IEH Institute of Environment and Health

Cranfield UNIVERSITY

Manganese Health Research Program:

Overview of Research into the Health Effects of Manganese (2008-2009)

December 2009

The Institute of Environment and Health (IEH) was established at Cranfield University in November 2005. The research and consultancy activities of the Institute are principally funded through specific grants, contracts and awards from UK Government Departments and Agencies.

This document is a report by the Institute of Environment and Health for the Manganese Health Research Program (MHRP)

Literature reviewed and report compiled by C Tam

Edited by R Bevan and L Levy

Institute of Environment and Health
Cranfield Health
Cranfield University
Cranfield
Bedfordshire
MK43 0AL
UK
http://www.cranfield.ac.uk/health/researchareas/environmenthealth/ieh/page19562.jsp

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 in the period March 2008 to February 2009, and have been included in the quarterly update service on the MHRP website.

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 2008 and February 2009.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

It is well established that occupational exposure to excess Mn can cause toxicity, and during this period of review, two occupationally-linked studies have been reported. In the first study, Bouchard et al (2008) evaluated the association between a Mn cumulative exposure index (CEI) and self-reported symptoms in Mn alloy production plant workers; data was obtained using questionnaires completed whilst actively working at the plant (initial examination) and repeated fourteen years following plant closure (second examination; Bouchard *et al.*, 2008). At the initial examination, fatigue, autonomic nervous system, hearing and movement disorders were described. Hearing and movement disorders were also reported at second examinations, with additional symptoms of memory and low concentration spans being recorded; severity of symptoms was found to be exposure related, with individuals having the highest CEI values exhibiting the most pronounced symptoms. The authors conclude that in former Mn workers, symptoms associated with Mn exposure were still apparent 14 years following termination of exposure. It should be noted however, that individuals participating in the study were not "blinded" to exposure, and therefore the possibility of bias in the results cannot be excluded (Bouchard *et al.*, 2008).

Baldwin et al., (2008) conducted a retrospective exposure assessment, again utilising a cumulative exposure index, in the Mn alloy production plant described by (Bouchard *et al.*, 2008) and reported that during the 18 years of production at the alloy plant, levels of estimated exposure were significantly higher than that measured when the plant was first closed. That is, there was a reduction in the exposure over the 18 year period when the plant was open due to changes in working practices and improved ventilation. The retrospective assessment revealed positive association with the number of neurobehavioral outcomes that were identified and increasing estimated levels of Mn exposure. The authors suggest that historical cumulative exposure assessment can be helpful in evaluating the long-term exposure and associated behavioural effects of Mn (Baldwin *et al.*, 2008).

Mn levels in canned (21 samples) and non-canned (29 samples) drinks processed in Nigeria were determined using atomic absorption spectrophotometry. Of the drinks analysed, 43% of canned and 52% of non-canned drinks contained Mn at levels above the maximum permitted level set by the United States Environmental Protection Agency of 0.05 mg/L (0.001 – 0.7 and 0.001 – 0.21 mg/L in canned and non-canned drinks respectively). The source of Mn and potential impact following exposure at the levels measured was not elucidated in the study (Maduabuchi *et al.*, 2008).

It has been previously reported in the literature that schizophrenic patients have lower levels of Mn in plasma than control patients(Yanik *et al.,* 2004 cited from Rahman *et al.,* 2009); a recently published study investigating levels of Mn in scalp hair showed no significant difference between schizophrenic patients and healthy controls (Rahman *et al.*, 2009). This supports the findings of a previous study investigating the use of hair as a correlate of blood/plasma Mn levels (Selikhova *et al.*, 2008); as no correlation was found between Mn levels in blood, plasma or hair of healthy individuals, it was considered by the authors that hair is not appropriate to use as a biomarker for the assessment of Mn deficiency or exposure (Rodrigues *et al.*, 2008).

3. Health Effects

3.1 Neurological Effects

In contrast to the findings reported by Rodrigues *et al.,* (2008; section 2), in a preliminary study conducted by Standridge and colleagues, a positive correlation was identified between Mn concentrations in hair and ambient environmental exposure to Mn. Assessment of Mn in hair samples from individuals living in close proximity (within 10 mile radius of plant for at least 3 previous consecutive years) to a ferromanganese alloy production plant showed a positive correlation with subclinical impairment of postural balance; however, blood Mn levels did not consistently show the same positive correlation. The authors interpreted these results as showing blood Mn levels to be less reliable as a marker of impaired postural balance compared with hair Mn levels (Standridge *et al.*, 2008).

Manganese has been implicated in the development of a neurologic disorder through the use of methcathinone (also referred to as ephedrone) as a drug of abuse. Individuals actively using methcathinone (parenteral injection) develop movement disorders that are similar to, but distinct from, Parkinson's disease. Mean blood Mn levels in individuals injecting methcathinone was shown to be approximately 4 times (831 nmol/L) that of the normal level (<209 nmol/L). Following cessation of use of the drug, the neurological disorders were not seen to be resolved, and were associated with continued mean high blood levels of Mn (mean of 346 nmol/L; Stepens *et al.*, 2008). Levels of Mn remained raised for up to one year following cessation of methcathinone use. These findings are supported by a separate study in which raised levels of Mn were detected in pubic hair of individuals affected by neurological disorders. Characterisation of disorders included dysarthria, gait disturbance, dystonia and levodopa-unresponsive bradykinesia and were considered to be most similar to progressive supranuclear palsy (a condition similar but distinct from Parkinson's disease) (Selikhova *et al.*, 2008).

