

Manganese and Compounds Reference Exposure Levels

1. Summary

Acute inhalation of high levels of manganese results in a nonspecific pulmonary edema, while chronic manganese inhalation leads to a characteristic neurotoxicity known as manganism with strong similarities to Parkinson's disease. Manganism is characterized by motor deficits (dystonia, altered gait, fine tremor, generalized rigidity) and may include psychiatric disturbances. At low manganese levels and in the absence of frank manganism, subtle deficits in cognitive and neurobehavioral functions have been reported in both adults and children. Neurodevelopmental deficits have been associated with early life exposure to excessive manganese and include impaired intellectual performance and behavioral disinhibition. [The studies described in this document include those published through the Spring of 2008. The RELs below are applicable to all respirable inorganic manganese compounds.](#)

1.1 Manganese Acute REL

An acute REL for manganese was not developed at this time.

1.2 Manganese 8-Hour REL

Reference Exposure Level
Critical effect(s)

0.17 $\mu\text{g}/\text{m}^3$

Impairment of neurobehavioral function in humans

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Hazard index target

Nervous system

1.3 Manganese Chronic REL

Reference Exposure Level
Critical effect(s)

0.09 $\mu\text{g}/\text{m}^3$

Impairment of neurobehavioral function in humans

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Hazard index target(s)

Nervous system

2. Physical and Chemical Properties

Table 2.1 Manganese and Manganese Species*

<i>Molecular Formula</i>	<i>Synonyms</i>	<i>Molecular Weight</i>	<i>CAS Reg. No.</i>
Mn	elemental manganese; colloidal manganese; cutaval	54.94 g/mol	7439-96-5
MnO	manganese oxide; manganese monoxide; manganosite	70.94 g/mol	1344-43-0
MnO ₂	manganese dioxide; black manganese oxide	86.94 g/mol	1313-13-9
Mn ₃ O ₄	manganese tetroxide; trimanganese tetraoxide; manganomanganic oxide	228.82 g/mol	1317-35-7
MnCl ₂	manganese chloride; manganese dichloride; manganous chloride	125.84 g/mol	7773-01-5
<u>MnSO₄</u>	<u>manganese sulfate</u>	<u>151.00 g/mol</u>	<u>7787-85-7</u>
<u>KMnO₄</u>	<u>potassium permanganate</u>	<u>158.03 g/mol</u>	<u>7724-64-7</u>
<i>Description</i>	Lustrous, gray-pink metal (Mn); green (MnO), black (MnO ₂) or pink (MnCl ₂ , <u>MnSO₄</u>), <u>purple (KMnO₄)</u> crystals; brownish-black powder (Mn ₃ O ₄)		
<i>Molecular formula</i>	see Table 2.1		
<i>Molecular weight</i>	see Table 2.1		
<i>Density (in g/cm³)</i>	7.21-7.4 (Mn – depending on allotropic form); 5.43-5.46 (MnO); 4.88 (Mn ₃ O ₄); 2.977 @ 25°C (MnCl ₂)		
<i>Boiling point</i>	2095°C (Mn); not available (MnO); not available (Mn ₃ O ₄); 1190°C (MnCl ₂); <u>850°C (MnSO₄)</u>		
<i>Melting point</i>	1246°C (Mn); 1839°C (MnO); 1567°C (Mn ₃ O ₄); 650°C (MnCl ₂) (CRC, 2005); <u>700°C (MnSO₄)</u>		
<i>Vapor pressure</i>	1 torr @ 1292°C (Mn); non-volatile at room temperature (Mn ₃ O ₄); not available (MnO; MnCl ₂)		
<i>Solubility</i>	Sol. in dil. acids and aq. solns. of Na- or K-bicarbonate (Mn); sol. in NH ₄ Cl, insol. in H ₂ O (MnO); insol. in H ₂ O, HNO ₃ , or cold H ₂ SO ₄ (MnO ₂ (Merck, 1976)); insol. in H ₂ O, sol. in HCl (Mn ₃ O ₄); 72.3 g/100 ml H ₂ O @ 25°C (MnCl ₂); <u>sol in 1 part H₂O (MnSO₄); 72.3 g/100 ml H₂O (KMnO₄)</u>		
<i>Conversion factor</i>	Not applicable (dusts or powders)		

3. Occurrence and Major Uses

Metallic manganese is used in the manufacturing of steel, carbon steel, stainless steel, cast iron, and superalloys to increase hardness, stiffness, and strength (HSDB, 2006). Manganese chloride is used in dyeing, disinfecting, batteries, and as a paint drier and dietary supplement. Manganese oxide (MnO) is used in textile printing, ceramics, paints, colored glass, fertilizers, and as food additives [and supplements](#). Manganese dioxide is used in batteries and may also be generated from the welding of manganese alloys. [Use of manganese-containing welding rods is a major source of occupational exposure to welders](#). Manganese tetroxide may be generated in situations where other oxides of manganese are heated in air (NIOSH, 2005). [Manganese is also released into the air during the erosion of manganese-containing rock and alloys. Relatively high levels of manganese have been measured in subways \(428 ng/m³ vs 9.7 ng/m³ ambient\), presumably from the frictional erosion of manganese-containing steel \(Crump, 2000\). As methylcyclopentadienyl manganese tricarbonyl \(MMT\), manganese has found use as an octane enhancer in some unleaded gasolines and is released during fuel combustion as manganese sulfate, phosphate, and oxides. Manganese exposure may also be significant among farm workers using the fungicide Maneb \(manganese ethylene-bis-dithiocarbamate\).](#)

[Manganese is present in ambient air as particles, often associated with other metals or organic material. The size of these particles depends on their source, history and contents. For example, Singh et al. \(2002\) compared the metal contents and size distributions of particles at two sites in the Los Angeles Basin. The first site, Downey, is in the vicinity of downtown Los Angeles. Particulate matter \(PM\) at this “source” site was primarily from vehicular, and to a lesser extent, industrial sources. The second site is Riverside, 70 km east of Los Angeles, where the PM is mainly formed by secondary photochemical reactions, with some contribution from dust from the nearby deserts, and/or derived from PM blown in from Los Angeles. In Downey, 40% of the manganese was in fine particles of 0.35-1.0 μm, and less than 20% in the coarse \(PM 2.5-10 μm\) fraction. By comparison, in Riverside, less than 10% of the manganese was in fine PM in the 0.35-1.0 range, while nearly 80% was in the 2.5-10 μm fraction. In contrast to Riverside, manganese measured in other urban settings tends to be mainly in the respirable fine fraction. In urban aerosols in Seville, Spain, manganese was found predominantly in fine particles < 0.61 μm \(44%\), with smaller amounts in coarse particles \(> 10 μm, 17.8%; 4.9-10 μm, 18.3%\) \(Espinoza et al., 2001\). A bimodal distribution of sizes was also found for manganese-containing aerosols in Tihany, Hungary. The bulk of the manganese was found bound to organic matter and silicates in particles of approximately 0.118 μm, with smaller amounts in the 1.4-2.8 μm range \(Hlavay et al., 1998\). In cities in which the gasoline contains MMT, manganese is also predominantly in the fine \(PM 2.5\) fraction \(Pellizzari et al., 2001\).](#)

The 2004 annual statewide emissions of manganese reported in the most recent California Toxics Inventory (CARB, 2005a) were estimated to be 1,055 tons. For 2002, the mean statewide ambient level was 31.5 ng/m³.

4. Metabolism / Toxicokinetics

Environmental manganese can enter the body primarily by oral and inhalation routes. Dermal absorption of manganese is insignificant through intact skin; however, broken skin would obviously allow more access to manganese (e.g., potassium permanganate) and other poorly dermally absorbed compounds. Parenteral exposures have occurred through parenteral feeding and more recently i.v. drug abuse, leading to human disease. Manganese is an essential element normally absorbed from the intestinal tract as part of the diet. It is estimated that 2 to 5% of ingested manganese is retained in the adult body (Andersen et al., 1999). Retention can be up to 41% in breast-fed infants, and 20% in formula-fed infants (Dorner et al., 1989). Manganese absorption is increased (along with iron absorption) when there is a deficiency of iron in the diet (Davis et al., 1992). Ascorbic acid, calcium and phosphorus also affect manganese utilization (ibid).

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As part of the normal manganese homeostatic mechanism, high levels of dietary manganese diminish absorption from the intestinal tract. Manganese appears to be absorbed from the gut largely in the divalent form, with approximately 80% of absorbed manganese subsequently bound in plasma to β_1 -globulin and albumin (Foradori et al., 1967). These manganese-protein complexes are efficiently removed from the blood by the liver and returned to the gut in bile for elimination, thus establishing an entero-hepatic circuit for manganese. In the blood, unbound manganese may be converted by ceruloplasmin to the trivalent cation which is then bound by transferrin. Transferrin-manganese complexes are much less efficiently removed by the liver and thus survive first pass elimination to circulate throughout the body (Gibbons et al., 1976). In the brain, transferrin receptors in the capillary beds may mediate uptake in regions with efferents to the nucleus accumbens and the caudate putamen. Other mechanisms also appear to contribute to brain uptake of manganese including a divalent metal transporter (DMT-1), and a less well-defined non-saturable mechanism. From these sites, manganese is thought to move by neuronal transport to the pallidum, thalamic nuclei, and substantia nigra, areas involved with motor control and movement (Aschner et al., 2005). While at normal plasma levels, manganese enters the brain mainly across the capillary epithelium, at elevated levels of manganese in the blood, transport across the choroid plexus becomes more prominent (Aschner, 2000).

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The mechanisms mentioned above are thought to apply generally to the transport of manganese across the blood brain barrier of adults. However, in the fetus and neonate, the blood brain barrier is characterized as having greater permeability to many substances, including manganese, and a different distribution of molecular transporters (Erikson et al., 2007b). In mice following a single parenteral administration of $MnCl_2$ on postnatal days 7, 14, or 42, maximum retention of manganese in the brain occurred 24 hours after exposure and was 3.5%, 2.5%, and 0.3%, respectively, of the administered dose. For manganese administered on day 0, the maximum brain concentration (2.9%) occurred 43 days later, suggesting a lack of perinatal homeostatic control (Valois and Webster, 1989). The approximately ten-fold higher brain levels following dosing on days 0-14 compared with day 42 indicate a more rapid and extensive uptake of manganese from the blood in neonates compared with adults. The drop in maximum brain levels

between days 14 and 42 is thought to reflect attainment of adult blood brain barrier function on day 21.

Manganese may be introduced directly into the blood during parenteral feeding or during injection of illicit designer drugs contaminated with permanganate. As with the inhalation route described below, parenteral administration of manganese avoids first pass clearance of manganese by the liver, and may result in high exposure of all organs to manganese.

Manganese exposure via the pulmonary route leads to more rapid absorption with higher efficiency, and with greater transfer to the brain compared with other routes (Drown et al., 1986; Roels et al., 1997). In experiments in rats, Roels et al. (1997) used intratracheal instillation as a surrogate for inhalation for comparison with the oral route (gavage). Intratracheal instillation of MnCl₂ (1.22 mg/kg, once weekly for four weeks) raised the steady state manganese levels 68% in blood, 205% in the striatum, 48% in the cortex, and 27% in the cerebellum compared to controls. By gavage, a much higher dose of MnCl₂ (24.3 mg/kg) was required to achieve the same blood levels (68%). However, by this route, manganese levels in the striatum and cerebellum were not affected, and levels in the cortex were raised by only 22% (Table 4.1). In animals given a single intratracheal dose of MnCl₂ (1.22 mg/kg bw), blood manganese levels peaked within 30 min at 7,050 ng/100 ml. This was followed by a gradual decline but blood levels remained elevated over controls for at least 24 hours. By comparison, the single oral administration of 24.3 mg MnCl₂/kg bw resulted in a five-fold lower peak blood level of 1,660 ng/100 ml after one hour, followed by a return to control levels in 12 hours. Thus, compared to ingestion, inhalation of a relatively water soluble form of manganese leads to a rapid increase in blood levels that remain higher for longer, and results in higher brain manganese levels.

