IEH Institute of Environment and H

Cranfield UNIVERSITY

Manganese Health Research Program:

Overview of Research into the Health Effects of Manganese (2002-2007)

December 2007

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)

Written by S Bagga and L Levy

Institute of Environment and Health
Cranfield Health
Cranfield University
Silsoe
Bedfordshire
MK45 4DT
UK
http://www.silsoe.cranfield.ac.uk/ieh/

PREAMBLE AND PURPOSE

This report is a summary review of some of the established thinking on the toxicology of manganese and its inorganic compounds plus an incorporation of some more recently published studies that have appeared in the scientific literature and has been reported on the MHRP database. It has been written for the use of a wide readership, including researchers, interested scientists and health professionals. It may also be of value to any laypersons who may wish to have an overview of manganese toxicity and recently published research.

1. Introduction

Manganese is a widely used transition metal residing in the periodic table within the d-block alongside other metals of interest and biological and toxicological relevance such as iron, chromium and copper. It is not a naturally occurring metal in its pure state, and thus exists as the oxide, carbonate or silicate derivatives. It can exist in eleven different oxidation states of which 2, 4 and 7 are the most common. It is an essential element in the human diet with a normal dietary intake calculated to be approximately 2 - 5 mg/day (Jankovic, 2005). Deficiency or accumulation of large concentrations of manganese can have repercussions on the central nervous system (Crossgrove & Yokel 2004). Manganese is required for the regulation of reproduction, carbohydrate and lipid metabolism and normal brain function (Greger 1999; Keen *et al.,* 1999; Schroeder, Balassa & Tipton 1966). Manganese is also an important co-factor in the brain for several enzymes, such as the anti-oxidant enzyme superoxide dismutase and is involved in neurotransmitter synthesis and metabolism (Golub *et al*., 2005).

Maganese intoxication (mainly of historical interest now), often termed “manganism”, is related to long-term high level occupational exposure to the metal or its inorganic compounds. Manganese is known to affect most critically the central nervous system and it aggregates into non-haem iron regions of the brain such as the globus pallidus, substantia nigra and subthalamic nuclei (Aschner, Lukey & Tremblay, 2006). Neurological symptoms vary and can range from a reduction in general response, irritability, compulsive behaviours, to a Parkinson’s-like syndrome type. Although manganism and true idiopathic Parkinson’s disease cause very similar deficits within the CNS, they do differ in the neurotransmitters upon which they act, manganese toxicity lies heavily on the degeneration of GABAergic neurones in the globus pallidus whilst Parkinson’s disease is more associated with the dopaminergic neurons in the basal ganglia (Roth, 2006).

Routes of exposure

For the majority of the population, their essential requirements of manganese intake will be met by their diet although deficiencies may occur in some circumstances. For those occupationally exposed, manganese exposure will vary within different working environments where manganese and its inorganic compounds may be used. It has been used in the glass and ceramics industries, for dyes and pigments and food and soil supplements (Gerber, Léonard & Hantson, 2002) but it is well reported that the primary source of manganese-induced intoxication in humans, is by means of occupational exposure in miners, smelters, welders and dry-cell battery employees (Bowler *et al*., 2006; Chandra *et al.,* 1981; Myers *et al*., 2003). As noted above, other forms of entry into the human system can occur through diet and water and milk (infants; Erikson *et al*., 2007).

Biological effects of manganese

Determination of manganese in human blood, urine, serum or plasma is a good measure or end point for determining the likelihood of any biochemical imbalances *in vivo*. However, great care is needed in the interpretation on these measures of biological monitoring as measurements of manganese in these biological matrices are generally poorly correlated with each other and also poorly correlated. It is always difficult to measure accurately the exact concentration of metals in bodily fluids for various reasons, but some authors have had success in producing viable models and techniques which have given estimate proportions of manganese levels in whole blood and urine. A supported liquid membrane technique (Soko *et*

al., 2003) has been used for whole blood analysis, as has electrothermal atomic absorption spectrometry (ETAAS) in biological samples (Ohta *et al*., 1992). Another study performed in blood and breast milk samples amongst plant workers and non-workers concluded that levels of manganese were found to be greatest in both biological matrices in plant workers, more so than mercury and lead (Sharma & Pervez, 2005).

2. Toxicokinetics

The toxicokinetics (pharmacokinetics) of manganese has been comprehensively reviewed by Aschner (2005), and the reader is referred to this paper should they require a detailed overview of current knowledge in this area.

2.1 Absorption

The respiratory and gastro-intestinal tracts provide the main routes of manganese absorption with only a small amount (1 - 5 %) absorbed under normal conditions (Finley, Johnson & Johnson, 1994). The extent of absorption partly depends on the solubility of the manganese compound. Other factors relevant to the extent of absorption are dietary manganese levels (Finley, Johnson & Johnson 1994), age and state of the individual (Keen, Bell & Lonnerdal, 1986) and iron status. After intestinal absorption, manganese is transported to the liver via the hepatic portal vein, resulting in further metabolism and oxidation of the metal. Inhalation of manganese via the respiratory tract represents the major toxic pathway, especially as this pathway is more efficient in delivering the metal in high concentrations to the brain and CNS (Dorman *et al*., 2001). The particles absorbed will vary depending on solubility and particle size, larger particles usually impacting on the lining cells the upper respiratory tract, smaller particles, penetrating into the alveoli. The solubility parameter of manganese and its inorganic compounds has also been under investigation in earlier studies, one such investigation using $MnCl_2$ and MnO_2 to detect the extent of penetration (Roels *et al*., 1997). This study concluded that $MnCl_2$ being a soluble salt, was absorbed more rapidly and thus taken up into the bloodstream more readily, whereas the insoluble MnO_2 given at similar doses was poorly absorbed and distributed.