Raised levels of Mn have been reported in human blood and brain tissue samples from individuals with Creutzfeldt–Jakob disease (CJD), compared with normal individuals whose Mn levels show little variation (Hesketh *et al.*, 2008). It was also reported by the authors that Mn levels were only raised following disease infection, and not during disease pathogenesis, in mice and sheep; it is proposed that Mn may be a potential marker for CJD, for which there is currently none (Hesketh *et al.*, 2007). Deposition of Mn in the basal ganglia and other parts of the central nervous system has been associated with the development of acquired hepatocerebral degeneration, although the exact pathogenesis of this condition is currently poorly understood. It has been proposed by the authors that Mn may be involved in the pathogenesis of this condition (Papapetropoulos *et al.*, 2008).

3.2 Reproductive and Developmental effects

Evaluation of the influence of Mn levels in maternal and umbilical cord blood on intrauterine growth retardation was carried out in non-complicated pregnancies in 542 women aged 18-35 years. A significant correlation was reported between intrauterine growth retardation and Mn levels in both maternal and umbilical cord blood; however, the study could not fully elucidate the relationship as intrauterine growth can be a consequence of a wide range of factors that were not included as part of the study (Vigeh *et al.*, 2008).

Other developmental studies that have been published during this reporting period and include a meeting abstract detailing an assessment of the influence of blood Mn levels in children with attention deficit/hyperactivity disorder (ADHD); the authors reported no

positive correlation (Cheong *et al.*, 2008) In a further study, available only as a meeting abstract, the effects of Mn exposure on children aged 7 – 11 years living close to (exact proximity not detailed) a Mn processing plant district in Mexico was examined. Chronic Mn exposure [exact assessment of chronic exposure was not characterised] was measured in scalp hair and showed that exposed children had a mean Mn concentration 20 fold higher than controls. The authors proposed a positive correlation between Mn exposure and children with an impaired IQ, as measured in verbal, performance and total performance scales (Riojas-Rodriguez *et al.*, 2008).

3.3 Other effects

An isolated case of Mn-induced occupational asthma has also been reported. A male employee who had worked in a train factory for 20 years, and had been exposed to a range of aerosols including Mn, developed occupational asthma; the diagnosis was made on the basis of a positive clinical response to specific challenge test with Mn chloride, and morphological changes following evaluation of sputum samples (Wittczak *et al.*, 2008).

A hypothesis has also been proposed for the perturbation of metabolism of several trace elements, including Mn, in diabetes mellitus patients (type 2). Analysis of human blood, urine or scalp hair from diabetics showed that Mn (as well as other metals) levels were decreased from normal in blood and scalp hair, and increased in urine. From these results, it was suggested that as Mn is required for normal insulin synthesis and secretion (Naga *et al.,* 2006, cited from Kazi *et al.,* 2008), increased levels may act to disturb these processes (Kazi *et al.*, 2008).

4. Mechanisms of toxicity

4.1 Toxicokinetic and metabolic considerations

The distribution of injected Mn to brain tissue was assessed in Marmosets (*Callithrix jacchus*, common marmoset) and rats (Sprague-Dawley) given a series of four i.v. injections of 30 mg/kg $MnCl_24H_20$ in the tail vein, 48 hours apart. MRI scans, carried out 48 hours following the final injection, showed that Mn levels in brain were considerably higher in the marmoset than in the rat, when compared with normalised data (e.g. to body weight). This was considered by the authors to be due to cerebrospinal fluid (CSF)-brain transport from large lateral ventricles, which may increase the susceptibility of non human primates to Mn neurotoxicity; the authors suggest that this finding may also be relevant for humans (Bock *et al.*, 2008).

Inhalation exposure to Mn [form of Mn not stated] via manual metal-arc stainless steel welding fumes of a maximum total suspended particulate = 62 mg/m^3; of up to 1.95 mg/m^3 for up to 8 months was assessed in male cynomologus monkeys. Results showed that accumulations of Mn were reversible in tissues examined including the brain, liver, kidneys, spleen, with the exception of the lung, confirming that solubility of Mn particles was a key factor in the reversal of Mn accumulation; where Mn accumulation was insoluble – i.e. the lung, Mn was seen to be more persistent (Han *et al.*, 2008).

Health effects following inhalation of Mn from airborne-manganese sulphate ($MnSO_4$) were assessed in Rhesus monkeys at concentrations of 1.5 mg/m^3 for up to 65 days followed by a treatment-free period of up to 90 days. Toxicity was monitored by measurement of biomarkers related to neurotoxicity, including total glutathione and tyrosine hydroxylase. Inhalation of Mn was shown to have varying effects within different areas of the brain; e.g. tyrosine hydroxylase levels in the globus pallidus were reduced, however, glutathione levels were increased in the putamen but decreased in the caudate. Although differences in effects were observed, most of the changes in biochemical markers levels were reversible (Erikson *et al.*, 2008).

Mn administered to rats in drinking water (for up to 60 days, at 1 g Mn/L; as $MnCl_2$) was reported to cause increased extracellular gamma-aminobutyric acid (GABA) concentrations, as a consequence of decreased GABA receptor and transporter protein expression. These changes led the authors to consider that observed GABA related effects may be part of the mechanism of neurological toxicity associated with manganism (Anderson *et al.*, 2008).

In rats administered $MnCl_2$ (10 µg) through intracerebellar injection (ICV), luteinizing hormone releasing hormone (LHRH) secretion was induced via activation of the hypothalamic nitric oxide (NO)/cyclic guanosine monophosphate/protein kinase G pathway. These results were supported *in vitro* by isolation and incubation of the medial basal hypothalamus of these rats brains with $MnCl_2$ (500 µM). However, NO also acts to release GABA which has an inhibitory action on the release of LHRH; it is therefore possible that Mn can act to both stimulate and inhibit the induction of LHRH secretion (Prestifilippo *et al.*, 2008). In a further study, rats were administered $MnCl_2$ (10 or 20 mg/kg) from a set period before pregnancy (15 – 20 days), through pregnancy, lactation and for one month after parturition. Increased levels of Mn in the brain of the rat pups, some damaged neurons and marked gliosis with associated functional impairments in behaviour and emotional state of the animals were reported (Lazrishvili Lazriev *et al.*, 2009). However, despite the findings reported from animal studies, the relevance to neurotoxicity in humans following Mn exposure is still unclear.

