IEH Institute of Environment and Health

Cranfield UNIVERSITY

Manganese Health Research Program:

Overview of Research into the Health Effects of Manganese (2007-2008)

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Corrigenda

The following amendments apply to:

Section 3.4 Reproductive and Developmental effects

The fourth paragraph of this section should be at the end of section **3.3. Other effects**

The fifth and sixth paragraphs of this section should be at the end of section **3.1 Neurological effects**

Section 4.1 Toxicokinetic and metabolic considerations

The third paragraph of this section should also be considered with section **2 Exposure Measurement and Modelling**

Section 4.2 Oxidative stress as a mechanism of neurotoxicity

The penultimate paragraph of this section should also be considered with section **2 Exposure Measurement and Modelling**

August 2009

-End-

PREAMBLE AND PURPOSE

This report is an overview of key recent publications, including full peer-reviewed papers and abstracts that have appeared in the scientific literature or conference proceedings, and have been included in the quarterly update service on the MHRP website in the period March 2007 to February 2008.

It has been written for the use of a wide readership including researchers, interested scientists and health professionals. It may also be of interest to laypersons who may wish to have an overview of recent study findings on the effects of manganese on human health.

1. Introduction

Manganese (Mn) is a widely used transition metal. In its pure state it is not a naturally occurring metal and exists as the oxide, carbonate or silicate derivatives. It is an essential element in the human diet and thus deficiency can lead to negative health outcomes. However, excess exposure and accumulation of large concentrations of manganese can have repercussions on a number of organ systems, including the central nervous system. For a full description and background to manganese, please see the previous reports.

This report summarises the published literature relating to human exposure to and potential health effects of, manganese and manganese-containing inorganic compounds, between March 2007 and February 2008. The published literature is recorded into the following sections:

Section 2 - EXPOSURE MEASUREMENT AND MODELLING: Papers relating to the measurements or modelling of environmental and occupational Mn exposure, the development of biomarkers of exposure or effect.

Section 3 - HEALTH EFFECTS: Papers on the influence of Mn on health, disease and dysfunction.

Section 4 - MECHANISMS: Papers on the physiological, biochemical and cellular mechanisms underlying the toxic effects of Mn.

Section 5 - HUMAN SUSCEPTIBILITY: Papers relating to assessment of the influence of genetic and epigenetic factors on human susceptibility to the effects of Mn.

Section 6 - TREATMENT AND IMAGING: Papers on the development and implementation of new medical approaches to the treatment of excessive Mn exposure.

Section 7 - MISCELLANEOUS: Other papers considered of interest or potential relevance to the study of the health effects of Mn.

An overview of the reported literature is presented as well as a comprehensive reference list for this report.

2. Exposure Measurement and Modelling

Humans require Mn and normally obtain this via the diet. However, exposure to high levels of Mn may occasionally occur through exposure to contaminated environments (e.g. water and soil) or, more frequently, through employment in occupations such as miners, smelters and welders via inhalation. Michalke *et al*., (Michalke, Halbach & Nischwitz 2007) reviewed the human exposure to Mn and suggested that Mn could come from natural sources, occupational sources as well as anthropogenically-caused environmental sources (e.g. use of methylcyclopentadienyl manganese tricarbonyl; MMT).

Further evidence of environmental exposure to high levels of Mn through exposure to contaminated water has been reported during the period under consideration. Concentrations of trace elements were measured in borehole, well and river water samples and urine samples from mine workers (n=15) and non-mine workers (n=17) in Tarkwa, a historic mining town in Ghana, were compared with tap water and urine samples from non-mine workers (n=4) in Accra (Asante et al. 2007). The mean water Mn concentrations (and standard deviations) were 797 (1380) µg/l in borehole samples, 162 (101) µg/l in well samples, and 682 (742) µg/l in river samples in samples taken from Tarkwa compared to 3.26 µg/l in tap water from Accra; the WHO drinking water guideline for Mn is 400 µg/l. The mean urine Mn concentrations were 8.16 µg/l in mine workers (standard deviation 5.57µg/l) whilst non-mine workers in Tarkwa had a mean Mn concentration in urine of 29.8µg/l (standard deviation 64.6µg/l) compared to the mean Mn urine concentration of non-mine workers in Accra of 4.08µg/l (standard deviation 0.87µg/l). There was a significant increase in the total mean urine Mn concentration of both mine and non-mine workers in Tarkwa compared with that of workers in Accra.

The presence of high levels of manganese has also been reported in bottled mineral waters. Samples from commercially-available mineral water in Italy (n=40) were analysed for carbon dioxide, total hardness and dissolved cations and anions, and were also assessed for ecotoxicity (determined as percentage of immobile *Daphnia magna* after shaking for 30 minutes). The Mn concentrations of the bottled water samples ranged from 0.1 µg/l to 645.2 µg/l, with the median concentration of 1.15 µg/l and the mode concentration of 0.3 µg/l. The legal limit in Italy is 500 µg/l) and so it is apparent that one sample had a Mn concentration greater than the legally permitted level. A further three samples were greater than 100 µg/l (Signorile et al. 2007).

Other environmental exposures have been reported around industrial areas. Historical measurement of Mn concentrations in 206 municipalities surrounding ferroalloy industries in the province of Brescia in Northern Italy showed that the Mn concentrations in settled dust was significantly higher in the areas surrounding the industrial plants and those downwind of the industrial plants (airborne Mn concentrations were 0.69 µg/m3 (range 0.2-1.8) 2km from plant and 0.08 µg/m3 (range 0.05 to 0.03) 50 km away from the plant) whilst the plant was active, which decreased again 2 years after the plant ceased to be active (0.03 µg/m3, range 0.00 to 0.11 µg/m3) (Lucchini et al. 2007). The authors noted the effect of Mn accumulation in blood of specific subpopulations (e.g. iron deficient individuals). The relationship between blood Mn and prolactin concentrations in 230 individuals in a population surrounding a mine and a mineral processing plant in Mexico suggested that there was a positive relationship between blood Mn concentrations and prolactin concentrations (Montes et al. 2008). Negative associations were shown between Mn concentrations and haemoglobin, age and lead levels. The blood Mn levels could be used to determine serum prolactin levels when corrected for gender and age.

In groups of Russian senior school children and students either trained skiers (31 senior trained, 110 low order student skiers, and 39 high order student skiers) or untrained (17 untrained students and 60 students not going in for sports) with seasonally-variable dietary Mn intake below the recommended values, the blood plasma Mn concentration and the corpuscular Mn concentration was determined (Nasolodin, Gladkikh 2007). The content of Mn in the blood and corpuscles were found to relate to the pattern of muscle activity rather than season, the Mn level in young skiers and high-order athletes were higher than the other populations, especially in periods of increased training. Whilst this study suggests that Mn intake and blood concentration is greater in populations that are more active, there is little to suggest that this is indicative of the general population.

Occupational exposure to Mn can occur in a number of situations at varying exposure levels (see Table 1). Welders exposed to Mn in welding fumes (geometric mean concentration 121 $\mu g/m^3$) had significantly increased blood Mn concentrations (8.6 µg/l) compared with age-matched controls (6.9 µg/l) (Ellingsen et al. 2008).

Bridge welders in confined space welding (n = 43) with little or no personal protection equipment had a cumulative exposure index calculated based on the Mn concentration in air, duration and type of welding (Bowler et al. 2007). The mean time-weighted average of Mn concentration in air ranged from 0.11 to 0.46 mg/m^3 with 55% exposed to concentrations greater than 0.20 mg/m^3. The mean Mn-air concentration was 0.21 mg/m^3 (± 0.08) and the mean CEI was 2.56 mg/m^3 month (± 1.2). The mean blood Mn concentration was 9.6 (± 2.5) µg/l with 43% of workers having a Mn blood concentration greater than 10 µg/l. Mn concentration in urine, as well as lead concentrations in blood, and copper and iron concentrations in plasma, were normal. Welders still actively welding at the time of the study (n = 21) had a significantly higher mean (± SD) blood Mn concentration than those who had stopped working 1 or more months prior to the study (n = 16), 10.3 (2.82) µg/l compared to 8.1 (1.74) µg/l.

The MRI imaging of the globus pallidus and blood Mn concentrations of male welders (n = 20) were compared to age- and gender-matched non-welding production workers (n = 10) (Choi et al. 2007). There was a significant increased in mean airborne Mn levels from 0.027 (± 0.028) mg/m^3 for non-welders and 0.399 (±0.205) mg/m^3 for welders, as well as an increase in the cumulative exposure index (CEI) of Mn from 0.592 (± 0.61) mg/m3 year to 6.85 (± 2.01) mg/m^3 year, but were no significant differences between the blood Mn concentrations of non-welders (1.05 ± 0.21 µg/dl) and welders (1.42 ± 0.65 µg/dl), nor in haemoglobin, serum iron, or liver enzyme levels. The visual grading of the MRI scans (Palliadal index, PI, the ratio of intensity of the globus pallidus to the frontal white matter) was positively correlated with CEI. However, visual grading was considered to only be useful over a narrow range of Mn concentrations due to the non-linear nature of the dose response relationship seen.

