. Relative liver weight was reduced in the highest dose group while relative and absolute kidney weights were increased in the same dose group. Gestational parameters: with the exception of an increase in the number of late resorptions (4, 8, 16 mg/kg) no other effects were seen. Pups: Reduced bodyweight was observed with increasing doses of manganese chloride. A statistically significant increased incidence of wavy ribs and reduced/delayed ossification was observed in the highest dose levels. No external or visceral effects were seen.

Executive summary: female mice were administered either 2, 4, 8, or 16 mg/kg of manganese chloride daily from day 6-15 of gestation. Maternal toxicity was observed at the highest dose level with significant mortality (32%). There was also a significant effect on body weight (decrease), organ weight (liver and kidney), as well as reduced food consumption. The only gestational parameter affected by manganese chloride treatment was an increase in late resorptions. In pups, increasing concentrations of manganese chloride caused decreasing bodyweight. Relatively minor skeletal effects were also observed.

Evaluation: the conclusions of this study are strengthened by the use of multiple dose groups. Since severe maternal toxicity was observed it can not be excluded that the effect on late resorptions and on pups is due to this effect.

3. Neonatal and Juvenile Offspring Development

Two studies on humans are available, and several studies have been conducted in animals. None of the studies investigated manganese metal, all have been conducted with manganese compounds. Only 10 out of 22 studies could be evaluated as reliable, while the other studies, in most cases did not provide enough details to judge on their quality.

One **human** epidemiology study established a correlation between manganese concentration in water from local wells and infant mortality in an area in Bangladesh [Hafeman *et al.*, 2007]. The cohort was part of a study on the effects of arsenic in drinking water and the size of the cohort and the information available on the individual subjects were considerable. There were some significant limitations of the study, including limited information on the well water (such as contamination by bacteria and organic compounds) and the different exposure scenarios (breast milk vs. direct exposure to drinking water). The authors acknowledged these limitations and suggested future studies to establish a more reliable relationship. A second epidemiology study [He *et al.*, 1994] described lower scores of behavioural tests in children with higher manganese exposure (as determined in hair); however, the article is in Chinese and it cannot be determined if the results of this study were affected by confounders.

The majority of **animal** studies examined the effects of manganese salts on offspring and their development, using neurological tests for the assessment of their effects. Since neurotoxicity is one of the symptoms observed in humans exposed to manganese, developmental neurotoxicity is a valid endpoint in offspring. The relevance to humans of some of the methods used, and the reliability of some of the studies can be questioned. Administration of manganese to lactating dams produced appreciable amounts of manganese in their suckling offspring, often at higher levels than those found in the dams [Seth *et al.*, 1977; Dorman *et al.*, 2000]. An effect on enzymes involved in neurochemistry and neurological pathways was observed in a number of studies [Seth *et al.*, 1977; Chandra *et al.*, 1978; Deskin *et al.*, 1981; Pappas *et al.*, 1996; Dorman *et al.*, 2000; Lai *et al.*, 1984]. The magnitude of these effects was relatively modest, and therefore no trend was established. The relevance to humans is unclear, and the studies can be seen as mode-of-action investigations. Results from behavioural and activity tests of offspring following exposure indicated reduced activity in animals exposed to manganese dioxide and trimanganese tetroxide by maternal inhalation [indicated by Lown *et al.*, 1984; Gray *et al.*, 1980], while hyperactivity and increased acoustic startle reflex was reported following oral exposure to manganese chloride [Pappas *et al.*, 1996, Dorman *et al.*, 2000]. Several studies have investigated the effect of various manganese forms on hormone status. The most consistent effects in these studies have been an increase in levels of LH, FSH, LHRH and testosterone/estradiol. In making an evaluation of the hormonal effects of manganese it should be considered that the majority of these studies (3 of 4) have come from the same group [Lee *et al.*, 2006, Lee *et al.*, 2007, and Pine *et al.*, 2005] and using the same

experimental system. Nevertheless, the experimental systems and relatively clear effects seem sound and reliable. Conflicting results have been seen with regards to results from histopathological examination of brains from exposed pups. The most reliable study [Dorman *et al.*, 2000] did not demonstrate any histopathological effect following 21 days of direct peroral (gavage) dosing of 25 or 50 mg/kg manganese chloride, while Chandra *et al.* (1978) observed relatively severe neurodegeneration in the cerebral and cerebellar cortex following administration of 50 µg/rat (1-1.25 mg/kg at start of study) of manganese chloride by gavage. No changes in histopathology were seen in the studies performed by Seth *et al.* (1977), where neonates were exposed indirectly via milk, or by Kristenssen *et al.* (1986) following high doses (150 mg/kg) of manganese chloride delivered directly to the neonate by oral gavage. When taken into consideration the relatively low level of manganese chloride used for exposures in the study by Chandra *et al.* (1978), compared to Dorman *et al.* (2000), and the general reliability of the study, it is believed that the severe neurodegeneration observed by Chandra *et al.* (1978) is not directly related to manganese chloride. This is further supported by unexplained deaths of treated animals in the study by Chandra.

Focusing on the studies considered reliable, indications of hyperactivity/increased acoustic sensitivity in exposed pups are reported [Pappas *et al.*, 1997; Dorman *et al.*, 2001], but of little prominence. Inhalation exposure of pregnant animals led to reduced activity in pups [Lown *et al.*, 1984]. While oral administration of manganese chloride had no effect on neurotransmitter levels in the brain [Pine *et al.*, 2005; Dorman *et al.*, 2000; Kontur *et al.*, 1988; Lee *et al.*, 2006] direct injection into the brain led to increased sexual hormone levels in blood [Pine *et al.*, 2005; Lee *et al.*, 2006]. This was confirmed *in vitro* on hypothalami from immature rats [Lee *et al.*, 2006/7; Pine *et al.*, 2005]. No alterations in neuropathology were not observed in the studies considered reliable.

In conclusion, the only significant trend relating to manganese exposure in neonates is a general increase of sex hormones in both males and females, in the absence of neuropathological changes. Slight hyperactivity/hypersensitivity might occur, while other studies reports reduced activity. However, a suitable study (i.e. developmental neurotoxicity study) is not available to complete the view on post-natal developmental effects of manganese exposure.

3.1 Human Data

manganese compound	Reference	Data Quality acc. Klimisch
Mixed exposure: mining	Hafeman <i>et al.</i> , 2007	2
Undefined exposure: drinking water	He <i>et al.</i> , 1994	4

Study: Hafeman *et al.*, 2007

Type of Study : retrospective epidemiology study, cohort analysis

Data Quality: Klimisch 2

Number of Individuals in exposed/control group: the sample population was 3824 infants and 84.5% of this group was exposed to water containing manganese > 0.4 mg/ L. The concentration of manganese was also taken into consideration

Sex: male and female infants

Manganese compound: the concentration of manganese was determined by HR-ICP-MS but no information on the manganese species was included. It can be assumed that the majority of manganese was in the form of Mn²⁺

Exposure conditions: manganese in water from local wells (1508). Median manganese concentration in wells was 1.28 mg/ L. Exposure of infants was either direct or maternal.

Correction for confounding factors: yes, the most important being water arsenic level in drinking water, socioeconomic status, maternal weight, maternal dietary intake, occupation, child sex, birth order. And additional analysis (n=479) of wells with information on the concentration of 29 other metals was also performed.

Results: the infant mortality odds ratio was determined for infants exposed to a Mn concentration above the World Health Organization (WHO) standard of 0.4 mg/ L. Additionally, the association between infant mortality and [Mn] quintiles were determined (0.1-0.5, 0.5-1.0, 1.0-1.6, 1.6-2.1, 2.1-8.6 mg/ L of Mn). An elevated mortality risk in the first year of life was seen in infants exposed to manganese in the drinking water at a concentration at or equal to 0.4 mg/L. No dose-response effect was observed.

Executive summary: the reproductive history and description of well use was available in a cohort of 11'749 people from Araihasar, Bangladesh. A total of 3'824 live births satisfied the inclusion criteria of the study. Manganese levels in water from 1508 wells in the same area were also available. An association between infant mortality and Mn concentration in drinking water (above the WHO standard of 0.4 mg/L and Mn concentration quintiles) was investigated. After correcting for confounding factors known to affect infant mortality and for arsenic levels (and 29 other metals) in the drinking water, a *possible* association between manganese in drinking water and infant mortality was found. No clear dose-response was found, and half of the analysed subsets did not achieve significance (Odds ratio including 1.0).

Evaluation: the cohort analyzed was part of a wider study on the health effects, including reproductive history, of arsenic in well water. The study is based on a relatively large sample size and a thorough analysis was performed. Adjustment for the most important confounding factor affecting infant mortality was done. Access to healthcare was not mentioned in the study. The authors also excluded the possibility that the association seen between manganese levels and infant mortality was due to other metals (29 other metals analyzed) in drinking water. However, known common contaminants of organic or microbial nature were not taken into consideration. There was also limited information on exposure routes, such as breastfeeding versus direct exposure to well water. The authors acknowledged "methodologic limitations" of the study and a need for further confirmatory studies.