2.2 Distribution

Manganese is transported to the CNS and mammalian tissues fairly homogenously. The greatest concentrations of manganese are found in bone, liver, pancreas, kidney and other tissues which are rich in mitochondria and pigments (Cotzias, Papavasiliou & Miller, 1964; Dorman *et al*., 2006). Transport of manganese into the CNS has been intensely studied, with three sites of particular interest, which might allow the passage of manganese to take place. The cerebral capillaries, cerebrospinal fluid, (CSF) and the olfactory nerve are considered to be the locations of manganese import into the CNS. (Brenneman *et al*., 2000; Crossgrove & Zheng, 2004). The concentration of manganese present in the CNS may be affected in the presence or absence of iron. Iron homeostasis is thought to play a crucial role in manganese uptake, regulation and transport (Li *et al*., 2006). Both manganese and iron share very similar properties in that both these transition metals can occupy oxidation states of +2 and +3. Iron is known to act as a co-factor within certain enzymes and manganese can possibly interfere with iron's binding properties. At the cellular level, interference in iron metabolism can be associated with changes in manganese toxicity (Zheng & Zhao, 2001). Transport of manganese across the blood-brain barrier (BBB) occurs by means of a series of transporters. Movement can take place by facilitated diffusion (Rabin *et al*., 1993), active transport (Aschner & Gannon, 1994) via divalent metal transport (Garrick *et al*., 2003) and transferrin

(Tf)-dependent transport (Aschner & Gannon, 1994). Systemic distribution of manganese appears to favour this latter form of transport (influenced by the oxidation state of the metal), with evidence supporting the movement of the metal into the brain and other organs (Aschner & Gannon 1994; Andersen, Gearhart & Clewell, 1999).

2.3 Elimination

Biliary excretion represents the main mechanism by which manganese is eliminated from the body. The amount excreted will depend on the intake of the metal, as seen in animal studies where excretion of manganese increases with increases in dietary intake. Smaller amounts of the metal are also eliminated through the pancreas, urine and milk.

Recent advances have also been made in understanding the potential effects of manganese pharmacokinetics in susceptible populations (Dorman *et al*., 2006).This investigations reports on evidence and studies which have considered aged individuals, individuals with abnormal biliary function or neonates.

3. Toxicodynamics

3.1 General toxicity

Manganese appears to be one of the least toxic metals from a nutritional perspective and since manganese levels are generally very low *in vivo* in non manganese using-occupational groups, small amounts obtained from the diet are essential for some biological processes. Generally, manganese does not appear to cause any unwanted health effects in the general population and does not prove to be a great risk, at low doses. Long-term exposure at high concentrations does however prove a greater risk. Acute effects have been noted after administration of potassium permanganate to experimental animals, toxicity often seen within the cardiovascular system. Inhalation of manganese has resulted in pulmonary oedema and tracheobronchitis (Nemery, 1990). Chronic severe toxicity is more highly associated with the CNS, especially through inhalation and long-term exposure, and is considered much more vital than the acute effects. (Huang *et al.*, 1989).Common symptoms arising from chronic manganism are anorexia, weakness and apathy. Symptoms often arise in so called phases, after this initial one, a second phase of hallucinations, delusions and insomnia become prevalent. During the later stages of chronic toxicity, parkinson-like symptoms such as tremor and muscle rigidity take place.

3.2 Organ and systems toxicity

Respiratory effects

Over the years, studies have been carried out to assess the effects, if any, of manganese on the respiratory system. Individuals who are exposed to manganese through prolonged and high occupational exposure are considered to be most at risk. Thus, welders, miners and metal-plant industry workers have exhibited increased conditions of pulmonary distress, resulting in conditions such as pneumonia and bronchitis (Sarić & Piasek, 2000). The lung epithelium functions as a barrier to infection and its auto-immune action to respond to foreign antigens is disrupted in the presence of manganese along with many other heavy metals. In a study by Roth and Garrick (2003), manganese was regarded as the second most responsible metal to cause inflammation in the lung tissue after copper. This inflammation is thought to occur by means of cytokine release (interleukins) as opposed to being immunoglobulin (IgE) mediated.

Inflammatory changes in the lungs in cases of manganese exposure have been noted even at non-cytotoxic levels, a suggestion implying that even modest doses of the metal are sufficient to affect lung physiology (Pascal & Tessier, 2004).