Table 4.1 Increase in Tissue Manganese by Route and Chemical Form

Chemical Form and Route	Increase in Tissue Manganese (%)			
	Blood	Striatum	Cortex	Cerebellum
MnCl ₂ Intratracheal (1.2 mg/kg)	68	205	48	27
MnCl ₂ Gavage (24.3 mg/kg)	68	0	22	0
MnO ₂ Intratracheal (1.2 mg/kg)	41	48	34	31
MnO ₂ Gavage (24.3 mg/kg)	0	0	0	0

Using the same exposure protocol with the less soluble MnO₂, intratracheal instillation raised manganese levels 41% in blood, 48% in striatum, 31% in cerebellum, and 34% in cortex. By contrast, neither blood nor brain levels were increased following oral exposure (Table 4.1). As with MnCl₂, Mn blood levels following intratracheal MnO₂ reached a higher peak value (1,760 ng Mn/100 ml; 200% increase) than that achieved after gavage (900 ng/100 ml; 27% increase). Blood levels rose more slowly than with MnCl₂, starting at 48–72 hr after intratracheal instillation and peaking at 168 hr. By gavage, blood levels rose gradually to peak at 144 hr (Roels et al., 1997). In these studies, the solubility of the manganese complexes influenced the rate of absorption by

either route, but in both cases inhalation resulted in substantially higher blood and brain levels.

In a further demonstration of the dependence of tissue distribution on oxidation state and route of exposure, Reaney et al. (2006) exposed rats to 0, 2, or 6 mg/kg Mn(III)-pyrophosphate or Mn(II)Cl₂ intraperitoneally (i.p.) for five weeks. Significantly higher blood manganese levels were seen with Mn(III) vs equimolar Mn(II). A dose-dependent increase in brain manganese was observed, with Mn(III) producing levels that were 25% higher than following Mn(II). This may be related to the higher blood levels of manganese achieved with Mn(III) vs Mn(II) via the i.p. route. Examination of the striatum, globus pallidus, thalamus, and cerebral cortex by PIXE (particle induced x-ray emission; an x-ray fluorescence technique) revealed no differences in the distribution of manganese across these brain regions. There were, however, differences among regions in response to the concentration and oxidation state of the manganese. In the globus pallidus, the highest cumulative dose (90 mg/kg) of both forms of manganese increased GABA levels compared to controls (15-30%, $p = 0.037$). By contrast, dopamine levels in globus pallidus at this dose were increased by 60% with Mn(III), but decreased by 40% with Mn(II). The mechanism behind this differential effect is not clear but suggests that manganese oxidation states are important in manganese toxicity.

Drown and colleagues studied the distribution of soluble ⁵⁴MnCl₂ and insoluble ⁵⁴Mn₃O₄ after instillation into the rat lung (Drown et al., 1986). Initially the soluble form of manganese distributed more rapidly from the lung to the peripheral tissues than did the insoluble form. After two weeks the rates of distribution of the two forms became almost equal. Manganese (⁵⁴Mn) reached higher concentrations in the liver, kidney, and gastrointestinal tissues, but persisted longer in the heart, brain, and bone. The manganese was eliminated mainly in bile with very little elimination in urine.

The influence of solubility on tissue distribution was examined with aerodynamically similar aerosols of three manganese compounds of differing solubilities (MnPO₄, MnSO₄, and Mn₃O₄) in rats following inhalation (6 hr/day, 14 days) at 0, 0.03, 0.3, and 3.0 mg/m³. At comparable dose levels, animals exposed to MnSO₄ had lower lung manganese levels than those exposed to either Mn₃O₄ (Dorman et al., 2001) or MnPO₄ (Vitarella et al., 2000), suggesting more rapid pulmonary clearance of the most soluble form. Consistent with this observation, after exposure to 3 mg/m³, manganese levels in the olfactory bulb and striatum were highest with MnSO₄, followed by Mn₃O₄, then MnPO₄. As observed by Drown et al. (1986) and Roels et al. (1997) for intratracheal instillation, the more soluble forms of manganese accumulate in the brain more quickly following inhalation than do the less soluble compounds (Normandin et al., 2004).

For humans with occupational and/or environmental exposures, the main route of exposure is via inhalation. In both cases the manganese is usually in the form of particulates of various sizes. Manganese deposited in the lung can be absorbed directly into the blood stream, or can migrate (by mucociliary transport) into the upper respiratory tract and then be swallowed for possible absorption in the GI tract. In experimental animals, inhaled manganese may be transported via olfactory nerves directly to the brain

following absorption from nasal passages (Brenneman et al., 2000; Dorman et al., 2002a; Elder et al., 2006; [Dorman et al., 2006a](#)). Neither pulmonary nor gastrointestinal absorption is required for this route of exposure, and the blood-brain barrier is bypassed. Evidence for absorption of particulate manganese oxide from the nose and transport to the brain was provided by Elder et al. (2006) in rats. Manganese concentrations in the olfactory bulb increased 3.5-fold following 12 days of intranasal instillation of ultrafine manganese oxide particles (3-8 nm) in both nares. With occlusion of the right nostril and instillation in the left naris, manganese accumulated almost exclusively in the left olfactory bulb. In this experimental paradigm, instillation of either the soluble manganese chloride or the insoluble manganese oxide particles (solubilization rate 1-1.5% per day) in the patent naris resulted in comparable levels of manganese in the ipsilateral side of the olfactory bulb. This, in conjunction with the observation that an increase in manganese in the olfactory bulb was detectable within 30 minutes of the instillation, suggests that particulate rather than dissolved manganese was the form transported to the brain. While instillation is not inhalation, this study does indicate absorption of manganese ultrafine particles occurs across the nasal epithelium with direct transport to the brain.

[More evidence for direct nose to brain transport in primates was provided by magnetic resonance imaging \(MRI\) studies in rhesus monkeys exposed by inhalation to manganese sulfate for 6 hours/day, 5 days/week for 13 weeks \(Dorman et al., 2006a\). Increases in signal intensity on T1-weighted images of various brain regions were well correlated with manganese levels measured upon necropsy. Increases in mean pallidal manganese concentrations of approximately 1.7-, 2.7-, and 6-fold over air-exposed controls were seen following exposure to MnSO₄ at 0.19, 0.97, and 4.55 mg/m³, respectively. The particles at these three concentrations had mass median aerodynamic diameters of 1.73, 1.12, and 2.12 μm, respectively. As expected, much higher increases in manganese concentrations were observed in olfactory epithelium, bulb, tract, and cortex. Lower but statistically significant \(p < 0.05\) increases were also observed in the putamen, white matter, and cerebellum. This study provided no evidence for translocation of manganese from the olfactory bulb to other brain regions, consistent with uptake from the blood as the source of manganese in the globus pallidus. However, the resolution of the MRI used in this study did not allow visualization of individual nerve tracts to rule out direct transfer of manganese between brain regions. Nevertheless, this study provides evidence for axonal transport from the nasal epithelium at least as far as the olfactory bulb in primates.](#)

The major route of excretion of manganese is via bile, although a lesser amount is excreted via urine (Davis et al., 1993). That the liver maintains homeostasis of manganese can be seen by the fact that patients with cirrhosis of the liver accumulate abnormally high levels of manganese in their brains, especially in the globus pallidus (Rose et al., 1999). Similarly, rats that have a liver bypass also show high levels of manganese in the brain, especially in the globus pallidus (Rose et al., 1999).

Neonatal humans do not excrete manganese for the first two to three weeks of life. The intestinal barrier to manganese absorption is also immature in premature and neonatal infants (Cawte, 1985).

The toxicokinetics of manganese may also influence and be influenced by other metals. There is evidence that manganese uptake from the intestinal tract (Mena, 1974; Erikson et al., 2002), lungs (Brain et al., 2006), and nose (Thompson et al., 2007), is enhanced by iron deficiency. Rats rendered anemic by periodic bleeding absorbed significantly higher amounts of manganese (Brain et al., 2006). In each of these studies, brain levels of manganese were increased by iron deficiency. Inasmuch as iron deficiency is a widespread condition that disproportionately affects the young (Beard et al., 2001), children represent a more susceptible population.

Manganese may exist in eleven different valence states (-3 to +7) and may participate in a variety of oxidation-reduction reactions as a pro- or anti-oxidant. In biological systems, while the divalent (Mn^{2+}) and trivalent (Mn^{3+}) forms are most abundant, the trivalent form predominates in many tissues and appears to be responsible for manganese's pro-oxidant properties, possibly by its participation in Fenton-type reactions (HaMai et al., 2001). In vitro studies attempting to emulate conditions in the brain have shown that Mn^{3+} , but not Mn^{2+} (both as pyrophosphate), oxidizes dopamine, DOPA (a dopamine precursor), norepinephrine, and epinephrine to quinones and other products, with reduction of Mn^{3+} to $Mn(OH)_2$ (Archibald and Tyree, 1987). Polymerization of these catecholamine-derived quinones to form neuromelanin, from which the substantia nigra derives its name, is an O_2^- -generating, auto-oxidative process that in turn enhances oxidation of Mn^{2+} to Mn^{3+} , thus increasing cellular oxidative stress.

The role of oxidative stress in manganese toxicity has been inferred in part from changes in cellular markers of oxidative stress upon exposure to high levels of manganese. As a result of participation in reactions with reactive oxygen species (ROS), levels of GSH in manganese-exposed cells decrease. Where metals are involved in the generation of ROS, metallothionein levels typically rise. Other markers, such as glutamine synthetase and tyrosine hydroxylase, are used due to their sensitivity to cellular oxidation states. In the hypothalamus of rats, subchronic inhalation exposure to $MnSO_4$ (0.03, 0.3, 3.0 mg/m^3) has been associated with a decrease in GSH and an increase in metallothionein mRNA, while the olfactory bulb experienced an increase in glutamine synthetase (Dobson et al., 2003). Similarly, subchronic inhalation of $MnSO_4$ (0.06, 0.3, 1.5 mg/m^3) by rhesus monkeys resulted in decreased tyrosine hydroxylase, glutamate transporter-1, glutamate/aspartate transporter, and glutamine synthetase (Erikson et al., 2007a). These changes reflect exposure to oxidative stress that impairs neurotransmitter synthesis, while increased metallothionein mRNA in all the brain regions examined (caudate, cerebellum, frontal cortex, globus pallidus, olfactory cortex, putamen) is a cellular response to ameliorate the effects of reactive oxygen species. Many of these effects were significantly different from controls starting at the lowest manganese concentration tested (0.06 mg/m^3).

The effects of manganese on the markers of oxidative stress described above show an age and sex dependency. Juvenile (8 weeks) male and female rats, and senescent males (18 mo) breathed atmospheres containing MnPO₄ at 0.099 or MnSO₄ at 0.01, 0.098, or 0.478 mg Mn/m³ for 6 hr/day, 5 day/wk for 13 weeks (Erikson et al., 2004). In young males, but not females or senescent males, there was an increase in glutamine synthetase levels in the hippocampus, but a decrease in the hypothalamus with both forms of manganese that was significant (p < 0.05) with exposure to MnPO₄. With exposure to the medium dose of MnSO₄, female and old male, but not young, male rats showed significant decreases in glutamine synthetase levels in the hippocampus. Total GSH levels significantly decreased in the olfactory bulb of young males, but increased in females. In the striatum, GSH levels were significantly decreased in females and old males at all doses of MnSO₄, but were largely unchanged in young males. This is interesting in light of the observation by Dorman et al. (2004) that neither old age nor gender influenced delivery of manganese to the striatum. The decrease of GSH in the striatum in aged rats may be a result of the age-related loss of dopaminergic neurons, while the effect in females is suggested to be related to differences in levels of sex hormones between males and females. These data indicate that toxicokinetic and toxicodynamic characterizations of manganese must take age and gender into account.

That the chemical form and oxidation state of manganese is critical to its toxicokinetics is evident from the foregoing. No less critical to the toxicokinetics is the presence of manganese on or as nanoparticles versus free Mn₃O₄ in solution. The ability of manganese to cause oxidative stress in cultured human lung epithelial cells was assessed by measurement of reactive oxygen species (ROS) (Limbach et al., 2007). Nanoparticles (20-75 nm) of pure manganese, or silica doped with 0.5 and 1.6 wt % manganese, were suspended in culture medium at 30 ppm for comparison with the more soluble Mn₃O₄ at comparable concentrations. Compared with pure silica nanoparticles, particles doped with as little as 1.6 wt% manganese increased ROS in cultured cells by 2,500% while the free Mn₃O₄ increased ROS by only 400%. For comparison, in similar experiments Co₃O₄ particles were less than half as potent. In cell-free culture medium, ROS production was not different between the particles and the dissolved salt. This suggests that it is the dissolved metal, not the particles per se, that is responsible for the ROS generation, and that the nanoparticles function to facilitate manganese uptake by the cell.