Study: He *et al.*, 1994

Type of Study : epidemiologic

Data Quality: Klimisch 4 (abstract, limited information)

Number of Individuals in exposed/control group: 92 matched pairs

Sex: not defined

manganese compound: not defined

Exposure conditions: manganese in drinking water

Correction for confounding factors: none stated

Results: children in area exposed to high levels of manganese (concentrations in drinking water and hair were available) had significantly lower scores in the following tests: digit span, Santa Ana manual dexterity, digit symbol, Bento visual-retention test and pursuit-aiming test. Analysis of hair samples from the subjects revealed higher concentration of manganese when living in an area of high manganese levels.

Executive summary: the concentration of manganese in hair and the scores from developmental/behavioural tests were assessed in age-matched pupils living in an area of high level manganese sewage irrigation and in a control area. Manganese in hair was significantly higher in children living in the high level manganese irrigation area. The scores on behavioural and developmental tests were also lower in these children.

Evaluation: this study is presented as an abstract and it is difficult to decide on the reliability of the study since background information is limited. As for the results, no mention of confounding factors is mentioned and the sample size is limited.

3.2 Animal Data

manganese compound	Reference	Data Quality acc. Klimisch
MnCl ₂	Chandra <i>et al.</i> , 1978	4
	Deskin <i>et al.</i> , 1981	3
	Seth <i>et al.</i> , 1977	3
	Pappas <i>et al.</i> , 1996	2
	Dorman <i>et al.</i> , 2000	2
	Lee <i>et al.</i> , 2007	2
	Kristenssen <i>et al.</i> , 1986	4
	Lee <i>et al.</i> , 2006	2
	Pine <i>et al.</i> , 2005	2
	Leung <i>et al.</i> , 1993	4
	Lai <i>et al.</i> , 1984	4
	Lai <i>et al.</i> , 1991	4
	Kontur <i>et al.</i> , 1985	2
	Zhang <i>et al.</i> , 1999	4
MnO ₂	Lown <i>et al.</i> , 1984	2
	Massaro <i>et al.</i> , 1980	4

Mn ₃ O ₄	Laskey <i>et al.</i> , 1985	4
	Gray <i>et al.</i> , 1980	4
undefined	Zhang <i>et al.</i> , 1998	4
	Zhang <i>et al.</i> , 2001	4

Study: Chandra *et al.*, 1978

Type of Study: oral gavage study of manganese chloride in rats from day 21 over a 60 day time period

Data Quality: Klimisch 4 (limited materials & methods section, expected variation in actual dosing expected to be large and not accounted for)

Test animals: male albino rats, 21 days old and weighing 40-50g

Housing conditions: 10 animals per cage in an air-conditioned room, diet and water *ad libitum*

Sex: Male

Test item: manganese chloride (purity or source not stated)

Substance administration: oral gavage at dose of 50 µg/rat (dissolved in 1 ml of distilled water) for 60 days, control group was gavaged with distilled water

Dose group size: 30 rats in treated group and 30 rats in control group

Observations during treatment: weight was taken every 15 days

Sacrifice: six rats were sacrificed by decapitation every 15 days for the duration of the study

Post-mortem studies: the brain was removed from animals following sacrifice and divided in half. One half of the brain was used for enzymatic assays (acetylcholinesterase, AChE and monoamine oxidase, MAO) and the other half was used for histopathological examination

Results: six animals in the treated group died during the study. The cause was not identified. There was an increase in the activity of MAO at 15 and 30 days with no further increase until termination. No effect on AChE was seen. No changes were observed in the histopathology after 15 days, but a focal neuronal degeneration was observed in the cerebral cortex of the frontal region and cerebellar cortex after 30 days in all treated rats. After 45/60 days this effect had increased to include more

regions of the brain. Degenerated neurons with swollen or little chromatin, and in some cases the nuclei appeared to be absent, were observed.

Executive summary: developing rats were administered manganese chloride over a 60 day period from the age of 21 days. Every 15 days thereafter a group of rats was sacrificed and histopathological analysis and enzyme activity of AChE and MAO of the brain was assessed. There was a significant increase in MAO activity after 15 and 30 days after which there was no further increase in enzyme activity. No effects on AChE were observed. There was significant neuronal degeneration in the cerebral and cerebellar cortex observed from day 30 and onwards in treated animals. This effect increased with duration of exposure.

Evaluation: the experimental approach is poorly described with limited information provided on the animals and the test item. Dosing was not indicated as relative to body weight (50 µg/rat) and based on the range of bodyweights listed (starting weight 40-50 g) a variation in dose of up to 25% is expected. The dose/kg bodyweight was reduced with age. Although the study demonstrates relatively severe neuronal degeneration it is not known whether this represents the mean observation or outliers.

Study: Zhang D, 1998

Type of Study: effect on manganese on offspring following maternal exposure

Data Quality: Klimisch 4 (abstract, limited information)

Test animals: rats

Housing conditions: not stated

Sex: not stated

Test item: not stated

Substance administration: not stated

Dose group size: not stated

Observations during treatment: behavioural tests (Morris Water Maze Test)

Sacrifice: not stated

Post-mortem studies: body and brain weight, amount of glial fibrillary acid protein (immunoreactivity)

Results: weight gain of body and brain was reduced, behavioural tests: slower response, higher levels of glial fibrillary acid protein

Executive summary: animals were exposed to manganese (unknown species) and subjected to behavioural tests, body weight analysis and quantitative determination of glial fibrillary acid protein. Slower response times were noted in the behavioural tests

Evaluation: there is not enough information presented to determine the reliability and validity of the study.

Study: Zhang D, 1999

Type of Study: administration of manganese chloride in drinking to pregnant animals and effect of offspring

Data Quality: Klimisch 4 (abstract)

Test animals: rats

Housing conditions: not stated

Sex: not stated

Test item: manganese chloride

Substance administration: in drinking water as 2 g/ L or 10 g/ L

Dose group size: not stated

Observations during treatment: behavioural tests (Morris Water Maze Test, Elevated Plus Arm Maze),

Sacrifice: not stated

Post-mortem studies: Glial fibrillary Acid Protein (GFAP), Tyrosine Hydroxylase (TH) levels and location

Results: elevated Plus Arm Maze; latent period of running from centre field to any arm was significantly reduced in animals exposed to manganese . No effect on the Water maze test was observed. A significant increase in GFAP in nucleus caudate and accumbens was observed. TH was reduced in substantia nigra, ventral tegmentum area of midbrain and nucleus caudate in exposed animals.

Executive summary: animals were exposed to manganese chloride via maternal exposure through drinking water at concentrations of 2 or 10 mg/ L. No effect was seen in the Morris Water Maze Test but a longer latency period was observed in the Elevated Plus Arm Maze. Levels of GFAP were increased while TH was reduced in the regions of the brain studied.

Evaluation: this study is presented as an abstract and there is not enough information to determine the validity and reliability of the study.

Study: Zhang D, 2001

Type of Study: effect of manganese on post-natal development following maternal exposure

Data Quality: Klimisch 4 (abstract), no enough information to evaluate validity of the study)

Test animals: mice

Housing conditions: not stated

Sex: not stated

Test item: not stated

Substance administration: not stated. "High" and "low" dose

Dose group size: not stated

Observations during treatment: behavioural effects: Morris Water Maze test

Sacrifice: not stated

Post-mortem studies: weight of body and brain, "protein content" of brain, acetylcholinesterase activity, level of glial fibrillary acid protein (GFAP) as measured by immunoreactivity.

Results: body weight gain and brain weight were significantly decreased in "high dose", latency period in finding hidden platform in the water maze test was increased on day 5, protein content of brain and acetylcholinesterase activity was decreased. Immunoreactivity of GFAP in the hippocampus area was significantly higher than in control group.

Executive summary: offspring were exposed to manganese from maternal exposure to drinking water. Two different concentrations of manganese in drinking water were used. There was an effect in manganese-treated animals with reduced brain- and bodyweight. One of the behavioural indices indicated an increased latency period.

There were also effects on proteins in brain with activity of AChE decreased and apparent increased immunoreactivity of GFAP in the hippocampus.

Evaluation: this study is presented as an abstract and does not contain enough information to determine the reliability of the study.

Study: Deskin *et al*, 1981

Type of Study: oral gavage of manganese chloride to neonatal rats from birth to 24 day post-partum.

Data Quality: Klimisch 3 (low number of determinations in analytical methods, discrepancy of conclusions and data presented)

Test animals: CD rats from Charles River Laboratories

Housing conditions: room temperature 21-23°C, relative humidity 40-60%, 12 hr light cycle, food and water *ad libitum*

Sex: male

Test item: manganese chloride (purity or source not indicated)

Substance administration: neonatal male rats were administered by gavage a dose of either 10, 15, or 20 µg/g body weight/day of manganese chloride in a 5% sucrose solution. Control group only received vehicle

Dose group size: 10 animals per dose

Observations during treatment: colonic temperature measured from day 12 to day 23

Sacrifice: animals were sacrificed on day 25 by decapitation

Post-mortem studies: the brain was removed and the level of serotonin and activity of AChE was measured in the corpus striatum and hypothalamus.

Results: at the highest dose level, there was an increase in serotonin level in the hypothalamus. This effect was not observed in the corpus striatum. AChE was reduced in the corpus striatum at the highest dose but no effect was seen in the hypothalamus. There was no effect of manganese on body temperature.