There is evidence that exposure to Mn can be reduced by personal protection equipment and appropriate control measures. Hovde and colleagues determined that ultrafine particle generation varied with different voltage levels during the welding using a particle sampler (cellularose ester membrane filters, which was then analyzed for Mn, iron and total particulate matter) and an ultrafine condensation particle counter (Hovde, Raynor 2007). The concentration of ultrafine particles (0.5 to 0.7µm) was more than three times greater at 23.5V (82800 particles cm^3) compared with 16V (9800 particles/cm^3) and the Mn concentration was also increased by the increased voltage (1.7mg/m^3 at 16V compared to 6.4mg/m^3 at 23.5V), leading the authors to suggest that welders should choose to use the lower voltage setting when possible.

Table 1. Associations between Mn airborne exposure and blood concentrations identified from studies on occupational exposure published in period February 2007 to February 2008.

Occupation	Mn exposure	Blood Mn concentration	Source
Welder (n = 96)	121 µg/m^3	8.6 µg/l	(Ellingsen et al. 2008)
Welder (n = 43)	100 - 460 µg/m^3	9.6 µg/l	(Bowler et al. 2007)
Factory workers (n = 121)		5.5 µg/l	(Chuang et al. 2007)
Welders (n = 59)	160 µg/m^3		(Halatek, Sinczuk-Walczak & Rydzynski 2008)
Welders (n = 20)	399 µg/m^3	14.2 µg/l	(Choi et al. 2007)Choi
Mine workers (n = 15)	797 µg/l, 162 µg/l, 682 µg/l (in water)	8.16 µg/l	(Asante et al. 2007)Asante

Other sources of exposure are also known. For example, foetal exposure to Mn may occur through maternal blood (Ericson et al. 2007), and it has been suggested from a study of Mn concentrations in children's teeth (n=27; 11 boys, 16 girls) that tooth levels might give an indication of maternal (i.e. offspring's prenatal) exposure to Mn at certain time points (20th and 62nd – 64th gestational week). Levels were determined by ion mass spectrometry (IMS) analysis but, regrettably, the concentrations detected were not reported in the paper.

In a cohort of 68 preterm infants in Hampshire, UK, the blood levels of zinc, copper, selenium and Mn were measured and compared to growth parameters and dietary intake (Zn and Cu only) of the infant (Marriott et al. 2007). The mean (± SD) birth weight was 1.47 (± 0.434) kg and the mean gestation was 31.4 (± 2.9) weeks. Mean Mn blood levels at term and 6 months old were 320 (± 189) nmol/l and 211 (± 68) nmol/l, respectively. Whilst there were no significant associations between dietary intake and blood levels for Mn, Zn or Cu, there was an association between Cu levels at term and head circumference.

The determination of Mn exposure in individuals is normally determined on the basis of blood Mn concentration. However, this parameter does not provide an indication of variations in concentration over a period of time. This is not the case with measurement for Mn deposits in tooth enamel (Ericson et al. 2007) or hair. Measurement of transition metals (including iron and Mn) in the blood and hair from ferro-alloy workers had higher Mn concentrations (and other metals) present in the hair than concentrations in the blood, suggesting that hair concentrations of transition metals are not necessarily indicative of short-term exposures but may be more representative of long-term exposure (Mishra, Ramteke & Wate 2007). Salivary Mn concentrations in male welders were higher than those of non-metal exposed controls and associated with airborne Mn levels, and were also higher in welders with 5 – 10 years employment than those with less than 5 years employment (Wang, Du & Zheng 2008). Mn concentrations in saliva may reflect welders' exposure to airborne Mn as well as their years of welding experience.

Other biomarkers for Mn concentration and accumulation in the body have been investigated including brain imaging. However, no quantitative biomarker has yet been established that can replace the use of blood Mn concentration. Modelling exposure may provide some indication of body Mn concentrations and accumulation.

A physiologically-based pharmacokinetic (PBPK) model that accounts for Mn-tracer kinetics and steady-state tissue Mn in rats on normal diets (45ppm; form not stated in the paper;

preventing dose-dependent homeostasis that occurs at high dose rates) was developed that represented six tissues (brain, respiratory tract, liver, kidneys, bone and muscle) (Teeguarden et al. 2007b). However, the inhalation tracer estimates were modelled using an assumption that deposited Mn was absorbed into deep tissue stores in the lung before becoming available, via the circulation, to other tissues which meant that the observed and modelled values did not correspond to each other. Another model considered kinetic approaches and integrated bulk tissue Mn kinetics and hepato-intestinal control of oral-route Mn uptake into an integrated model structure connecting systemic and oral Mn. Hepatic extraction of orally absorbed Mn in rats was 19, 54 and 78% at dietary exposures of 1.5, 11.2 and 100 ppm and the hepatic extraction of systemic Mn, predicted through the simulation of elimination kinetics, was less 0.004, 0.005 or 0.009% at dietary levels of 2, 10, 100 ppm (Teeguarden et al. 2007a). The differences in hepatic processing of blood Mn were considered to arise from differences in the dose route, and would need to be accounted for in more complete PBPK models. Whilst both models may be appropriate to support risk assessment calculations for Mn, they are not considered sufficient for use alone.

3. Health Effects

3.1 Neurological Effects

A range of health effects of Mn have been widely reported, with the most obvious effect being neurological/neuropsychological impairment, and the development of a disease similar to Parkinson's Disease (PD) called manganism.

Neurobehavioral effects where investigated in 96 welders exposed to 121 μg/m^3 Mn in welding fume (individual means from 2 successive days of sampling). A comparison was made with 96 age matched controls and with diagnosed manganism patients (n = 27) who were former welders (Ellingsen et al. 2008, Ellingsen et al. 2007). Whole blood Mn concentrations were significantly greater in the current welders (8.6 μg/l) compared to controls (6.9 μg/l) but were not different to those of the manganism patients (8.7 μg/l). Welders with the highest concentration of blood Mn (12.6 μg/l) were noted to have statistically significantly lower scores on a digit symbol test. Furthermore, welders exposed to the highest Mn concentration (423 μg/m^3, range 204 – 2322) had statistically poorer finger tapping test score compared with controls.

The use of MRI scoring has been investigated. The signal intensity of the T1-weighted MRI imaging of the globus pallidus was compared with that of the frontal white matter, and grading the signals (grade I being slightly increased and grade III being the same as subcutaneous fat) and calculating the pallidal index (PI) was used as an assessment of tissue Mn concentration to determine the effects of Mn on neurobehavioral function following occupational exposure of workers ((Shin et al. 2007). The neurobehavioral function was determined using the World Health Organization Neurobehavioral Core Test Battery and computerized finger tapping speed was used to assess motor speed. The mean blood Mn concentration increased in individuals with grade III signals (2.46 ± 1.18 μg/dl) compared to those with grade I (1.46 ± 0.28 μg/dl) or no increased signals (1.23 ± 0.37 μg/dl), and similar results were seen with the airborne Mn (calculated) (0.07 ± 4.62 μg/m3, 0.21 ± 3.59 μg/m3 and 0.73 ± 2.81 μg/m3, respectively) and PI (103.5 ± 2.77, 110.05 ± 2.63, 125.2 ± 4.32, respectively). Signal intensity was related to neurobehavioral performance in Mn exposed workers.

Behavioural issues have also been noticed with Mn administration. The Mn, lead and iron concentrations in the enamel of children's teeth (11 boys, 16 girls, sites within the USA) were determined by IMS (the Mn concentration measured was not reported in the paper) at the 20th and 62nd-64th gestational week (by using the concentration at different points on the molar teeth) as a method of determining in utero exposure to Mn, and assessing if there is any relationship to subsequent behavioural performance measures (Ericson et al. 2007). Levels of Mn in the 20th week were found to be positively correlated with measurements of behavioural disinhibition (play with a forbidden toy at 36 months, impulsive errors on a continuous performance at 54 months and with a children's' Stroop test at 54 months), parents' and teachers' ratings of externalizing and attention problems on the Child Behaviour Checklist (1st and 3rd grades) and teacher ratings on the Disruptive Behaviour Disorders Scale. In contrast, the Mn level at 62nd-64th gestational week only correlated with the teachers' reports of externalizing behaviour (1st and 3rd grades). The source and extent of Mn exposure and Mn concentration in teeth were not reported. However, the authors suggested that over-absorption of Mn could occur as a consequence of gestational iron-deficiency anaemia.

Other studies relating Mn exposure to persistent deficits in neuromotor functions in a study of 71 Mn workers and 81 controls (Bouchard et al. 2007a), and links to other neuropsychiatric symptoms, such as depression and anxiety, have been suggested (Bouchard et al. 2007b). Some reports of neuropsychological impairment have, however, been subject to criticism. Rohling and Demakis reviewed two published articles by Bowler and colleagues (2003, 2006) which considered the occupational Mn exposure of welders and the incidence of neuropsychological impairment, compared with a common control group (Rohling, Demakis 2007). The claim by Bowler and colleagues that chronic Mn exposure in welders resulted in a specific and identified pattern of neuropsychological impairment was considered not to be supported by the reported data because of the variation of, and pattern of, effect size differences between welders and controls in the two studies.