5. Acute Toxicity of Manganese

Acute inhalation exposure to high levels of manganese as its oxides is associated with pulmonary edema and impaired function (Shiotsuka, 1984). The very small body of literature on acute toxicity includes two animal experiments involving acute exposures by inhalation. One is a two-hour exposure of 200 female CD-1 mice to manganese oxide (Mn₃O₄) aerosols (Adkins et al., 1980) that resulted in a NOAEL of 2.9 mg/m³ based on respiratory effects (edema). The other is a 24 hr exposure of guinea pigs to 22 mg/m³ MnO₂ (Bergstrom, 1977) that examined the effects of manganese exposure on pulmonary leukocytes, macrophages, and the clearance of bacteria from the lungs. However, since no dose response in lung wet:dry weight ratios was observed, no LOAEL was reported in the Adkins study, and the Bergstrom study employed a single exposure level. The

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accumulation of manganese in brain structures following acute inhalation exposure (Newland et al., 1987; Brennehan et al., 2000; Dorman et al., 2002a), and following intranasal instillation (Gianutsos et al., 1997) has been described; however, the toxicological consequences of these exposures were not reported. Neurobehavioral effects have been observed in mice following acute subcutaneous injections (Dodd et al., 2005), and in rats after a single oral administration of manganese (Shukakidze et al., 2003), but it is not clear how these routes of exposure compare to inhalation. No studies of acute manganese inhalation were located that demonstrated a dose-response or evaluated other toxicological endpoints.

6. Chronic Toxicity of Manganese

6.1 Chronic Toxicity to Adult Humans

Exposure of humans to manganese by inhalation leads to a suite of neurological effects called “manganism” (Lucchini et al., 1999). Frank manganism is a progressive disease that involves symptoms similar to those of Parkinson’s disease. Manganism is characterized by altered gait, fine tremor and occasionally psychiatric disturbances. The psychiatric disturbances are seldom seen in Parkinson’s disease, although dementia sometimes occurs late in this disease. Despite their similarities, the symptoms of manganism and Parkinson’s disease differ somewhat (Barbeau, 1984; Calne et al., 1994). Both manganism and Parkinson’s disease involve generalized bradykinesia and widespread rigidity. However, tremor is less frequent and dystonia more frequent in manganism. Manganism is also distinguished by a propensity to fall backward, failure to achieve a sustained therapeutic response to levodopa, and failure to detect a reduction in fluorodopa uptake by positron emission tomography (Calne et al., 1994). In Parkinsonism, the damage appears to be confined to the substantia nigra, whereas in manganism the damage is more widespread, involving other parts of the basal ganglia (Huang et al., 1998).

Manganese accumulates in certain brain structures, especially the extrapyramidal system. Structures rich in dopaminergic neurons show a heightened sensitivity to manganese toxicity. Within these tissues, manganese is found preferentially in mitochondria where it disrupts oxidative phosphorylation and mitochondrial function (Gavin et al., 1999). Cytochrome c, released from damaged mitochondria, leads to apoptosis and loss of neurons (Malecki, 2001). Trivalent manganese can promote the formation of reactive oxygen species (HaMai et al., 2001) that can cause oxidative stress, which in turn has been shown to lead to apoptosis of neurons in the rat brain (Dobson et al., 2003). While individuals exposed to massive amounts of manganese show frank neurological symptoms as in the Groote Eylandt studies (Kilburn, 1987) and the industrial workers studies, individuals exposed to lesser amounts of manganese show more subtle neurological deficits in neurobehavioral tasks (Wennberg et al., 1992; Lucchini et al., 1999).

Adverse effects may occur at manganese exposure levels that are too low to cause frank manganism. Lucchini and his co-workers studied a group of 61 Italian ferroalloy workers who had been exposed to low levels of manganese dust by inhalation (Lucchini et al.,

1999). These workers did not exhibit the frank signs of manganism, but they did exhibit subtler neurofunctional changes. The workers were exposed to a “current overall value” of $54 \mu\text{g Mn per m}^3$ air at the time of the study, and an estimated average of $70.83 \mu\text{g Mn dust/m}^3$ per year over an average 15.17 years of exposure. Earlier exposures were higher. In order to obtain a measure of cumulative exposure the investigators calculated a “cumulative exposure index” (CEI) for each worker based on their exposure history in the factory. For the purposes of analysis, workers were separated into three CEI groups of low CEI ($< 500 \mu\text{g/m}^3 \times \text{yrs}$), mid CEI ($500\text{-}1800 \mu\text{g/m}^3 \times \text{yrs}$), and high CEI ($> 1800 \mu\text{g/m}^3 \times \text{yrs}$). The CEIs correlated positively with blood manganese levels. The workers were subjected to symptom questionnaires and neurobehavioral and neurophysiological testing for the purpose of finding whether neurological effects correlated with cumulative exposure. In multiple regression analyses, positive correlations were found between the log of the CEI and the following tests of the Swedish Performance Evaluations System: finger tapping in the dominant ($R = 0.32, p = 0.01$) and non-dominant ($R = 0.32, p = 0.01$) hands, Symbol Digit ($R = 0.33, p = 0.01$) and Digit Span ($R = 0.44, p = 0.004$). The moderate but significant correlation coefficients reported in this study suggest that manganese is an important contributor to these effects but likely not the only one. In addition, these results demonstrate that subtle neurological changes are taking place in workers exposed to relatively low levels of manganese in the absence of frank manganism. To identify safe exposure levels, the authors took the geometric mean of the mid CEI group ($1113 \mu\text{g/m}^3 \times \text{yrs}$) and divided this by the geometric mean exposure time (11.51 yrs) to derive a value of $96.71 \mu\text{g/m}^3$. When the low CEI group is used as the control group, this value represents the LOAEL for the observed neurobehavioral symptoms.

A battery of neurofunctional tests was also employed by Mergler et al. (1994) to document early nervous system dysfunction among workers with long-term (mean 16.7 yr) manganese exposure in a ferromanganese and silicomanganese alloy plant. Subjects ($n = 115$) were matched by age, educational level, and number of children to workers in the same geographical region but without exposure to metals or other neurotoxicants in the workplace. The test batteries assessed motor function (range, speed, stability, grip strength, manual dexterity, graphomotor) and sensory function (visual acuity, chromatic discrimination, contrast sensitivity, olfactory and vibrotactile threshold). A third battery assessed speech initiation and regulation, attention, concentration and memory, cognitive flexibility, and affect. Environmental manganese levels were measured with personal monitors for total dust and manganese content ($0.014 - 11.48 \text{ mg Mn/m}^3$), while stationary monitors measured manganese in respirable ($0.001 - 1.273 \text{ mg/m}^3$) and nonrespirable dust. Manganese measured in blood was higher among manganese workers (1.03 vs $0.68 \mu\text{g}/100 \text{ ml}$, $p < 0.0001$), while urine levels were not significantly different.

On the tests of motor functions, the performance of manganese-exposed workers was significantly worse than controls ($p < 0.001$), with the greatest differences associated with tests requiring rapid, alternating, coordinated movements. While in the context of speech initiation and regulation there were no overall differences between groups, the manganese-exposed workers took significantly longer on the second trial of the digit

naming test ($p = 0.05$) and made more errors ($p < 0.001$). Cognitive flexibility was also worse with manganese exposure ($p < 0.002$). Attention, concentration, and memory functions were similar between groups. In the comparison of mood states, manganese workers displayed significantly more tension ($p < 0.01$), anger ($p = 0.01$), fatigue ($p < 0.001$), and confusion ($p = 0.01$) than controls. The cross-sectional nature of this study precludes assigning symptoms of manganese toxicity to specific environmental levels of manganese since levels in the plant varied widely both spatially and likely historically. However, there was a strong association between blood levels of manganese and subtle neurobehavioral deficits.

Whether the neurobehavioral effects associated with occupational exposure to manganese are permanent or transitory has been the subject of several follow-up studies of occupational cohorts. Occupational exposure at the ferroalloy plant featured in the study above by Mergler et al. (1994) ceased with its closure in 1990. Fourteen years later, 69 of the original workers and 68 referents were re-examined with many of the same assessment tools used in the 1994 study (Bouchard et al., 2007a). After controlling for age, education, alcohol consumption, and smoking, manganese workers performed significantly worse on tests of motor function (Luria Motor Scale) than did referents in both the initial ($p < 0.001$) and follow-up ($p < 0.05$) evaluations, although the differences at follow-up were not as striking. The motor deficits that persisted between the initial and follow-up studies included slowing of simple and alternate movements, and poorer quality of form drawing. Deficits in hand steadiness observed in the initial study remained but were less pronounced at follow-up. These deficits were significantly associated with increasing levels of cumulative manganese exposure ($p < 0.05$). Although in the initial study, several tests of cognitive function showed significant deficits among the exposed workers, these differences were no longer evident at follow-up. In general, measures of mood states showed improvement over time. However, compared to referents, manganese workers tended to report more feelings of anger and hostility in both the initial and follow-up studies ($p < 0.1$). Feelings of confusion and bewilderment, while not different between groups in the initial study, were significantly more pronounced among manganese workers ($p < 0.05$) at follow-up and significantly associated with cumulative exposure ($p < 0.01$). In a neuropsychiatric profile, former manganese workers were significantly ($p < 0.05$) more likely to experience feelings of anger and depression than the referents (Bouchard et al., 2007b). In addition to the effects on the nervous system, in the present study former manganese workers had a ten-fold increase in risk of respiratory problems. These two studies suggest that with cessation of manganese exposure, there is improvement in some neurological functions, but deficits in others remain.

While studies of the effects of manganese have tended to emphasize occupational exposures, a similar constellation of neurobehavioral effects has been found in a community study from which those with occupational exposures were excluded. Mergler et al. (1999) assessed nervous system functions in 273 individuals (151 women, 122 men) randomly selected from those living in proximity to a former manganese production plant in Southwest Quebec. A battery of tests similar to those of the occupational study above (Mergler et al., 1994) was used to profile nervous system function in relation to blood

manganese levels. Motor skills and coordination, learning and recall, visual perception and speed, verbal naming, and cognitive flexibility were assessed. Blood manganese levels ranged from 2.5 to 15.9 $\mu\text{g/l}$, with results stratified by blood levels (low $< 7.5 \mu\text{g/l}$ vs high $> 7.5 \mu\text{g/l}$), age and sex. Elevated blood manganese ($> 7.5 \mu\text{g/l}$) was associated with poorer upper limb coordination ($p = 0.04$) and deficits in learning and recall, which was stronger in men ($p = 0.002$) than in women ($p = 0.04$). These deficits were more pronounced in older subjects with elevated blood manganese. The neurobehavioral effects reported here were observed at blood manganese levels lower than those of the occupational study above ($0.75 \mu\text{g}/100 \text{ ml}$ vs $1.03 \mu\text{g}/100 \text{ ml}$). The authors thus suggest that “manganese neurotoxicity can be viewed as a continuum of dysfunction, with early, subtle changes at lower exposure levels, progressing to more severe neurological disorders at the high exposure levels that have been observed in mining, industry and agriculture.”

Male workers ($n = 92$, plus 101 matched controls) in an alkaline battery plant in Belgium exposed to manganese dioxide dust were the subjects of a cross-sectional epidemiological study (Roels et al., 1992). Total manganese concentrations and manganese dust were measured in the workers' breathing zones with personal samplers. Lifetime integrated respirable dust levels (LIRD) ranged from 0.04 to 4.43 $\text{mg Mn/m}^3 \cdot \text{year}$, with a geometric mean of 0.793 $\text{mg Mn/m}^3 \cdot \text{year}$. The average age of control and exposed groups was 30 years with a mean manganese exposure time of 5.3 years (0.2 to 17.7 years) for the latter group. In exposed workers, the geometric mean levels of blood and urine manganese (corrected for creatinine) were significantly higher ($p < 0.001$) than in controls. The subjects were also evaluated for neurobehavioral function, lung function, and hematological parameters. There were no significant differences in respiratory symptoms between those exposed and controls, and hematological parameters were in the normal range for both groups. In neurobehavioral tests, significant decrements in performance were found in exposed workers on tests for visual reaction time ($p < 0.001$), five measures of eye-hand coordination ($p < 0.005$), and in two of three tests of hand tremor ($p < 0.03$). The data for individuals in this study were used in a BMD analysis to calculate the 8-hr and chronic RELs.