Cytotoxic effects

Cell damage can occur by a number of mechanisms and removal of damaged or broken cells can be done by either programmed cell death (apoptosis) or accidental cell death (necrosis). Metals have the ability to interfere with cellular activity resulting in cell lysis, cell phagocytosis or cell death. Progress is continually being made regarding the mechanisms by which manganese can induce cell death. The concentration levels of manganese inside cells and the variety of signal processing mechanisms involved in cell removal and repair are closely implicated to apoptosis and necrosis, both of which have been linked to manganese-induced cell toxicity. Strong evidence has suggested that apoptosis contributes to manganese toxicity due to certain cell death signalling pathways being triggered in cells treated with manganese. (Roth, 2006).

Other studies have demonstrated that manganese toxicity is not solely due to apoptosis and that there are other related cytotoxic events, which in part, are responsible for manganese-initiated cell death. Disruption of mitochondrial function (Gavin, Gunter & Gunter, 1992), and ATP depletion (Zhang, Fu & Zhou, 2005) have also been noted, such effects being cell-specific, whereby apoptosis and necrosis have caused the ultimate demise of the cell.

Cardiovascular toxicity

The effect of manganese on the circulatory system in humans or animals has not been documented in detail. Manganese has been reported to have a toxic effect on cardiac cells and tissues from animals *in vitro* but this has not been demonstrated in whole animals or humans (Crossgrove & Zheng, 2004).

Hepatotoxicity

Studies performed in humans with regards to manganese-related hepatotoxicity are rare. Biliary excretion is the major elimination pathway for manganese, accounting for > 90 % manganese excretion. (Crossgrove & Zheng, 2004). This figure would be decreased in the case of liver disease, resulting in elevated levels of manganese in the plasma.

Reproductive toxicity (and teratogenicity)

Early studies have looked at the embryonic effects of manganese, using animal models (Orent & McCollum 1931). A more complete investigation was performed in the late 1980s which concluded that manganese deficiency during prenatal periods would give rise to skeletal abnormalities, ataxia reduced litter size and increase in stillborns (Hurley, 1981). The effect of manganese in humans has been linked in a similar fashion to animal models, often thought to decrease fertility and increase foetal abnormalities (Crossgrove & Zheng 2004). Decreased fertility amongst manganese exposed male workers has been previously suggested (Lauwerys *et al.,* 1985); such individuals have significantly fewer children than non-exposed individuals. This has not been reported in other studies.

Genotoxicity and carcinogenicity

The experimental and epidemiological evidence based on the mutagenicity of manganese is limited.

Manganese, although a possible mutagen in some experimental studies is not an established animal carcinogen (Gerber, Léonard & Hantson, 2002; NTP, 1993), and the US-EPA has

classified manganese as a Group D carcinogen, *not classifiable to cause human carcinogenicity*. (USEPA, 1992).

Neurotoxicity

Although the precise mechanisms by which manganese induces toxic effects within the central nervous system are a matter of continuing debate, there are a number of reports which highlight possible associations between manganese and other trace elements such as iron (Verity, 1999), copper (Lai *et al*., 1999), and aluminium (Olanow *et al*., 1996). Manganese demonstrates most of its toxicity within the neuronal pathways, including the brain and neurotransmitter regions. Postulated mechanisms of manganese-induced neurotoxicity include:

1) Production of radicals, (reactive oxygen species, ROSs) such as superoxide (SO), hydrogen peroxide (H_2O_2) and hydroxyl ions (OH) (Cohen 1984).

2) Neuronal degeneration by means of activation of glutamate-gated channels (Brouillet *et al.,* 1993).

3) Manganese in a divalent (or higher) oxidation state exerting toxicity on dopamine (DA; Archibald & Tyree, 1987).

4) Dopamine oxidation by manganese causing oxidative DNA damage (Oikawa *et al.,* 2006).

5) Production of 6-hydroxydopamine (or other toxic catecholamines; Graham, 1984).

Oxidative stress (Radical production)

Oxidative stress is an essential biomarker in cell survival and death. Oxygen related radicals have a strong ability to cause long-term or even permanent damage to cellular components, particularly within the mitochondrial electron transport system (ETS). Studies have demonstrated that manganese has the capability of inducing oxidative stress and radical formation. (Chen & Liao, 2002). Manganese within the cell has a tendency to bind within the inner mitochondrial membrane where the production of oxygen and oxygen radicals will result in superoxide radicals converting the divalent manganese (Mn^{2+}) to the trivalent species, (Mn^{3+}; Normandin & Hazell, 2002). One report has shown that inhibition of Complex 1 of the ETS is a direct result of treatment of PC12 cell cultures with manganese chloride (Brouillet *et al.,* 1993). Other reports have demonstrated that the ATPase complex is inhibited at low concentrations and complex I (NADH:ubiquinone oxidoreductase) at high concentrations (Gavin, Gunter & Gunter, 1999). Separate reports have tried to distinguish between the toxic effects of the divalent and trivalent cation, proposing that the former oxidation state of manganese is the predominant species within cells bound to ATP with the latter oxidation state of manganese is more influential and effective in causing the inhibition of complex I (Archibald & Tyree, 1987; Chen *et al.,* 2001).

