Manganese Health Research Program:

Overview of Research into the Health Effects of Manganese (2009-2010)

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

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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 2009 to December 2010, 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 has previously been well reported that occupational exposure to excess Manganese (Mn) could increase incidence of neurodegenerative diseases, and during this period of review two occupationally-linked studies have been published. In the first study, Stampfer (2009) examined mortality rates from neurodegenerative diseases amongst 107,773 subjects with welding-related occupations in the USA between 1985 and 1999. The authors reported that there was no increased mortality from either Parkinson's disease (PD) or Alzheimer's disease (AD); furthermore there was no evidence to support an association with mortality from presenile dementia. However, there are several limitations within the study that may have biased an association to the null. Similarly, Firestone *et al.* (2010) reported results of a population-based case-control study of PD patients with past exposure to a range of industrial toxicants, including Mn. The authors did not find evidence of increased risk associated with metal working, however, the numbers of cases specifically exposed to Mn was very low (3/404 PD and 5/526 controls) thereby limiting the power of the study considerably.

An association between high levels of non-occupational exposure to Mn and attention impairments in an adult population from a mining district in Hidalgo State, Mexico, has also been described (Solís-Vivanco *et al.*, 2009).

During this reporting period, a number of environmental exposure studies have been described with a focus on the effects of Mn exposure in children. Menezes-Filho *et al.* (2009) demonstrated increased levels of Mn in hair samples of children living near a ferro-manganese alloy production plant in Bahia, Brazil; proximity to the plant, gender and duration of *in utero* exposure were factors associated with children's current Mn blood levels. Support for the significance of *in utero* exposure to Mn, was described in a study to assess placental permeability of several environmental toxicants using paired maternal and cord blood samples from South African women. Mn levels in cord blood were found to be twice as high as those of mothers, demonstrating high placental permeability (Rudge *et al.*, 2009) Mn exposure during pregnancy and lactation has also been assessed in rural Bangladeshi women exposed to high levels of the metal through drinking water. Although urine and blood levels of Mn were elevated, being comparable to those found in occupationally exposed groups, breast milk did not contain high levels of the metal (Ljung *et al.*, 2009). The importance of using age-specific parameters to assess adverse health effects in children was illustrated in a study by Brown & Foos (2009) evaluating exposure to Mn through drinking water in different age groups of children. Winder (2010) addressed differences in toxicodynamics between children and adults, demonstrating that children, particularly neonates, are at greater risk for Mn neurotoxicity associated with inhalation. Potential detrimental effects on the intelligence of children following co-exposure to environmental Lead and Mn were highlighted in a study by Kim *et al.* (2009) who proposed an additive interaction and subsequent effect modification between the two metals. Children living in the Taxco mining district in Southern Mexico were shown to be environmentally co-exposed to several metals, with levels of some, including Mn, above reference values. The authors stress the need for assessment of simultaneous exposure to toxic metals (Moreno *et al.*, 2010).

An *in vivo* study in rats repeatedly exposed (over 7 weeks) by intratracheal instillations to welding fume particulates with high and low Mn content has been described. Repeated exposure resulted in accumulation of Mn in the brain, persistent molecular alterations in dopaminergic markers, affected neurotransmission factors, induced oxidative stress and neuroinflammation. It was proposed that such sustained events may elicit neurobehavioural and neurodegenerative manifestations (Sriram *et al.*, 2010).

In the context of exposure measurements, Lidén & Surakka (2009) describe the use of a head-mounted mini sampler for the accurate measurement of occupational manganese exposure from welding aerosols. In addition, Laohaudomchok *et al.* (2010) provided an assessment of a portable X-ray fluorescence spectrometer for measurement of several metals, including Mn, *in situ*. The importance of establishing background reference levels for essential trace elements from environmental exposure has been highlighted by Lech & Dudek-Adamska (2009) who carried out measurements of both Zinc and Mn levels in human post-mortem tissues, including brain, liver, kidney, stomach, small-intestine, lung and spleen and in blood, urine and bile. Nunes *et al.*(2010) have detailed the development of a rapid and simple high-throughput MS-based method for the determination of background blood levels of several metals, including Mn. In addition, Olmedo *et al.* (2010) have described the validation of methodology to quantify several metals, in blood, urine, saliva and hair using electrothermal atomic absorption spectrometry.