Deleted: The LOAEL for this study, based on neurobehavioral effects, corresponds to 0.15 $\text{mg respirable manganese dust/m}^3$ (geometric mean of the LIRD divided by the average exposure time = $0.793 \text{ mg Mn/m}^3 \cdot \text{year} / 5.3 \text{ year} = 0.15 \text{ mg Mn/m}^3$).

In 1999, Roels et al. (1999) published a follow-up study of the cohort in the Roels et al. (1992) study described above. During the course of the present study, covering the years 1988-1995, the cohort dropped from 92 to 34 workers. Three neurobehavioral assessments were made. Eye-hand coordination was tested yearly with an orthokinesimeter. Starting in 1991, yearly assessments were also made of visual reaction time, and hand steadiness with a hole tremometer. Respirable manganese dust exposure was measured in a manner similar to that of the 1992 study using personal air monitors. Three levels of exposure (low, medium, and high), with average exposures from 1987-1992 of 400, 600, and 2,000 $\mu\text{g Mn/m}^3$, respectively, were compared with unexposed controls in a nearby chemical plant. After 1992, there was a substantial decline in manganese levels with the mean manganese levels dropping by the end of the study to 119, 181, and 744 $\mu\text{g/m}^3$ for the low, medium and high groups, respectively. In the low exposure group, the test of eye-hand coordination showed improvement with the decreasing manganese levels, and results were normal by the end of the study, while the

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effects in the higher exposure groups persisted. The time courses of the hand steadiness and visual reaction time tests showed no improvement and suggested irreversible impairment. Similarly, in neurobehavioral assessments of workers who had ceased manganese exposure, eye-hand coordination significantly improved, but deficits in hand steadiness and visual reaction time remained. These studies suggest that some of the neurological deficits improve when manganese exposure decreases, while others may be permanent.

Another follow-up among workers in the same plant covered by Roels et al., 1992 and 1999 was conducted by Crump and Rousseau (1999) for the years 1985-1996. This study covered 213 workers including 114 of the 140 originally tested by Roels. In this study the metric for manganese exposure was blood and urine levels, neither of which was associated with memory or eye-hand coordination tests. There were, however, marginally significant associations between manganese levels and poorer hand steadiness ($p = 0.05$). As in the Roels et al. (1999) follow-up, some neurobehavioral deficits improved with time and lower manganese levels; others appeared to be more permanent.

Welding in confined spaces represents a setting in which significant occupational inhalation exposure to manganese may occur. In an evaluation of 43 welders working on the San Francisco Bay Bridge, Bowler et al. (2007) documented decrements on neurological, neurophysiological, and pulmonary tests associated with exposure to a time-weighted average manganese dust level of 0.11-0.46 mg/m³ for an average of 16.5 months. Manganese blood levels exceeded 10 µg/l in 43% of the workers. Multiple regression analyses against blood manganese and/or the individual's cumulative exposure index (CEI) revealed significant inverse dose-effect relationships with IQ ($p \leq 0.05$), executive function ($p \leq 0.03$), sustaining concentration and sequencing ($p \leq 0.04$), verbal learning ($p \leq 0.01$), working memory ($p \leq 0.04$), and immediate memory ($p \leq 0.02$) after adjustment for demographics and years of welding before working on the Bay Bridge. Spirometric measurements, taken at three time points, indicated declining lung function with manganese exposure. The first time point was after working on the bridge for an average of 1.5 months, the second after 10.8 months, and the third after 20.9 months. Between the first and third time points, measures of lung function decreased: 7% for FEV₁, 2% for FVC, and 21.2% for the FEV₁:FVC ratio ($p < 0.05$). In tests of mood and affect, the levels of clinical depression and anxiety among welders were greater than two standard deviations above the normative mean. Neuropsychological tests of parameters characteristic of parkinsonism found that tremor was present 39-90% of the time on three different tests, postural sway was increased in about half of the welders, and motor dexterity and speed were impaired 52-95% of the time. Additional symptoms reported by the welders that showed significant negative correlations with the CEI included sexual function ($p < 0.05$), fatigue ($p < 0.05$), depression ($p < 0.01$), and headache ($p < 0.05$). Compared to test norms, olfaction was impaired in 88% of the welders. This study suffers from the absence of an unexposed control group for comparison. However, all welders were prescreened during the hiring process to ensure good health and fitness. Blood levels of copper, iron, and lead were also measured and considered to be in the normal range. This, coupled with the significant correlations between both blood and air

manganese levels and the physiological and neurological decrements, strongly implicates manganese as a causative agent.

That an association between exposure to welding fumes and symptoms of neurotoxicity may be due to manganese was corroborated by magnetic resonance imaging (MRI) detection of characteristic bilateral hyperintense T1-weighted signals in the globus pallidus of eight welders referred for neurological assessment (Josephs et al., 2005). Among the six cases with multiple MRI follow-up scans, the intensity of the MRI signal among the four for whom manganese exposure was discontinued either remained the same (1 case) or faded (3 cases), indicating a loss of pallidal manganese. In the remaining two with continued exposure, the signal remained the same or increased in intensity. All cases presented multiple symptoms characteristic of manganism including postural tremors, reduced arm swing, ataxia, altered gait, multifocal myoclonus, and cognitive impairment. In addition, several cases reported irritability, memory loss, headaches, slurred speech, and reduced sexual drive. All cases had elevated or high normal serum manganese levels. This constellation of symptoms in association with manganese exposure and characteristic MRI images suggests a role for welding fumes in the development of manganism. Substantially similar symptomologies have been reported elsewhere in case studies of welders (Sadek et al., 2003). However, it is important to note that welding fumes are a mixture of metals, many of which are also neurotoxic and may contribute to the reported neurological symptoms.

The neurotoxic effects of exposure to welding fumes may be accompanied by pulmonary damage. Clara cells lining the airways normally secrete Clara cell protein (CC16) that has anti-inflammatory properties. Pulmonary damage that includes the Clara cells results in decreased recovery of CC16 in bronchoalveolar lavage fluid, as well as an increase in serum levels of CC16. The former effect is presumably due to reduced production by the affected Clara cells, while the latter is attributed to damage to the bronchoalveolar/blood barrier (Hermans et al., 1999). In ship welders, measures of serum CC16 levels, blood, urine and air manganese levels, pulmonary function (vital capacity), and subclinical neurological effects (EEG and visual evoked potential (VEP) were compared with unexposed controls (Halatek et al., 2005; 2008). During examinations that assessed both subjective and objective neurological status, 66% of the 59 workers reported subjective central nervous system symptoms, while 29% had abnormal VEP results and 41% had abnormal EEGs. Among welders showing neurological symptoms, blood manganese was significantly elevated (12.2 vs 6.1 $\mu\text{g/l}$, $p < 0.05$) while vital capacity was significantly depressed (84.5%, $p < 0.05$) (Halatek et al., 2008). Multiple linear regression analysis revealed strong partial correlations between abnormal VEP and EEG, and both blood manganese (0.72, $p = 0.03$) and an index of cumulative manganese exposure (0.66, $p = 0.01$) (Halatek et al., 2005). Levels of CC16 were significantly correlated (0.82, $p = 0.015$) with abnormal VEP, EEG and CNS symptoms. The CC16 levels were significantly lower (9.6 $\mu\text{g/l}$, $p < 0.05$) among the younger welders who had fewer years of exposure (3 years) but higher blood manganese levels (13.7 $\mu\text{g/l}$), abnormal VEP and EEG results, and depressed vital capacity (83%). The authors suggest that the elevated CC16 levels indicate that welding fume exposure compromises pulmonary function,

including that of the bronchoalveolar barrier. This in turn facilitates manganese access to the blood and brain, with the attendant subclinical neuropathological changes.

As mentioned in the study above, in addition to neurotoxicity, manganese inhalation may lead to symptoms of pulmonary toxicity. Indeed, the incidence of respiratory disease is higher among manganese-exposed workers than those not exposed (Boojar and Goodarzi, 2002). In a case report, Wittczak et al. (2008) present the study of a 42-year-old non-smoking welder with suspected occupational asthma. At admission, the patient presented with a recurrent nonproductive cough, and dyspnea with wheezing that usually occurred after 30-60 minutes of welding. Compared with non-work days, on the days the patient was exposed to welding fumes, he exhibited a greater than 20% variability in his peak expiratory flow rate (PEFR). Histamine challenge revealed significant bronchial hyperreactivity ($PC_{20} = 0.5$ mg/ml). In contrast to a placebo inhalation challenge (1% KCl solution), five minutes following a challenge with 0.1% MgCl solution, dyspnea occurred and forced expiratory volume (FEV1) dropped 45%. At one hour, FEV1 was 55% below resting levels, and only recovered to 35% below resting levels by 24 hours. At 4 and 24 hours post-exposure, changes in the proportions of eosinophils (8% and 10% resp.) and basophils (1% and 3%, resp.) were observed in induced sputum. None of these effects was observed in non-exposed controls after similar challenges. This constellation of symptoms and sensitivity to manganese challenge supports a role for manganese in occupational asthma.

Another occupational study of lower exposures was done in Sweden (Wennberg et al., 1992). In this study workers had been exposed for a year or more to manganese dust at mean concentrations of 0.18 mg/m^3 at one smelter, and 0.41 mg/m^3 at another. They were compared to workers at similar industrial plants without manganese exposure via a suite of neurological tests, including electroencephalogram, brainstem auditory evoked potential, event related auditory evoked potential, and diadochokinesometry (a test of the subject's ability to rotate a handle rapidly). Of these tests, the only one that produced significantly different results in the exposed subjects was the diadochokinesometry. The manganese-exposed workers were unable to rotate the handle as quickly as the control workers. This is interpreted as evidence of a "preclinical" effect of low-level manganese exposure.

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▼ A major study of non-industrial human exposures is the study of the natives of Groote Eylandt, a large island off the coast of Australia. The inhabitants of this island are Australian Aborigines. The island is so rich in manganese that the environment has been described as a "manganese ecology" (Kilburn, 1987). The inhabitants are exposed by virtually all routes of exposure, but especially by ingestion of food and water high in manganese. Kilburn studied the natives of Groote Eylandt and compared them to a control group of Australian Aborigines living in another part of Australia. This paper does not quantitate the manganese exposures or body levels of manganese in the study population, and it would be difficult to quantitate exposures in this complex environmental situation. Kilburn reports certain congenital abnormalities, such as deformations of the foot (talipes equinovarus), closed anus (imperforate anus), and anorectal malformations, and neurobehavioral problems, including progressive muscle

wasting (amyotrophy) and failure of muscle coordination (ataxia), that apparently occur with greater frequency in the islanders than in the control groups, but these could also be due to genetic factors present in this small population. Indeed all of the problems were seen in just two pedigrees. A likely interpretation would be that the adverse health effects observed reflect gene-environment interactions.

Exposure to excess manganese may occur via the parenteral route, especially in individuals receiving total parenteral nutrition (TPN). Unfortunately, patients receiving TPN are often those with liver damage and/or gastrointestinal disorders, both of which compromise the hepatobiliary circuit by which the body regulates retention of dietary manganese. Manganese intoxication in these cases typically manifests as confusion, dysarthria, rigidity, gait disturbances, and hypokinesia, and is generally confirmed by marked hyperintensity of the globus pallidus by magnetic resonance imaging (MRI) (Ono et al., 1995; Nagatomo et al., 1999). Symptomatic improvement and reversion to more normal T1-weighted MRI images following discontinuation of manganese supplementation support the diagnosis of manganism. While the parenteral route is thus involved in the increased risk of hypermanganesaemia during TPN, it is also involved in the recent rise in cases of manganism in adults associated with long-term intravenous use of illicit designer drugs. Sikk et al. (2007) described four cases of young adults presenting with symptoms of manganism (including impaired postural control, unsteady gait, manual dysfunction) in conjunction with long-term (7 months – 8 years) repeated use of the psychostimulant ephedrone. The synthesis of ephedrone involved the oxidation of pseudoephedrine with potassium permanganate, which remained in the injected solution. Based on the drug injection history of two of the cases, and an analysis of the manganese content of a similarly synthesized ephedrone preparation, the authors estimate a total body burden of manganese corresponding to 900 and 500 mg/kg body weight compared to a normal body burden of 10-20 mg/kg (Schroeder et al., 1966). Cranial MRI of two individuals with exposures and symptoms similar to those described by Sikk et al. revealed hyperintense patterns in the globus pallidus indicating an abnormally high accumulation of manganese (Meral et al., 2007).