Neuronal degeneration

Glutamate and gaba neurotoxicity

Manganese is thought to affect the neurotransmitter signalling pathways involving glutamate and GABA (γ-amino butyric acid), especially in the brain. Manganese is thought to facilitate the release of excessive amounts of glutamate into extracellular space v(Fitsanakis *et al.*, 2006), possibly leading to prolonged degenerative processes (Danbolt, 2001). Excess glutamate is removed by astrocytes, predominantly by the glutamate:aspartate transporter (GLAST). The effects of manganese exposure are thought to involve a large increase and release of glutamate into the extracellular space, inhibition or reduction of the ability of

astrocytes to remove any excess glutamate from this space, and the inhibition of mitochondrial activity, resulting in increased ROS production.

GABA is the main neurotransmitter in the brain, whose production is directly linked to glutamate regulation. Astrocytes remove glutamate from the synaptic cleft. Glutamate is converted to glutamine and then released back into the neurons. The re-conversion of glutamine to glutamate takes place in the neurons as does the conversion of glutamine to GABA (Figure 1; Fitsanakis *et al*., 2006).

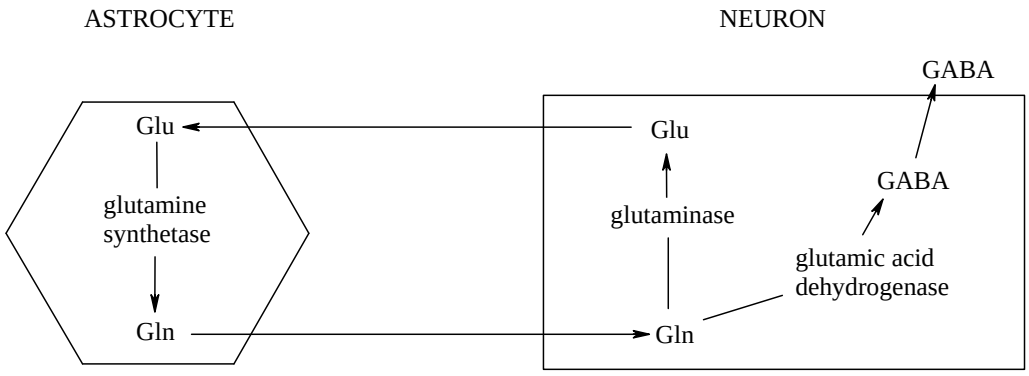

Figure 1 Interrelationship between glutamate, glutamine and GABA

Adapted from Fitsanakis *et al.* (2006)

There are no recent advances in understanding the toxicological effects of manganese on the GABAergic system. An early report of the effects of manganese on GABAergic systems was reported a few decades ago (Bonilla, 1978) which showed that manganese intoxication was responsible for increased brain GABA content. However, other reports have suggested the opposite, with intraperitoneal injections of manganese having been shown to result in diminished cerebellar GABA levels (Lipe *et al.*, 1999).

Dopamine neurotoxicity

Dopamine (DA) has been implicated in the neurological condition of idiopathic Parkinson's disease (Sistrunk, Ross & Filipov, 2007/5). In the presence of manganese, levels of DA have been shown to be experimentally reduced both *in vivo* (Parenti *et al.*, 1986) and *in vitro* (Vescovi *et al*., 1991) causing cell death of dopaminergic cells (Higashi *et al*., 2004). Reduction in DA has been hypothesised to occur in part by direct oxidation by manganese (Donaldson, McGregor & LaBella, 1982). The proposed mechanism of dopamine oxidation by manganese is shown in Figure 2.