A number of methods for the modelling of environmental and occupational Mn exposure have been reported. Zeng *et al.* (2009) described the spatial analysis of the probabilistic human health risk associated with ingestion of Mn from water sources in Huangxing Town, China. A physiologically based pharmacokinetic (PBPK) model for Mn in rats has been developed that can be scaled to predict exposure through inhalation and diet in nonhuman primates (Nong *et al.*, 2009). In addition, Andersen *et al.* (2010) reviewed the development of PBPK models for Mn and detailed their potential use in risk assessment. Douglas *et al.* (2010) also demonstrated the use of an 11-compartment whole body physiologically-based toxicokinetic (PBTK) model to predict Mn distribution using rat data.

Potential biomarkers for the detection of early onset neurobehavioural alterations in Manganism have been proposed by Cowan *et al.* (2009a). The authors demonstrated the efficacy of using Mn/Fe ratio (MIR) in biological samples to assess Mn exposure in smelting workers in Guizhou Province, China. In a further study, plasma and erythrocyte MIR were related to early changes in Mn induced motor and memory dysfunction amongst the same study cohort of smelters. Results indicated that both pMIR and eMIR reflected, to varying degrees, changes in fine movement coordination following Mn exposure (Cowan *et al.*, 2009b). Mn deposition in the brain is most commonly assessed using the Pallidal Index (PI), obtained through MRI. Chang *et al.* (2009) evaluated the relative sensitivities of 3D gradient-echo (FSPGR) and conventional spin-echo (SE) for obtaining the PI from male welders; results indicated that FSPGR showed better correlation with neurobehavioural performance indicators.

Bone Mn levels have been proposed as a biomarker of cumulative exposure, to be used in conjunction with blood Mn measurements as a marker of recent exposure, to assess adverse health effects (Pejović-Milić *et al.*, 2009). Placental monoamine oxidase (MAO) activity in placenta has been shown to correlate with Mn in maternal and cord blood at delivery. The authors propose that placental MAO may provide a marker for Mn toxicity in the newborn against which effects of exposure on psychomotor development could be assessed (Abdelouahab *et al.*, 2010).

In separate reviews, Santamaria & Sulsky (2010) and Boyes (2010) detailed a risk assessment strategy for Mn as an essential element. Revision of the US EPA's Reference Concentration for Mn in light of findings from recent epidemiology studies has been discussed by Bailey et al. (2009), and development of reference inhalation exposure levels for Mn by Winder et al. (2010)

3. Health Effects

3.1 Neurological Effects

In a review, Flynn & Susi (2009) discussed findings from studies examining an association between exposure to welding fumes and neurological disease. Meyer-Baron *et al.* (2009) also reviewed epidemiological studies published during the period 1987 and 2008 aimed at quantifying evidence of performance effects following chronic exposure to Manganese (Mn).

A study to assess neurochemical effects of chronic exposure to Mn in a cohort of welders in South Korea has been described by Chang *et al.* (2009). The study examined changes in cerebral metabolite ratios using proton magnetic resonance spectroscopy (MRS), investigated whether abnormal brain metabolism was associated with neurobehavioural changes and assessed potential implications of chronic Mn exposure. Several metabolites were measured, specifically, *N*-acetylaspartate (NAA), myoinositol (mI), total choline (tCho) and glutamine plus glutamate (Glx) and expressed as a ratio to total creatinine (tCr). Data showed no difference between ratios of NAA/tCr, Glx/tCr and tChol/tCr in welders or controls, and no correlation with blood Mn levels. However, mI levels were seen to be significantly lower in the anterior cingulated cortex (ACC) area of the brain in welders when compared to controls. In addition, the mI/tCr ratio in the frontal lobe area of the brain was found to be significantly correlated with verbal memory scores and blood Mn levels. The authors proposed that decreased levels of mI in welders chronically exposed to Mn may reflect glial cell swelling and detoxification processes in the brain.