Executive summary: manganese chloride was administered to neonatal rats from birth to day 24 post-partum. The level of serotonin and AChE activity in the hypothalamus and corpus striatum was assessed. Colonic temperature was measured on day 12-23. In the highest dose group (20 µg/g bodyweight) there was

an increase in serotonin in the hypothalamus and a decrease of AChE activity in the corpus striatum.

Evaluation: the conclusions of the study are based on assays with relatively low accuracies. Additionally, the measurements were only done in duplicate (10 animals in each dose group and a total of 20 assay determinations). This reduces the validity of the measurements. Furthermore, since the doses are relatively close (10-15-20 µg/g) one would expect to see more of a dose-response. There were also a few discrepancies between the results section and the conclusions made.

Study: Lown *et al*, 1984

Type of Study: inhalation exposure study (pre-conception, post-conception) to manganese dioxide dust in female mice and impact on post-natal development

Data Quality: Klimisch 2

Test animals: random bred female Swiss mice, approximately 1 month old

Housing conditions: clear plastic cage, temperature 21± 1°C, 12 hour light cycle, 5 animals per cage before pregnancy, after birth animals were kept single with litters. Offspring kept in suspended steel cages under the same environmental conditions. During exposure: 0.78 m³ "Rochester-type" inhalation chamber.

Sex: male and female offspring analyzed

Test item: manganese dioxide dust prepared from manganese dioxide powder (99%, Mallinkrodt), MMAD of 1.5 µm

Substance administration: Preconception exposure: inhalational exposure of females for 16 weeks pre-conception (Exposure was 7 hrs/day at the following concentrations: 49.1± 2.3 mg/m³ for the first 12 weeks and then 85.3± 15.6 mg/m³). Groups of 5 females exposed or control animals were then bred to unexposed male. Postconception exposure: On day 1 of gestation, pregnant animals (exposed preconception and controls) were assigned to either further exposure or filtered air for the next 17 days.

Dose group size: groups of 5 females were exposed to manganese dioxide powder and bred to a single unexposed male. Following parturition, litters were removed, pooled, and reassigned to parturient mothers of the same or different treatment group. The newly formed litters consisted of 3 males and 3 females. The following exposed groups were generated (exposure history of biological mother/exposure history of foster mother): Mn-Mn/ Mn-Mn, C-C/ Mn-Mn, Mn-Mn/C-C, C-C/C-C, Mn-Mn/

Mn-C, Mn-Mn/C-Mn, C-C/C-Mn, and C-Mn/C-C. C indicates control and Mn indicates exposed to Mn.

Observations during treatment: offspring weighed post-partum day 3, 7, 12, and 21. Additional locomotor and behavioural observations were made; gross locomotor activity was assessed in pups on postpartum day 7 and 12, maternal retrieval latency on post-partum day 3, and on post-partum day 45 pups were subject to behavioural testing

Sacrifice: all animals sacrificed on post-partum day 45

Post-mortem studies: the brain was isolated following sacrifice and mitochondrial levels of manganese in the cerebrum, cerebellum, and brainstem analyzed

Results: litter size was significantly larger in animals exposed to manganese dioxide during pre-conception than in animals only exposed to filtered air. This effect was not observed in animals exposed post-conception. Animals exposed to manganese dioxide during and after gestation had reduced body weight, activity, exploratory behaviour and rearing frequency. This effect was observed in offspring from animals exposed pre-conceptionally or pre-natally but not in animals exposed prenatally but suckled by control mothers. Maternal retrieval latency for offspring exposed to manganese dioxide during gestation was significantly shorter than in animals only receiving filtered air.

Executive summary: to determine the effect of post-natal development, pregnant female mice were exposed to manganese dioxide dust pre-conception and post-conception. The litters were either raised by mothers of the same treatment group (fostering) or a different treatment group (cross-fostering). The pups were subject to a battery of behavioural/activity tests and weighed on regular intervals. Litter size was larger from animals exposed to manganese dioxide dust during pre-conception while pre-natal exposure caused reduced neonatal activity and weight (~-7% at day 7). A continued exposure from suckling caused neonatal activity to stay at a lower levels compared to control animals (or even a slight additional reduction), while fostering by a (non-exposed) control mother led to recovery. This effect was confirmed in adult sexually mature offspring.

Evaluation: the study is relatively detailed in explanation of animal background and exposure scenario. Furthermore, inhalation is a relevant exposure route for humans. The study cannot be used for a quantitative risk assessment, as only one exposure condition was tested.

Study: Seth *et al.*, 1977

Type of Study: exposure to manganese from breast milk in developing rats and effect on selected brain enzymes and levels of manganese in brain

Data Quality: Klimisch 3 (limited information on background on animals and substance, number of exposed animals small, uncertainty of analytical methods used)

Test animals: Albino rats, average weight 220 g

Housing conditions: not stated

Sex: male and female offspring

Test item: manganese chloride (purity or source not indicated)

Substance administration: dams were given 15 mg manganese chloride (in saline)/kg daily by intubation starting on second day of lactation. Control group received only saline in a similar manner

Dose group size: not clearly stated. It can be inferred that analysis was based on 6 pups and 6 dams from treated and control group were analyzed on day 15 and a similar number on day 30

Observations during treatment: animals were weighed daily from day 15-30

Sacrifice: either day 15 or day 30

Post-mortem studies: analysis of brain: enzyme assays (succinic dehydrogenase, adenosine triphosphatase, adenosine deaminase, acetylcholine esterase, monoamine oxidase,), level of manganese, and histopathological studies

Results: no effect on bodyweight and other developmental parameters (opening of eyes, body hair, walking movements) was observed. Significant accumulation of manganese in brain was observed in pups. With the exception of monoamine oxidase, the activity of all enzymes was reduced in treated animals. No histopathological changes were noted.

Executive summary: suckling rats were exposed to manganese chloride in breast milk following oral administration of manganese chloride to nursing dams. There was significant accumulation of manganese in the brains of pups but to a much lesser extent in dams. Depressed activity of brain enzymes was observed. There was no effect on body weight or other developmental parameters.

Evaluation: the validity and relevance of the study is difficult to assess since there is limited experimental information. Animal background and test substance were not

clearly defined. The number of animals used was relatively small in both the treated and control groups. There is also no information on the number of determinations for each sample in the enzymatic assays and determination of manganese in the brain. This further reduces the reliability of the conclusions of the study.

Study: Pappas *et al*, 1996

Type of Study: oral exposure to manganese chloride in drinking water to dams and litters from conception through post-natal day 30

Data Quality: Klimisch 2

Test animals: Sprague-Dawley rats

Housing conditions: 12 hour light cycle

Sex: female parent, male offspring

Test item: manganese chloride (Sigma, purity not stated)

Substance administration: manganese chloride was administered to pregnant females from gestation day 1 in drinking water as either 2 or 10 mg/ml. On post-natal day 22, pups were removed from dams and were further exposed to manganese chloride in drinking water until post-natal day 30.

Dose group size: 50 pups (males) per group

Observations during treatment: behavioural testing of pups at post-natal day 17, bodyweight on day of birth and every 3 days until day 24

Sacrifice: animals were sacrificed at different time points depending on analysis (see below)

Post-mortem studies: histological analysis on animals from post-natal days 32 and 90, choline acetyl transferase (ChAT) activity, brain catecholamines and serotonin on animals from post-natal day 32

Results: no evidence of any deformities was seen. No difference was observed in birth weight on day 3. At later time points, the animals exposed to the highest concentration weighed less than the two other groups. After 90 days, there was no difference in bodyweight between the different groups. Rats in the highest dose group were also hyperactive in the activity test at 17 days of age. The level of manganese in the highest dose group was significantly higher (150% increase) than the two other dose groups. No difference was observed between animals exposed to 2 mg/ml and

the control group. No difference in activity of any of the enzymes studied or on monoamine levels was seen. Cortical thinning was observed in both treated groups but it is not known if this is directly due to manganese exposure or due to perinatal malnutrition.

Executive summary: the impact of manganese on behaviour, neurochemistry and histopathology was studied in rats exposed to manganese chloride from conception through to post-natal day 30. Exposure was either from suckling or directly from drinking water. No effect on parturition and no physical abnormalities were observed in the offspring. In animals exposed to the highest dose (10 mg/ ml) a reduced weight gain was observed from day 9-24 and hyperactivity on day 17. There was also a significantly elevated level of manganese in the brain in these animals. No effect on neurochemistry was observed at either dose group. In both of the treated groups, thinning of the cerebral cortex was observed.

Evaluation: this study includes two different exposure scenarios; exposure from suckling and from drinking water following weaning of animals. Accordingly, the exposure is much higher when directly from drinking water than when received from milk. This complicates establishing the actual dose levels. The study helps in assessing the impact of manganese in offspring following maternal exposure.