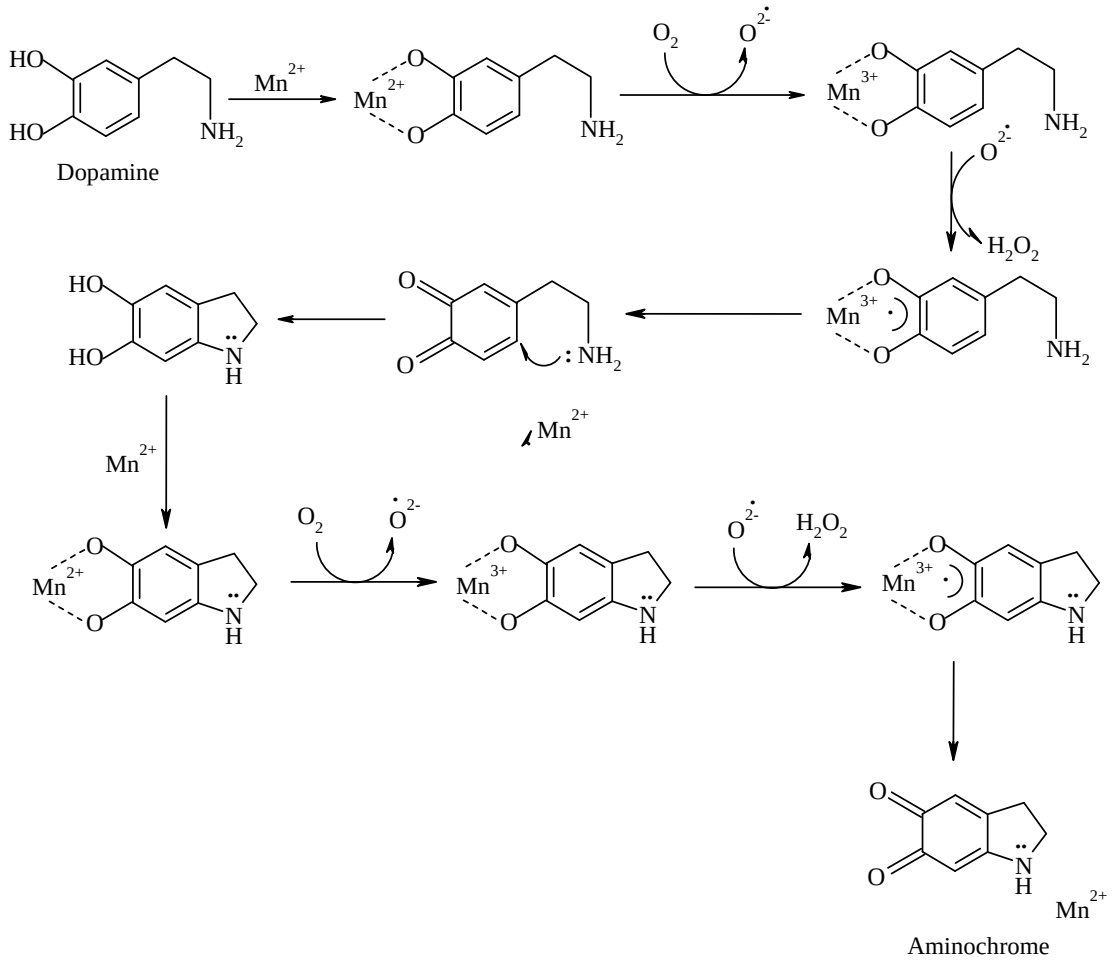

Figure.2 Proposed mechanism of dopamine oxidation by manganese

Adapted from Lloyd (1995)

Figure 2 shows the two different valence states of manganese undergoing redox cycling forming radical intermediates. Manganese toxicity has been suggested to relate to the formation of the trivalent cation, which contains four unpaired d-orbital electrons which are thermodynamically unstable compared to the three more favourably paired electrons present in the divalent cationic state. Production of ROS from impairment of mitochondrial function has been explained above, but ROS may also be generated from the oxidation of dopamine in the presence of copper (Cu (II)), as proposed in Figure 3.

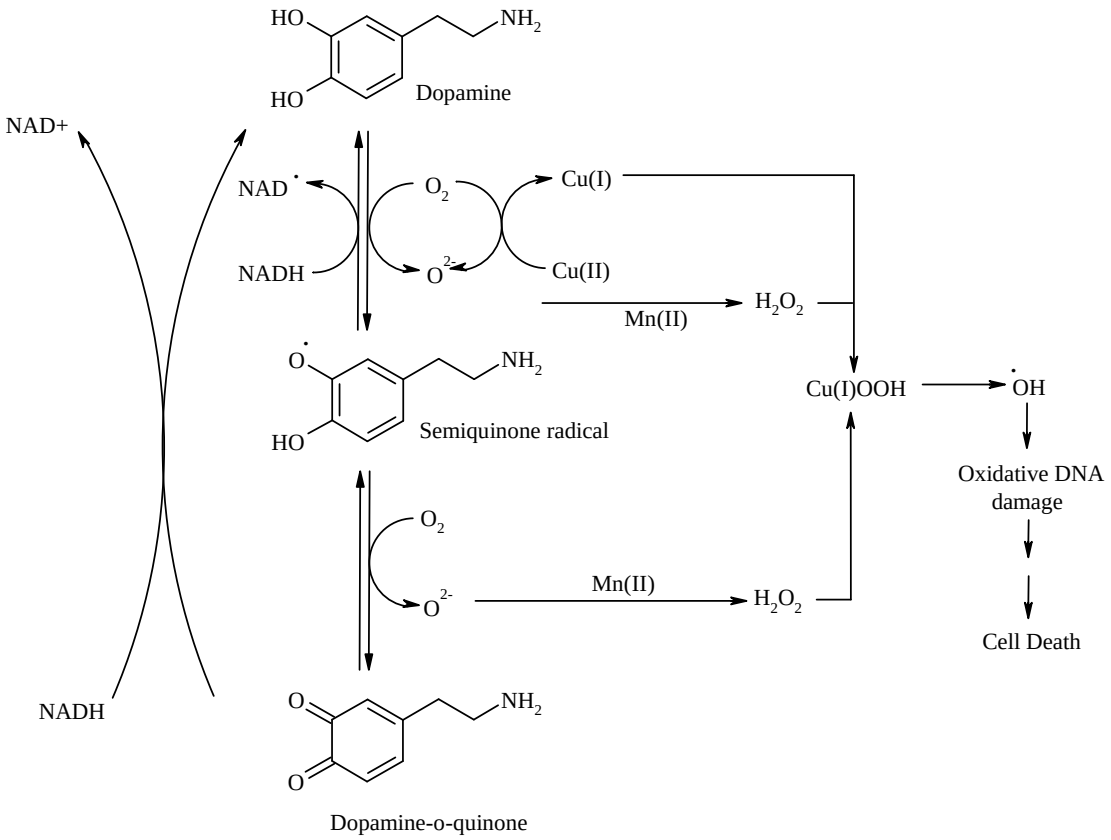

Figure 3 Proposed mechanism of DNA damage through DA oxidation by manganese and copper

Adapted from Oikawa *et al*. (2006)

The above proposed mechanism illustrates how DNA damage may occur through DA oxidation. It relies on the presence of manganese and copper to form intermediate radical species which initiate cell death. Evidence and possible theories of cellular defects arising from manganese intoxication have been presented here and it is vital to consider the effects which are seen on the brain, upon transportation of manganese into the central nervous system.