Exposure to Mn through sub-cutaneous injections of $MnCl_2$ given on 3 alternating days at 100mg/kg per injection was shown to cause accumulation of Mn in the otic capsule (skeletal element enclosing the inner ear) and olfactory epithelium isolated from the ears of C57BL/6J and DBA/2J mice, as well as expression of metal transporters some of which have high affinity for Mn transport, e.g. ZIP8. In this study, elevated Mn levels persisted for 2 weeks following administration of the last dose, and the authors propose that this supports a hypothesis for Mn exposure-related hearing loss either alone or alongside excess noise exposure in Mn exposed individuals (Ma *et al.*, 2008).

Disruption of Fe homeostasis, leading to generation of Fe –initiated reactive oxygen species generation, has been implicated in the aetiology of Parkinson's disease (PD). An *in vitro* study has been reported with exposure of rat choroid plexus to $MnCl_2$ or $FeSO_4$ (at 1 or 10 µM for 1 hr at 37°C). Increased transferrin receptor protein expression and metal transport protein-1 were reported, which may be indicative of early tissue responses to Mn and/or Fe, and suggests the involvement of microtubule dependent intracellular trafficking of transferrin and metal transporter protein-1. The authors propose that these responses may be part of the mechanism for Mn-induced disruption to Fe homeostasis (Wang *et al.*, 2008). The role of Fe homeostasis in the accumulation of Mn in brain tissue has been investigated in a further study whose authors hypothesise that as deposition of Mn increases during Fe depletion, it is likely that the two metals use the same transporters. It is therefore possible that Fe supplementation in the diet of rats administered Mn would lead to decreased Mn accumulation in the brain. In support of this hypothesis, rats were fed either a diet supplemented with, or deficient in Fe and exposed by i.v. injection to $MnCl_2$ (3 mg/kg) for 14 weeks. Although results showed Mn accumulation in specific regions of the brain, these were indistinguishable between Fe deficient or supplemented diets (Fitsanakis *et al.*, 2008).

There is a general consensus in the literature which considers that Mn induces upregulation of inflammatory cytokines, e.g. (Crittenden & Filipov, 2008). This has been discussed in detail in a previous report (Manganese Health Research Program Overview 2007-2008). Further evidence in support of this finding has been reported in an *in vitro* study by Moreno *et al.,*2008 which showed that in mouse cortical astrocytes, exposure to near-physiological levels of Mn (form not stated;10µM) led to increased cytokine production, including tumour necrosis factor-α (TNF- α). This was also associated with NO production via soluble guanylyl cyclase activating extracellular response kinase (ERK) leading to increased induction of nuclear factor kappa-B (NFκB) signalling (Moreno *et al.*, 2008). In a separate study, $MnCl_2$ (and other metals) was shown to inhibit lipopolysaccharide (LPS) binding in rat Kupffer cells exposed to LPS. Furthermore in the presence of Mn, the concentration of TNF-α produced by Kupffer cells following LPS exposure was reduced. The authors' proposed that binding of Mn (and other transition metals) to LPS results in neutralization of negatively charged phosphate groups on LPS, thereby facilitating insertion of LPS in to plasma membrane with subsequent absorptive pinocytosis. Together with the inhibition of LPS-induced TNF-α production, the authors suggest that Mn (and other transition metals) may be potentially useful as therapeutic agents in sepsis patients (Thomas *et al.*, 2008), however, as the hypothesis proposed by Thomas *et al.,* is in contrast with others hypotheses in relation to Mn-induced cytokine release, this must remain a point of contention at the present time.

An i*n vitro* model of Mn-induced lung toxicity using human lung adenocarcinoma (A549) cells has been described. Incubation of A549 cells with $MnCl_2$ at concentrations up to 2 mM for a maximum of 72 hours led to inhibition of cell proliferation and induction of apoptosis which occurred via interruption of normal cell cycling at G0/G1 and S phases (Zhao *et al.*, 2008). In a further *in vitro* study, cultured human lymphocytes were incubated with $MnCl_2$ at concentrations of 15, 20 or 25 µM for pulses of 1 and 6 hours during G1, G1/S, S and G2 phases. Cytotoxicity was reported at all concentrations for all phases with the exception of G2, for which only the highest concentration of $MnCl_2$was cytotoxic. Mn was found not to affect formation of the mitotic spindle, but at the highest concentration, clastogenicity and

DNA damage was reported with chromosomal aberrations in G2 only (Lima *et al.*, 2008). Due to the associated cytotoxicity observed in this study, these results may be of limited value for extended interpretation.

4.2 Oxidative stress as a mechanism of neurotoxicity

Exposure of mitochondrial DNA from isolated rat hepatic mitochondria to $MnCl_2$ (up to 1 nmol/L for 30 mins) was shown to induce single strand breaks. *In vivo*, Sprague-Dawley rats administered $MnCl_2$ (i.p. injections; up to 20 mg/kg/day) for 3 months exhibited increased breaks in mitochondrial DNA isolated from brain and liver. In addition, decreased levels of glutathione (GSH) were detected in a dose related manner in rat hepatic mitochondria and brain homogenates. The authors proposed Mn-induced oxidative stress to be the most probable mechanism behind this toxicity (Jiao *et al.*, 2008).