Results from human epidemiology studies designed to assess the relationship between cognitive deficits, including learning and memory, and chronic Mn exposure have, to date, been inconsistent. In an attempt to address these inconsistencies, Schneider *et al.* (2009) carried out an *in vivo* study in cynomolgus macaques monkeys, to evaluate the effects of chronic exposure to Mn sulphate at concentrations between 15-20 mg/kg/week, on memory functioning. Exposed animals were shown to develop mild deficits in spatial working memory, more significant deficits in non-spatial memory and no deficits in reference memory. Indeed, for many of the brain regions studied, a significant inverse relationship between working memory task performance and brain Mn concentration was found.

Although epidemiological studies have reported associations between elevated dietary manganese exposure and neurobehavioural and neurocognitive deficits in children, the relationship is still not well defined. In an *in vivo* study, neonate rats were exposed to Mn (oral administration) at levels of either 25 or 50 mg Mn/kg/day during the postnatal period 1-21 days (Kern *et al.*, 2010). Preweaning exposure to Mn caused deficits in behavioural inhibition and spatial and associative learning that were linked with significant changes to dopamine receptors and dopamine transporter levels in prefrontal cortex, nucleus accumbens, and dorsal striatum regions of the brain. The authors concluded that the data supports findings from epidemiological studies in children showing associations between elevated Mn exposure and cognitive deficits and ADHD-like behaviours.

A gene-environment interaction screening method utilising the ST*Hdh* mouse striatal cell line model of Huntington's disease (HD) has been used to identify common pathophysiological targets or mechanisms for the disease (Williams *et al.*, 2010). Cells were exposed to a range of metals, specifically, Fe(III)chloride, Mn(II) chloride, Cd(II) chloride, Co(II) chloride, Cu(II) chloride, Pb(II) chloride, Ni(II) sulphate and Zn(II) chloride. The authors report that striatal cells expressing mutant *Huntingtin* showed increased sensitivity to

cadmium toxicity but resistance to manganese toxicity, due to significant impairment in manganese accumulation; this was not found with any other metal tested.

3.2 Reproductive and Developmental effects

No relevant articles have been identified in this period.

3.3 Other effects

Primary biliary cirrhosis (PBC) is an autoimmune chronic cholestatic liver disease which is associated with debilitating fatigue in around 50% of sufferers. Recent studies of PBC have shown a prevalence of abnormalities in autonomic nervous system function, which is controlled largely by the globus pallidus (GP) and basal ganglia (BG). In Parkinson's disease (PD), lesions in the BG have been associated with disruption in cerebral pathways that are perceived as fatigue. In addition, the BG and GP have been shown to be susceptible to accumulation of Mn in liver cirrhosis patients. To fully assess whether fatigue in PBC is associated with Mn deposition in the GP, Hollingsworth *et al.* (2009) carried out magnetization transfer ratio (MTR) measurements of the globus pallidus area of the brains of 30 early-stage PBC patients, and 4 end-stage PBC patients. The authors reported that MTR measurements correlated well with age in early-stage PBC and control subjects, but did not correlate with Mn deposition or fatigue severity.

Although a recent review by ATSDR found no evidence of cancer effects in humans exposed to Mn, Spangler & Reid (2010) argue that long-term studies of large populations have, to date, been scarce. The authors reported an assessment of age-adjusted all-cause and cancer death rates for North Carolina counties (n=8,049,313) correlated with groundwater and air Mn levels, over the period 1997-2001. Data showed positive associations between groundwater Mn and rates of all-site cancers, colon and lung cancers; associations between air Mn and cancer rates was less well defined. The authors highlight the importance of defining these relationships due to the global practice of replacing lead in petrol with Mn.