Study: Dorman *et al*, 2000

Type of Study: oral administration of manganese chloride to neonatal rats throughout the period of lactation from day after birth to day of weaning

Data Quality: Klimisch 2

Test animals: six week old CD rats from Charles River Laboratories

Housing conditions: polycarbonate housing with cellulose-fiber chip bedding, NIH-07 pelleted diet and filter-purified tap water available *ad libitum*, 20±1°C, 50±10% air humidity, 12 hour light cycle, HEPA filtered air

Sex: male and female

Test item: manganese chloride (Sigma, no purity stated)

Substance administration: manganese chloride was dissolved in Nanopure water (1 ml/ kg) and administered by gavage to adult rats and by micropipette to pups. The dose levels studied were 0, 25, 50 mg/ kg bodyweight

Dose group size: adults: 20 rats per dose, Pups: 10 litters (4 animals per sex) per dose

Observations during treatment: bodyweight, behavioural studies including motor activity (automated photobeam), passive avoidance, acoustic startle, functional observational battery

Sacrifice: post-natal day 21

Post-mortem studies: brain catecholamine assay, manganese concentrations in regions of the brain (striatum, hypothalamus, hindbrain, cerebellum, hippocampus, rest of brain), neuropathology (analysis of olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain and cerebellum)

Results: there was a significantly decreased bodyweight gain in pups in the highest dose group. This effect was not seen in adult rats. The only behavioural index found to be statistically significant was the acoustic startle reflex. There was an increase in the magnitude of the startle reflex for both groups of treated pups (118 and 121% of control, statistically significant $p < 0.05$) but not in adults. Pups exposed to the highest dose of manganese chloride had a statistically significant increase in striatal concentrations of dopamine and dopamine metabolite DOPAC. Other changes in neurotransmitters were minor in pups and adults. Statistically significant increases in manganese concentrations in the majority of brain regions in pups (striatal, hippocampal, hindbrain, and cortical) and adults (striatal, cerebellar, and brain residue) were observed. No neuropathological effects were observed.

Executive summary: adult and neonatal rats were administered manganese chloride via the oral route at a dose of either 0, 25, or 50 mg/kg bodyweight. The concentration of manganese delivered to the pups was 100-fold higher than in milk from lactating dams. Reduced bodyweight gain and increased acoustic startle reflex were observed in pups in the highest dose group. No other changes in behavioural parameters or effect on neuropathology were seen. The levels of manganese in most regions of the brain were increased in pups and adults following manganese exposure.

Evaluation: this study is relatively detailed in description of background and the results observed. The goals of the study are appropriately demonstrated.

Study: Laskey *et al*, 1985

Type of Study: oral exposure of particulate trimanganese tetraoxide to male rat from birth to day 21 with assessment of hypothalamic, pituitary and testicular function

Data Quality: Klimisch 4 (no apparent indication of dose group size, background of animals and observation during treatment not indicated)

Test animals: Long-Evans rats

Housing conditions: not stated

Sex: male

Test item: particulate trimanganese tetraoxide (no details on purity, source, or size of particles)

Substance administration: pups were dosed by gavage with 0, 71 or 214 µg/g bodyweight of trimanganese tetraoxide daily from day 1-21 post-partum. Trimanganese tetraoxide was delivered at a rate of 1 µl/g bodyweight/day in a 50% sucrose solution. Study 1: randomly selected rats were castrated on day 18. One group of rats were injected with 500 ng LH-RH (Luteinizing Hormone Releasing Hormone) 2 hours prior to sacrifice to stimulate pituitary FSH (Follicle Stimulating Hormone) secretion, another group of rats were injected with hCG (Human Chorionic Gonadotropin, 2 hrs or 7 days prior to sacrifice on d21 or d28) to stimulate testosterone production. Study 2: randomly selected rats were castrated on day 21. Randomly selected rats were dosed with 500 ng LH-RH 15 minutes before being sacrificed (d28) or injected with hCG 2 hours before being sacrificed.

Dose group size: not indicated

Observations during treatment: none indicated

Sacrifice: 21 or 28 days post-partum

Post-mortem studies: serum prepared from cardiac blood, tissues weighed including hypothalamus, testes and seminal vesicles. The levels of testosterone, FSH, and LH were determined together with tissue levels of manganese.

Results: Study 1: no effect on bodyweight was observed. The only treatment-related effect was a significant reduction in testosterone levels following 7 days of hCG stimulation. Study 2: no effect on FSH or LH was observed. After 7 days of hCG stimulation, testicular growth and testosterone was reduced in the highest dose group.

Executive summary: Long-Evans rats were administered trimanganese tetraoxide orally from birth to day 21 and the effect on hypothalamic, pituitary and testicular function was assessed by determining the levels of FSH, LH and testosterone. There were no effects on LH or FSH at any dose level studied. After 7 days of hCG stimulation there was a decrease in serum testosterone levels in animals exposed to manganese. This effect was not observed in animals following 2 hours of hCG stimulation.

Evaluation: the materials and methods section of this study is poorly described and lacks key information on important parameters. Most importantly, there is no apparent group size indicated for the treatment making evaluation of the results difficult. Additionally, trimanganese tetraoxide is marginally soluble and the relevance of oral exposure is not known. The concentrations obtained are not certain.

Study: Lee *et al*, 2007

Type of Study: *in vitro* study on the effect of Mn^{2+} on luteinizing hormone-releasing hormone (LHRH)

Data Quality: Klimisch 2

Test item: manganese chloride (Sigma, purity not indicated)

Substance administration: the hypothalamus was isolated from immature female (30 days old) Sprague-Dawley rats. The effect of Mn^{2+} on release of LHRH was assessed by determining concentrations of LHRH immunologically. To determine an eventual mechanistic pathway, these studies were also performed with inhibitors of enzymes known to be involved in formation of LHRH.

Results: manganese chloride induced release of LHRH *in vitro* from hypothalamus at concentrations of 50, 100, and 250 μM . Furthermore, co-incubation with NMMA (inhibitor of NOS) did not block this effect. Co-incubation with ODC (inhibitor of guanylyl cyclase, sGC) blocked Mn^{2+} -stimulated release of LHRH. There was also a slight but significant increase in cGMP levels following Mn^{2+} treatment.

Executive summary: the hypothalamus from immature 30 day old female rats was isolated and exposed to various concentrations of manganese chloride to determine the effect on release of LHRH. In addition, further studies were performed to determine the pathway that Mn^{2+} is postulated to affect. There was a marked increase in LHRH release at all concentrations of Mn^{2+} studied (50, 100, 250 μM). This increase was not affected by co-incubation with an inhibitor of NOS while co-incubation with an inhibitor of sGC blocked Mn^{2+} -stimulated release. The mechanism of action of Mn^{2+} seems to be activation of sGC with corresponding generation of cGMP and release of LHRH.

Evaluation: this study is adequate to demonstrate and propose a mechanism of action of Mn^{2+} *in vitro* in isolated rat hypothalamus. The relevance to humans and particularly at the dose levels used in the study can not be determined.

Study: Lai *et al.*, 1984

Type of Study: administration of manganese chloride in drinking water from conception and onwards

Data Quality: Klimisch 4 (information on dose group size is not reported clearly, some of the endpoints were studied at seemingly random time points)

Test animals: Wistar rats

Housing conditions: not stated

Sex: male and female

Test item: manganese chloride tetrahydrate (purity and source not stated)

Substance administration: in drinking water “usually” at a concentration 1 mg/ml of drinking water

Dose group size: not clearly defined.

Observations during treatment: not stated

Sacrifice: up to 2 years

Post-mortem studies: 1) Effect of manganese on brain regional activities of Na-K-ATPase, Mg-ATPase, isocitric dehydrogenase, glucose-6-phosphate dehydrogenase, glutamic acid carboxylase, choline acetyltransferase, and acetylcholinesterase. 2) Uptake of dopamine, noradrenaline, serotonin and cholin by synaptosomes

Results: manganese exerted an effect on synaptosomal uptake of dopamine (decrease in hypothalamus, striatum and mid-brain) and choline (decrease in hypothalamus, increase in striatum). There were only relatively minor changes in the enzymes studied; a minor decrease in activity of acetylcholinesterase was observed in cerebellum and mid-brain of 2 month old rats.

Executive summary: rats were exposed to manganese chloride in drinking water from conception and onwards. The only significant effect seen was on synaptosomal uptake of dopamine and choline. Only relatively minor and transient changes were seen in activity of the enzymes studied.

Evaluation: The reporting of experimental background and treatment groups is limited and evaluation of results is difficult. Both the number of animals exposed and the animals used in each study are not clearly defined. The scope of the study is not clearly defined and it is difficult to compare endpoints since they were performed at different time points.

Study: Lai *et al.*, 1991

Type of Study: administration of manganese chloride in drinking water from conception and onwards

Data Quality: Klimisch 4

Test animals: Wistar rats

Housing conditions: not stated

Sex: male and female

Test item: manganese chloride tetrahydrate (purity and source not stated)

Substance administration: drinking water at concentration of 1 mg or 10 mg/ ml of drinking water

Dose group size: not clearly defined. Manganese determination in brain based on n=6-10

Observations during treatment: not stated

Sacrifice: 120 days for brain determination of manganese

Post-mortem studies: determination of manganese level in brain, effect of manganese on brain regional activities of Na-K-ATPase

Results: levels of manganese in brain were significantly increased with the most significant increase being in the hippocampus, hypothalamus, and striatum. There was no significant effect of chronic manganese treatment on the activity of Na-K-ATPase in brain.

Executive summary: rats were exposed to manganese chloride in drinking water from conception and onwards. The only significant effect was an increase in manganese levels in the brain. Only transient changes were seen in the activity of Na-K-ATPase in regions of the brain and no significant effect of manganese was seen on development of this enzyme in offspring.

Evaluation: evaluation of this study is difficult since there is limited information on the experimental approach. Furthermore, dose groups are not clearly defined and the enzymatic measurements are based on a relatively limited number of samples.