Ceruloplasmin is an oxidase protein that is postulated to be involved in the mediation of Fe and Mn oxidation loading on to plasma transferrin, and cellular iron efflux. In an *in vivo* study, a single tracer dose of radiolabelled Mn (^{54}Mn) or repeat doses (3 times a week, for four weeks of 0, 7.5 or 15 mg/kg s.c) was given to wild type or genetically modified aceruloplasminemic mice. Results showed that ceruloplasmin was not involved in the loading of Mn on to plasma transferrin, or in the partitioning of Mn between plasma and whole blood. However, ceruloplasmin was shown to affect retention of Mn in the blood, and therefore tissue distribution of Mn. Increases in the levels of Mn-induced oxidative stress in the brain were also noted which may indicate the oxidation of Mn to be important to its toxicity and also suggests oxidase proteins may be involved in the neurotoxicity of Mn (Jursa & Smith, 2009).

Rats administered 10 or 25 mg/ml $MnCl_2$ in drinking water for 30 days showed impaired locomotor activity. The study authors suggest that this was due to increased oxidative stress following increases in lipid peroxidation and inhibition of δ-aminolevulinate dehydratase (an enzyme sensitive to pro-oxidant situations) which, in turn, causes inhibition of heavy Ca^{2+} influx in to the striatum (Ávila *et al.*, 2008).

The ability of $MnCl_2$ (concentrations up to 500 μM) to induce oxidative stress related toxicity has been evaluated in a number of *in vitro* studies using a range of cell types. In isolated rat ventricular myocytes, hydrogen peroxide was shown to be the main intermediate in Mn-induced reactive oxygen species production. Decreases in GSH and loss of mitochondrial membrane potential were also noted following Mn exposure (Yang *et al.*, 2009). Primary astrocyte cultures from new born Sprague Dawley rats were used to assess the effect of $MnCl_2$ (concentrations up to 1mM) exposure on ERK and caspase-3 precursor protein activation. Mn exposure led to phosphorylation of the ERK triggering cleavage of caspase-3, with consequential apoptosis. A further study has described Mn-induced disruption of the mitochondrial membrane potential and increased reactive oxygen species (ROS) generation causing activation of the ERK pathway (Yin *et al.*, 2008). In PC12 cells (derived from rat adrenal medulla pheochromocytoma) incubated with $MnCl_2$ (up to 1.8 mM), decreased cell viability, and induction of apoptosis was observed which was thought to be via caspase-3 activity. This mechanism is potentially involved in the degeneration of dopaminergic neurones following Mn exposure (Deng *et al.*, 2008).

Incubation with 1mM $MnCl_2$ for up to 24 hours has been shown to target mitochondria in rat cortical astrocytes, with subsequent induction of mitochondrial membrane depolarisation and cleavage of poly (ADP-ribose) polymerase-1 (PARP-1) and caspases 3, 6 and 7. These results

illustrate that Mn may shift the balance of cell death / survival via influence on Bcl-2 family proteins to induce apoptosis of astrocytes (Gonzalez *et al.*, 2008).

In primary culture of rat mesencephalic cells, $MnCl_2$ exposure of up to 500 µM for 24 hours led to apoptotic cell death. Co-incubation with dopamine (DA) led to the acceleration of apoptosis in these cells. The potential mechanism was investigated using a series of inhibitors including, 7-nitroindazole as a NO inhibitor and vitamin E as an inhibitor of NFκB. In the presence of DA, Mn exposure led to ROS generation, inducible nitric oxide synthase (iNOS) upregulation, and oxidative stress induced NFκB activation leading to apoptotic cell death. All these elements are considered by the authors to be implicated in Mn-induced neurotoxicity (Prabhakaran *et al.*, 2008).

Administration of $MnCl_2$ to Wistar rats (50 mg/kg) for 1 week by intra-peritoneal (i.p.) injections led to significant decreases in the total anti-oxidant status, and increases in acetylcholinesterase activity of the rat brain. Co-administration of L-cysteine with $MnCl_2$ led to partial reversal of this effect. The authors comment that this effect may be considered of potential therapeutic use as a neuroprotective agent following chronic Mn exposure. The authors also noted that Mn inhibited Mg^{2+}-ATPase; possibly by replacing Mg^{2+} although Na+K+-ATPase was unaffected by Mn (Liapi *et al.*, 2008).

N-Acetyl-cysteine (NAC), a precursor for GSH synthesis, and stimulator of enzymes required for GSH regeneration was shown to be an effective antioxidant against Mn-induced oxidative stress in mitochondria and microvessel endothelial cells isolated from rat brain and incubated with $MnCl_2$ or $MnSO_4$ concentrations up to 800 µM for 24 hours. NAC is also thought to be mitochrondrial protective (Marreilha dos Santos *et al.*, 2008). In a further study, Mn toxicity was evaluated in mitochondria isolated from rat brain with and without pre-incubation with NAC ($MnCl_2$ up to 1000 µM for 2 hours). Mn was shown to inhibit energy metabolism, increase free radical production and disrupt mitochondrial membrane potential thereby inducing apoptosis. A reduction in the effects of Mn-induced accumulation of Mn in the mitochondrial matrix was noted following pre-treatment with NAC. NAC is considered to be of low toxicity and the authors suggest NAC, to be of therapeutic potential in the treatment of Mn toxicity [specific type of toxicity not stated] (Zhang *et al.*, 2008).