It has been proposed that Mn may play a role in the pathogenesis of prion diseases such as transmissible spongiform encephalopathy (TSE; (Li *et al.*, 2009)). An assessment of the effects of copper depletion and/or Mn enhancement in the diet on susceptibility to Scrapie and progression of the disease has also been reported using genetically modified murine models (Hortells *et al.*, 2010).

4. Mechanisms of toxicity

In a general review, Lucchini *et al.*(2009) explore current literature regarding potential subclinical effects and biological pathways, impairment and development of Parkinsonism and manganism following exposure to manganese (Mn) through inhalation and ingestion. A further review discusses evidence for common biochemical and molecular mechanisms in the pathogenesis of manganism and Parkisonism (Roth, 2009). Recent advances in the neuropathology associated with Mn exposure and treatments to attenuate neurodegenerative effects are reviewed by Aschner *et al.* (2009).The importance of the interdependency of Mn and Fe transport and regulation, and examples of transport proteins currently under investigation are highlighted in a review by (Fitsanakis *et al.* (2010). A review of several neurotoxic metals, including Mn, with a focus on trace element speciation analysis with relevance to neuroscientific research has also been published (Michalke *et al.*, 2009).

4.1 Toxicokinetic and metabolic considerations

In a review of current literature, Yokel (2009) assessed potential mechanisms for Mn flux across the blood-brain barrier (BBB). Mishra *et al.* (2009) also proposed a role for Mn in altering BBB permeability, thereby potentiating the neurotoxicity of *Lathyrus sativus* (a legume commonly known as the grass pea) leading to the neurodegenerative disorder Neurolathyrism.

The toxicity of Mn nanoparticles has been assessed in male Wistar rats exposed to a nanosuspension of MnO_2 (23nm particles) of either 2.63 or 5.26 mg Mn/kg by tracheal instillation for between 3 and 9 weeks. At the end of the exposure period, treated rats had lower body and liver weight, and increased lung weight compared with control rats. Mn content of the nanoparticles was shown to have accessed both lung and brain tissues. A number of electrophysiological and behavioural parameters were also significantly changed in the treated group (Sarkozi *et al.*, 2009).

The role of the excitatory neurotransmitter glutamate (Glu) in the mechanisms underlying excitotoxicity induced by manganism have been investigated in a rat study (Deng *et al.*, 2009a). Rats were exposed to $MnCl_2$ at concentrations between 50 and 200 µmol/kg by i.p. injection and levels of Mn and activities of glutamine synthesis (GS), phosphate-activated glutaminase (PAG), Na^+-, K^+- and Ca^{2+}-ATPase in striatum were assessed. $MnCl_2$ was shown to inhibit activities of GS, Na^+-, K^+- and Ca^{2+}-ATPase and elevate PAG activity and Mn levels. The authors conclude that the data confirms that dysfunction of Glu transportation and metabolism associated with excitotoxicity play an important role in manganism. In a similar study, Xu *et al.* (2010) demonstrated that *in vivo* Mn exposure in rats lead to significantly increased Glu content in the striatum, thereby increasing extracellular Glu leading to excitotoxicity. In a further study, Deng *et al.* (2009b) evaluated the effects of exposure to $MnCl_2$ (8 – 200 µmol/kg) on the morphology and neurological ultrastructural features in the striatum of rats, with measurements of levels of Mn, Glu and Glutamine (Gln), activities of Na^+- and K^+-ATPase, GS and PAG, mRNA and protein expression of GS, GLAST and GLT-1. Data showed that exposure to Mn caused elevated Mn and Glu levels and PAG activity; changed morphological and ultrastructural features; inhibited Gln levels, GS and Na^+- and K^+-ATPase activities, expression of GS, GLAST and GLT-1 mRNA. The authors reported that the results confirmed the hypothesis that disruption of the Glu-Gln cycle, associated with excitotoxicity may play a key role in manganism. Sidoryk-Węgrzynowicz *et al.* (2009) reported a study using rat primary astrocytes to identify Gln transport routes and Gln transporters that contribute to impairment of astrocyte Gln transport following Mn exposure. The authors demonstrated that Mn is a strong inhibitor of expression of the principal carriers

of Gln, thereby decreasing transport into cells. Taken together with Mn-induced disturbances in Gln and Glu metabolism, this could lead to glutamatergic neurotransmission impairment which is one of the most prominent features of Mn neurotoxicity.