Historical measurement of Mn particulate concentrations in 206 municipalities surrounding ferroalloy industries in the province of Brescai in Northern Italy were studied in respect of the incidence of 2667 Parkinsonian cases in 903,997 residents (with a crude prevalence of 296 per 100,000 residents; (Lucchini et al. 2007). Bayesian-smoothed standardized morbidity ratios (SMR) were calculated for the province and each municipality, and were related to vicinity of industrial sites. There were significantly higher SMRs in 37 municipalities near industrial plants (324 cases in 77,708 residents), when compared to the other 169 municipalities of the province. SMR's were also associated with the Mn concentration in settled dust (collected by brushing marble window sills on ground floor residences, does not state whether the sills are inside or outside the home). The authors concluded that the relationships shown in the study suggest that environmental exposure to Mn associated with an increased prevalence of Parkinsonian disturbances (two out of the four cardinal signs of PD; rest tremor, bradykinesia, rigidity, and impaired postural reflexes). However, the study did not distinguish between the occupation of the subjects (e.g. welder or homemaker) in the study nor by other environmental conditions (e.g. smokers and non-smokers). There is therefore a need for further supporting evidence before the relationship between Mn and increased prevalence of PD can be confirmed. Environmental Mn exposure, in the form of methylcyclopentadienyl manganese tricarbonyl (MMT) in car exhaust fumes from Canadian cities has also been shown to be associated with increased incidence of PD-like symptoms from physician diagnosis and prescriptions of L-Dopa (Finkelstein, Jerrett 2007) although again individual conditions and occupational exposures were not considered.

A review and critical analysis of published studies conducted on Mn-exposed occupational cohorts during the last 100 years was performed by Santamaria and colleagues (Santamaria et al. 2007). The exposure data that were considered for the review indicated that Mn exposure in welders was lower than that in miners and smelter workers showing clinical neurotoxicity and manganism, and that many such exposures were below the current US OSHA permissible exposure limit (PEL) of 5 mg/m^3. The reviewers concluded that, although manganism was observed in highly-exposed workers, the exposure-response data available for welders does not support the conclusion that welding is associated with clinical neurotoxicity. Indeed, they suggested that there were no epidemiological studies showing an association between PD and welding although there is an increased likelihood of PD in welders. The authors recommended that further studies on the association between Mn exposure and neurotoxic effects should include consideration of: 1) whether subclinical effect proceed to clinical effects in the absence of additional Mn exposures (i.e. long term follow-up of Mn cohorts); 2) the mechanistic basis for Mn as a risk factor for PD; and 3) the development of a standardized suite of objective diagnostic criteria for the evaluation of subclinical effects of Mn exposure on humans.

Mn intoxication in two individuals with symptoms resembling Parkinsonism as a result of parenteral administration of a psycho-stimulant substance (a combination of acetylsalicylic acid, ephedrine HCl, potassium permanganate, and vinegar melted in tap water) was reported (Meral et al. 2007). The source of Mn exposure was the potassium permanganate which,

when administered intravenously, will result in Mn overexposure. Mn intoxication is known to persist after cessation of exposure, but a deterioration in the Parkinsonian symptoms has been reported to occur during the initial 5 to 10 years, which does not occur in idiopathic PD (Huang 2007). However, Mn intoxication has also been shown to persist 18 years after cessation of exposure (Huang et al. 2007).

A review of 31 studies considering occupational (n=18), environmental (n=7) and childhood (n=6) exposure, reinforced the known neurobehavioral effect of Mn (Zoni, Albini & Lucchini 2007).The authors suggested that a test battery including tests of motor function, response speed, cognitive functions, intellectual abilities, mood and symptom questionnaires should be included in future studies as a core battery to determine any Mn effect.

There is some evidence to suggest that metabolic disorders may also play a role in Mn accumulation (Sahni et al. 2007). A 6-year old child showed severe Mn neurotoxicity, iron deficiency and elevated cobalt levels as a result of seasonal ingestion Mn exposure from well-water which had Mn concentrations exceeding recommended guidelines. However other family members also had elevated plasma Mn levels but were asymptomatic. Whilst the exposure was confirmed, this was considered to be insufficient to explain the toxicity observed as other siblings with identical exposures remained asymptomatic. Therefore a metabolic disorder involving divalent metals (Mn, Fe, Co) interacting with environmental exposures, was considered the most likely explanation.

3.2 Hormonal effects

Hormone expression has also been shown to be affected by Mn exposure. Prolactin (PRL) and Inhibin B concentrations were measured in blood from current welders (with exposure to 121 $\mu g/m^3$ Mn in welding fume, averaged over 2 days sampling, n = 96) and from control subjects (turners/fitters at same plants, age and sex matched, n=96) and manganism patients (n = 23, 4 to 7 years since welding cessation) (Ellingsen et al. 2007). Data on exposures to Mn for the welders and exposure levels for former welders were not available, but it was noted that there were similar numbers of smokers and non-smokers in each group. The Mn concentration in blood and urine, as well as serum PRL and Inhibin B concentrations, were measured. Serum PRL concentration is indicative of dopamine synthesis, linked to Parkinson's disease. Therefore, PRL production and secretion may be targets for Mn toxicity. Smokers have a lower serum PRL than non-smokers, and current welders had higher serum PRL concentrations than the referents. Higher blood Mn levels were also associated with higher serum PRL levels, suggesting a dose-response relationship. A study comparing 251 welders (cumulative Mn exposure index of 1.4 ± 1.1 mg/m^3 year) and 100 age-matched office workers showed increases in thyroid stimulating hormone releasing hormone (TSH; as stated and defined in paper), follicle stimulating hormone (FSH) and luteinizing hormone (LH) from welders (5.08 pg/ml, 7.40 mIU/ml, and 4.91 mIU/ml, respectively) compared to office workers (3.91 pg/ml, 6.15 mIU/ml, and 4.00 mIU/ml, respectively); dopamine concentrations were lower in the welders(Kim et al. 2007b). The authors suggest that Mn suppresses the inhibitory feedback control of dopamine on the hypophyseal-pituitary axis, and further postulate that this may be responsible for the increased PRL levels noted in the welders.

3.3 Other effects

A hospital-based case-control study was carried out to determine the relationship of blood metal concentration (lead, Mn, arsenic and selenium) and hearing function in factory workers in Taiwan (Chuang et al. 2007). The control subjects (n = 172) had normal hearing whilst the case subjects (n = 121) had an average hearing threshold of over 25 decibels (considered to be the threshold for occupational hearing loss). The individuals hearing threshold was tested over

6 frequencies (0.5, 1, 2, 3, 4, and 6 kHz) 16 hours after the working day. There was no significant difference between the mean (± SD) blood Mn concentration of controls (5.34 ± 1.36µg/l) and case subjects (5.5 ± 1.57µg/l), and no relationship was found between the blood Mn concentration and hearing loss.

3.4 Reproductive and Developmental effects

Impairment of fertility has been suggested to relate to Mn exposure. In a study comparing Mn blood concentration and sperm motility, concentration and morphology in 200 male patients attending infertility clinics, high Mn concentrations (>14µg/l; n = 70) were associated with increased low sperm motility and concentration (Wirth et al. 2007). However, an association between low Mn concentration (<10µg/l; n = 130) and low sperm motility and concentration were noted although the magnitude of effect was less than that observed at high Mn levels. Subjects with occupations suggesting Mn exposure (n = 22 out of 200) had metal levels and semen parameters similar to the rest of the sample population, which contradicts other studies which suggest that blood Mn concentrations increases with occupational exposure. There were no normal control subjects in the study. The authors suggested that Mn adversely affects both sperm motility and concentration. However, the study was limited by the lack of normal controls.

In three overlapping studies of 590 Bangladeshi children aged between 6 and 10 years old considering exposure to lead, Mn and arsenic by drinking water, 141 were selected to take part in further studies concerning water Mn exposure (mean level 797 µg/l) on the grounds of low arsenic concentration in their well water (Wasserman et al. 2008). Water Mn concentration was found to negatively associate with Verbal, Performance and Full Scale measures of intellectual function, independent of stunting. However there was an indication that the mortality risk amongst Bangladeshi infants (n = 3824) in their first year of life increased if there was exposure to water Mn levels greater than the WHO standard of 4 mg/l (Hafeman et al. 2007). The study was limited due to its method of inclusion and exclusion of subjects and the result, which suggests no indication of a link between environmental Mn exposure and childhood growth, contradicts other studies (e.g., (Wasserman et al. 2008)).

The inhalation of airborne Mn (along with other metals) as a particulate pollutant in schoolchildren (n = 43) associated with a decrease in peak expiratory flow rate (PEFR), recorded three times a day, was not influenced by genetic polymorphisms in glutathione s-transferase (M1 and T1) (Hong et al. 2007). The association in PEFR was also evident with lead or ambient particle concentration.

The serum and urine concentrations of Mn (and of zinc, copper and magnesium) were determined in residents of Chandigarh, India, and compared with the occurrence of essential hypertension (EH) (Taneja, Mandal 2007). Serum levels of Zn, Mg and Mn were significantly higher in subjects with hypertension than in normotensive subjects (number of individuals not stated). Positive correlations were reported between serum Mn level and systolic and diastolic pressures (r = 0.876 and 0.326, respectively), and for urine Mn level and systolic and diastolic pressures (r = 0.681 and 0.461, respectively).

The possible role of Mn (and Cu) imbalance in the occurrence of Creutzfeldt-Jakob Disease (CJD) was investigated in two Slovakian towns, one with a focal accumulation of CJD cases (Ovara, 18 houses sampled) and the other acting as a control (Zahorie, 16 houses sampled). Significantly higher Mn concentrations were found in samples from Ovara when compared with Zahorie, and significantly higher Mn/Cu ratios were also noted for Ovara (Mašánová et al. 2007)

. The authors propose that these results suggest that increased Mn concentration in foodstuffs might be an exogenous CJD risk factor, which has been suggested by other studies that have detected increased Mn concentrations in the brain of CJD cases.