4.3 Neurotoxicity associated with dopamine

In a chronic administration study, cynomologus monkeys were given $MnSO_4$ (3.3 – 5 mg/kg/week intravenously (i.v.) via saphenous vein) once a week, for 40 weeks. Results showed Mn accumulation in the frontal cortex, which was considered by the authors to be the first such report. It was proposed that this region of the brain accumulates lower concentrations of Mn than the basal ganglia, and additionally, showed that the frontal cortex was susceptible to neurodegeneration. Exposure to Mn was shown to cause changes in gene expression of many of the genes associated with cellular stress, producing neurodegeneration and diffuse amyloid –β plaques. Copper dysregulation in the brain was also reported, and thought to be a consequence of toxic levels of Mn in the brain; which is important as accumulation or dysregulation of brain copper homeostasis has been associated with amyloid –β plaques in Alzheimer's disease. The authors conclude that Mn is itself neurotoxic and also that the increase of Mn in the brain may lead to dysregulation of other elements such as copper. Thus two possible mechanisms of neurodegeneration may arise from one initial imbalance (Guilarte *et al.*, 2008b). In a further study in cynomologus monkeys, $MnSO_4$ exposure (up to 10 mg/kg once a week i.v. via the saphenous vein for 7 – 59 weeks) led to reduced dopamine (DA) release in the striatum of exposed animals. Although associated motor function deficits were also reported, these changes were seen in the absence of changes to normal dopamine levels and nigrostriatal dopamine synapses. This led the authors to conclude that the Mn-induced decrease in dopamine release was due to presynaptic inhibition

of DA neurotransmission; the motor function deficits observed in this study were proposed to be due to inhibition of DA release (Guilarte *et al.*, 2008a).

Fischer 344 rats were exposed by inhalation to $MnSO_4$ at 1.5 mg/m^3, for six hours a day, 5 days a week for 4 or 13 weeks, leading to decreased DA levels in the hypothalamus. Increases in prolactin levels, prolactin mRNA expression and pituitary specific transacting factor-1 (Pit-1; transacting factor of the prolactin gene) mRNA levels in the rat brain were also reported. Thus, the authors suggest that Pit-1 may regulate and coordinate DA concentration and prolactin expression, and their findings support the hypothesis [currently there is conflicting support in the literature] that serum prolactin may be used as a biological marker for Mn exposure (Kim *et al.*, 2008).

The effect of oral exposure of $MnCl_2$ (750 µg/day) to Sprague Dawley rats during post-natal days 1 – 21 was evaluated on postnatal day 90. Decreases in dopamine transporter protein expression, radiolabelled dopamine uptake in to the striatum and nucleus accumbens and long-term reductions in striatal dopamine efflux were reported. Functionally, the behaviour of the rats was affected by Mn exposure as demonstrated in a range of associative and non-associative tests that suggested alterations in striatal dopaminergic functioning (McDougall *et al.*, 2008).

In a proposed model of Parkinson's disease (PD), CD-1 mice were exposed by inhalation to a mixture of $MnCl_2$ (0.04 M) and Mn $(OAc)_3$ (0.02 M) for one hour twice a week for 5 months. [No details of exposure to $MnCl_2$ and Mn $(OAc)_3$ given separately are described.] Results showed decreased tyrosine hydroxylase immunopositive neurons in the *substantia nigra pars compacta*; decreased motor performance shown as akinesia, postural instability and action tremor were also reported. All changes were considered to be signs of PD, and although the authors conclude this to be a model of PD, the mode of dopamine involvement is not fully clarified in this study (Ordoñez-Librado *et al.*, 2008). In line with the ambiguity concerning the role of dopamine in this proposed PD model, it is a generally held view that whilst symptoms of PD and manganism have similarities, there are distinct pathophysiological differences between the two conditions (Santamaria, 2008).

In mice (C57Bl/6) administered $MnCl_2$ (i.p.) at 20 or 40 mg/kg for 5 days followed by ten treatment free days, decreased motor co-ordination and/or learning was seen to develop at the end of day 5 of dosing and these effects persisted on day 10 of the treatment free period (i.e. 15 days after the initiation of treatment). Striatal tyrosine hydroxylase and dopamine receptors D3 and D4 were not altered following Mn treatment. However, dopamine receptor D2 mRNA and protein levels were significantly increased. The authors concluded that the Mn-induced neurotoxicity observed in this study involved dopamine via the dopamine D2 receptor subtype. Ten days after the end of treatment, the motor effects were still significantly reduced from controls. However, the controls throughout the study, and particularly at day 10, varied considerably from pre-treatment control levels (Nam & Kim, 2008).

Cells (SN4741) from genetically modified mouse embryos with targeted immortalization of substantia nigra dopamine neurons, were exposed to $MnCl_2$ (500uM) for 24 hours. Results showed that p38a mitogen activated protein kinase promoted cell viability but that c-Jun N-terminal kinase (JNK) mitogen activated protein kinase mediated cell death following Mn exposure. These results led the authors to suggest that Mn-induced cell death may involve multiple pathways (Kim *et al.*, 2008). Incubation with Mn ($MnSO_4$) at 50 or 100 µM in PC12 cells was carried out for up to 24 hours. Results showed an increased expression of amyloid precursor protein (APP) and beta site APP cleaving enzyme-1 (Lin *et al.*, 2008).

5. Human Susceptibility

No relevant articles have been identified in this period.