Study: Kontur *et al.*, 1985

Type of Study: intubation of neonatal rats daily with 0, 25, 50 µg manganese chloride for 14 or 21 days

Data Quality: Klimisch 2

Test animals: Long-Evans hooded rats

Housing conditions: 21°C, 12 hour light cycle

Sex: male and female

Test item: manganese chloride tetrahydrate (JT Baker, no purity indicated)

Substance administration: manganese chloride was delivered by gastric intubation from birth through weaning at a dose of 25 or 50 µg in distilled water. Actual dose of manganese : 6.9 and 13.8 µg /day

Dose group size: measurement of manganese: male and female from each of 4-5 litters (n=8-10), measurement of monoamines: one male from 4-6 litters (n=4-6)

Observations during treatment: weight

Sacrifice: day 14 or 21

Post-mortem studies: level of manganese in regions in the brain, measurement of monoamines, norepinephrine, dopamine and serotonin.

Results: there was no significant effect of manganese on weight gain (body or regions of brain). Levels of manganese in brain increased with exposure concentration in all regions of the brain with levels slightly lower after 21 days than after 14 days. There were no effects on monoamine levels following exposure to manganese.

Executive summary: neonatal rats were exposed to manganese chloride from the day of birth until day 14 or 21 when they were sacrificed and the levels of manganese in regions of the brain were analyzed. At these time points the levels of norepinephrine, dopamine, and serotonin were also analyzed. Treatment with manganese chloride did not cause an effect on body weight or weight of the brain. There were also no changes in norepinephrine, dopamine or serotonin following manganese exposure. There was a significant increase in manganese in all regions of the brain.

Evaluation: the study is well reported with enough background and results to draw a conclusion on the effects of manganese chloride following intubation. The conclusions of the study are also based on relatively sound statistical data and animal numbers.

Study: Lee *et al.*, 2006

Type of Study: 1) Oral gavage study. Manganese chloride was administered to male rats from day 15 until day 48/55 followed by assessment of development of spermatogenesis. 2) Direct delivery of manganese chloride into the third ventricle of the brain and analysis of LH secretion. 3) *In vitro* study of LHRH release from hypothalamus.

Data Quality: Klimisch 2 (limited information on observation of animals, no weight data on whole animals)

Test animals: immature Sprague-Dawley rats

Housing conditions: 12 hour light cycle, 23°C, food and water *ad libitum*

Sex: male

Test item: manganese chloride tetrahydrate (sigma, purity not indicated)

Substance administration: 1) Oral gavage; 10 or 25 mg/kg manganese chloride. 2) Direct administration of 1, 2.5, 10, or 25 µg/3 µl of manganese chloride into third ventricle of the brain 3) *In vitro* incubation of hypothalamus at concentrations of manganese chloride of 50, 250, or 500 µM.

Dose group size: depending on study and treatment: oral administration n=13/14, direct administration n= 6-16

Observations during treatment: not stated

Sacrifice: 1) Oral gavage: day 48 or 55

Post-mortem studies: 1) Oral gavage: determination of LH and FSH, analysis of spermatogenic development. 2) Direct administration: LH secretion

Results: 1) Oral gavage: animals administered 25 mg/kg manganese chloride until 55 days of age showed increased levels of LH, FSH, and testosterone. Daily sperm production per gram parenchymatic tissue and per testes were increased in the same dose group. 2) Direct administration: a significant dose-dependent increase in LH was observed in treated animals. This effect was not observed in animals pre-treated with the LHRH receptor agonist, acyline 3) *In vitro* incubation of hypothalamus with manganese chloride at 250 or 500 µM caused a dose-dependent stimulation of release of LHRH.

Executive summary: animals were treated with manganese chloride either by gavage or by direct administration of manganese chloride into the third ventricle of the brain. A further *in vitro* study on the effects of manganese chloride on isolated hypothalamus was also performed. Following oral administration of manganese chloride there was a small increase in sperm production and efficiency of spermatogenesis with a concomitant increase in LH, FSH, and testosterone in these animals. There was also an increase in LH release in animals administered manganese chloride directly into the brain. This effect was not seen when animals were pre-treated with acyline indicating that manganese chloride acts on the hypothalamus and not on the pituitary gland. When the hypothalamus was isolated and incubated with manganese chloride there was a dose-dependent increase in LHRH release.

Evaluation: this study is relatively detailed in description of materials and methods and the results. The results indicated a dose-dependent increase in release of LH, FSH, testosterone (*in vivo*) and LHRH (*in vitro*) following manganese chloride exposure. The effects of manganese chloride on spermatogenesis and sperm production in developing animals were not so clear with the difference between control and treated animals being ~10%. The biological significance of this observation is not apparent.

Study: Kristensson *et al*, 1986

Type of Study: daily administration of manganese chloride by gastric intubation to 3-day old Sprague-Dawley rats for 41 days

Data Quality: Klimisch 4 (Limited information on animals and treatment, size of dose groups and repeats for the analytical section)

Test animals: Sprague-Dawley rats

Housing conditions: not stated

Sex: not stated

Test item: manganese chloride (source or purity not stated)

Substance administration: animals were given manganese chloride in aqueous solution by gastric intubation at a dose of 150 mg Mn²⁺/kg bodyweight

Dose group size: not clear. The manganese levels in whole blood and brain were based on n=3 at each time point

Observations during treatment: general observation of behaviour

Sacrifice: at different time points depending on analysis: 15, 20, 43, 60 days

Post-mortem studies: determination of manganese in brain, brain histopathology, biochemical analysis including content of monoamines and monoamine metabolites (dopamine, serotonin) in various brain regions, activity of catechol-O-methyl transferase (COMT)

Results: histopathological examination revealed no differences between treated and control rats. Clinical signs in manganese-treated rats included a rigid and unsteady gait. There was significant accumulation of manganese in blood and brain of 15- and 20-day old exposed animals, however after 60 days the manganese level was reduced to 3-times the control level. With the exception of the dopamine metabolite, homovanillic acid (HVA), there was no impact of Mn^{2+} treatment; HVA was decreased in the hypothalamus and striatum (42 and 32%, respectively). The activity of COMT was not affected

Executive summary: manganese chloride was delivered by gastric intubation at a dose of 150 Mn^{2+} /kg bodyweight to developing rats from day 3 after birth. Brain histopathology, levels of manganese and monoamines in brain were determined at various time points. Histopathology, including analysis of neurons and axonal growth, revealed no effect on brain but clinical signs included rigid and unsteady gait (day 15-22, no effect at day 44) indicative of neurotoxicity. There was a slight impact on HVA. This effect was not due to an effect on COMT as the activity of this enzyme was normal. No further effect was seen on dopamine or serotonin. A reversible increase in the level of manganese in brain of exposed animals was also observed.

Evaluation: the validity of the study is difficult to assess since there is limited information on the experimental background. Furthermore, the strength of the conclusions is difficult to determine since the number of animals seem relatively low or are not indicated properly. Nevertheless, the study adds to the overall evaluation of developmental neurotoxicity of manganese since it was performed at a high dose level of manganese and no morphologic effect on the brain was observed. The clinical signs were similar to those observed in humans with manganism. Unfortunately, adult animals were not dosed in this study and therefore it can not be determined whether the effect is restricted to developing animals.

Study: Massaro *et al*, 1980

Type of Study: inhalational exposure of manganese dioxide dust to female pregnant mice before pregnancy and until day 18 of gestation

Data Quality: Klimisch 4 (abstract, very limited information on all sections)

Test animals: female HA/ICR mice

Housing conditions: not stated

Sex: male/female pups

Test item: manganese dioxide dust (particle size, purity not indicated)

Substance administration: manganese dioxide was delivered via inhalation 7 hr/day, 5 days/week for 4 months before pregnancy and then until day 18 of gestation.

Dose group size: 3 pups of each sex

Observations during treatment: behavioural studies including rearing /exploration, motor control and fatigue, bodyweight

Sacrifice: not indicated. Pups were allowed to mature.

Post-mortem studies: none stated.

Results: 1) Adult offspring of mothers exposed to manganese dioxide showed lower body weight. 2) Offspring of mothers exposed to manganese dioxide followed by fostering of these offspring by mothers exposed to manganese dioxide, showed lower rearing and exploration than offspring of mothers exposed to manganese dioxide, followed by fostering of these offspring by control mothers. 3) There was an effect observed on the control offspring fostered to mothers exposed to manganese dioxide.

Executive summary: female mice were exposed to manganese dioxide by inhalation for 4 months and up until day 18 of gestation. The effect of manganese dioxide before, during, and after gestation, was determined by distributing pups to mothers exposed to manganese dioxide or to control mothers. There was an effect on body weight with adult offspring of exposed mothers having reduced weight. Additional behavioural parameters were also changed. Gestational and post-partum exposure of manganese dioxide affects pup development.

Evaluation: the study is presented as an abstract and the available information is not sufficient to determine the validity and reliability of the study.