Ljung and Vahter reviewed the scientific background used to establish the current WHO's guideline value of Mn in drinking water (400 µg/l) and concluded that the increasing number of studies reporting associations between neurologic symptoms and Mn exposure in children, as well as the questionable scientific background data used to set the WHO guideline value, warrant a re-evaluation of the guideline (Ljung, Vahter 2007).

4. Mechanisms of toxicity

4.1 Toxicokinetic and metabolic considerations

Recent developments in the understanding of Mn transport into the CNS, as well as brain imaging and neurocognitive studies, have been reviewed and general conclusions drawn (Aschner et al. 2007). The identified potential mechanisms for Mn transfer across the blood brain barrier (BBB) include: facilitated diffusion; active transport; divalent metal transport 1 (DMT-1)-mediated transport; ZIP8-mediated transport; and transferrin (Tf)-dependant transport, although the predominant mechanism has not been determined (Crossgrove, Yokel 2004). It is likely that certain mechanisms, such as the DMT-1 mediated transport, are not as important as others for Mn transfer (Park et al. 2007). In addition to these transport mechanisms, inhibition of the dopamine transporter (DAT) by the specific DAT inhibitor GBR12909 decreases Mn accumulation in striatal synaptosomes, an observation confirmed in the globus pallidus of rats fed 10 mg/kg Mn in diet for 4 weeks and administered GBR12909 (1 mg/kg, 3 times a week for 4 weeks), when compared with controls treated with saline (Anderson, Cooney & Erikson 2007). This suggests that the DAT may play a role in the transport of Mn across the BBB. However, other studies have suggested that DAT does not play a role in Mn cytotoxicity in dopaminergic cells (Hirata et al. 2008) and that Mn treatment does not affect MPTP neurotoxicity in male C57BL/6 mice (Baek et al. 2007).

Serum samples and cerebrospinal fluid (CSF) from healthy humans not exposed to Mn (n = 5) were analysed by capillary electrophoresis coupled to CZE-inductively coupled plasma (ICP) dynamic reaction cell (DRC) mass spectrometry (MS) (Michalke et al. 2007). The Mn concentration in serum was 1.7 ± 0.8 µg/l with MS peaks at 165, 70 and below 7 kDa, which correspond to Mn enzymes (e.g. oxalate oxidase and Mn-α2-macroglobulin), Mn carriers (Mn-transferrin and Mn-albumin) and low molecular weight Mn species (e.g. citrate), respectively. The Mn concentration in CSF was 2.6 µg/l, in the upper normal range of other reported studies. The majority of the Mn species had an MS peak in the range 640-680 Da which was attributed to Mn-citrate. The study thus suggests that the main Mn-carriers in serum were Mn-albumin and Mn-transferrin, whilst LMW carriers were found in CSF (such as Mn-citrate) and are likely to be the species that will cross neural barriers directly without interaction with the transferring receptor mechanism.

The possible role of Mn and Cu imbalance of the food chain in focally increased occurrence of Creutzfeldt-Jakob Disease (CJD) was investigated in two Slovakian towns, one with a focal accumulation of CJD cases (Ovara , 18 houses sampled) and the other as a control (Zahorie, 16 houses sampled). The Mn and Cu concentrations in soil, drinking water and foodstuffs collected from households were analyzed (mean ± SD). Significantly higher Mn concentrations were found in samples from Ovara when compared to concentrations in samples from Zahorie including soil 634 (±470) mg/kg compared to 191 (±62) mg/kg; honey, 2.62 (±1.98) mg/kg compared to 0.34 (±0.37) mg/kg; bread, 9.1 (±1.4) compared to 6.32(±1.14); potatoes, 1.38 (±0.69) mg/kg compared to 1.05 (±0.27) mg/kg; and apples, 0.366 (±0.121) mg/kg compared to 0.256 (±0.106) mg/kg. Significantly higher Mn/Cu ratios were also found in the soil (49.3 versus 21.1) and local potatoes (2.09 versus 1.07) in Ovara compared to Zahorie (Mašánová et al. 2007). These results suggest that the Mn/Cu ratio and increased concentrations in foodstuffs could be possible exogenous CJD risk factors, but the possible mechanism was not determined and further investigation would be necessary to elaborate on this.

Mn neurotoxicity also disturbs amino acid metabolism and cellular iron regulation. Using a neural-derived cell line (AF5), Mn administration (MnCl in cell culture medium) significantly increased glutamate release (to 174% of that released in untreated cells) but inhibited aconitase activity (specifically m-aconitase). Mn treatment caused c-aconitase to be converted to iron regulatory protein 1 (IRP1), which increased the amounts of IPR2. The increased expression of IRP1 and IRP2 led to reduced H-ferritin expression, increased Tf receptor expression and increased uptake of Tf, affecting iron homeostasis (Crooks, Welch & Smith 2007).

The neurotoxic effects of Mn have been visualized through the demonstration of focal neural damage in the globus pallidus and frontal cortex using NMR spectroscopy. This was achieved through administration of ^{13}C glucose and ^{13}C acetate to male Sprague-Dawley rats following a 4 day Mn treatment (50 mg/kg/day manganese) (Zwingmann, Leibfritz & Hazell 2007). Dosing with Mn resulted in a decrease of glutamine in the globus pallidus (67% of control value) but an increase in the frontal cortex (+ 56%), whereas the accumulation of ^{13}C-labeled γ-amino butyric acid (GABA) occurred in the globus pallidus but not the frontal cortex. Mn administration also resulted in a decrease of N-acetyl-aspartate in the globus pallidus. Glutamate homeostasis may also be preferentially affected over GABA in AF5 cells (derived from foetal rat mesencephalic tissue) during low-level Mn treatment (Crooks, Welch & Smith 2007), which suggests a mechanism by which Mn-induced toxicity may differ in different regions of the brain. Juvenile rhesus monkeys exposed to airborne Mn at 0.06, 0.3, or 1.5 mg/m^3 for 65 days had reduced pallidal GS protein, decreased glutamate transporters in the caudate (GLT-1) and pallidal GLAST, and increased olfactory cortical tyrosine hydroxylase (TH) mRNA levels (Erikson et al. 2007).Thus increased inhalation Mn exposure differentially effects biomarkers in different brain region. This is supported by findings from Struve *et al.* (Struve et al. 2007). Striatal GABA concentrations have also been shown to be elevated in rats exposed to Mn but no consistent effect on dopamine concentration have been established (Gwiazda, Lucchini & Smith 2007). It is suggested that alterations in neuronal metabolic function and regional differences may become significant in the early phase of Mn neurotoxicity and be important for determining the severity of cellular injury.

4.2 Oxidative stress as a mechanism of neurotoxicity

Dobson and Ashner reviewed the available literature on the role of oxidative stress in the brain due to Mn accumulation (Dobson, Aschner 2007). There have been a number of studies that support this hypothesis, including those that suggest that Mn enhances the production of lipopolysaccharide (LPS)-induced pro-inflammatory cytokines (IL-6 and TNF-α) (Crittenden, Filipov 2008), and that Mn cytotoxicity in dopaminergic neurons is associated with proteasome inhibition connected with oxidative damage (Cai et al. 2007). Reactive oxygen species (ROS) generation, measured as hydrogen peroxide (H_2O_2) production, has also been shown to occur after *in vitro* Mn administration (via EBDC fungicide mancozeb) in mesencephalic cells (Domico et al. 2007). The ROS generation is likely to occur from redox cycling by extracellular and intracellular oxidases.

Rat microglia produced H_2O_2 in response to Mn administration *in vitro* in amounts that were linearly related to time and concentration of exposure, with the possible regulation by mitogen-activated protein kinases (MAPK) (Zhang, Zhou & Fu 2003). This finding supports an observation that reactive oxygen species (ROS) are responsible for the onset of Mn toxicity in neural stem cells (Tamm, Sabri & Ceccatelli 2008). However, an *in vivo* study of gavage Mn administration to neonatal rats for 20 days reported an increase in cerebral cortex Mn concentration but no increase in oxidative stress parameters (Weber et al. 2002). (Morello et al. 2008)

The accumulation of Mn in astrocyte mitochondria {{19825 Morello,M. 2008}} has been shown to associate with astrocyte swelling which is mediated by oxidative stress and mitochondria permeability transition (mPT) (Rama Rao et al. 2007)(Norenberg, Rao 2007/6). The administration of Mn to astrocytes selectively affects the cell cycle progression and expression of hypoxia-response genes, as well as increasing the pro-inflammatory factors (Sengupta et al. 2007). Production of oxidative stress factors (such as nitric oxide) as a response to Mn administration has been shown to be inhibited by the peroxisome proliferation activated receptor (PPAR) (Tjalkens et al. 2008). It has also been suggested that oxidative DNA damage is induced by dopamine in the presence of Mn and that it is possibly linked to the degeneration of dopaminergic neurons (Oikawa et al. 2006).

Neonatal female and male rats exposed to airborne Mn during gestation and postnatal d1-18 (0.05, 0.5, 1 mg/m3) showed a decrease in brain glutamine synthetase (GS) protein levels, but only the highest dose (1 mg/m^3) caused the GS mRNA levels to increase in the hypothalamus and olfactory bulb of male rats and decrease in the striatum of female rats (Erikson et al. 2005). Whilst metallothioein (MT) mRNA levels were lower in the hippocampus, hypothalamus and striatum in males given the lowest dose, the median dose caused decreased MT mRNA in hippocampus and hypothalamus of males and in the olfactory bulb of the females. However, there were no effects at the highest dose. Mn exposure did not affect total glutathione (GSH) concentrations. However, cerebella in males showed significant reductions in total GSH when compared to controls. Other research has suggested that glutathione S-transferase (GST) activity by hydroperoxides (e.g. fatty acid hydroperoxides, cumene hydroperoxide and phospholipid hydroperoxides) is increased by Mn administration (Casalino et al. 2004) which may account for the reduction in GSH levels after Mn administration. The reduced amount of GSH is also indicative of increased oxidative stress.