6.2 Chronic Toxicity to Infants and Children

Manganese is an essential nutrient, but it has toxic effects if exposure is excessive or prolonged, especially if exposure is by the inhalation route. A number of studies have reported correlations between early life exposure to excessive manganese and symptoms of impaired neurodevelopment as revealed on neurobehavioral tests and in poorer academic performance. In a prospective study of the neurobehavioral effects of *in utero* exposure to manganese, Takser et al. (2003) reported an inverse correlation between cord blood manganese at birth and three subscales of psychomotor development (McCarthy scales of children's abilities) measured at three years of age (n = 126): attention (partial r = -0.33, p < 0.01), nonverbal memory (partial r = -0.28, p < 0.01), and hand skills (partial r = -0.22, p < 0.05). The adverse effects of manganese on neurodevelopment in these children persisted after adjustment for gender and maternal education, although the effects of manganese on hand skills were only observed in boys. Similarly, Collipp et al. (1983) used a battery of tests, including cognitive and projective tests, psycho-educational evaluation, speech, language and hearing evaluations, and social services

evaluations, to identify 16 children who were hyperkinetic and exhibited learning disabilities. In comparison with 44 normal children of the same age, significantly elevated levels of hair manganese (0.434 $\mu\text{g/g}$; measured at 8 years of age) were reported in children with learning disabilities and hyperactivity compared with normal children (0.268 $\mu\text{g/g}$) ($p < 0.05$). An association between poorer performance in school and elevated hair manganese (1.242 $\mu\text{g/g}$) has also been observed among children in China compared with children with more normal manganese levels (Zhang et al., 1995).

Wasserman et al. (2006) reported adverse effects of manganese in 10-year old children ($n = 142$) in Bangladesh who had been exposed to manganese in their drinking water ($< 200, 200\text{-}499, 500\text{-}999, > 1,000 \mu\text{g/l}$). Comparing the lowest and highest dose groups (< 200 vs. $> 1,000 \mu\text{g/l}$), significant decrements in intellectual function at 9.5-10.5 years of age were revealed in scores on the Wechsler Intelligence Scale for Children-III with increasing daily intake of manganese (full scale, $p < 0.0001$; performance, $p < 0.0001$; verbal, $p < 0.02$). The scores of children with intermediate manganese exposures were also lower than those of the lowest dose group, but not significantly so. In this study, confounding by co-exposure to arsenic was limited by including only children whose drinking water contained $< 10 \mu\text{g As/l}$. Scores were adjusted for maternal education and intelligence, house type, television, child height and head circumference. Blood levels of manganese, arsenic and lead were also determined and added to the core model. In this case, only blood lead was correlated with decreased intellectual performance. However, in a simultaneous analysis of water manganese, water arsenic, and blood lead, the negative association between manganese water levels and intellectual function test scores remained (Full-Scale $\beta = -4.56, p < 0.01$; Performance $\beta = -3.82, p < 0.01$).

The uptake of metals into developing teeth provides a record of gestational exposure to manganese. In multiple regression analyses, after controlling for lead, high levels of manganese incorporated into teeth during the 20th week of gestation were positively correlated with behavioral disinhibition at 36 months of age ($R = 0.48, p < 0.01$) and, at 54 months, with impulsive errors on the Mirsky Continuous Performance Test ($R = 0.48, p < 0.01$) and the Children's Stroop Test ($R = 0.38, p < 0.01$). Positive correlations with manganese were also seen in ratings made by both parents and teachers of externalizing and attention problems on the Child Behavior Checklist in the 1st ($R = 0.40 - 0.47, p < 0.05$) and 3rd grades ($R = 0.38 - 0.48, p < 0.05$), and in the 3rd grade with the teachers' ratings on the Disruptive Behavior Disorders Scale ($R = 0.44, p < 0.05$), ADHD ($R = 0.48, p < 0.01$), and hyperactivity – impulsivity ($R = 0.55, p < 0.01$). In contrast, manganese levels in tooth enamel formed in the 62-64th week of gestation (i.e., postnatally) were correlated only with teachers' reports of externalizing behaviors in the 1st ($R = 0.40, p < 0.05$) and 3rd grades ($R = 0.57, p < 0.01$). It thus appears that high prenatal manganese exposure may adversely affect behaviors expressed postnatally. There was, however, no correlation between tooth manganese and cognitive ability as measured on the Woodcock-Johnson Psycho-Educational Battery (Ericson et al., 2007).

Subtle neurobehavioral effects were seen in a case report of a 10-year old boy exposed for five years to elevated manganese in the family's drinking water (Woolf et al., 2002). The boy's hair manganese was high (3,091 ppb vs normal reference < 260 ppb), as was

that of his 16 year-old brother (1,988 ppb). Neuropsychological tests on the 10 year-old revealed intact global cognitive skills but striking deficits in visual and verbal memory (< 20th percentile in the Wide Range Assessment of Visual-motor Abilities). No obvious neurobehavioral problems were noted for either the parents or the older sibling.

As with adults, children receiving long-term parenteral nutrition are at greater risk of hypermanganesaemia. This is especially problematic since it is often the premature infants that require TPN. Among infants receiving parenteral nutrition containing supplemental manganese, MRI scans revealed bilaterally symmetrical hyperintense signals in the globus pallidus associated with movement disorders (dystonia and abnormal posturing) (Fell et al., 1996), and in basal ganglia, brainstem, and cerebellum associated with seizures (Komaki et al., 1999). In one infant these effects developed within eight months (Fell et al., 1996). While an abnormally high T1-weighted MRI signal suggests high brain manganese levels, especially when removal of manganese results in gradually diminishing signal intensity, it should be noted that not all patients with elevated brain manganese develop overt neurological symptoms. Kafritsi et al. (1998) reported on siblings receiving parenteral nutrition for 63 and 23 months that resulted in elevated blood manganese levels of 323 nmol/l and 516 nmol/l, respectively (normal = 73-210 nmol/l) and hyperintense signals in the globus pallidi. The signal intensity reverted to normal following cessation of manganese supplementation with no evidence of abnormal neurological development at three years of follow up. Whether either child had subclinical effects or effects that will manifest only later in life is unknown.

6.2.1 Potential for Differential Effects in Children

Infants and children may be more susceptible than adults to manganese toxicity for the following [toxicodynamic and toxicokinetic](#) reasons:

1. [As noted in the previous section, manganese exposures in childhood are associated with impaired neurodevelopment including decrements in intellectual function. Thus a major toxicodynamic factor that differs between adults and children, namely development of the central nervous system, presents hypersensitive targets for toxicity in the developing infant and child.](#)
2. [Early life manganese exposures may predispose to manifestations of neurological damage in adulthood following subsequent exposure to environmental toxicants \(Barlow et al., 2004\).](#)
3. Newborns absorb and retain more manganese from the gastrointestinal tract than do adults (Dorner et al., 1989; Davis et al., 1993).
4. The liver of newborns has not yet developed the ability to maintain safe levels of manganese in the bloodstream and brain tissues by excreting excess manganese in the bile, i.e., homeostasis of manganese has not yet developed (Miller et al., 1975).
5. Some infant formulas and foods are high in manganese (Tran et al., 2002a,b).
6. The newborn's brain is still developing and the blood-brain barrier is not completely formed (Chan et al., 1992).
7. Modeling of the inhalation dosimetry of particles (0.001-10 μm), comparing [infants](#) (3 mo) and adults, in four regions of the respiratory tract (extra-thoracic, tracheo-upper bronchi, bronchiolar, pulmonary), suggests that differences in the dose per unit surface area between neonates and adults are dependent on particle size and respiratory tract region (Ginsberg et al., 2005). In addition, infants and young children experience overall higher deposition of particles than adults.
8. Manganese absorption from the nose (Thompson et al., 2007), lungs (Brain et al., 2006), and intestinal tract (Erikson et al., 2002) is enhanced by low iron levels, a condition more prevalent among children than adults.

6.3 Animal Studies of Chronic Toxicity

Animal studies of the toxic effects of chronic manganese exposure have focused on altered neurobehavior and the effects of manganese on the associated brain structures. These studies indicate that differences in age at exposure, route, and chemical form of the metal are critical to the distribution of manganese, and the type and extent of the adverse effects.

[Early life exposure to air borne manganese may occur by multiple routes: in utero via the mother, and perinatally via milk ingestion and inhalation. To examine the effects of these exposures on tissue concentrations of manganese in adult and young rats, adult male and female rats were exposed to \$\text{MnSO}_4\$ \(0.05, 0.5, or 1 mg Mn/m³\) or air 28 days prior to breeding, for up to 14 days during the mating period, during gestation days 0-19, and](#)

from one day post-partum through postnatal day (PND) 18 (Dorman et al., 2005a). Exposures were for 6 hr/day, 7 days/week. While these exposures did not affect maternal brain, lung, pancreas, or liver weights, the high dose (1 mg/m³) was associated with decreased brain weights in pups on PND 14, female pups on PND 19, and male pups on PND 45. Measurements of manganese in the striatum, cerebellum, and olfactory bulb of neonatal rats on PND 19 showed statistically significant and dose-dependent increases relative to controls. On PND 18, maternal olfactory bulb, striatum, and cerebellum also had significantly ($p < 0.05$) elevated manganese from exposures to 0.5 mg/m³ and above. Measurements made 27 days following exposure cessation on PND 45 showed that all tissue manganese levels had returned to control values. However, some pups on PND 45 still showed decreased brain weights suggesting that high early-life manganese exposure may result in prolonged alterations in brain size. By PND 63, all brain weights were at control values, but whether structural or functional deficits were present was not determined in this study.

Some consequences of early life exposure to manganese may not manifest until later in life. This appears to be the case for prenatal exposure to the manganese-containing fungicide Maneb (manganese ethylene-bis-dithiocarbamate), followed later in life by exposure to the pesticide paraquat and the development of symptoms of manganism. It has been reported previously (Thiruchelvam et al., 2000) that mice treated with Maneb twice a week for six weeks showed reduced motor activity immediately after treatment, with recovery of function in 24 hours. This effect was not seen with paraquat alone but was enhanced by co-exposure to paraquat. In these mice, co-exposure reduced tyrosine hydroxylase and dopamine transporter immunoreactivity in dorsal striatum. Similarly, only the combined Maneb/paraquat exposure decreased striatal tyrosine hydroxylase protein levels, caused reactive gliosis in dorsal-medial but not ventral striatum, and reduced tyrosine hydroxylase immunoreactivity and cell counts in the substantia nigra but not ventral tegmental area.

Barlow et al. (2004) extended these observations to early life susceptibility with the treatment of pregnant mice with Maneb, paraquat, or saline on gestation days 10-17. As adults, these mice received challenge exposures for eight days to either Maneb or paraquat on postnatal days 45-55. Prior to the challenge exposures, locomotor activity was evaluated but no significant differences among groups were found. On the eighth day of challenge exposures, locomotor activity was depressed in all animals exposed to Maneb as adults, but recovered to control levels within one week of the last challenge exposure, except for males prenatally exposed to Maneb and subsequently exposed to paraquat as adults. These males showed a 95% reduction in locomotor activity, while similarly exposed females showed no effects. In the context of dopaminergic neurochemistry, neither prenatal exposure to Maneb alone nor adult exposure to paraquat alone caused significant change in either gender. However, compared to male controls and paralleling the locomotor effects, males receiving Maneb prenatally and paraquat as adults had 50% lower striatal dopamine levels, 35% lower 3,4-dihydroxyphenylacetic acid (DOPAC, a dopamine metabolite) levels, and 40% greater dopamine turnover. In the substantia nigra pars compacta, these males showed a loss of tyrosine hydroxylase-positive neurons of 30% compared with saline-treated males ($p < 0.001$), 30% compared

with males receiving Maneb prenatally and saline as adults ($p < 0.001$), and 21% compared with males receiving paraquat as adults only ($p < 0.05$). The reduction in tyrosine hydroxylase positive neurons only occurred with Maneb followed by paraquat, not following Maneb alone. These results suggest that prenatal exposure to Maneb causes damage to the nigrostriatal region of the male brain that is only revealed in adulthood following another neurotoxic insult in the form of paraquat. While these experiments do not demonstrate that it is the manganese in Maneb that is responsible for the observed neurotoxicity, the types of toxicity described are similar to those observed for other manganese compounds.