Study: Pine *et al.*, 2005

Type of Study: 1) Administration of manganese chloride directly into the third ventricle of the brain and effect on LH secretion, 2) Effect of manganese chloride on LHRH release from hypothalamus *in vitro*, 3) Administration of manganese chloride by

gastric gavage to female pups from post-natal day 12 to 29 and effect on onset of puberty

Data Quality: Klimisch 2

Test animals: Sprague-Dawley rats

Housing conditions: 12 hour photoperiod, 23°C, food and water *ad libitum*

Sex: female

Test item: manganese chloride tetrahydrate (Sigma Chemical company, purity not stated)

Substance administration: 1) Directly to third ventricle of brain at a dose of 1, 2.5, 5.0, 10.0, or 25 µg/3 µl of manganese chloride. 2) *In vitro* incubation of isolated hypothalamus with 0, 50, 250, or 500 µM manganese chloride in medium, 3) Gastric gavage of 5, 10, 20, or 100 mg/kg manganese chloride in saline.

Dose group size: depending on study: 1) n=7, 2) n=11, 3) n= 7

Observations during treatment: not stated

Sacrifice: animals administered manganese chloride by gastric gavage were sacrificed on day 29

Post-mortem studies: 1) LH secretion, 2) LHRH release (*in vitro*), 3) Levels of sex hormones LH, FSH and estradiol

Results: 1) A significant increase in release of LH was observed in animals dosed manganese chloride directly into brain. This effect was seen at all doses except the 1 µg dose. In animals pre-treated with acyline, an LHRH receptor antagonist, this effect was not observed. 2) A significant increase in release of LHRH was observed from isolated hypothalamus incubated in 50, 250, and 500 µM of manganese chloride. 3) Females administered manganese chloride from day 12-29 had increased serum levels of LH, FSH and estradiol. A concomitant reduction in age of vaginal opening was also observed.

Executive summary: animals were exposed to manganese chloride either as a direct administration into the third ventricle of the brain or via gastric gavage. In animals dosed directly with manganese chloride into brain, a significant increase in circulating LH was observed. An increase was also seen in levels of LH, FSH, and estradiol in females dosed from day 12-29 with a corresponding reduction in the age of vaginal opening. These results indicate that manganese acts by facilitating the normal onset of puberty.

Evaluation: this study is thoroughly reported and the results demonstrate a dose-dependent stimulating effect of manganese chloride on sex hormones, and further, that it may facilitate early onset of puberty. Since the conclusions are partially based on direct dosing of manganese chloride to parts of the brain, the relevance to humans is not certain.

Study: Gray *et al.*, 1980

Type of Study: administration of trimanganese tetroxide in the diet from day 15 of lactation

Data Quality: Klimisch 4 (exposure length not clearly defined, test item not properly, uncertainty about group size)

Test animals: Mouse (CD-1)

Housing conditions: transparent plastic cage, 12 hour light period

Sex: male and female

Test item: trimanganese tetroxide

Substance administration: administered in diet at 50 ppm as manganese sulfate (control) or 1050 ppm as trimanganese tetroxide from day 15 of lactation

Dose group size: 26 litters assigned to either manganese sulfate or trimanganese tetroxide, exact number of animals not stated

Observations during treatment: behavioural, growth, general appearance

Sacrifice: day 58, day 73, day 90

Post-mortem studies: weight of reproductive organs including seminal vesicle, testes and preputial gland, body weight. Liver and kidney weights were measured on animals sacrificed on day 73 and 90

Results: no effect was seen on growth and general appearance of manganese-treated animals. No effect on body weight, liver or kidney weight following manganese treatment. The only effects seen were reduced activity level of treated animals after 73 days and retarded growth of testes and accessory sex organs.

Executive summary: animals were exposed to trimanganese tetroxide in the diet at 1050 ppm from day 15 of lactation. Activity levels and size of reproductive organs were determined on day 58, 73, or 90. There was no effect on body weight or general

appearance but there was a statistically significant effect on activity and on the weight of reproductive organs.

Evaluation: key information necessary to evaluate the reliability of this study is lacking, most notably, group size and exposure length. The relevance of oral administration of trimanganese tetraoxide, a poorly soluble manganese oxide, can also be questioned.

Study: Leung *et al.*, 1993

Type of Study: administration of manganese chloride in drinking water from conception and onwards

Data Quality: Klimisch 4 (no information on size of dose groups, observations during treatment, analytics based on limited sample size and determinations)

Test animals: Wistar rats

Housing conditions: not stated

Sex: male/female

Test item: manganese chloride

Substance administration: 1 or 10 mg of manganese chloride per ml of drinking water

Dose group size: not clearly defined. Analysis is based on pooled material from 2-4 animals

Observations during treatment: none stated

Sacrifice: 80-90 days

Post-mortem studies: dissection of brain and preparation of homogenate for enzymatic analysis

Results: chronic exposure to manganese chloride did not significantly alter age-related changes in monoamine oxidase A and B

Executive summary: rats were exposed to manganese chloride in drinking water from conception until day 80-90 and the brain regional activities of monoamine oxidase A and B was determined. There was no impact on manganese chloride on age-related changes in monoamine oxidase A and B activities

Evaluation: the study presents a very limited background including no obvious sample size. The analytical results are also based on a limited material pooled from 2-4 animals.

Implications of Toxicokinetics for hazard assessment of Manganese exposure

For the purpose of hazard assessment of test materials with respect to reproductive toxicity toxicokinetics can only serve as supplementary evidence. Hazard identification is primarily to establish whether an effect upon reproductive performance or embryofetal/neonatal development is real or not. It is generally rare for human epidemiological data to be conclusive, even though this data should be regarded as the most pivotal when risk to the human population is considered. The use of animal models therefore has its merits. In order to take account of extrapolation from experimental animal to human a conservative approach to exposure is adopted with animal studies generally using dose levels well above potential human exposure.

Despite these limitations toxicokinetics can establish the relationships between dosage and human/animal response. The mechanisms underlying response and exposure can also serve to explain potential toxicity. In the case of manganese the identified characteristics associated with its toxicokinetics may be used to support the hazard assessment.

Absorption

It has been concluded that humans and animals show similarities in the rate of absorption from the gastrointestinal tract. Although gastric absorption is not the anticipated primary route of human exposure, it will nonetheless be of impact because of the side effect of inhalation exposure where material may end up in the stomach following passage of inhaled material via the mucocilliary escalator into the gut. Absorption of manganese will take place in animals and man following inhalation but direct passage of manganese into the brain of humans via the neurones associated with the olfactory lobes of the brain, as has been shown in experimental animals has not been established. Absorption of manganese salts is solubility dependent. This is of note because much experimental work for reproductive effects has been conducted using soluble salts such as chloride and sulphate. Inhalation exposure to manganese has been shown to result in higher tissue levels than oral exposure. This may well be the result of factors involved in gastric absorption such as diet and concentrations of transporter proteins/other ions.

Distribution

Manganese is widely distributed and crosses both the placental and blood brain barriers. Manganese accumulates in specific brain regions.

Metabolism

Manganese may undergo metabolism from Mn^{2+} to Mn^{3+} in the liver. It has been shown that the body may adapt to increased manganese uptake particularly in the liver. As a consequence, the lack of first pass metabolism in chronic exposure via inhalation may mean that tissue accumulation will be enhanced compared with oral ingestion.

Excretion

Biliary excretion is the primary route for manganese removal. The process is biphasic as a consequence of the rapid tissue distribution of manganese.

In conclusion experimental studies in animals and man indicate a number of common pathways which show that manganese has the potential to be absorbed and distributed systemically. Similar processes exist for all species and any toxic hazard associated with manganese cannot be dismissed following the application of toxicokinetic factors.

Appendix 1 Literature Search Strategy

The authors of the IEH/IOM report (2002) commissioned a literature search using Datastar:

Medline (1966+), Embase (1974+) and Toxfile (1966+) in December 2001, with a further update search being performed in August 2002.

Search terms used.

The terms used for manganese and its compounds are listed below; terms were searched for in the 'Title', 'Abstract' and 'Descriptors' fields.

BaMnO₄
 Barium manganate
 Braunite
 Cianciulliite
 Femanganese
 Ferromanganese
 Ferrosilicomanganese
 FeSimanganese
 Hausmannite
 KMnO₄
 manganese
 manganese chloride
 manganese II chloride
 manganese II nitrate
 manganese II oxide
 manganese II sulphate
 manganese III oxide
 manganese III sulphate
 manganese IV oxide
 manganese IV sulphate
 manganese nitrate
 manganese ore\$1
 manganese oxide\$1
 manganese sulphate
 manganese with steel*
 Manganous salt\$1
 Mn₂O₃
 Mn₃O₄
 Mn₃O₇
 Mn₅O₈
 Mn(NO₃)₂
 Mn(SO₄)₂
 Mn₂(SO₄)₃
 MnCl₂
 MnO
 MnO₂
 manganese sulfate
 Na₃MnO₄
 Polianite
 Potassium manganate VII
 Potassium permanganate
 Potassium VII manganate

Pyrochroite
 Pyrolusite
 Ramsdellite
 Silicomanganese
 SiMn
 Sodium manganate

Terms for manganese and its compounds were searched for using the Boolean search term 'AND', combined with SET 1 below. The toxicity terms in SET 2 below were searched for 'in the same sentence' as the terms for manganese. No year limits were applied.

Medline and Toxfile Embase

SET 1

Toxicology#
 Toxicity-tests#
 Mutagenicity-tests#
 Toxic\$8.ti,de,ab.
 Teratogen\$5.ti,de,ab.
 Genotoxic\$5.ti,de,ab.
 Health effect\$1.ti,ab.
 Poison\$3.ti,de,ab.