Summary

Manganese is an essential mineral present in small quantities *in vivo*. It is important in the processing of biological processes but does present a potential toxic risk at excess concentrations as it has the ability to disrupt homeostatic mechanisms, especially those which involve elemental iron. Severe manganese neurological toxicity - or manganism - is correlated closely to the symptoms of Parkinson's disease. Although not entirely alike, there is considerable overlap in the biological effects and some of the symptoms observed. . Manganese absorption appears most relevant via the inhalation route where particle size and solubility will have an impact on the severity of the metal in the airways. It is rapidly absorbed and distributed by a series of transporter systems, primarily DMT-1, into the brain where it exerts its neurological effects. Oxidation of the divalent cation to its trivalent state also results in chronic neurological effects. The transitions between oxidation states also has an effect on the cellular-related toxicities of manganese.

High doses of manganese cause an increase in glutamate secretion and reduction in dopamine release, actions which are postulated to be direct consequences of manganese toxicity. The production of radicals (such as the superoxide ion) are also relevantly linked to manganese oxidation and subsequent genetic instabilities.

Abnormally high or low levels of manganese are associated with disease states in humans. The evidence base on the pharmacokinetics, transport of the metal, dietary and environmental factors and tissue distribution is increasing but it is still difficult to attempt to establish an accurate assessment of the risk to humans. The development of models for risk assessment is problematic, as there is very little data on non-human primates, and extrapolation of existing animal data to humans is difficult due to interspecies differences. Recent studies, in particular those cited above, have informed on a number of key aspects regarding the mechanisms by which manganese may exert neurological effects, and research is underway that may inform on the situation over the course of the next few years.

References

Andersen, M.E., Gearhart, J.M. & Clewell, H.J. (1999) Pharmacokinetic data needs to support risk assessments for inhaled and ingested manganese. *Neurotoxicology*, 20(2-3), 161-171.

Archibald, F.S. & Tyree, C. (1987) Manganese poisoning and the attack of trivalent manganese upon catecholamines. *Archives of Biochemistry and Biophysics*, 256(2), 638-650.

ASCHNER, M. & GANNON, M. (1994) Manganese(mn) transport across the rat blood-brain barrier: Saturable and transferrin dependent transport mechanisms. *Brain Research Bulletin*, 33(3), 345-349.

Aschner, M., Lukey, B. & Tremblay, A. (2006) The manganese health research program (MHRP): Status report and future research needs and directions. *Neurotoxicology*, 27(5), 733-736.

Bonilla, E. (1978) Increased GABA content in caudate nucleus of rats after chronic manganese chloride administration. *Journal of Neurochemistry*, 31(2), 551-552.

Bowler, R.M., Gysens, S., Diamond, E., *et al.* (2006) Manganese exposure: Neuropsychological and neurological symptoms and effects in welders. *Neurotoxicology*, 27(3), 315-26.

Brenneman, K.A., Wong, B.A., Buccellato, M.A., *et al.* (2000) Direct olfactory transport of inhaled manganese ((54)MnCl(2)) to the rat brain: Toxicokinetic investigations in a unilateral nasal occlusion model. *Toxicology and Applied Pharmacology*, 169(3), 238-248.

Brouillet, E.P., Shinobu, L., McGarvey, U., *et al.* (1993) Manganese injection into the rat striatum produces excitotoxic lesions by impairing energy metabolism. *Experimental Neurology*, 120(1), 89-94.

Chandra, S.V., Shukla, G.S., Srivastava, R.S., *et al.* (1981) An exploratory study of manganese exposure to welders. *Clinical Toxicology*, 18(4), 407-416.

Chen, C. & Liao, S. (2002) Oxidative stress involves in astrocytic alterations induced by manganese. *Experimental Neurology*, 175(1), 216-225.

Chen, J.Y., Tsao, G.C., Zhao, Q., *et al.* (2001) Differential cytotoxicity of Mn(II) and Mn(III): Special reference to mitochondrial [fe-S] containing enzymes. *Toxicology and Applied Pharmacology*, 175(2), 160-168.

Cohen, G. (1984) Oxy-radical toxicity in catecholamine neurons. *Neurotoxicology*, 5(1), 77-82.

Cotzias, G.C., Papavasiliou, P.S. & Miller, S.T (1964) Manganese in melanin. *Nature,*, 2011228-1229.

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*, 25(3), 451-460.

Crossgrove, J. & Zheng, W. (2004) Manganese toxicity upon overexposure. *NMR in Biomedicine*, 17(8), 544-553.

Danbolt, N.C. (2001) Glutamate uptake. *Progress in Neurobiology*, 65(1), 1-105.