The relative sensitivity of neonatal and adult CD rats to manganese-induced neurotoxicity was studied by administering manganese dichloride orally to rats at doses of 0, 25, and 50 mg/kg per day (Dorman et al., 2000). Adults and pups were dosed for 21 consecutive days, and then were evaluated with behavioral tests such as pulse elicited startle response amplitude, and in terms of manganese levels in striatum, hippocampus, hindbrain, and cortex. Neonatal rats exposed at the highest level of manganese showed a statistically significant increase in amplitude of acoustic startle response. They also showed increases in brain levels of manganese. The results suggest that neonates may be at greater risk for manganese-induced neurotoxicity when compared to adults receiving high oral levels of manganese. The authors state that there are known pharmacokinetic processes that may relate to the increase in brain manganese concentration in neonatal rats including increased manganese absorption from the juvenile gastrointestinal tract, an incompletely formed blood-brain barrier, and a virtual absence of excretory mechanisms until weaning.

The effects of manganese inhalation on levels of the metal in various tissues has been explored in Rhesus monkeys. In a study by Dorman et al. (2006b), Rhesus monkeys inhaled manganese sulfate (60, 300 or 1,500 $\mu\text{g}/\text{m}^3$) 6 hours per day, 5 days per week for 13 weeks. At termination, tissue manganese levels were significantly ($p < 0.05$) elevated in all tissues examined, except testes, in animals exposed to the highest dose (1,500 $\mu\text{g}/\text{m}^3$). Even at the lowest dose (60 $\mu\text{g}/\text{m}^3$), manganese levels were significantly elevated in four of the eight brain regions examined, globus pallidus, putamen, white matter and cerebellum (Table 6.3.1). For comparison, the table includes manganese levels in these same brain regions reported below by Schneider et al. (2006) and Guilarte et al. (2006) for monkeys displaying neurobehavioral toxicity. To facilitate comparison with human occupational studies, an annual dose is also presented showing the calculated air concentration had the dose levels been spread over a period of one year. In the study by Roels et al (1992), used for the development of the REL (Section 8), a similar measure of the annualized exposure was calculated as the lifetime integrated respirable dose (LIRD). In the Roels study, neurotoxicity was observed in individuals with LIRDs in the range of 60 to 3,715 $\mu\text{g}\cdot\text{yr}/\text{m}^3$, a range that overlaps the concentrations used by Dorman that resulted in brain levels associated with neurotoxicity by Schneider and Guilarte. Although the brain levels of manganese were not measured in workers showing neurotoxicity in the Roels study, these studies in primates provide support that the air concentrations to which the workers were exposed was sufficient to result in brain manganese levels with which neurotoxicity is associated in primates.

Table 6.3.1 Manganese Levels in Primate Brain After Inhalation or IV Exposure

	<u>Dorman et al., 2006</u>			<u>Schneider et al., 2006/</u>
	<u>Inhalation level</u>			<u>Guilarte et al., 2006</u>
	<u>60 µg/m³</u>	<u>300 µg/m³</u>	<u>1,500 µg/m³</u>	<u>3.26-4.59 mg Mn/kg</u>
<u>Caudate</u>	<u>0.47 µg/g</u>	<u>0.69 µg/g</u>	<u>1.72 µg/g</u>	<u>1.18 µg/g</u>
<u>Putamen</u>	<u>0.58</u>	<u>0.75</u>	<u>1.81</u>	<u>1.50</u>
<u>Globus pallidus</u>	<u>0.80</u>	<u>1.28</u>	<u>2.94</u>	<u>3.30</u>
<u>White matter</u>	<u>0.25</u>	<u>0.39</u>	<u>0.87</u>	<u>0.57</u>
<u>Annual dose</u>	<u>15 µg*yr/m³</u>	<u>75 µg*yr/m³</u>	<u>375 µg*yr/m³</u>	

Neurobehavioral effects may be preceded by changes in brain chemistry. Such changes were studied in four female rhesus monkeys exposed in an inhalation chamber to 30 mg/m³ respirable manganese dust for five hours/day, five days/week (Bird et al., 1984). After two years the animals were sacrificed and compared to unexposed controls. The exposed monkeys showed decreased dopamine in the caudate and globus pallidus, as well as a 60 to 80 percent increase in manganese levels in the basal ganglia of the brain. However, the exposed monkeys did not exhibit any of the movement disorders that are characteristic of Parkinson's disease.

In another study of the effects of manganese inhalation on neurotransmitters in rhesus monkeys, Struve et al. (2007) found that subchronic (13 wk) exposure to manganese sulfate resulted in statistically significant increases in mean manganese concentrations in the pallidus and putamen at 0.06, 0.3, and 1.5 mg/m³, and in the caudate at ≥ 0.3 mg/m³. Marginally statistically significant (p < 0.1) changes in neurotransmitter levels were seen only at the highest manganese concentration (1.5 mg/m³) in the globus pallidus for GABA and 5-HIAA, and in the caudate for norepinephrine. This is consistent with the suggestion that manganese neurotoxicity derives in part from dis-regulation of GABA-ergic neurons (Fitsanakis et al., 2006), possibly related to the observed decreases in tyrosine hydroxylase and glutamine synthetase by manganese (Erikson et al., 2007a).

The distribution of manganese in primate brain, and its neurobehavioral and cognitive effects in Cynomolgus macaques following weekly intravenous injection of MgSO₄ (10-15 MgSO₄ or 3.26-4.89 mg Mn/kg) for 39 weeks were investigated by Guilarte and associates. Neurobehavior, as rated on a modified Parkinsonian symptoms scale, activity levels measured with an activity monitor, and fine motor skills, assessed as the number of errors while trying to retrieve objects from wells of different sizes, all showed significant decrements (p < 0.05) at the end of the experiment compared with baseline (Guilarte et al., 2006a). Over this same period, stereotypical or compulsive-like behaviors, such as licking/biting fingers and grooming, significantly increased in frequency with manganese exposure (p < 0.01) (Schneider et al., 2006). The levels of manganese were significantly (p < 0.05) elevated in exposed monkeys compared to controls in the globus pallidus (3.3 µg/g tissue), caudate (1.18 µg/g), putamen (1.50 µg/g), and frontal white matter (0.57 µg/g) (Table 6.3.1). Imaging studies were performed at 128 days and 157 days after the start of manganese exposure, and included T-1 weighted magnetic resonance imaging

(MRI), magnetic resonance spectroscopy (H-MRS), and positron emission tomography (PET). As assessed by PET, manganese decreased the ability of amphetamine to stimulate dopamine release in the striatum, apparently without the loss of dopaminergic terminals. The authors speculate that the inhibition of dopamine release may alter the excitability of nigrostriatal dopaminergic neurons and/or may alter dopamine compartmentalization. The former case may contribute to the behavioral symptoms while, in the latter case, the probability of dopamine oxidation and consequent neuronal damage may be increased (Guilarte et al., 2006a). Neuronal loss or dysfunction in these monkeys was suggested by a change in brain metabolites with chronic manganese exposure. Specifically, significant decreases in the N-acetylaspartate: creatinine ratio in parietal cortex ($p = 0.028$), and a near significant ($p = 0.055$) decrease in the white matter were observed.

Concern for the consequences of exposure to the combustion products of methylcyclopentadienyl manganese tricarbonyl (MMT) has fueled investigation of the bioaccumulation and neurobehavioral effects following subchronic exposure to manganese as the free metal, conjugates of sulfate and phosphate, and a mixture of the two conjugates. In a collection of related studies (Normandin et al., 2002; Salehi et al., 2003; Normandin et al., 2004; Tapin et al., 2005), rats were exposed to aerosolized manganese or its conjugates for 6 hours/day, 5 days/week for 13 weeks at target levels of 30, 300, and 3,000 $\mu\text{g}/\text{m}^3$. Following exposure, locomotor activity over a 36 hr period was recorded as resting time, distance traveled, and total ambulatory count. The animals were then sacrificed and the manganese levels in various tissues and brain regions measured. These studies consistently showed significant ($p < 0.05$) dose-dependent increases in manganese in the lungs for all forms of the metal. The highest dose of the manganese conjugates, separately or mixed, resulted in significantly ($p < 0.05$) elevated levels in all tissues except the liver, reflecting the liver's role in manganese homeostasis. The sulfate and phosphate conjugates were better assimilated into all extra-pulmonary tissues than was the un-conjugated metal (Normandin et al., 2004). Uptake of manganese into brain tissue was more efficient from the combined sulfate and phosphate exposure than from exposure to metallic manganese or the phosphate conjugate alone, presumably due to the higher solubility of the sulfate conjugate. This difference was reflected in the significantly ($p < 0.05$) lower ambulatory count for the animals exposed to the conjugate mixture compared to controls (Normandin et al., 2004). For rats exposed to MnSO_4 alone, the total distance traveled in the locomotor studies increased at all manganese concentrations concomitant with an increase in total resting time, suggesting shorter bursts of activity. These rats also showed a dose-dependent decrease in total ambulatory counts over 36 hours, as well as a dose-dependent loss of cells in the globus pallidus and caudate putamen (Tapin et al., 2005). Similarly, the highest exposure to the Mn sulfate/phosphate mixture produced a significant increase in motor activity and a significant decrease in total ambulatory counts (Saleh et al., 2003). In contrast, no behavioral changes were noted with exposure to the phosphate conjugate (Normandin et al., 2002). Collectively these studies suggest that behavioral neurotoxic effects are associated with inhalation of manganese conjugates and that the sulfate conjugate is more toxic than the phosphate or metallic forms, consistent with its greater solubility.

In addition to neurotoxicity, pulmonary dysfunction may be associated with inhalation of manganese. In a subchronic chamber study, young, male rhesus monkeys were exposed to manganese sulfate at 0.06, 0.3, or 1.5 mg Mn/m³ for 6 hours/day, 5 days/week, for 65 exposure days (Dorman et al., 2005b). The mass median aerodynamic diameters of the particles in these aerosols were 1.73, 1.89, and 2.12, respectively. Another set of monkeys, exposed to 1.5 mg Mn/m³ by this regimen, was held for 45 or 90 days prior to evaluation. A third set was exposed to 1.5 mg Mn/m³ and evaluated after 15 or 30 days of exposure. The evaluations included histopathological assessments of the lungs, and measurements of the manganese content of lungs and olfactory epithelium. Manganese levels were significantly elevated ($p < 0.05$) in olfactory epithelium with all exposures, and in lungs at exposures of 0.3 mg/m³ and above. In animals exposed to 1.5 mg/m³ and evaluated after 15 or 33 days, significantly ($p < 0.05$) elevated manganese levels were found in both olfactory epithelium and lungs, however, these levels returned to control levels 45 and 90 days after exposure was discontinued. Significant bronchiolitis and alveolar duct inflammation was seen only in the animals exposed to 1.5 mg Mn/m³, but these effects were apparently reversible as they were no longer present at 45 and 90 days post exposure. Increased bronchus-associated-lymphoid tissue was also observed only with the highest exposure. Thus the inflammatory changes in small airways and increased manganese lung concentrations were only associated with the highest exposure levels used (1.5 mg Mn/m³), and were apparently reversible following cessation of exposure, suggesting that the lungs are a less sensitive target for manganese toxicity than is the central nervous system.

The pulmonary toxicity of manganese, as measured by induction of an inflammatory response, is relatively high compared with a number of the transition metals found in particulate pollution. Intratracheal instillation of the sulfates of copper, vanadium, nickel, iron, zinc, and manganese (0.1, 1.0 $\mu\text{mol/kg}$) was used to assess the relative inflammatory potential of these metals in rats. Bronchoalveolar lavage (BAL) at 4, 16 and 48 hours following exposure provided the medium in which markers of inflammation were measured (Rice et al., 2001). Lactate dehydrogenase activity and total protein levels in the lavage fluid, used as general indicators of toxicity, showed copper at 1 $\mu\text{mol/kg}$ to be the most toxic at all time points, followed by nickel and manganese. Whereas with copper LDH activity peaked at 16 hours post-exposure, then declined by 48 hours, manganese-induced LDH activity was significantly ($p < 0.05$) elevated at all time points and continued to increase with time. Similarly, the numbers of leukocytes recovered from BAL fluid were highest for manganese compared with the other metals at 16 and 48 hours. At these same time points, neutrophilia was seen at the low dose only with copper, while at the higher dose, manganese was the most potent. Significant eosinophilia was observed for manganese, copper, iron, and nickel at 16 and 18 hours, but eosinophil numbers were the highest with manganese by two to three-fold. Lymphocyte levels were not elevated by metal treatment except with low-dose copper at 16 hours, and high dose manganese at 48 hours. Thus manganese and copper were found to be the most proinflammatory of the metals tested but presumably by different signaling pathways. The effects with copper tended to appear earlier and at lower exposure levels, while manganese was more effective at stimulating the appearance of immune cells at a higher dose and later time points.