6. Treatment and imaging

A case report has been published that describes a 38 year old man with alcoholic liver cirrhosis presenting with tremors of tongue, jaw and both hands. Investigations revealed moderate global cognitive impairment and whole blood Mn levels of 38 µg/L (normal = <8 µg/L) and urinary copper excretion levels of 94 µg (normal range: 38 – 70 µg). MRI scans revealed hyperintense lesions in the bilateral globus pallidus, cerebral peduncle, pontine tegmentum and dentate nuclei of the cerebellum. Treatment with levodopa/carbidopa had no therapeutic benefit, however, treatment with trientine (a chelator of copper and other metals – normally used for treatment of Wilson's disease) at 2000 mg/day reduced tremors. Following four months of treatment, blood Mn levels fell to 5.6 µg/L with tremors and other 'Parkinsonism' symptoms significantly reduced. After one year of treatment, MRI scans showed significantly decreased high signal intensities in (generally) the same brain regions that had previously showed hyperintensity. The patient was diagnosed with acquired hepatocerebral degeneration (AHD). As there is no established treatment regime for AHD at present, the authors suggest that trientine could be used in this way (Park *et al.*, 2008).

7. Miscellaneous

A review discussing the need for re-evaluation of Mn as a routine supplement in parenteral nutrition has been published. Due to impaired elimination processes in individuals requiring parenteral nutrition and occasional oral intake of Mn, the requirement of routine supplementary Mn is discussed. Finally it is suggested by the authors that current Mn dose guidelines need to be reconsidered and that monitoring of patients receiving parenteral nutrition – paediatric and long-term home patients in this case, should be frequently conducted (Hardy *et al.*, 2008).

A review of excessive Mn exposure and associated toxicity by Santamaria has also been published in this period which generally discusses the essential nature, toxicity and possible exposure biomarkers. The threshold level for Mn induced neurotoxicity and regulatory levels for environmental Mn are also reviewed (Santamaria, 2008).

Overview

This document reviews publications (papers and abstracts) relating to human exposure to, and potential health effects from Mn and Mn-containing inorganic compounds, published during the period March 2008 to February 2009.

Exposure measurements, taken both during and following cessation of exposure to Mn have provided useful information regarding harmful health effects. A single and unusual report of a link between occupational exposure to Mn and respiratory disturbance (asthma) has been reported; it should be noted, however, that such an occurrence is considered to be very rare.

Biomonitoring of Mn in human tissues remains problematical and continues to be unresolved at this time. The reliability of hair sampling for assessment of Mn levels has been discussed, with conflicting opinions in the literature. Although hair and blood sampling are still widely used measures to assess Mn levels it has been reported during this period that hair and blood levels do not correlate. Furthermore, the reliability of blood levels to measure recent exposure to Mn has also been highlighted. Although this opinion conflicts with many other studies where Mn blood levels have been used effectively as a disease indicator/correlate of symptoms, no further suggestions of alternative biomarkers were proposed by the authors. Other studies have proposed the use of serum prolactin as a marker for Mn toxicity, and Mn itself may be useful as a marker for CJD, as animal studies have shown that levels of Mn are disrupted before any behavioural effects are noted.

The majority of papers published regarding mechanism of toxicity of Mn during this period support the hypothesis that Mn induces enhanced cytokine release and associated downstream inflammatory mediators. The role of DA in Mn toxicity has also been discussed with the D2 receptor identified as being increased following Mn exposure. The role of Mn in inducing oxidative stress as a mechanism of neurotoxicity is heavily supported in the literature reported here, with the antioxidant N-Acetyl-cysteine (NAC) being suggested to have therapeutic potential in Mn toxicity.

A final observation of interest from the literature reported here is in the use of trientine, a metal chelator used for treating Wilson's disease, which has been shown to be efficacious in treating symptoms (movement and cognitive disorder) associated with increased Mn levels.

References

Anderson, J.G., Fordahl, S.C., Cooney, P.T., *et al.* (2008) Manganese Exposure Alters Extracellular GABA, GABA Receptor and Transporter Protein and mRNA Levels in the Developing Rat Brain. *NeuroToxicology,* 29(6), 1044-1053.

Ávila, D.S., Gubert, P., Fachinetto, R., *et al.* (2008) Involvement of Striatal Lipid Peroxidation and Inhibition of Calcium Influx into Brain Slices in Neurobehavioral Alterations in a Rat Model of Short-Term Oral Exposure to Manganese. *NeuroToxicology,* 29(6), 1062-1068.

Baldwin, M., Bouchard, M., Larribe, F., *et al.* (2008) Past Occupational Exposure to Airborne Manganese in a Manganese Alloy Plant. *Journal of Occupational and Environmental Hygiene,* 5(7), 426-437.

Bock, N.A., Paiva, F.F., Nascimento, G.C., *et al.* (2008) Cerebrospinal Fluid to Brain Transport of Manganese in a Non-Human Primate Revealed by MRI. *Brain Research,* 1198, 160-170.

Bouchard, M., Mergler, D., Baldwin, M.E., *et al.* (2008) Manganese Cumulative Exposure and Symptoms: A Follow-Up Study of Alloy Workers. *NeuroToxicology,* 29(4), 577-583.

Cheong, H., Kwon, H., Kim, E., *et al* (2008) Blood Manganese Level and Attention Deficit/Hyperactivity Disorder in Early School Age Children. *Epidemiology,* 19(6 Supp), S223-S224. [Abstract]Presented at the 20th ISEE Annual Conference, October 12-16, 2008, Pasadena, California, USA.

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,* 22(1), 18-27.

Deng, Y., Luan, Y., Qing, H., *et al.* (2008) The Formation of Catechol Isoquinolines in PC12 Cells Exposed to Manganese. *Neuroscience Letters,* 444(2), 122-126.

Erikson, K.M., Dorman, D.C., Lash, L.H., *et al.* (2008) Duration of Airborne-Manganese Exposure in Rhesus Monkeys is Associated with Brain Regional Changes in Biomarkers of Neurotoxicity. *Neurotoxicology,* 29(3), 377-385.