An animal model, mimicking human exposure conditions to welding fumes has been described (Antonini *et al.*, 2009). Rats were exposed by inhalation to 40mg/m^{3}of gas metal arc-mild steel (MS) welding fumes for 3h/day for 10 days. Analysis of the fumes showed a complex of iron and Mn with a high proportion of particles in the nanometer size range. Following inhalation, manganese was shown to translocate from the lungs to kidney and accumulated in the olfactory bulb, cerebellum and cortex regions of the brain; subtle changes in neuroinflammatory cell markers and astrogliosis were also observed.

It has previously been reported that Mn-citrate complex is the most abundant Mn carrier in blood, however data concerning the effects of Mn compounds on human erythrocytes has not been well studied. Suwalsky *et al.* (2010) reported investigations of the molecular interaction of Mn with cell membranes using intact human erythrocytes, isolated unsealed human erythrocyte membranes and molecular models of the erythrocyte membrane. Both Mn^{2+} and Mn-citrate complex were shown to perturb lipid bilayer structures of membranes, thereby potentially affecting their shape, permeability and functionality. The authors propose that this provides new insight into possible mechanisms of toxicity of Mn at the entry level.

The role of the cytoplasmic iron exporter ferroprotein (Fpn) as a Mn exporter to attenuate Mn toxicity has been investigated (Yin *et al.*, 2010). Results demonstrated that increased Fpn protein expression is associated with decreased Mn concentration and attenuated cytotoxicity in human embryonic kidney (HEK293) cells. The authors propose that this establishes a role for Fpn in Mn efflux from mammalian cells.

The role of Mn/zinc transport system in mammalian cells in uptake of cadmium has been reviewed (Himeno *et al.*, 2009). In addition, it has been reported that the Mn transport protein Smf1p is down regulated in both response to high physiological levels and when cells are subject to toxic levels of the metal (Jensen *et al.*, 2009).

4.2 Oxidative stress as a mechanism of toxicity

Exposure to elevated levels of Mn is known to cause neuronal damage in the midbrain leading to development of Parkinsonism symptoms. The role of microglia activation and subsequent production of reactive nitrogen and oxygen free radicals has been shown to contribute to the neurodegeneration of dopaminergic neurons associated with Parkinson's disease (PD) pathogenesis (Zhang *et al.*, 2009). The effect of Mn on the Olfactory Bulb (OB) has been evaluated *in vivo* using mice. Data showed ultrastructural changes of the OB in response to chronic Mn exposure, possibly caused through oxidative stress; the authors proposed that as the OB connects to the brain limbic region, the initial psychiatric symptoms of Mn poisoning in humans may originate in the olfactory-limbic region (Villalobos *et al.*, 2009)

The underlying mechanisms of Mn neurotoxicity have been investigated using *in vitro* and *in vivo* assays to assess the effects of Mn on reactive oxygen species formation, high-energy phosphates, neuroinflammation mediators and neuronal dysfunctions. *In vitro* cultures of primary cortical neuronal cells exposed to between 100 and 1000 μM Mn showed concentration-dependent alterations in F$_2$-isoprostanes, mitochondrial dysfunction (ATP) and increases in levels of prostaglandin E$_2$. These findings were verified through an *in vivo* study in mice which additionally showed progressive spine degeneration and dendritic damage to medium spiny neurons. The authors suggested that the findings indicate that oxidative stress,

mitochondrial dysfunction and neuroinflammation are underlying mechanisms in Mn-induced neurotoxicity and that mediation of these may have a therapeutic role in slowing the neurodegenerative process (Milatovic *et al.*, 2009).