6.4 Dietary Exposure to Manganese

Newborns and infants may be exposed to more manganese in their diets than are adults. Infant formulas based on cow's milk have about 16 times more manganese than human milk (Dorner et al., 1989). Soy based formulas have even higher levels of manganese – about 40 times the manganese of human milk (Tran et al., 2002a; Tran et al., 2002b). Formula usage can lead to significantly elevated body burdens of manganese. For example, the hair manganese in normal infants at birth was reported to be 0.19 µg/g hair and, in breast-fed infants, increased to 0.330 µg/g at four months of age. By comparison, hair manganese levels in infants on a formula diet reached 0.965 µg/g at six weeks of age, and 0.685 µg/g at four months (Collipp et al., 1983). In addition, infants can have a less varied diet than adults and may consume more of certain foods that are high in manganese (e.g., sweet potatoes, 2.6 mg/cup; spinach, 1.8 mg/cup; oatmeal, 1.4 mg/cup; (NWU, 2006)).

6.5 Nutritional Requirement

Manganese is an essential nutrient involved in the formation of bone, and in amino acid, cholesterol, and carbohydrate metabolism (FNB, 2004). It is required in a number of metalloenzymes, including arginase, glutamine synthetase, phosphoenolpyruvate decarboxylase, and superoxide dismutase (FNB, 2004). Levels of manganese in adult tissues are maintained at stable levels by homeostatic mechanisms that involve regulation of both uptake and excretion (Aschner and Aschner, 2005). Manganese homeostasis is not maintained in newborn infants, and it is not clear how long it takes for it to develop (FNB, 2004); homeostasis in mice takes 17 to 18 days to become effective (Fechter, 1999). Rat pups born to manganese-exposed mothers (dosed with 2000 ppm Mn in drinking water throughout pregnancy and for 11 days of lactation) have seven times the manganese (whole body) as controls (Kostial et al., 2005). By weaning (11 days after birth) the manganese concentration in both groups is virtually the same, indicating that in rat pups manganese homeostasis may begin shortly after birth and become effective by weaning (Kostial et al., 2005).

Adequate intakes (AI) of manganese have been established by the Food and Nutrition Board of the Institute of Medicine (FNB, 2004). They are given in Table 6.5.1 below. This table also contains tolerable upper intake levels (UL) for manganese consumption. It is of note that in many cases the UL is not very far above the AI level. For children one to three years of age the UL is less than twice the AI.

The AI for infants 0 to 6 months was set based on the amount of manganese in human milk and the average amount of milk consumed. There are no reports of nursing infants showing any symptoms of manganese deficiency (FNB, 2004). The AI for infants 7 to 12 months of age is based on the manganese content of a typical diet including human milk and other foods. This AI is much higher than the one for infants 0 to 6 months because the manganese content of other foods is generally much higher than the manganese content of human milk (FNB, 2004).

Table 6.5.1 Adequate Intakes and Tolerable Upper Intake Levels for Manganese for Different Age Groups

Group	Adequate Intake (AI) (mg/day)	Tolerable Upper Intake Level (UL) (mg/day)
Infants, 0-6 months	0.003	“not possible to establish”
Infants, 7-12 months	0.6	“not possible to establish”
Children, 1-3 years	1.2	2
Children, 4-8 years	1.5	3
Boys, 9-13 years	1.9	6
Boys, 14-18 years	2.2	9
Girls, 9-13 years	1.6	6
Girls, 14-18 years	1.6	9
Men, 19 to >70 years	2.3	11
Women, 19 to >70 years	1.8	11
Pregnant women, 14-18 yrs	2	9
Pregnant women, 19-50 yrs	2	11
Lactating mothers, 14-18 years	2.6	9
Lactating mothers, 19-50 years	2.6	11

7. Developmental and Reproductive Toxicity

While data are scarce on the developmental effects of perinatal manganese exposure in humans, rats exposed to supplemental manganese (50, 250, 500 µg/day) beginning at birth show decreased dopamine in the striatum and poorer performance on behavioral tests (Tran et al., 2002b). This is consistent with studies examining manganese levels in various brain regions following developmental exposure. Female rats were exposed to MnCl₂ in drinking water (10 mg/ml) from the time of mating through weaning. The female offspring were similarly treated until sacrifice at 5, 10, 22, or 120 days postpartum (Chan et al., 1992). These time points represented the early postnatal period (day 5), the period of active myelination (days 10- 22), and sexual maturation (days 22-120). As shown in Table 7.1, manganese levels in all regions of the 5-day-old brain, except the cerebellum, are significantly elevated relative to unexposed controls. During the period of myelination (days 10-22), the manganese concentrations decreased. However, compared with controls, the concentrations in the treated rats were 2-13-fold higher, with the greatest difference in the striatum, followed by the midbrain. The differences in levels between groups decreased through sexual maturation. These data suggest that manganese distribution in the developing brain is heterogeneous and age-dependent, with the striatum and midbrain as potentially more susceptible regions for metal accumulation with high exogenous exposure.

Table 7.1 Brain Regional Mn Concentrations ($\mu\text{g/g}$ wet wt.) fr. Chan et al. (1992)

Brain Region		Postnatal Age (days)			
		5	10	22	120
Hypothalamus	Control	2.50 ± 0.33^c	0.52 ± 0.05^c	0.42 ± 0.01^c	0.30 ± 0.03
	Mn	$4.52 \pm 0.72^{c,e}$	$0.99 \pm 0.11^{c,e}$	$2.23 \pm 0.20^{c,e}$	1.11 ± 0.22^e
Cerebellum	Control	5.73 ± 0.28^c	3.97 ± 0.19^c	0.47 ± 0.16	0.38 ± 0.05
	Mn	4.95 ± 0.95^c	$6.16 \pm 0.11^{c,e}$	$1.32 \pm 0.09^{b,e}$	0.94 ± 0.07^e
Pons & medulla	Control	9.56 ± 1.16^c	4.73 ± 0.50^c	0.42 ± 0.01^b	0.35 ± 0.04
	Mn	$13.86 \pm 0.53^{c,e}$	5.00 ± 0.28^c	$1.46 \pm 0.05^{b,e}$	1.07 ± 0.23^e
Striatum	Control	12.05 ± 0.10^c	1.78 ± 0.10^c	0.12 ± 0.02^c	0.24 ± 0.03
	Mn	$12.86 \pm 0.54^{c,d}$	$3.72 \pm 0.13^{c,e}$	$1.57 \pm 0.24^{b,e}$	1.13 ± 0.24^e
Midbrain	Control	1.96 ± 0.27^c	1.51 ± 0.08^c	0.19 ± 0.04^c	0.38 ± 0.07
	Mn	$6.43 \pm 0.51^{c,e}$	$2.49 \pm 0.03^{c,e}$	$2.15 \pm 0.04^{b,e}$	1.35 ± 0.32^e
Cerebral cortex	Control	0.85 ± 0.20^c	1.15 ± 0.10^c	0.19 ± 0.04^b	0.34 ± 0.07
	Mn	$4.42 \pm 0.21^{c,e}$	$2.56 \pm 0.05^{c,e}$	$1.39 \pm 0.16^{c,e}$	0.62 ± 0.16^d

^a Values are the means \pm SD of 6-10 female rats ^b $P < 0.05$ compared to day 120 by ANOVA

^c $P < 0.01$ compared to day 120 by ANOVA ^d $P < 0.01$ compared to age-matched controls by t-test

^e $P < 0.001$ compared to age-matched controls by t-test

In children on long-term parenteral nutrition resulting in blood manganese levels of 615-1840 nmol/l (vs reference range of 72-210 nmol/l), elevated manganese levels have been seen in globus pallidus and subthalamic nuclei (Fell et al., 1996), suggesting an enhanced potential for neurological damage. This is consistent with the decrements in intellectual function in children exposed to manganese in drinking water reported by Wasserman et al. (2006).

The effects of manganese on reproduction in humans have been reported in epidemiological studies of workers with occupational exposure to manganese. The results have been mixed with Gennart et al. (1992) reporting no effect on fertility among workers exposed to a median manganese dust level of 0.71 mg/m^3 , while those exposed to $0.07\text{-}8.61 \text{ mg/m}^3$ (geometric mean 0.94 mg/m^3) in a study by Lauwerys et al. (1985) sired a statistically significant lower number of children during the period of paternal exposure. However, workers in the Gennart et al. study were exposed to the relatively insoluble manganese oxide and had mean urine manganese levels of $0.82 \text{ }\mu\text{g/g}$ creatinine. By comparison, the workers in the study by Lauwerys et al. were exposed to the more soluble manganese salts in addition to the oxide, and had mean urinary manganese levels of $4.37 \text{ }\mu\text{g/g}$ creatinine. Thus the differences in the effects of manganese on reproduction reported in these two studies may be due to the significant differences in manganese exposures.

Adverse changes in reproductive parameters and behaviors have been seen in studies of rodents exposed to high levels of manganese. In immature female rats (23 days old), manganese ($1\text{-}25 \text{ }\mu\text{g MnCl}_2$) introduced into the third ventricle of the brain significantly and dose-dependently stimulated the release of luteinizing hormone (LH). This effect

was apparently at the level of the hypothalamus as pretreatment with the LH releasing hormone (LHRH) receptor antagonist, acyline, prior to manganese exposure blocked the release of LH (Pine et al., 2005). These authors further reported that serum LH, follicle stimulating hormone, and estradiol were all elevated by 29 days of age in rats that had received $MnCl_2$ by gavage starting on postnatal day 12. In these animals manganese altered the timing of reproductive events resulting in a significantly ($p < 0.001$) earlier onset of puberty as measured by vaginal opening.

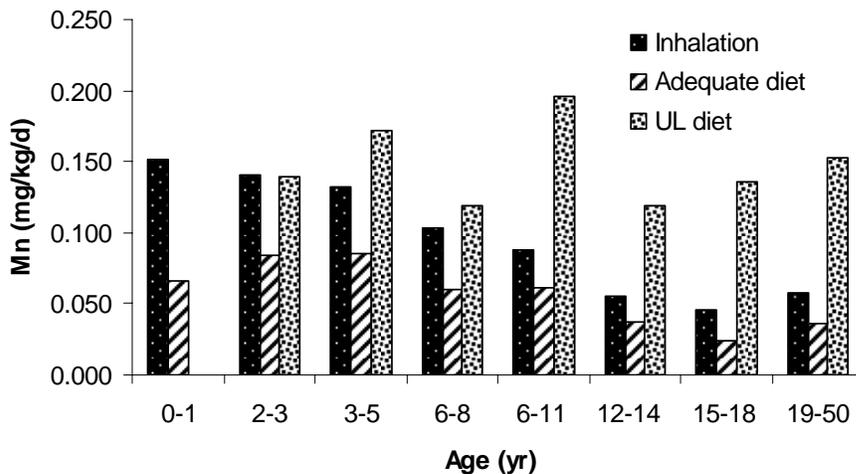
In adult male rats, exposure to 1,000 ppm manganese sulfate in drinking water for 12 weeks significantly suppressed sexual performance compared with controls as measured by prolonged ejaculatory latencies ($p < 0.001$), and increased post-ejaculatory intervals ($p < 0.05$). Displays of aggressive behaviors (lateralizations, boxing bouts, and fights with stud males) were also reduced ($p < 0.001$). The extent to which the altered behaviors represent neurological effects versus effects on testes and androgen production is not clear. However, among females mated to the manganese-treated males, the total number of resorptions was significantly increased ($p < 0.025$), suggesting a testicular effect. This is supported by a significant ($p < 0.001$) reduction in absolute and relative testes weights, and absolute seminal vesicle weights among manganese-exposed males (Bataneh et al., 1998). An effect of manganese on male reproductive organs was also investigated in mice following 43 days of oral manganese acetate (7.5 – 30 mg/kg/d) (Ponnapakkam et al., 2003). Unlike the study with rats above, there was no significant change in testicular weight or pathology with manganese exposure in the mice. Nor was there evidence of abnormal mating behavior. However, epididymal weights were significantly lower ($p < 0.05$) and there was a significant ($p < 0.001$) dose-dependent decrease in sperm number and motility.