Donaldson, J., McGregor, D. & LaBella, F. (1982) Manganese neurotoxicity: A model for free radical mediated neurodegeneration? *Canadian Journal of Physiology and Pharmacology*, 60(11), 1398-1405.

Dorman, D.C., Struve, M.F., James, R.A., *et al.* (2001) Influence of particle solubility on the delivery of inhaled manganese to the rat brain: Manganese sulfate and manganese tetroxide pharmacokinetics following repeated (14-day) exposure. *Toxicology and Applied Pharmacology*, 170(2), 79-87.

Dorman, D.C., Struve, M.F., Clewell, H.J., *et al.* (2006) Application of pharmacokinetic data to the risk assessment of inhaled manganese. *eurotoxicology*, 27(5), 752-764.

Erikson, K.M., Thompson, K., Aschner, J., *et al.* (2007) Manganese neurotoxicity: A focus on the neonate. *Pharmacology & Therapeutics*, 113(2), 369-77. Epub: 2006 Sep 22.

Finley, J.W., Johnson, P.E. & Johnson, L.K. (1994) Sex affects manganese absorption and retention by humans from a diet adequate in manganese. *The American Journal of Clinical Nutrition*, 60(6), 949-955.

Fitsanakis, V.A., Au, C., Erikson, K.M., *et al.* (2006) The effects of manganese on glutamate, dopamine and gamma-aminobutyric acid regulation. *Neurochemistry International*, 48(6-7), 426-433.

Garrick, M.D., Dolan, K.G., Horbinski, C., *et al.* (2003) DMT1: A mammalian transporter for multiple metals. *Biometals: An International Journal on the Role of Metal Ions in Biology*, 16(1), 41-54.

Gavin, C.E., Gunter, K.K. & Gunter, T.E. (1999) Manganese and calcium transport in mitochondria: Implications for manganese toxicity. *Neurotoxicology*, 20(2-3), 445-453.

Gavin, C.E., Gunter, K.K. & Gunter, T.E. (1992) Mn2+ sequestration by mitochondria and inhibition of oxidative phosphorylation. *Toxicology and Applied Pharmacology*, 115(1), 1-5.

Gerber, G.B., Léonard, A. & Hantson, P. (2002) Carcinogenicity, mutagenicity and teratogenicity of manganese compounds. *Critical Reviews in oncology/hematology*, 42(1), 25-34.

Golub, M.S., Hogrefe, C.E., Germann, S.L., *et al.* (2005) Neurobehavioral evaluation of rhesus monkey infants fed cow's milk formula, soy formula, or soy formula with added manganese. *Neurotoxicology and Teratology*, 27(4), 615-627.

Graham, D.G. (1984) Catecholamine toxicity: A proposal for the molecular pathogenesis of manganese neurotoxicity and Parkinson's disease. *Neurotoxicology*, 5(1), 83-95.

Greger, J.L. (1999) Nutrition versus toxicology of manganese in humans: Evaluation of potential biomarkers. *Neurotoxicology*, 20(2-3), 205-212.

Higashi, Y., Asanuma, M., Miyazaki, I., *et al.* (2004) Parkin attenuates manganese-induced dopaminergic cell death. *Journal of Neurochemistry*, 89(6), 1490-1497.

Huang, C.C., Chu, N.S., Lu, C.S., *et al.* (1989) Chronic manganese intoxication. *Archives of Neurology*, 46(10), 1104-1106.

Hurley, L.S. (1981) Teratogenic aspects of manganese, zinc, and copper nutrition. *Physiological Reviews*, 61(2), 249-295.

Jankovic, J. (2005) Searching for a relationship between manganese and welding and parkinson's disease. *Neurology*, 64(12), 2021-2028.

Keen, C.L., Bell, J.G. & Lonnerdal, B. (1986) The effect of age on manganese uptake and retention from milk and infant formulas in rats. *The Journal of Nutrition*, 116(3), 395-402.

Keen, C.L., Ensunsa, J.L., Watson, M.H., *et al.* (1999) Nutritional aspects of manganese from experimental studies. *Neurotoxicology*, 20(2-3), 213-223.

Lai, J.C., Minski, M.J., Chan, A.W., *et al.* (1999) Manganese mineral interactions in brain. *Neurotoxicology*, 20(2-3), 433-444.

Lauwerys, R., Roels, H., Genet, P., *et al.*. (1985) Fertility of male workers exposed to mercury vapor or to manganese dust: A questionnaire study. *American Journal of Industrial Medicine*, 7(2), 171-176.

Li, G.J., Choi, B.S., Wang, X., *et al.* (2006) Molecular mechanism of distorted iron regulation in the blood-CSF barrier and regional blood-brain barrier following in vivo subchronic manganese exposure. *Neurotoxicology*, 27(5), 737-744.

Lipe, G.W., Duhart, H., Newport, G.D., *et al.* (1999) Effect of manganese on the concentration of amino acids in different regions of the rat brain. *Journal of Environmental Science and Health.Part.B, Pesticides, Food Contaminants, and Agricultural Wastes*, 34(1), 119-132.