SET 2

Mutagen\$5.ti,de,ab.
 Carcinogen\$5.ti,de,ab.
 Cytotoxic\$5.ti,de,ab.
 Neurotoxic\$5.ti,de,ab.
 Adverse effect\$1.ti,de,ab.

SET 3

manganese -poisoning#

SET 1

Toxicology#
 Toxicity#
 Toxicity-Testing#
 Toxic\$8.ti,de,ab.
 Teratogen\$5.ti,de,ab.
 Genotoxic\$5.ti,de,ab.
 Health effect\$1.ti,de,ab.
 Adverse effect\$1.ti,de,ab.

SET 2

Mutagen\$5.ti,de,ab.
 Carcinogen\$5.ti,de,ab.
 Cytotoxic\$5.ti,de,ab.
 Neurotoxic\$5.ti,de,ab.

Supplementary searches for information on manganese exposure were conducted using on-line and CD-based databases including Medline, HSELine, CISDOC, NIOSHTIC, RILOX and EMBASE. Additional sources included NIOSH and ACGIH publications for reports. Search terms used were manganese, manganate and exposure\$ or occupational.

A second literature search was also commissioned by Harlan covering the period from 1959 to May 2009.

The databases Medline, Embase, Biosis, HCAPLUS and Toxcenter were searched using STN and relevant manganese substances searched by CAS no:

7439-96-5, 1344-43-0, 1313-43-0, 1317-35-7, 598-62-9, 7785-87-7, 10377-66-9, 7773-01-5, 18820-29-6, 69012-28-8, 69012-33-5, 69012-49-3, 796740-23-3 or 796740-22-2 or 12518-52-4, 161162-70-5 or 12032-85-8.

The following queries were used with the Boolean search term 'OR'

Query 1

Adverse effect#
Toxic?
Reproduct?
Neurotox?
Genotoxic?
Carcinogen?
Sensitizat?
Teratogen?
Irritat?

Query 2

Mutagen?
Fertility, Developmental, Health effect#
Toxicity-test###
Mutagenicity- test ###
Poison?
manganese -poisoning

A third query was also made in the same databases:

Sensitizat?
Irrit?
Local lymph node
Murine
Guinea pig maximization
LLNA
Skin
Dermal
Buehler
Magnusson (1W) kligman

Reference lists from various review documents were also referred to.

The following references were provided by the manganese consortium, but were not considered to be of direct relevance for this report because they contain no original data (reviews) or relate more to exposure rather than reproduction toxicity:

Thomassen. Y *et al.* J. Environ. Monitoring (2001) 3, 555-559
UK HSE report on the impact of occupational exposure limits.
Hudson. N.J. *et al.* Ann. Occup. Hyg. (2001) 45, 3, 187-192.
Gwiadza *et al.* University of California post prints.
Francis. A.A and Forsyth. C. Oak Ridge Laboratories Chemical Hazard Evaluation Group.
Sandstrom. B. Proceedings of Nutrition Society (1992) 51, 211-218.
Sierra. P. *et al.* Environ. Res. (1998) 79, 2 94-101.
Norwegian Labour Inspectorate.

Appendix 2 List of References

Agency for toxic substance and disease registry, U.S. Department of Health and Human services, (2000) Toxicological Profile for Manganese 1-463

Alessio L, Apostoli P, Ferioli A, Lombardi S (1989) Interference of Manganese on Neuroendocrinal System in Exposed Workers. *Biological Trace Element Research* 21, 249-252

Bataineh HN, Bataineh ZM, Daradka H (2007) Short-Term Exposure of Female Rats to Industrial Metal Salts:Effect in Implantation and Pregnancy. *Repro Med Biol.* 6:179-183

Bonde JPE (1990a) Subfertility in Relation to Welding: A Case Referent Study among Male Welders. *Danish Medical Bulletin* 37, 105-108

Bonde JPE (1990b) Semen Quality and Sex Hormones among Mild Steel and Stainless Steel Welders: A Cross Sectional Study. *British Journal of Industrial Medicine* 43, 508-514

Chandra SV, Shukla GS (1978) Manganese Encephalopathy in growing rats. *Environmental Research* 15, 28-37

Chandra SV, Ara R, Nagar N, Seth PK (1973) Sterility in Experimental Manganese Toxicity. *Acta biol. Med. Germ.*, 30 (6,) 857-862

Chandra SV (1971) Cellular Changes induced by Manganese in the rat Testis-Preliminary results (1971). *Acta pharmacol. et toxicol.*29, 75-80

Colomina MT, Domingo JL, Liobet JM, Corbella J, (1996) Effect of Day of Exposure on the Developmental Toxicity of Manganese in Mice. *Vet. Human Toxicol.*38 (1) 7-9

Deskin R, Bursian SJ, Edens FW (1981) The Effect of Chronic Manganese administration on some Neurochemical and Physiological Variables in Neonatal Rats. *Gen. Pharmac.* Vol. 12, 279-280

Dorman DC, Struve MF, Vitarella D, Byerly FL, Goetz J, Miller R (2000) Neurotoxicity of Manganese Chloride in Neonatal and Adult CD Rats Following Subchronic (21-Day) High-Dose Oral Exposure. *Journal of applied Toxicology* 20, 179-187

Dorman DC, McElveen AM, Marshall MW, Parkinson CU, Arden James R, Struve MF and Wong BA (2005) Maternal-fetal distribution of manganese in the rat following inhalation exposure to manganese sulfate. *Neurotoxicology* 26:625-632.

Doyle D, Kapron CM (2002) Inhibition of cell differentiation by manganese chloride in micromass cultures of mouse embryonic limb bud cells. *Toxicol. In vitro*, 16 (20), 101-106

Elbetieha A., Bataineh H., Darmani H., Al-Hamood M.H(2001), Effects of long term exposure to manganese chloride to fertility of male and female mice, *Toxicology letters* 119: 193-201

Ellingsen DG, Haung E, Ulvik RJ, Thomassen Y (2003a) Iron Status in Manganese Alloy Production Workers. *J Appl Toxicol* 23:239-247

Ellingsen DG, Hetland SM, Thomassen Y (2003b) Manganese Air Exposure Assessment and biological Monitoring in the Manganese Alloy Production industry. *J Environ Monit.* 5:84-90

Ellingsen DG, Chashchin V, Haug E, Chashchin M, Tkarenko V, Lubnina N, Bast-Pettersen R, Thomassen Y (2007) An Epidemiological Study of Reproductive Function Biomarkers in Male Welders. *Biomarkers* 12(5):497-509

Emara AM, El-Ghawabi SH, Madkour OI, El-Samra GH (1971) Chronic manganese poisoning in the dry battery industry. *Brit. J. Industr. Med.* (28), 78-82

Fechter LD (1999) Distribution of manganese in development. *Neurotoxicology* 20:197-201.

Gennart JP, Buchet JP, Roels H, Ghyselen P, Ceulemans E, Lauwerys R (1992) Fertility of Male Workers Exposed to Cadmium, Lead, or Manganese. *American Journal of Epidemiology* 135 (11), 1208-1219

Grant D, Hustvedt SO (1998) Developmental Toxicity of Manganese Chloride in the Rat. *NeuroToxicology* 19(3):469

Gray LE, Laskey JW (1980) Multivariate Analysis of the Effects of Manganese on the Reproductive Physiology and Behaviour of the males Mouse. *J Toxicol Env Health* 6:861-867

Hafeman D, Factor-Litvak P, Cheng Z, van Geen A, Ahsan H (2007) Association between Manganese Exposure through Drinking Water and Infant Mortality in Bangladesh. *Environmental Health Perspectives*, 115 (7), 1107-1112

Hameed BN, Ziad BM, Haytham D (2007) Short-term Exposure of Female Rats to Industrial Metal Salts: Effect on Implantation and Pregnancy. *Reproductive Medicine and Biology*, 6 (3); 179-183

He P, Liu DH, Zhang GQ (1994) Effects of high-level-manganese sewage irrigation on children's neurobehavior. *Zhonghua Yu Fang Yi Xue Za Zhi* 28(4):216-8.