Astrocytes perform a number of functions that are essential for normal neuronal activity, provide protection against oxidative stress and support neurons by fostering their survival, proliferation, differentiation, neurite outgrowth and synaptogenesis. An *in vitro* study using rat cortical astrocytes exposed to concentrations of Mn between 50 and 500 µM showed impaired ability to promote axonal and neurite outgrowth in hippocampal neurons; this process, which is vital during brain development and regeneration following neuronal injury, appeared to be mediated by oxidative stress (Giordano *et al.*, 2009). In an additional study, the role of Mn in AQP4 overexpression, leading to astrocyte swelling, was investigated (Rao *et al.*, 2010). The authors reported that Mn increased AQP4 protein in the plasma membrane of astrocytes; however, total cellular AQP4 protein and its mRNA levels were unchanged. Increased AQP4 in plasma membrane was shown to be temporarily associated with astrocyte swelling, indicating a key role for AQP4 in the process.

It has been proposed that endothelial cells of the BBB are a target for Mn-induced neurotoxicity (dos Santos *et al.*, 2010). Using an *in vitro* BBB model, immortalised rat brain endothelial (RBE4) cells were exposed to 200 or 800 µM $MnCl_2$ or $MnSO_4$ for 4 or 24 h. Results showed a significant decrease in cell viability in exposed cells associated with an increase in F_2-isoprostane levels and decreased membrane potential. The authors concluded that endothelial cells are a target of Mn-induced cytotoxicity, mediated through mitochondrial dysfunction, with oxidative stress as an underlying mechanism.

The subcellular distribution of manganese and its interactions with other trace metals has been assessed by Carmona *et al.* (2010) using synchrotron X-ray fluorescence nanoimaging and PC12 dopaminergic cells exposed to Mn at physiological concentrations. Mn was seen to accumulate in the Golgi apparatus (GA) which acted as a subcellular store, thereby preventing Mn cytotoxicity; however, the authors proposed that the effect of this may have been to alter vesicular trafficking of the dopamine cell. In addition, once the GA was altered, Mn was able to reach the cell nucleus and cytoplasm where genomic DNA and mitochondria could be compromised, leading to neuronal cell death. Mukhopadhyay *et al.* (2010) also identified a potential homeostatic mechanism for Mn in human HeLa cells.

Study of the toxicity of Mn using brain cells has to date been limited to a small number of cell types. Park & Park (2010) reported the use of human glioblastomas of T98G cells, derived from glioblasts and which develop into various brain cells with specific functions, to assess neurological dysfunctions following exposure to Mn. Incubation of T98G cells with $MnCl_2$ at concentrations between 100 and 800 µM was associated with an increase in ROS generation and decrease in intracellular GSH, suggesting oxidative stress. The increase in ROS production was linked to increased activity of caspase-3, leading to apoptosis, and pro-inflammatory responses with increased levels of IL-6 and IL-8.

4.3 Neurotoxicity associated with dopaminergic neurons

In vivo neuroimaging in nonhuman primate studies provides essential evidence of the effects of Mn on brain chemistry. In a review, Burton & Guilarte (2009) collate recent findings from a large nonhuman primate study addressing neurologic effects following chronic exposure to Mn. In a further review, mechanisms associated with neurodegeneration due to copper or Mn

toxicity to the dopaminergic system in basal ganglia are discussed; particular emphasis is given to Parkinsonism symptoms encountered in chronic liver failure (Butterworth, 2010).