The pulmonary toxicity of Mn inhalation was thought to be partially due to the activation of the hypoxia-inducible factor-1 (HIF-1) transcription factor family (which regulates a number of genes including vascular endothelial growth factor, VEGF). The *in vitro* administration of 0.25 mM Mn to human pulmonary epithelial cells altered the morphology and slowed the growth of human pulmonary epithelial cell lines whilst, *in vivo*, nasal inhalation of 2 mg/m^3 for 5 days (6h/day) by mice produced no significant pulmonary inflammation but did induce a 2-fold increase in pulmonary VEGF mRNA levels (Bredow et al. 2007). This suggests that short term exposure may alter gene expression and affect the susceptibility of the lungs to disease. A lack of pulmonary inflammation was also noted after the inhalation of Mn oxide ultrafine particles (30 nm, 500 mg/m^3 for 12 days exposure) by rats with either both nostrils or right nostril occluded (Elder et al. 2006). The Mn concentration in olfactory bulb, striatum, frontal cortex, cerebellum and lung increased after inhalation but lung lavage showed no indication of lung inflammation which was, however, apparent in the olfactory bulb (N.B. this may not be indicative of the human situation due to the differences between rat and human pathways). Subchronic Mn inhalation (6 h/d, 7 d/w) by male rhesus monkeys showed that high doses associated with increased lung Mn concentrations and small airway inflammatory changes in the absence of observable clinical signs (Dorman et al. 2005b). However, subchronic exposure to Mn at greater than or equal to 0.3 mg/m^3 was not associated with pulmonary pathology.

The neurotoxicity of Mn^{3+} *in vivo* is potentiated by DT-diaphorsase inhibition, suggesting that this enzyme could play a neuroprotective role in the nigrostriatal DA systems (Diaz-Veliz et al. 2004).

The Mn body burden in CD rats and their foetuses was assessed following inhalation of Mn (as MnSO4) at 0.05, 0.5 or 1 mg/m^3 for 6h/d 7d/w during pregnancy (Dorman et al. 2005a). Foetal liver Mn levels were higher after exposure to levels greater than or equal to 0.5 m g/m^3, all other foetal tissues did not have significantly different Mn concentrations to those of air–exposed controls. Therefore, the placenta partially sequesters inhaled Mn limiting

exposure although accumulation of Mn does occur in the foetal liver. Oral dosing of Mn also results in accumulation in the blood, cortex, hippocampus and parenchymal tissues of young adult rats (Weber et al. 2002). *In vivo* Mn sequestration in mitochondria of astrocytes has also been seen (Morello et al. 2008) and is suggested to lead to further dysfunction in Mn neurotoxicity.

Subclinical effects of Mn exposure on the nervous system have been shown to be associated with the relationship of biomarkers of exposure and effect in workers exposed to neurotoxic fumes(Halatek, Sinczuk-Walczak & Rydzynski 2008). Blood and urine Mn concentrations in samples from 50 shipyard welders, exposed to mean levels of 0.16 mg/m^3, were compared to age and smoking-habit matched non-exposed workers (n = 23) and subjected to tests to determine subclinical neurotoxic symptoms and Clara cell protein (CC16) levels. There was a significant increase in blood Mn concentration (12.2 ± 8.9 µg/l) and a decrease in vital capacity (VC%; 84.5 ± 12.2%) in Mn-exposed workers with neurological symptoms (measured on the visual evoked potentials, VEP) compared to those of the reference group (6.1 ± 2.3µg/l and 100.5 ± 11.7%, respectively). The expression of CC16 was unaffected in Mn-exposed workers with neurological symptoms (15.0 ± 6.3 µg/l) or those without neurological symptoms (14.5 ± 6.9 µg/l), when compared to the control group (15.6 ± 5.3 µg/l). The Mn-exposed workers with neurological symptoms were exposed to lower airborne Mn levels than those without neurological symptoms (0.53 ± 0.75 mg/m^3 compared to 0.73 ± 1.14 mg/m^3, respectively). The low respiratory performance in Mn welders is thought to enhance subclinical neurotoxic symptoms (especially VEP) related to exposure to airborne Mn and blood Mn concentrations.

PD and manganism in humans show differing distinct neuropathological affects; whilst Mn administration results in damage to globus pallidus, sparing the substantia nigra pars compacta and without Lewy bodies, PD shows preferential damage of dopamine neurones in the substantia nigra pars compacta (Perl, Olanow 2007). The effects of Mn administration have been shown to be different according to the age and gender of subjects (Prestifilippo et al. 2007, Ponnapakkam et al. 2003) which will also affect the mechanism and function. A further complication arises from mechanistic studies, as animal data indicates that Mn neurotoxicity may differ at lower concentrations compared to elevated exposures suggesting that existing animal model studies are of limited relevance for the risk assessment of chronic low-level Mn exposure to humans (Gwiazda, Lucchini & Smith 2007).

5. Human Susceptibility

The link between genetic polymorphisms, PD, occupational exposure to solvents, pesticides and metals (including Mn) has been investigated in a case-control study of 959 cases of parkinsonism (out of which 767 patients had Parkinson's disease) and 1989 controls. CYP2D6, PON1, GSTM1, STTT1, GSTM3, GSTP1, NQO1, CYP1B1, MAO-A, MAO-B, SOD2, EPHX, DAT1, DRD2, and NAT2 were genotyped in the case and control populations. The results were compared with environmental factors to determine whether there was a link between environmental risk factors and PD, whether the polymorphisms in a number of genes can modify disease progression/incidence or whether there was any evidence of gene-environment interaction in PD(Dick et al. 2007). There was a significant association reported between MAO-A polymorphism and disease risk and possible interaction effects between GSTm1 null genotype and solvent exposure (although this was stronger when limited to PD cases only). However, no relationship was observed between Mn exposure and the expression of the studied genes. There was insufficient evidence to suggest that Mn can modify PD disease risk.

6. Treatment and imaging

A case study of an individual reporting similar symptoms to manganism, but who did not have any occupational Mn exposure, was used to suggest a novel treatment. A case study of an alcoholic 53 year old man with liver damage and rapid onset parkinsonism-dementia complex was shown to have symptoms that may be associated with the accumulation of Mn (blood Mn levels were > 50µg/l) despite the apparent lack of any occupational or environmental exposure to Mn. The individual was treated by liver transplantation as a last resort after failure of treatment with carbidopa/L-dopa and memantine (500mg/day and 20mg/day, respectively). The Mn blood levels (> 50µg/l, no specific figure given) were reduced after the transplantation (no concentration given) leading to the suggestion from the author that liver transplantation can be used to treat Mn toxic effects when all other treatments have been exhausted (Fabiani et al. 2007). However, the patient developed sepsis and died on the 7th post operative day.

Other studies have shown that liver function affects the accumulation of Mn. The blood Mn concentration in patients with billary atresia (involving the blockage or closure of bile ducts in liver) was measured before and after patients undergoing Kasai's portoenterostomy surgery. The blood Mn levels were above normal in 3 out of 14 patients before the surgery, and were shown to be increased in 4 patients. However, globus pallidus hyperintensity (T1-weighted MRI image) was only seen with one patient. Therefore, whole blood Mn concentrations and MRI in patients with billiary atresia and undergoing Kasai's portoenterostomy can be used to determine prognosis and inform on whether liver transplant is necessary (Agarwal, Sharma & Bhatnagar 2008). Mn accumulation in patients with acquired and congenital diseases of the abdomen was also shown in the globus pallidus of the brain using T1-weighted but not T2-weighted images (Uchino et al. 2007).

MRI imaging has been used by a number of recent studies as an assessment of tissue Mn levels in cases of occupational or environmental exposure as discussed in Section 2 ((Shin et al. 2007)(Kim et al. 2007a)(Choi et al. 2007)(Agarwal, Sharma & Bhatnagar 2008). The comparison of the signal intensity of the T1-weighted image of MRI of the globus pallidus to that of the frontal white matter in long-term occupational exposure to manganese in welders (n = 20) compared to controls (office workers, n = 10), showed a dose-response relationship with the cumulative exposure index (r = 0.54). However, the small sample number and the lack of comparison of lifestyles between office workers and welders limits interpretation of the study (Kim et al. 2007a).

A relationship was observed between the occupational exposure of welders to Mn and the visual grading of areas of the brain (globus pallidus) of MRI scans (Choi et al. 2007) but this was considered to be only useful over a narrow range of Mn concentration due to non-linear nature of dose-response. The direct measurement of T1 relaxation time was determined to be a more reliable determination of tissue Mn concentration. The study only considered a small number of individuals, had no control subjects, and did not take into consideration other environmental and lifestyle differences.

7. Miscellaneous

A review and historical reconstruction of the research development that yielded modern understanding of lead and mercury neurotoxicity was conducted in an attempt to identify useful lessons for the prevention of Mn neurotoxicity (Alessio, Campagna & Lucchini 2007). The review concluded that further information is needed on the early neurotoxic and neurobehavioural effects after prolonged exposure to very low doses of Mn. The authors suggest that the precautionary principle should be used to prevent the onset of more severe health effects in the population - considered to be the most important lesson to be learned and applied from more than 30 years of occupational and environmental neurotoxicity of metals.