Fitsanakis, V.A., Zhang, N., Anderson, J.G., *et al.* (2008) Measuring Brain Manganese and Iron Accumulation in Rats Following 14 Weeks of Low-Dose Manganese Treatment using Atomic Absorption Spectroscopy and Magnetic Resonance Imaging. *Toxicological Sciences,* 103(1), 116-124.

Gonzalez, L.E., Juknat, A.A., Venosa, A.J., *et al.* (2008) Manganese Activates the Mitochondrial Apoptotic Pathway in Rat Astrocytes by Modulating the Expression of Proteins of the Bcl-2 Family. *Neurochemistry International,* 53(6-8), 408-415.

Guilarte, T.R., Burton, N.C., McGlothan, J.L., *et al.* (2008a) Impairment of Nigrostriatal Dopamine Neurotransmission by Manganese is Mediated by Pre-Synaptic Mechanism (s): Implications to Manganese-Induced Parkinsonism. *Journal of Neurochemistry,* 107(5), 1236-1247.

Guilarte, T.R., Burton, N.C., Verina, T., *et al.* (2008b) Increased APLP1 Expression and Neurodegeneration in the Frontal Cortex of Manganese-Exposed Non-Human Primates. *Journal of Neurochemistry,* 105(5), 1948-1959.

Han, J.H., Chung, Y.H., Park, J.D., *et al.* (2008) Recovery from Welding-Fume-Exposure-Induced MRI T1 Signal Intensities After Cessation of Welding-Fume Exposure in Brains of Cynomolgus Monkeys. *Inhalation Toxicology,* 20(12), 1075-1083.

Hardy, I.J., Gillanders, L. & Hardy, G. (2008) Is Manganese an Essential Supplement for Parenteral Nutrition? *Current Opinion in Clinical Nutrition and Metabolic Care,* 11(3), 289-296.

Hesketh, S., Sassoon, J., Knight, R., *et al.* (2008) Elevated Manganese Levels in Blood and CNS in Human Prion Disease. *Molecular and Cellular Neuroscience,* 37(3), 590-598.

Hesketh, S., Sassoon, J., Knight, R., *et al.* (2007) Elevated Manganese Levels in Blood and Central Nervous System Occur before Onset of Clinical Signs in Scrapie and Bovine Spongiform Encephalopathy. *Journal of Animal Science,* 85(6), 1596-1609.

Jiao, J., Qi, Y., Fu, J., *et al.* (2008) Manganese-Induced Single Strand Breaks of Mitochondrial DNA in Vitro and in Vivo. *Environmental Toxicology and Pharmacology,* 26(2), 123-127.

Jursa, T. & Smith, D.R. (2009) Ceruloplasmin Alters the Tissue Disposition and Neurotoxicity of Manganese, but Not its Loading Onto Transferrin. *Toxicological Sciences,* 107(1), 182-193.

Kazi, T.G., Afridi, H.I., Kazi, N., *et al.* (2008) Copper, Chromium, Manganese, Iron, Nickel, and Zinc Levels in Biological Samples of Diabetes Mellitus Patients. *Biological Trace Element Research,* 122(1), 1-18.

Kim, S., Park, E., Kim, S.J., *et al.* (2008) Differential Role of Mitogen-Activated Protein Kinases in Response to Manganese Treatment in Substantia Nigra Dopaminergic Neurons. *Journal of Health Science,* 54(2), 244-249.

Lazrishvili Lazriev, I.L., Shukakidze, A.A., Chkhartishvili, N.N., *et al.* (2009) Morphological Changes and Manganese Content in the Brains of Rat Pups Subjected to Subchronic Poisoning with Manganese Chloride. *Neuroscience and Behavioral Physiology,* 39(1), 7-12.

Liapi, C., Zarros, A., Galanopoulou, P., *et al.* (2008) Effects of Short-Term Exposure to Manganese on the Adult Rat Brain Antioxidant Status and the Activities of Acetylcholinesterase, (Na,K)-ATPase and Mg2-ATPase: Modulation Byl-Cysteine. *Basic & Clinical Pharmacology & Toxicology,* 103(2), 171-175.

Lima, P.D., Vasconcellos, M.C., Bahia, M.O., *et al.* (2008) Genotoxic and Cytotoxic Effects of Manganese Chloride in Cultured Human Lymphocytes Treated in Different Phases of Cell Cycle. *Toxicology in Vitro,* 22(4), 1032-1037.

Lin, R., Chen, X., Li, W., *et al.* (2008) Exposure to Metal Ions Regulates mRNA Levels of APP and BACE1 in PC12 Cells: Blockage by Curcumin. *Neuroscience Letters,* 440(3), 344-347.

Ma, C., Schneider, S.N., Miller, M., *et al.* (2008) Manganese Accumulation in the Mouse Ear Following Systemic Exposure. *Journal of Biochemical and Molecular Toxicology,* 22(5), 305-310.

Maduabuchi, J.M.U., Nzegwu, C.N., Adigba, E.O., *et al.* (2008) Iron, Manganese and Nickel Exposure from Beverages in Nigeria: A Public Health Concern? *Journal of Health Science,* 54(3), 335-338.

Marreilha dos Santos, A.P., Santos, D., Au, C., *et al.* (2008) Antioxidants Prevent the Cytotoxicity of Manganese in RBE4 Cells. *Brain Research,* 1236, 200-205.

McDougall, S.A., Reichel, C.M., Farley, C.M., *et al.* (2008) Postnatal Manganese Exposure Alters Dopamine Transporter Function in Adult Rats: Potential Impact on Nonassociative and Associative Processes. *Neuroscience,* 154(2), 848-860.