Hjollund NHI, Bonde JPE, Jensen TK, Ernst E, Henriksen TB, Kolstad HA, Giwercman A, Skakkebaek NE, Olsen Joern (1998) Semen Quality and Sex Hormones with Reference to Metal Welding. *Reproductive Toxicology* 12 (2), 91-95

Järvinen R, Ahlström A (1975) Effect of the Dietary Manganese Level on Tissue Manganese, Iron, Copper and Zinc Concentrations in female rats and their Fetuses. *Medical Biology* 53, 93-99

Jiang Y, Lu J, Xie P (1996) Effects of manganese on the sexual function and reproductive outcome of male exposed workers. *Chi J. Ind. Hyg. Occup. Dis.* 14:271-273 (chinese)

Kilburn CJ (1987), Manganese, Malformations and Motor Disorders: Findings in a Manganese-Exposed Population. *Neurotoxicology* 8 (3), 421-430

Koshida Y, Kato M, Hara T (1965) Distribution of Radiomanganese in Embryonic Tissues of the Mouse. *Annot Zool Jap.* 38(1):1-7

Klimisch HJ, Andreae M, Tillmann U (1997), A Systematic Approach for Evaluating the Quality of Experimental Toxicological and Ecotoxicological Data, *Regulatory toxicology and Pharmacology* (25), 1-5

Kontur PJ, Fechter LD (1988) Brain regional Manganese Levels and Monoamine Metabolism in Manganese-Treated Neonatal Rats. *Neurotox Teratol.* 10:295-303

Kristensson K., Eriksson H., Lundh B., Plantin L.O., Wachtmeister L., EL Azazi, Morath C., Heilbronn E., (1986), Effects of Manganese chloride on the rat developing nervous system, *Acta pharmacol. et toxocol.* 59: 345-348

Lai JCK, Leung TKC, Lim L (1984) Differences in the Neurotoxic Effects of Manganese During Development and Ageing: Some Observations on Barin Regional Neurotransmitter and non-Neurotransmitter Metabolism in a Developmental Rat Model of Chronic Manganese Encephalopathy. *Neurotox* 5(1):37-48

Lai JCK, Leung TKC, Lim L, Chan AWK, Minski MJ (1991) Effects of Chronic Manganese Treatment on Rat Brain Regional Sodium-Potassium-Activated and Manganese-Activated Adenosine Triphosphatase Activities During Development. *Metab Brain Disea.* 6(3):165-174

Laskey JW, Rehnberg GI, Hein JF, Laws SC (1985) Assessment of the male reproductive system in the preweanling rat following $Mn_3 O_4$ Exposure. *Journal of Toxicology and Environmental Health*, 15:339-350

Laskey JW, Rehnberg GL, Hein JF, Carter SD, (1982) Effects of chronic manganese (Mn_3O_4) Exposure on selected reproductive parameters in rats, *Journal of Toxicology and Environmental Health* 9:677-687

Lauwerys R, Roels H, Genet P, Toussaint G, Bouckaert A, De Cooman S (1985) Fertility of Male Workers Exposed to Mercury Vapor or to Manganese Dust, A Questionnaire Study. *American Journal of Industrial Medicine* (7), 171-176

Lee B, Pine M, Johnson L., Rettori V., Hiney JK, Les Dees W (2006), Manganese acts centrally to activate reproductive hormone secretion and pubertal development in male rats, *Reproductive Toxicology* 22: 580-585

Lee B, Hiney JK, Pine MD, Srivastava VK, Les Dees W (2007) Manganese stimulates luteinizing hormone releasing hormone secretion in prepubertal female rats: hypothalamic site and mechanism of action. *J Physiol* 578(3):765-772

Leung TKC, Lim L, Lai JCK (1993) Brain Regional Distributions of Monoamine Oxidase Activities in Postnatal Development in Normal and Chronically Manganese-Treated Rats. *Metab barin Disea.* 8(3):137-149

Lown BA, Morganti JB, D'Agostino R, Stineman CH, Massaro EJ (1984). Effects on the Postnatal Development of the Mouse of Preconception Postconception and/or Suckling Exposure to Manganese Via Maternal Inhalation Exposure to MnO_2 Dust. *Neurotoxicology* 5 (1), 119-131

Massaro EJ, D'Agostino RB, Stineman CH, Morganti JB, Lown BA, (1980), Alterations in the behaviour of adult offspring of female mice exposed to $Mn O_2$ Dust during Gestation; *Metals* II 39: 623

Meeker JD, Rossano MG, Protas B, Diamond MP, Puscheck E, Daly D, Nigel Paneth , Wirth JJ, (2009), Multiple metals predict prolactin and thyrotropin (TSH) levels in men, *Environmental Research* 109 869–873

Meeker JD, Rossano MG, Protas B, Padmanahban V, Diamond MP, Puscheck E, Daly D, Nigel Paneth, Wirth JJ, (2008) Environmental exposure to metals and male reproductive hormones: circulating testosterone is inversely associated with blood molybdenum, Article in Press 1-11

Mena I, Marin O, Fuenzalida S, Cotzias GC (1967) Chronic Manganese Poisoning, clinical picture and manganese turnover. *Neurology* 17 (2), 128-136

Miller RK, Mattison DR, Panigel M, Ceckler T, Bryant R, Thomfors P (1987) Kinetic assessment of manganese using magnetic resonance imaging in the dually perfused human placenta in vitro. *Environ Health Perspect.* 74: 81–91.

Mortensen JT (1988) Risk for reduced sperm quality among metal workers, with special reference to welders. *Scand J Work Environ Health* 14, 27-30

Murthy RC, Srivastava RS, Gupta SK, Chandra SV,(1980) Manganese induced testicular changes in monkeys, *Exp. Path.* 18, 24-244

Mutti A, Bergamaschi E, Alinovi, R, Lucchini R, Vettori MV, Franchini I (1996) Serum Prolactin in Subjects Occupationally Exposed to Manganese. *Annals of Clinical and Laboratory Science* 26, 10-17

NTP Technical Report (1993), Toxicology and Carcinogenesis, Studies of Manganese (II) Sulfate Monohydrate in F344/N Rats and B6C3F₁ Mice, NTP TR 428 NIH Publ. No. 94-3159 U.S. Department of Health and Human Services, 5- 272

NTP Technical report on the toxicology and Carcinogenesis Studies of Manganese (II) Sulphate Monohydrate (CAS No. 10034-96-5) in F344/N Rats and B6C3F₁ Mice (Feed Studies) (1993) NIH Publication No. 94-3159, NTP TR 428

Onoda K, Hasegawa A, Sunouchi M, Tanaka S, Takanaka A, Omori Y, Urakubo G (1978) Studies on the Fate of Poisonous Metals (VII) – Distribution and Transplacental Passage of Manganese in Pregnant Rat and Fetus. *J Food Hyg Soc.* 19(2):208-215

Pappas BA, Zhang D, Davidson CM, Crowder T, Park GAS, Fortin T (1997) Perinatal Manganese Exposure: Behavioral, Neurochemical and Histopathological Effects in the Rat. *Neurotoxicology and Teratology* 19(1), 17-25

Peñalver R (1955) Manganese Poisoning: the 1954 Ramazzini oration. *Industrial Medicine and Surgery*, 24, 1-7

Pine M, Lee B, Dearth R, Hiney JK, Les Dees W., (2005), Manganese Acts Centrally to Stimulate Luteinizing Hormone Secretion: A Potential Influence on Female Pubertal Development, *Tox. Sciences* 85, 880–885

Prestifilippo JP, Fernández-Solari J, Mohn C, De Laurentiis A, McCann SM, Dees WL, Rettori V (2007) Effect of Manganese on Luteinizing Hormone-Releasing Hormone Secretion in Adult Male rats. *Tox. Sciences*, 97(1) 75-80

Rodier J (1955) Manganese Poisoning in Moroccan Miners. *Brit. J. Industr. Med.* 12, 21-35

Roels H, Meiers G, Delos M, Ortega I, Lauwerys R, Buchet JP, Lison D (1997) Influence of the route of administration and the chemical form (MnCl₂, MnO₂) on the absorption and cerebral distribution of manganese in rats. *Arch. Toxicol.* 71, 223-230

Sánchez DJ, Domingo JL, Llobet JM, Keen CL, (1993), Maternal and developmental toxicity of manganese in the mouse, *Tox. Letters* 69, 45-52

Schuler P, Oyanguren H, Maturana V, Valenzuela A, Cruz E, Plaza V, Schmidt E, Haddad R (1957) Manganese Poisoning: Environmental and Medical Study at a Chilean Mine. *Industrial Medicine and Surgery* 167-173

Seth PK, Husain R, Mushtaq M, Chandra SV (1977) Effect of Manganese on Neonatal Rat: Manganese Concentration and Enzymatic Alterations in Brain. *Acta pharmacol. et Toxicol.* 40, 553-560

Seth PK, Nagar N, Husain R, Chandra SV, (1973), Effects on Manganese on Rabbit testes, *Environ. Physiol. Biochem.* 3, 263-267

Webster WS, Valois AA (1987) Reproductive Toxicology of Manganese in Rodents, including Exposure during the Postnatal Period. *Neurotoxicology* 8 (3), 437-444

Wirth JJ, Rossano MG, Daly DC, Paneth N, Puschek E, Potter RC, Diamond MP (2007) Ambient Manganese Exposure is negatively associated with Human Sperm Motility and Concentration. *Epidemiology* 18(2), 270-273

Wu W, Zhang Y, Zhang F (1996) Studies on the semen quality in workers exposed to manganese and electric welding. *Chin. J. Prev. Med.* 30: 266-268. (chinese)

Wu Y, Cui J, Zhang Y, Wang X, Ma M (2004) Study on mechanisms of subacute reproductive toxicity in male rats exposed to manganous chloride. *Chinese J. ind. Med.* 17(3): 183-185

Zhang D, He X, Zhang W, TanJ (1998) Effect of manganese on the growth and development of rat offspring. *Wei Sheng Yan Jiu* 27(4):237-40

Zhang D, He X, Zhang W, TanJ (1999) Effect of manganese on the brain extrapyramidal development of rat offspring. *Wei Sheng Yan Jiu* 28(4):214-7

Zhang D, He X, Hunag S, Li Y (2001) Effect of manganese exposure on brain development in postnatal mice. *Wei Sheng Yan Jiu* 30(5):260-2