The issue of toxic metal mixtures and children's health was reviewed by Hu and colleagues (Hu, Shine & Wright 2007) focusing on the specific example of mining waste at the Tar Creek Superfund Site in Northeast Oklahoma where residents are exposed to higher than normal levels of lead, cadmium, iron and Mn compared with a control site outside the area. The uncertainty over potential interactions of metals in environmental exposures in the test area was described and the routes of exposure and bioavailability were identified as requiring further investigation.

A review of the effect of transition metals on neurobiology was undertaken by Wright and Baccarelli (Wright, Baccarelli 2007) which suggested that, while transition metals are critical for cellular respiration, detoxification and metabolism, it is possible that early life exposures may also influence later life stages and adult disease phenotypes via epigenetic processes. Therefore epigenetic effects may be a critical pathway by which metals elicit health effects.

Overview

This document reviews publications (papers and abstracts) relating to human exposure to and potential health effects of manganese and manganese-containing inorganic compounds published from March 2007 to February 2008.

Studies of both environmental and occupational exposures to manganese have been reported for this period. Examples of exposures to Mn at concentrations greater than the WHO guideline value of 400 µg/l have been reported in a number of different areas during the period covered by this review; these include in samples of commercially-available bottled water in Italy and in food stuffs grown in areas surrounding industrial plants. One occupational study of welders highlighted the fact that Mn concentration in welding fumes has been shown to be affected by voltage (increased voltage lead to increased Mn concentration). This observation is in keeping with other studies of welding fumes that have consistently shown that welding fume concentrations can be markedly affected by the applied voltage and that a reduction of fume is best achieved by keeping the voltage at low as possible consistent with a good workpiece weld.

After human exposure to Mn, the Mn concentrations in urine, blood, saliva were shown to be increased. This increase in these Mn biomarkers was correlated with the increase in the MRI signal intensity in the globus pallidus, and concentrations in tooth enamel and hair, although these are not quantifiable. These biological Mn concentrations were shown to decrease over time after the cessation of exposure.

Papers on a number of potential mechanisms for Mn transfer across the blood brain barrier have been published; transport mechanisms considered included facilitated diffusion; active transport; DMT-1-mediated transport; ZIP8-mediated transport; DAT-mediated transport; and Tf-dependant transport. However, the predominant mechanism is not yet clearly established although it appears that certain mechanisms (e.g. DMT-1 mediated transport) may not be as important as others. However, there is some evidence that low molecular weight species, such as Mn-citrate, may pass directly across the blood-brain barrier without need of a transport facilitator.

Toxicological studies have shown that Mn exposure via inhalation may affect a range of biomarkers in different brain regions (e.g. striatal GABA and dopamine expression). The alteration in neuronal metabolic function and regulation has also been shown to differ significantly in the early phase of Mn neurotoxicity and this may be important in determining the severity of subsequent cellular injury.

Papers published have provided further information on the potential associations between Mn exposure/accumulation and effects on neurobehavioural, motor control, childhood behaviour and development, neuropsychological behaviour and respiratory function, as well as the controversial potentially-increased likelihood of PD development (although there are conflicting reports) and a speculative potential link with CJD. The respiratory effect of occupational Mn exposure may be due to the inhalation of particulate matter, but has been suggested to be linked to subclinical neurological symptoms and blood Mn concentrations.

Pre-existing metabolic disorders have been shown to affect Mn accumulation, which could in turn elicit secondary symptoms, and it has been suggested that liver transplantation may be an appropriate route to treat severe Mn accumulation and manganism, if all other options are exhausted.

Concerns have been expressed that data from the experimental Mn administration in animal models may be unrepresentative of the human Mn neurotoxicity that may occur at low concentrations, raising concerns that existing animal models are of limited relevance for the risk assessment of chronic low-level Mn exposure to humans. These concerns, if vindicated, would directly impact on the scientific background used to establish current guidelines (e.g. the WHO guideline value of Mn in drinking water (400 µg/l)). Furthermore, some authors have suggested that the increasing number of studies reporting associations between neurologic symptoms and Mn exposure in children warrant a re-evaluation of this guidance value.

References

Agarwal, G.S., Sharma, R. & Bhatnagar, V. 2008, "Assessment of latent manganese toxicity as a prognostic factor following surgery for biliary atresia", *European journal of pediatric surgery : official journal of Austrian Association of Pediatric Surgery [et.al] = Zeitschrift fur Kinderchirurgie*, vol. 18, no. 1, pp. 22-25.

Alessio, L., Campagna, M. & Lucchini, R. 2007, "From lead to manganese through mercury: Mythology, science, and lessons for prevention", *American journal of industrial medicine*, vol. 50, no. 11, pp. 779-787.

Anderson, J.G., Cooney, P.T. & Erikson, K.M. 2007, "Inhibition of DAT function attenuates manganese accumulation in the globus pallidus", *Environmental toxicology and pharmacology*, vol. 23, no. 2, pp. 179-184.

Asante, K.A., Agusa, T., Subramanian, A., Ansa-Asare, O.D., Biney, C.A. & Tanabe, S. 2007, "Contamination status of arsenic and other trace elements in drinking water and residents from Tarkwa, a historic mining township in Ghana", *Chemosphere*, vol. 66, no. 8, pp. 1513-1522.

Aschner, M., Guilarte, T.R., Schneider, J.S. & Zheng, W. 2007, "Manganese: Recent advances in understanding its transport and neurotoxicity", *Toxicology and applied pharmacology*, vol. 221, no. 2, pp. 131-147.

Baek, S.Y., Kim, Y.H., Oh, S.O., Lee, C.-., Yoo, C.I., Lee, J.H., Lee, H., Sim, C.S., Park, J., Kim, J.-., Yoon, C.S. & Kim, Y. 2007, "Manganese does not alter the severe neurotoxicity of MPTP", *Human and Experimental Toxicology*, vol. 26, no. 3, pp. 203-211.

Bouchard, M., Mergler, D., Baldwin, M., Panisset, M., Bowler, R. & Roels, H.A. 2007a, "Neurobehavioral functioning after cessation of manganese exposure: A follow-up after 14 years", *American Journal of Industrial Medicine*, vol. 50, no. 11, pp. 831-840.

Bouchard, M., Mergler, D., Baldwin, M., Panisset, M. & Roels, H.A. 2007b, "Neuropsychiatric symptoms and past manganese exposure in a ferro-alloy plant", *Neurotoxicology*, vol. 28, no. 2, pp. 290-297.

Bowler, R.M., Roels, H.A., Nakagawa, S., Drezgic, M., Diamond, E., Park, R., Koller, W., Bowler, R.P., Mergler, D., Bouchard, M., Smith, D., Gwiazda, R. & Doty, R.L. 2007, "Dose-effect relationships between manganese exposure and neurological, neuropsychological and pulmonary function in confined space

bridge welders", *Occupational and environmental medicine,* vol. 64, no. 3, pp. 167-177.

Bredow, S., Falgout, M.M., March, T.H., Yingling, C.M., Malkoski, S.P., Aden, J., Bedrick, E.J., Lewis, J.L. & Divine, K.K. 2007, "Subchronic inhalation of soluble manganese induces expression of hypoxia-associated angiogenic genes in adult mouse lungs", *Toxicology and applied pharmacology,* vol. 221, no. 2, pp. 148-157.

Cai, T., Yao, T., Li, Y., Chen, Y., Du, K., Chen, J. & Luo, W. 2007, "Proteasome inhibition is associated with manganese-induced oxidative injury in PC12 cells", *Brain research,* vol. 1185, pp. 359-365.

Casalino, E., Sblano, C., Landriscina, V., Calzaretti, G. & Landriscina, C. 2004, "Rat liver glutathione S-transferase activity stimulation following acute cadmium or manganese intoxication", *Toxicology,* vol. 200, no. 1, pp. 29-38.

Choi, D.S., Kim, E.A., Cheong, H., Khang, H.S., Ryoo, J.W., Cho, J.M., Sakong, J. & Park, I. 2007, "Evaluation of MR signal index for the assessment of occupational manganese exposure of welders by measurement of local proton T(1) relaxation time", *Neurotoxicology,* vol. 28, no. 2, pp. 284-289.

Chuang, H., Kuo, C., Chiu, Y., Ho, C., Chen, C. & Wu, T. 2007, "A case-control study on the relationship of hearing function and blood concentrations of lead, manganese, arsenic, and selenium", *The Science of the total environment,* vol. 387, no. 1-3, pp. 79-85.

Crittenden, P.L. & Filipov, N.M. 2008, "Manganese-induced potentiation of in vitro proinflammatory cytokine production by activated microglial cells is associated with persistent activation of p38 MAPK", *Toxicology in Vitro,* vol. 22, no. 1, pp. 18-27.

Crooks, D.R., Welch, N. & Smith, D.R. 2007, "Low-level manganese exposure alters glutamate metabolism in GABAergic AF5 cells", *Neurotoxicology,* vol. 28, no. 3, pp. 548-554.

Crossgrove, J.S. & Yokel, R.A. 2004, "Manganese distribution across the blood-brain barrier III. The divalent metal transporter-1 is not the major mechanism mediating brain manganese uptake", *Neurotoxicology,* vol. 25, no. 3, pp. 451-460.