Moreno, J.A., Sullivan, K.A., Carbone, D.L., *et al.* (2008) Manganese Potentiates Nuclear Factor-kappaB-Dependent Expression of Nitric Oxide Synthase 2 in Astrocytes by Activating Soluble Guanylate Cyclase and Extracellular Responsive Kinase Signaling Pathways. *Journal of Neuroscience Research,* 86(9), 2028-2038.

Nam, J. & Kim, K. (2008) Abnormal Motor Function and the Expression of Striatal Dopamine D2 Receptors in Manganese-Treated Mice. *Biological & Pharmaceutical Bulletin,* 31(10), 1894-1897.

Ordoñez-Librado, J.L., Gutierrez-Valdez, A.L., Colín-Barenque, L., *et al.* (2008) Inhalation of Divalent and Trivalent Manganese Mixture Induces a Parkinson's Disease Model: Immunocytochemical and Behavioral Evidences. *Neuroscience,* 155(1), 7-16.

Papapetropoulos, S., Tzakis, A., Sengun, C., *et al.* (2008) Case of Pediatric Acquired Chronic Hepatocerebral Degeneration. *Pediatric Neurology,* 38(1), 67.

Park, H.K., Kim, S.M., Choi, C.G., *et al.* (2008) Effect of Trientine on Manganese Intoxication in a Patient with Acquired Hepatocerebral Degeneration. *Movement Disorders,* 23(5), 768-770.

Prabhakaran, K., Ghosh, D., Chapman, G.D., *et al.* (2008) Molecular Mechanism of Manganese Exposure-Induced Dopaminergic Toxicity. *Brain Research Bulletin,* 76(4), 361-367.

Prestifilippo, J.P., Fernandez-Solari, J., De Laurentiis, A., *et al.* (2008) Acute Effect of Manganese on Hypothalamic Luteinizing Hormone Releasing Hormone Secretion in Adult Male Rats: Involvement of Specific Neurotransmitter Systems. *Toxicological Sciences,* 105(2), 295-302.

Rahman, A., Azad, M.A., Hossain, I.*, et al.* (2009) Zinc, Manganese, Calcium, Copper, and Cadmium Level in Scalp Hair Samples of Schizophrenic Patients. *Biological Trace Element Research,* 127(2), 102-108.

Riojas-Rodriguez, H., Rodriguez-Agudelo, Y., Schilmann, A.*, et al* (2008) Neuropsychological Effects of Manganese Exposure on Children Living in Communities Near Processing Plants in Mexico. *Epidemiology,* 19(6 Supp), S153-S154. [Abstract]Presented at the 20th ISEE Annual Conference, October 12-16, 2008, Pasadena, California, USA.

Rodrigues, J.L., Batista, B.L., Nunes, J.A.*, et al.* (2008) Evaluation of the use of Human Hair for Biomonitoring the Deficiency of Essential and Exposure to Toxic Elements. *The Science of the Total Environment,* 405(1-3), 370-376.

Santamaria, A.B. (2008) Manganese Exposure, Essentiality & Toxicity. *Indian Journal of Medical Research,* 128, 484-500.

Selikhova, M., Fedoryshyn, L., Matviyenko, Y.*, et al.* (2008) Parkinsonism and Dystonia Caused by the Illicit use of Ephedrone--a Longitudinal Study. *Movement Disorders : Official Journal of the Movement Disorder Society,* 23(15), 2224-2231.

Standridge, J.S., Bhattacharya, A., Succop, P.*, et al.* (2008) Effect of Chronic Low Level Manganese Exposure on Postural Balance: A Pilot Study of Residents in Southern Ohio. *Journal of Occupational and Environmental Medicine,* 50(12), 1421-1429.

Stepens, A., Logina, I., Liguts, V.*, et al.* (2008) A Parkinsonian Syndrome in Methcathinone Users and the Role of Manganese. *New England Journal of Medicine,* 358(10), 1009.

Thomas, P., Hayashi, H., Lazure, D.*, et al.* (2008) Inhibition of Lipopolysaccharide Activation of Kupffer Cells by Transition Metals. *The Journal of Surgical Research,* 148(2), 116-120.

Vigeh, M., Yokoyama, K., Ramezanzadeh, F.*, et al.* (2008) Blood Manganese Concentrations and Intrauterine Growth Restriction. *Reproductive Toxicology,* 25(2), 219-223.

Wang, X., Miller, D.S. & Zheng, W. (2008) Intracellular Localization and Subsequent Redistribution of Metal Transporters in a Rat Choroid Plexus Model Following Exposure to Manganese Or Iron. *Toxicology and Applied Pharmacology,* 230(2), 167-174.

Wittczak, T., Dudek, W., Krakowiak, A.*, et al.* (2008) Occupational Asthma due to Manganese Exposure: A Case Report. *International Journal of Occupational Medicine and Environmental Health,* 21(1), 81-83.

Yang, H., Gu, L., Zhang, L.*, et al.* (2009) Involvement of Hydrogen Peroxide in the Manganese-Induced Myocytes Mitochondrial Membrane Potential Loss. *Toxicology Mechanisms and Methods,* 19(1), 66-72.

Yin, Z., Aschner, J.L., dos Santos, A.P.*, et al.* (2008) Mitochondrial-Dependent Manganese Neurotoxicity in Rat Primary Astrocyte Cultures. *Brain Research,* 1203, 1-11.

Zhang, F., Xu, Z., Gao, J.*, et al.* (2008) In Vitro Effect of Manganese Chloride Exposure on Energy Metabolism and Oxidative Damage of Mitochondria Isolated from Rat Brain. *Environmental Toxicology and Pharmacology,* 26(2), 232-236.

Zhao, P., Zhong, W., Ying, X.*, et al.* (2008) Manganese Chloride-Induced G0/G1 and S Phase Arrest in A549 Cells. *Toxicology,* 250(1), 39-46.