The potential neurotoxicity of Mn nanoparticles has been demonstrated using the dopaminergic cell line, PC12, with oxidative stress and enzymatic alterations being linked to dopaminergic neurotoxicity (Wang *et al.*, 2009). *In vivo* and *in vitro* models have been used to investigate cellular and circuitry alterations induced by Mn exposure. Primary mesencephalic cultures were treated with $MnCl_2$ to show the effect on dopaminergic neurons, and resulted in notable changes to the neuronal cytoskeleton. In addition, mice repeatedly exposed to $MnCl_2$ (i.p. for 30 days) showed substantial reduction in SNpc (substantia nigra pars compacta) cell numbers and alteration to other areas of the basal ganglia, with overlapping features to PD (Stanwood *et al.*, 2009).

An *in vitro* study using Pheochromocytoma 12 cells (PC12) Posser *et al.* (2009) showed changes in dopaminergic cell function at low concentrations of Mn that did not induce oxidative stress; the authors propose that the results provide a potential new mechanism for Mn induced neuronal toxicity.

Dopaminergic neuronal injury by $MnCl_2$ has been shown to be associated with microglia activation leading to induction of the inflammatory factors nitric oxide synthatase, tumour necrosis factor-α and interleukin-1ß; these factors may cause neuronal injury either in isolation or in combination (Liu *et al.*, 2009).

It has been suggested that PD has a mutifactorial etiology, likely to include the innate vulnerability of the nigrostriatal dopaminergic pathway to oxidative damage, exposure to environmental toxicants, and potential genetic pre-disposition, all superimposed on the aging process. In support of this, an *in vitro* study using primary glial and neuronal cultures has shown synergistic dopaminergic neurotoxicity of Mn and an immune-stimulator, endotoxin lipopolysaccharide, at individually ineffective concentrations (Zhang *et al.*, 2010).

4.4 Gene expression studies

There are a number of possibilities and contradictions in the literature regarding mechanisms through which Mn induces apoptotic cell death. In an *in vitro* study , Xu *et al.* (2009) exposed primary neuronal cultures isolated from brains of neonatal Wistar rats, to Mn concentrations between 0 – 400 µM for up to 48 h. Results showed that Mn induced neuronal damage by increasing the concentration of intracellular Calcium and altering expression of N-methyl-$_D$-aspartate receptor subunit mRNAs and proteins.

There is a frequent clinical, pathological and biochemical overlap between Alzheimer's disease (AD), Parkinson's disease (PD) and Dementia with Lewy Bodies (DLB). Evidence has shown that AD is mediated by brain insulin and insulin-like growth factors (IGF) resistance and deficiency; Tong *et al.* (2009) examined whether these are also impaired in PD or DLB. The authors used quantitative RT-PCR to measure mRNA expression in samples from human post-mortem brain tissue of PD and DLB diagnosed patients. Data showed that IGF-I, IGF-II and neurotrophin signalling are impaired to a greater extent in DLB than in PD which correlates with the more pronounced neurodegeneration seen in DLS patients. In addition, exposure to Mn caused PD/DLB associated abnormalities in neurons of the CNS, which may contribute to molecular pathogenesis. Although the authors found that PD and DLB molecular abnormalities overlapped, these could be distinguished from those present in Alzheimer's disease.

A major transporter of Mn in mammals is the divalent-metal transporter (DMT1), however at present both Mn transport and DMT protein function(s) are not well understood. The lack of

clarity may stem from the existence of several DMT proteins in distinct tissues, which are differentially regulated at both transcriptional and post-translational levels. In an attempt to address some of these issues, Au *et al.* (2009) utilised the nematode, *C.elegans* and reported a complex integrated system of transcriptional and post-translational regulation of 3 DMT1-like transporters in adjacent tissues that regulate metal-content. The authors proposed that the mechanism is conserved from nematodes to man.

The synucleinopathy, α-Synuclein (α-Syn) plays a key role in the pathogenesis of familial and sporadic PD. Gitler *et al.* (2009) assessed functional connections between α-syn and other predisposition factors such as Mn exposure in an α-syn expressing yeast strain. The authors reported a connection between PD genetics and manganese as an environmental risk factor.