Diaz-Veliz, G., Mora, S., Gomez, P., Dossi, M.T., Montiel, J., Arriagada, C., Aboitiz, F. & Segura-Aguilar, J. 2004, "Behavioral effects of manganese injected in the

rat substantia nigra are potentiated by dicumarol, a DT-diaphorase inhibitor", *Pharmacology, biochemistry, and behavior,* vol. 77, no. 2, pp. 245-251.

Dick, F.D., De Palma, G., Ahmadi, A., Osborne, A., Scott, N.W., Prescott, G.J., Bennett, J., Semple, S., Dick, S., Mozzoni, P., Haites, N., Wettinger, S.B., Mutti, A., Otelea, M., Seaton, A., Soderkvist, P., Felice, A. & Geoparkinson Study Group 2007, "Gene-environment interactions in parkinsonism and Parkinson's disease: the Geoparkinson study", *Occupational and environmental medicine,* vol. 64, no. 10, pp. 673-680.

Dobson, A. & Aschner, M. 2007, "Manganese-induced oxidative stress" in *Oxidative Stress and Neurodegenerative Disorders,* eds. G.A. Qureshi & S.H. Parvez, Elsevier Science B.V., Amsterdam, pp. 433-450.

Domico, L.M., Cooper, K.R., Bernard, L.P. & Zeevalk, G.D. 2007, "Reactive oxygen species generation by the ethylene-bis-dithiocarbamate (EBDC) fungicide mancozeb and its contribution to neuronal toxicity in mesencephalic cells", *NeuroToxicology,* vol. 28, no. 6, pp. 1079-1091.

Dorman, D.C., McElveen, A.M., Marshall, M.W., Parkinson, C.U., Arden James, R., Struve, M.F. & Wong, B.A. 2005a, "Maternal-fetal distribution of manganese in the rat following inhalation exposure to manganese sulfate", *Neurotoxicology,* vol. 26, no. 4, pp. 625-632.

Dorman, D.C., Struve, M.F., Gross, E.A., Wong, B.A. & Howroyd, P.C. 2005b, "Sub-chronic inhalation of high concentrations of manganese sulfate induces lower airway pathology in rhesus monkeys", *Respiratory research,* vol. 6, pp. 121.

Elder, A., Gelein, R., Silva, V., Feikert, T., Opanashuk, L., Carter, J., Potter, R., Maynard, A., Ito, Y., Finkelstein, J. & Oberdörster, G. 2006, "Translocation of inhaled ultrafine manganese oxide particles to the central nervous system", *Environmental health perspectives,* vol. 114, no. 8, pp. 1172-1178.

Ellingsen, D.G., Chashchin, V., Haug, E., Chashchin, M., Tkachenko, V., Lubnina, N., Bast-Pettersen, R. & Thomassen, Y. 2007, "An epidemiological study of reproductive function biomarkers in male welders", *Biomarkers,* vol. 12, no. 5, pp. 497-509.

Ellingsen, D.G., Konstantinov, R., Bast-Pettersen, R., Merkurjeva, L., Chashchin, M., Thomassen, Y. & Chashchin, V. 2008, "A neurobehavioral study of current and former welders exposed to manganese", *NeuroToxicology,* vol. 29, no. 1, pp. 48-59.

Ericson, J.E., Crinella, F.M., Clarke-Stewart, K.A., Allhusen, V.D., Chan, T. & Robertson, R.T. 2007, "Prenatal manganese levels linked to childhood behavioral disinhibition", *Neurotoxicology and Teratology*, vol. 29, no. 2, pp. 181-187.

Erikson, K.M., Dorman, D.C., Lash, L.H. & Aschner, M. 2005, "Persistent alterations in biomarkers of oxidative stress resulting from combined in utero and neonatal manganese inhalation", *Biological trace element research*, vol. 104, no. 2, pp. 151-163.

Erikson, K.M., Dorman, D.C., Lash, L.H. & Aschner, M. 2007, "Manganese Inhalation by Rhesus Monkeys is Associated with Brain Regional Changes in Biomarkers of Neurotoxicity", *Toxicological Sciences*, vol. 97, no. 2, pp. 459-466.

Fabiani, G., Rogacheski, E., Wiederkehr, J.C., Khouri, J. & Cianfarano, A. 2007, "Liver transplantion in a patient with rapid onset parkinsonism-dementia complex induced by manganism secondary to liver failure", *Arquivos de Neuro-Psiquiatria*, vol. 65, no. 3A, pp. 685-688.

Finkelstein, M.M. & Jerrett, M. 2007, "A study of the relationships between Parkinson's disease and markers of traffic-derived and environmental manganese air pollution in two Canadian cities", *Environmental Research*, vol. 104, no. 3, pp. 420-432.

Gwiazda, R., Lucchini, R. & Smith, D. 2007, "Adequacy and Consistency of Animal Studies to Evaluate the Neurotoxicity of Chronic Low-Level Manganese Exposure in Humans", *Journal of Toxicology and Environmental Health - Part A*, vol. 70, no. 7, pp. 594-605.

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*, vol. 115, no. 7, pp. 1107-1112.

Halatek, T., Sinczuk-Walczak, H. & Rydzynski, K. 2008, "Early neurotoxic effects of inhalation exposure to aluminum and/or manganese assessed by serum levels of phospholipid-binding Clara cells protein", *Journal of Environmental Science and Health, Part A*, vol. 43, no. 2, pp. 118.

Hirata, Y., Suzuno, H., Tsuruta, T., Oh-hashi, K. & Kiuchi, K. 2008, "The role of dopamine transporter in selective toxicity of manganese and rotenone", *Toxicology*, vol. 244, no. 2-3, pp. 249-256.

Hong, Y.-., Hwang, S.-., Kim, J.H., Lee, K.-., Lee, H.-., Lee, K.-., Yup, S.-. & Kim, D.-. 2007, "Metals in Particulate Pollutants Affect Peak Expiratory Flow of Schoolchildren", *Environmental health perspectives*, vol. 115, no. 3, pp. 430-434.

Hovde, C.A. & Raynor, P.C. 2007, "Effects of voltage and wire feed speed on weld fume characteristics", *Journal of occupational and environmental hygiene*, vol. 4, no. 12, pp. 903-912.

Hu, H., Shine, J. & Wright, R.O. 2007, "The challenge posed to children's health by mixtures of toxic waste: the Tar Creek superfund site as a case-study", *Pediatric clinics of North America*, vol. 54, no. 1, pp. 155-175.

Huang, C.-. 2007, "Parkinsonism Induced by Chronic Manganese Intoxication-An Experience in Taiwan", *Chang Gung medical journal*, vol. 30, no. NUMB 5, pp. 385-395.

Huang, C., Chu, N., Lu, C., Chen, R., Schulzer, M. & Calne, D.B. 2007, "The natural history of neurological manganism over 18 years", *Parkinsonism & related disorders*, vol. 13, no. 3, pp. 143-145.

Kim, E.A., Cheong, H., Choi, D.S., Sakong, J., Ryoo, J.W., Park, I. & Kang, D.M. 2007a, "Effect of occupational manganese exposure on the central nervous system of welders: (1)H magnetic resonance spectroscopy and MRI findings", *Neurotoxicology*, vol. 28, no. 2, pp. 276-283.

Kim, E.A., Cheong, H., Joo, K., Shin, J., Lee, J.S., Choi, S., Kim, M., Lee, I.J. & Kang, D.M. 2007b, "Effect of manganese exposure on the neuroendocrine system in welders", *Neurotoxicology*, vol. 28, no. 2, pp. 263-269.

Ljung, K. & Vahter, M. 2007, "Time to Re-evaluate the Guideline Value for Manganese in Drinking Water?", *Environmental health perspectives*, vol. 115, no. 11, pp. 1533-1538.

Lucchini, R.G., Albini, E., Benedetti, L., Borghesi, S., Coccaglio, R., Malara, E.C., Parrinello, G., Garattini, S., Resola, S. & Alessio, L. 2007, "High prevalence of parkinsonian disorders associated to manganese exposure in the vicinities of ferroalloy industries", *American journal of industrial medicine*, vol. 50, no. 11, pp. 788-800.

Marriott, L.D., Foote, K.D., Kimber, A.C., Delves, H.T. & Morgan, J.B. 2007, "Zinc, copper, selenium and manganese blood levels in preterm infants", *Archives of Disease in Childhood - Fetal and Neonatal Edition*, vol. 92, no. 6, pp. F494-497.

Mašánová, V., Mitrova, E., Ursinyova, M., Uhnakova, I. & Slivarichova, D. 2007, "Manganese and copper imbalance in the food chain constituents in relation to Creutzfeldt-Jakob disease", *International Journal of Environmental Health Research*, vol. 17, no. 6, pp. 419.

Meral, H., Kutukcu, Y., Atmaca, B., Ozer, F. & Hamamcioglu, K. 2007, "Parkinsonism Caused by Chronic Usage of Intravenous Potassium Permanganate", *Neurologist*, vol. 13, no. 2, pp. 92-94.

Michalke, B., Berthele, A., Mistriotis, P., Ochsenkühn-Petropoulou, M. & Halbach, S. 2007, "Manganese species from human serum, cerebrospinal fluid analyzed by size exclusion chromatography-, capillary electrophoresis coupled to inductively coupled plasma mass spectrometry", *Journal of Trace Elements in Medicine and Biology*, vol. 21, no. Suppl 1, pp. 4-9.