Lloyd, R.V. (1995) Mechanism of the manganese-catalyzed autoxidation of dopamine. *Chemical Research in Toxicology*, 8(1), 111-116.

Myers, J.E., Thompson, M.L., Ramushu, S., *et al.* (2003) The nervous system effects of occupational exposure on workers in a South African manganese smelter. *Neurotoxicology*, 24(6), 885-894.

Nemery, B. (1990) Metal toxicity and the respiratory tract. *European Respiratory Journal*, 3(2), 202-219.

Normandin, L. & Hazell, A.S. (2002) Manganese neurotoxicity: An update of pathophysiologic mechanisms. *Metabolic Brain Disease*, 17(4), 375-387.

NTP (1993) *Toxicology and carcinogenesis studies of Manganese (II) Sulfate Monohydrate (CAS no. 10034-96-5) in F344/N rats and B6C3F$_1$ mice (feed studies).* NTP TR 428. NIH Publication No. 94-

3159. Research Triangle Park, NC, National Toxicology Program. Available at: http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr428.pdf

Ohta, K., Itoh, S., Kaneco, S., *et al.* (1992) Direct determination of manganese in biological materials using electrothermal atomic absorption spectrometry with a molybdenum tube atomizer. *Analytical Sciences*, 8(3), 423-426.

Oikawa, S., Hirosawa, I., Tada-Oikawa, S., *et al.* (2006) Mechanism for manganese enhancement of dopamine-induced oxidative DNA damage and neuronal cell death. *Free Radical Biology & Medicine*, 41(5), 748-756.

Olanow, C.W., Good, P.F., Shinotoh, H., *et al.* (1996) Manganese intoxication in the rhesus monkey: A clinical, imaging, pathologic, and biochemical study. *Neurology*, 46(2), 492-498.

Orent, E.R. & McCollum, E.V. (1931) Effects of deprivation of manganese in the rat. *Journal of Biological Chemistry*, 92(3), 651-678.

Parenti, M., Flauto, C., Parati, E., *et al.*. (1986) Manganese neurotoxicity: Effects of L-DOPA and pargyline treatments. *Brain Research*, 367(1-2), 8-13.

Pascal, L.E. & Tessier, D.M. (2004) Cytotoxicity of chromium and manganese to lung epithelial cells in vitro. *Toxicology Letters*, 147(2), 143-151.

Rabin, O., Hegedus, L., Bourre, J.M., *et al.* (1993) Rapid brain uptake of manganese(II) across the blood-brain barrier. *Journal of Neurochemistry*, 61(2), 509-517.

Roels, H., Meiers, G., Delos, M., *et al.* (1997) Influence of the route of administration and the chemical form (MnCl2, MnO2) on the absorption and cerebral distribution of manganese in rats. *Archives of Toxicology*, 71(4), 223-230.

Roth, J.A. (2006) Homeostatic and toxic mechanisms regulating manganese uptake, retention, and elimination. *Biological Research*, 39(1), 45-57.

Roth, J.A. & Garrick, M.D. (2003) Iron interactions and other biological reactions mediating the physiological and toxic actions of manganese. *Biochemical Pharmacology*, 66(1), 1-13.

Sarić, M. & Piasek, M. (2000) Environmental exposure to manganese and combined exposure to gaseous upper respiratory irritants: Mechanism of action and adverse health effects.*Reviews on Environmental Health*, 15(4), 413-419.

Schroeder, H.A., Balassa, J.J. & Tipton, I.H. (1966) Essential trace metals in man: Manganese. A study in homeostasis. *Journal of Chronic Diseases*, 19(5), 545-571.

Sharma, R. & Pervez, S. (2005) Toxic metals status in human blood and breast milk samples in an integrated steel plant environment in central India. *Environmental Geochemistry and Health*, 27(1), 39-45.

Sistrunk, S.C., Ross, M.K. & Filipov, N.M. (2007/5) Direct effects of manganese compounds on dopamine and its metabolite dopac: An in vitro study. *Environmental Toxicology and Pharmacology*, 23(3), 286-296.

Soko, L., Chimuka, L., Cukrowska, E., *et al.* (2003) Extraction and preconcentration of manganese(II) from biological fluids (water, milk and blood serum) using supported liquid membrane and membrane probe methods. *Analytica Chimica Acta*, 485(1), 25-35.

USEPA. Integrated Risk Information System (2002) *Manganese* (CASRN 7439-96-5). Available at: http://www.epa.gov/iris/subst/0373.htm

Verity, M.A. (1999) Manganese neurotoxicity: A mechanistic hypothesis. *Neurotoxicology*, 20(2-3), 489-497.

Vescovi, A., Facheris, L., Zaffaroni, A., *et al.* (1991) Dopamine metabolism alterations in a manganese-treated pheochromocytoma cell line (PC 12). *Toxicology*, 67(2), 129-142.

Zhang, S., Fu, J. & Zhou, Z. (2005) Changes in the brain mitochondrial proteome of male sprague-dawley rats treated with manganese chloride. *Toxicology and Applied Pharmacology*, 202(1), 13-17.

Zheng, W. & Zhao, Q. (2001) Iron overload following manganese exposure in cultured neuronal, but not neuroglial cells. *Brain Research*, 897(1-2), 175-179.