A potential detrimental role of the BNIP3 gene in Mn-induced neuronal cell death has been proposed (Prabhakaran *et al.*, 2009). Expression of BNIP3 by MES 23.5 cells was shown to be increased following Mn exposure, leading to apoptotic cell death. The authors suggest that BNIP3 acts to regulate mitochondrial functions during Mn-induced neurotoxicity.

Institute of Environment and Health Report to MHRP, March 2011

5. Human Susceptibility

In a review article, Curran *et al.* (2009) described The National Institute for Occupational Safety and Health (NIOSH) strategy for its current review of recommendations for worker exposure to Manganese. Of particular interest is an attempt to quantify inter-individual susceptibility using data generated from the Human Genome Project and experimental data to identify genetically based biomarkers of exposure, disease and susceptibility.

Institute of Environment and Health Report to MHRP, March 2011

6. Treatment and imaging

A discussion on current therapeutic aspects of Manganese (Mn) exposure has been included in a review by Aschner *et al.* (2009). In a case-study Sikk *et al.* (2010) describe the diagnosis and treatment of 4 patients with a history of chronic Mn-ephedrone exposure.

Magnetic resonance imaging (MRI) of the brain in Parkinsonian disorders, including Mn-induced Parkinsonism, has been the subject of a review by Sitburana & Ondo (2009).

Mn-enhanced MRI (MEMRI) has been assessed as a tool to evaluate delayed neuronal death following hypoxic-ischemic injury (HI). Wistar rat pups (7 d) were subjected to HI and subsequently injected with $MnCl_2$ prior to MR-scanning; following scanning, pups were sacrificed and brain slices taken for immunohistochemical staining. The authors reported that during the first days after HI, no increased Mn-enhancement was detected on MRI in areas that showed massive neuronal death using immunohistochemical staining; therefore the method was not applicable to detect neuronal death in general. However, late increased Mn enhancement was seen in areas showing delayed neuronal cell death and inflammation, thought to be caused by accumulation of Mn in activated microglia (Widerøe *et al.*, 2009).

Interest in the potential neurotoxicity of some contrast agents (CAs) used in MRI has been increasing in recent years. Bertin *et al.* (2010) have reported an *in vitro* toxicological evaluation using rat primary neurons, to provide a neurotoxicity ranking of CAs as a function of paramagnetic ion, chemical engineering and molecular structure. The authors concluded that the architecture of the CA did not have an important role in determining *in vitro* neurotoxicity, however, the cage structure was found to play a greater role.

7. Miscellaneous

A review discussing clinical manifestations, pathogenesis, diagnosis and treatment of acquired hepatocerebral degeneration and the potential involvement of Manganese (Mn) exposure has been published (Ferrara & Jankovic, 2009).

An evaluation of drinking water testing and treatment plants in western Bangladesh has been reported (Frisbie *et al.*, 2009) in an attempt to decrease exposure of the population to elements, including Mn, in drinking water.

George *et al.* (2009) have produced a comprehensive summary of the putative causes and risk factors involved in Parkinson's disease, current trends in therapeutics and new therapeutic strategies.

The incorporation of Mn as a trace element in parenteral nutritional supplements has been highlighted (Hardy, 2009). A potential source of ingestion of heavy metals, including Mn, from paediatric syrups in Nigeria has also been reported (Nduka & Orisakwe, 2009).

The perceived health risks of Mn exposure by the general public of the Molang mining district in Mexico have been evaluated (Catalán-Vázquez *et al.*, 2010) to inform the risk management plan for the area.

The potential use of Mn(III)-salen derivatives as novel anti-tumour agents has been proposed (Ansari *et al.*, 2009). A novel Mn complex has been reported to be effective as a superoxide anion scavenger, with potential therapeutic use against cell and tissue oxidative injury (Failli *et al.*, 2009). Mn(III) corroles have also been shown to protect rat pancreatic beta cells against intracellular nitration by peroxynitrite, and subsequent cell death (Okun *et al.*, 2009).

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