Michalke, B., Halbach, S. & Nischwitz, V. 2007, "Speciation and toxicological relevance of manganese in humans", *Journal of Environmental Monitoring*, vol. 9, no. 7, pp. 650-656.

Mishra, S., Ramteke, D.S. & Wate, S.R. 2007, "Quantification of transition metals in biological samples and its possible impact on ferro-alloy workers", *Journal of Environmental Biology*, vol. 28, no. 4, pp. 851-856.

Montes, S., Riojas-Rodríguez, H., Sabido-Pedraza, E. & Ríos, C. 2008, "Biomarkers of manganese exposure in a population living close to a mine and mineral processing plant in Mexico", *Environmental Research*, vol. 106, no. 1, pp. 89-95.

Morello, M., Canini, A., Mattioli, P., Sorge, R.P., Alimonti, A., Bocca, B., Forte, G., Martorana, A., Bernardi, G. & Sancesario, G. 2008, "Sub-cellular localization of manganese in the basal ganglia of normal and manganese-treated rats: An electron spectroscopy imaging and electron energy-loss spectroscopy study", *NeuroToxicology*, vol. 29, no. 1, pp. 60-72.

Nasolodin, V.V. & Gladkikh, I.P. 2007, "[Manganese provision in trained and untrained schoolchildren and students in different seasons]", *Gigiena i sanitariia*, , no. 1, pp. 59-61.

Norenberg, M.D. & Rao, K.V.R. 2007/6, "The mitochondrial permeability transition in neurologic disease", *Neurochemistry International*, vol. 50, no. 7-8, pp. 983-997.

Oikawa, S., Hirosawa, I., Tada-Oikawa, S., Furukawa, A., Nishiura, K. & Kawanishi, S. 2006, "Mechanism for manganese enhancement of dopamine-induced

oxidative DNA damage and neuronal cell death", *Free radical biology & medicine,* vol. 41, no. 5, pp. 748-756.

Park, J., Kim, K., Kim, D., Choi, S., Choi, B., Chung, Y.H., Han, J.H., Sung, J.H., Kwon, I.H., Mun, J. & Yu, I.J. 2007, "Tissue distribution of manganese in iron-sufficient or iron-deficient rats after stainless steel welding-fume exposure", *Inhalation toxicology,* vol. 19, no. 6, pp. 563-572.

Perl, D.P. & Olanow, C.W. 2007, "The Neuropathology of Manganese-Induced Parkinsonism", *Journal of Neuropathology & Experimental Neurology,* vol. 66, no. 8, pp. 675-682.

Ponnapakkam, T.P., Bailey, K.S., Graves, K.A. & Iszard, M.B. 2003, "Assessment of male reproductive system in the CD-1 mice following oral manganese exposure", *Reproductive Toxicology,* vol. 17, no. 5, pp. 547-551.

Prestifilippo, J.P., Fernández-Solari, J., Mohn, C., De Laurentiis, A., McCann, S.M., Dees, W. & Rettori, V. 2007, "Effect of manganese on luteinizing hormone-releasing hormone secretion in adult male rats", *Toxicological sciences,* vol. 97, no. 1, pp. 75-80.

Rama Rao, K.V., Reddy, P.V.B., Hazell, A.S. & Norenberg, M.D. 2007, "Manganese induces cell swelling in cultured astrocytes", *NeuroToxicology,* vol. 28, no. 4, pp. 807-812.

Rohling, M.L. & Demakis, G.J. 2007, "Potential Neuropsychological Profiles in Welders Occupationally Exposed to Manganese: An Examination of Effect Size Patterns", *Journal of Clinical and Experimental Neuropsychology,* vol. 29, no. 8, pp. 813-822.

Sahni, V., Leger, Y., Panaro, L., Allen, M., Giffin, S., Fury, D. & Hamm, N. 2007, "Case report: a metabolic disorder presenting as pediatric manganism", *Environmental health perspectives,* vol. 115, no. 12, pp. 1776-1779.

Santamaria, A.B., Cushing, C.A., Antonini, J.M., Finley, B.L. & Mowat, F.S. 2007, "State-of-the-science review: Does manganese exposure during welding pose a neurological risk?", *Journal of toxicology and environmental health - Part B,* vol. 10, no. 6, pp. 417-465.

Sengupta, A., Mense, S.M., Lan, C., Zhou, M., Mauro, R.E., Kellerman, L., Bentsman, G., Volsky, D.J., Louis, E.D., Graziano, J.H. & Zhang, L. 2007, "Gene expression profiling of human primary astrocytes exposed to manganese

chloride indicates selective effects on several functions of the cells", *Neurotoxicology*, vol. 28, no. 3, pp. 478-489.

Shin, Y.C., Kim, E., Cheong, H., Cho, S., Sakong, J., Kim, K.S., Yang, J.S., Jin, Y., Kang, S. & Kim, Y. 2007, "High signal intensity on magnetic resonance imaging as a predictor of neurobehavioral performance of workers exposed to manganese", *Neurotoxicology*, vol. 28, no. 2, pp. 257-262.

Signorile, G., Neve, A., Lugoli, F., Piccinni, M.C., Arena, R. & Di Marino, R. 2007, "Evaluation of toxic chemical parameters and ecotoxicity levels in bottled mineral waters", *Journal of preventive medicine and hygiene*, vol. 48, no. 1, pp. 10-16.

Struve, M.F., McManus, B.E., Wong, B.A. & Dorman, D.C. 2007, "Basal ganglia neurotransmitter concentrations in rhesus monkeys following subchronic manganese sulfate inhalation", *American journal of industrial medicine*, vol. 50, no. 10, pp. 772-778.

Tamm, C., Sabri, F. & Ceccatelli, S. 2008, "Mitochondrial-Mediated Apoptosis in Neural Stem Cells Exposed to Manganese", *Toxicological Sciences*, vol. 101, no. 2, pp. 310-320.

Taneja, S.K. & Mandal, R. 2007, "Mineral Factors Controlling Essential Hypertension-a Study in the Chandigarh, India Population", *Biological trace element research*, vol. 120, no. 1-3, pp. 61-73.

Teeguarden, J.G., Dorman, D.C., Nong, A., Covington, T.R., Clewell, H.J. & Andersen, M.E. 2007a, "Pharmacokinetic modeling of manganese. II. Hepatic processing after ingestion and inhalation", *Journal of toxicology and environmental health - Part A*, vol. 70, no. 18, pp. 1505-1514.

Teeguarden, J.G., Gearhart, J., Clewell, H.J., Covington, T.R., Nong, A. & Andersen, M.E. 2007b, "Pharmacokinetic modeling of manganese. III. Physiological approaches accounting for background and tracer kinetics", *Journal of toxicology and environmental health - Part A*, vol. 70, no. 18, pp. 1515-1526.

Tjalkens, R.B., Liu, X., Mohl, B., Wright, T., Moreno, J.A., Carbone, D.L. & Safe, S. 2008, "The peroxisome proliferator-activated receptor-? agonist 1,1-bis(3?-indolyl)-1-(p-trifluoromethylphenyl)methane suppresses manganese-induced production of nitric oxide in astrocytes and inhibits apoptosis in cocultured PC12 cells", *Journal of neuroscience research*, vol. 86, no. 3, pp. 618-629.

Uchino, A., Noguchi, T., Nomiyama, K., Takase, Y., Nakazono, T., Nojiri, J. & Kudo, S. 2007, "Manganese accumulation in the brain: MR imaging", *Neuroradiology,* vol. 49, no. 9, pp. 715-720.

Wang, D., Du, X. & Zheng, W. 2008, "Alteration of saliva and serum concentrations of manganese, copper, zinc, cadmium and lead among career welders", *Toxicology Letters,* vol. 176, no. 1, pp. 40-47.

Wasserman, G.A., Liu, X., Factor-Litvak, P., Gardner, J.M. & Graziano, J.H. 2008, "Developmental impacts of heavy metals and undernutrition", *Basic & clinical pharmacology & toxicology,* vol. 102, no. 2, pp. 212-217.

Weber, S., Dorman, D.C., Lash, L.H., Erikson, K., Vrana, K.E. & Aschner, M. 2002, "Effects of manganese (Mn) on the developing rat brain: oxidative-stress related endpoints", *Neurotoxicology,* vol. 23, no. 2, pp. 169-175.

Wirth, J.J., Rossano, M.G., Daly, D.C., Paneth, N., Puscheck, E., Potter, R.C. & Diamond, M.P. 2007, "Ambient manganese exposure is negatively associated with human sperm motility and concentration", *Epidemiology,* vol. 18, no. 2, pp. 270-273.

Wright, R.O. & Baccarelli, A. 2007, "Metals and Neurotoxicology", *Journal of Nutrition,* vol. 137, no. 12, pp. 2809-2813.

Zhang, S., Zhou, Z. & Fu, J. 2003, "Effect of manganese chloride exposure on liver and brain mitochondria function in rats", *Environmental research,* vol. 93, no. 2, pp. 149-157.

Zoni, S., Albini, E. & Lucchini, R. 2007, "Neuropsychological testing for the assessment of manganese neurotoxicity: A review and a proposal", *American journal of industrial medicine,* vol. 50, no. 11, pp. 812-830.

Zwingmann, C., Leibfritz, D. & Hazell, A.S. 2007, "Nmr spectroscopic analysis of regional brain energy metabolism in manganese neurotoxicity", *Glia,* vol. 55, no. 15, pp. 1610-1617.