The available data suggest that manganese is a reproductive toxicant in animals (both males and females) albeit at relatively high doses. Neurobehavioral toxicity manifests at levels encountered in the environment (Wasserman et al., 2006). Whether this decrement in intellectual function represents a true developmental effect with permanent consequences is not clear.

8. Derivation of Reference Exposure Levels

The determination of safe exposure levels to manganese is complicated by its status as an essential nutrient. However, as described above, inhalation of manganese results in a qualitatively and quantitatively different exposure compared to oral intake, with inhalation resulting in more rapid uptake and higher blood and brain levels. While dietary manganese levels moderate intestinal absorption of manganese, there appears to be no effect of dietary manganese on the pharmacokinetics of inhaled manganese (Dorman et al., 2002b). To provide perspective on potential manganese exposure from inhalation relative to the suggested upper limits for age-dependent dietary intake, we compared the potential manganese internal dose from inhalation with that from the recommended dietary levels for the various age groups indicated (Fig. 8.1). For this comparison, we used an air level of manganese of 0.215 mg Mn/m³, the average level of respirable manganese dust to which workers were exposed in an occupational study of the effects of manganese exposure (Roels et al., 1992). It thus represents a high but real world exposure level associated with neurotoxicity. Adequate dietary intake and the recommended upper limit levels, above which toxic effects may be observed, are those set by the Food and Nutrition Board (FNB, 2004). The data for inhalation represent the amount of manganese taken up by the different groups, age-adjusted for average breathing rates per kilogram body mass per day (OEHHA, 2000; Arcus-Arth and Blaisdell, 2007). For this example, we assumed that uptake from the lungs is 100% of the inhaled manganese, and that absorption from dietary intake is 41% for 0-1 yrs, 10% for 2-3 yrs, and 5% for all other ages. The inhaled manganese becomes the age-specific breathing rate multiplied by the air concentration. We compared the inhaled dose to the internal dose of manganese expected from an adequate diet, and from intake at the upper limit. This analysis suggests that among neonates and children through age 8, the manganese uptake from inhalation of this high level of manganese would substantially exceed the adequate dietary amount and may approach or exceed the levels beyond which toxicity may be expected. Therefore, compared with adults at the same exposure level, a child's manganese inhalation is a larger proportion of the maximum recommended levels. This is due to the higher breathing rates, lower body mass, and greater absorption of manganese by children. Thus, for comparable air exposures, children are more at risk for exceeding safe levels than are adults.

Figure 8.1 Internal Manganese Dose from Inhalation and Diet by Age



Age- and breathing rate-adjusted internal manganese dose following chronic inhalation of 0.215 mg/m³ manganese (Inhalation), consumption of recommended minimum daily intake (Adequate diet), or upper limit level beyond which toxic effects may be observed (UL diet). Data expressed as mg Mn/kg body weight/day.

8.1 Manganese Acute Reference Exposure Level

Acute Reference Exposure Levels (RELs) are levels at which intermittent one-hour exposures are not expected to result in adverse health effects (see Section 5 of the Technical Support Document (TSD)). Pulmonary damage and inflammation are the principal endpoints associated with acute inhalation exposure to manganese. However, at present the database is insufficient to allow the development of an acute REL for manganese based on inhalation studies. No studies were located that reported dose-response data for acute inhalation exposures, nor was it possible to determine both LOAELs and NOAELs from the available data.

8.2 Manganese 8-Hour Reference Exposure Level

<i>Study</i>	Roels et al., 1992
<i>Study population</i>	92 workers in a battery plant
<i>Exposure method</i>	Inhalation of workplace air
<i>Exposure continuity</i>	
<i>Exposure duration</i>	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
<i>Critical effects</i>	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	72 $\mu\text{g}/\text{m}^3$
<i>Time-adjusted exposure</i>	51 $\mu\text{g}/\text{m}^3$ (72*5/7)
<i>LOAEL uncertainty factor (UF_L)</i>	Not applicable
<i>Subchronic uncertainty factor (UF_s)</i>	$\sqrt{10}$ (default 8-12% of lifetime)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1 (default: human study)
<i>Toxicodynamic (UF_{A-d})</i>	1 (default: human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10 (greater absorption and lung deposition in children)
<i>Toxicodynamic (UF_{H-d})</i>	10 (greater susceptibility of children to neurotoxicity)
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	0.17 $\mu\text{g}/\text{m}^3$

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The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures (see Section 6 in the TSD).

The proposed 8-hr REL for manganese is 0.17 $\mu\text{g}/\text{m}^3$ based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992) described in Section 6.1. Data on the lifetime integrated exposure to respirable dust (LIRD) for each of the 92 workers and whether or not their response scores were abnormal on each of the three tests (visual reaction time, eye-hand coordination, and hand steadiness), were originally compiled by Dr. Roels, and were provided to OEHHA by Dr. J. Michael Davis of the US EPA. Abnormal scores were defined as those values that exceeded the 95th percentile value of the parameter as estimated from the cumulative frequency curves of the control group as in Roels et al. (1982). The LIRD was estimated for each worker based on the current airborne manganese concentration characteristic of each job multiplied by the number of years the worker had spent in that job. The result for each job held by an individual worker was then summed to obtain the LIRD for the individual. In our analysis, using US EPA's BMDS version 1.4.1b software, the LIRD (in $\mu\text{g}/\text{m}^3 \times \text{yr}$), divided by the total years of manganese exposure for each individual, was compared with his performance on each of the three tests (scored as 0 if the test was normal and 1 if abnormal). In this treatment, the group size is 1. Eye-hand coordination (EHC) and hand steadiness (HST) are the two most critical endpoints; the incidence of

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changes in the visual reaction time in the study population was consistently lower. For both these endpoints, the best fitting models were the Probit and Logistic models; the quality of fit was not distinguishable on statistical grounds. The lower 95% confidence bound benchmark confidence level (BMCL₀₅) for the Probit fit to the EHC data was selected as the most health protective value among these relatively closely spaced results.

<u>Eye Hand Coordination (EHC)</u>					
<u>Model</u>	<u>BMC₀₅</u>	<u>BMCL₀₅</u>	<u>BMC/BMCL</u>	<u>P for fit</u>	<u>AIC</u>
<u>Probit</u>	<u>97</u>	<u>72</u>	<u>1.4</u>	<u>0.3797</u>	<u>93.9124</u>
<u>Logistic</u>	<u>105</u>	<u>78</u>	<u>1.4</u>	<u>0.3874</u>	<u>93.8307</u>
<u>Hand Steadiness (HST)</u>					
<u>Model</u>	<u>BMC₀₅</u>	<u>BMCL₀₅</u>	<u>BMC/BMCL</u>	<u>P for fit</u>	<u>AIC</u>
<u>Probit</u>	<u>162</u>	<u>115</u>	<u>1.4</u>	<u>0.2201</u>	<u>68.2214</u>
<u>Logistic</u>	<u>175</u>	<u>125</u>	<u>1.4</u>	<u>0.2389</u>	<u>67.9531</u>

This analysis yielded a BMCL₀₅ of 72 µg Mn/m³. A time correction was applied since the workers in the study were only exposed 5 days per week, whereas the 8-hour REL is designed to protect against daily exposures, as noted in the TSD. A cumulative UF of 300 was applied, comprising a $\sqrt{10}$ for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty, resulting in an 8-hour REL of 0.17 µg Mn/m³. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF_{H-k} of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 µm size range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, neonates and infants more efficiently absorb and retain manganese than do adults (Dorner et al., 1989).

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A UF_{H-d} of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects.

The development of these RELs is based on a benchmark concentration analysis of data from an occupational study as described above. An alternative approach involves the use of physiologically-based pharmacokinetic (PBPK) models, the development of which in rats has been described in a recent series of papers by workers at the Chemical Industry Institute of Toxicology, now the Hamner Institutes for Health Sciences (Teeguarden et al., 2007a; Teeguarden et al., 2007b; Teeguarden et al., 2007c); (Nong et al., 2008). While these papers represent significant progress in the modeling of manganese pharmacokinetics, they have yet to be extended to and validated in humans or non-human primates. For this reason, they were not used for the estimation of these REL values.

8.3 Manganese Chronic Reference Exposure Level

<i>Study</i>	Roels et al., 1992
<i>Study population</i>	92 workers in a battery plant
<i>Exposure method</i>	Inhalation of workplace air
<i>Exposure continuity</i>	
<i>Exposure duration</i>	8 hr/day, 0.2-17.7 yr (mean 5.3 yr)
<i>Critical effects</i>	Impaired neurobehavior: visual reaction time, eye-hand coordination, hand steadiness
<i>NOAEL</i>	not observed
<i>Benchmark concentration</i>	<u>72</u> $\mu\text{g}/\text{m}^3$
<i>Time-adjusted exposure</i>	<u>26</u> $\mu\text{g}/\text{m}^3$ (<u>72</u> $\mu\text{g}/\text{m}^3$ x 10/20 x 5/7)
<i>LOAEL uncertainty factor (UF_L)</i>	Not applicable
<i>Subchronic uncertainty factor (UF_s)</i>	$\sqrt{10}$ (default 8-12% of lifetime)
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1 (default: human study)
<i>Toxicodynamic (UF_{A-d})</i>	1 (default: human study)
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10 (greater absorption and lung deposition in children)
<i>Toxicodynamic (UF_{H-d})</i>	10 (greater susceptibility of children to neurotoxicity)
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	<u>0.09</u> $\mu\text{g}/\text{m}^3$

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The chronic Reference Exposure Level is a concentration at which adverse noncancer health effects would not be expected from continuous lifetime exposures (see Section 7 in the Technical Support Document).

The proposed chronic REL for manganese of 0.09 $\mu\text{g}/\text{m}^3$ is based on impairment of neurobehavioral function in humans in the occupational study of Roels et al. (1992). The benchmark dose approach was used, as described above for the 8-hour REL, to derive a BMCL₀₅ of 72 $\mu\text{g}/\text{m}^3$. This corresponded to a time-adjusted concentration of 26 $\mu\text{g}/\text{m}^3$ (based on an 8 hour TWA occupational exposure to 10 m^3 manganese-contaminated air per day out of 20 m^3 total air inhaled per day over 5 days/week).

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A cumulative UF of 300 was used, comprising a $\sqrt{10}$ for subchronic to chronic conversion (average exposure duration = 5.3 yr; Section 4.4.6 of the TSD), and 10 each for intraspecies toxicokinetic (UF_{H-k}) and toxicodynamic (UF_{H-d}) uncertainty. This REL is based on healthy adult male workers with adjustments for the potentially greater susceptibility of children. The intraspecies UF_{H-k} of 10 was chosen in part to reflect the 3-4-fold greater deposition of inhaled particulates in the 1-10 μm range in the lungs of neonates relative to adults exposed to similar particulate levels in ambient air (Ginsberg et al., 2005). In addition, neonates and infants more efficiently absorb and retain manganese than do adults (Dorner et al., 1989). It should also be noted that the effects

reported in the Roels study were to a relatively insoluble form of manganese, MnO_2 . As shown in Table 4.1 above, exposures to similar levels of the more soluble $MnCl_2$ by the same route result in higher manganese brain levels.

A UF_{H-d} of 10 is used to address the expectation that the still-developing brain of newborn and infant children is more sensitive to the effects of manganese and that injuries to the nervous system during development are anticipated to have lasting effects. This REL was developed with specific consideration of the potentially greater susceptibility of children to manganese neurotoxicity. For comparison, the RfC for chronic manganese inhalation developed by the US EPA is $0.05 \mu g/m^3$ (U.S.EPA, 1993) and is also based on Roels et al. (1992).

8.4 Manganese as a Toxic Air Contaminant

In view of the potential for high exposure coupled with a lower ability to regulate manganese, and enhanced neurodevelopmental susceptibility leading to differential impacts in infants and children identified in Section 6.2.1, OEHHA recommends that manganese be identified as a toxic air contaminant which may disproportionately impact children pursuant to Health and Safety Code, Section 39669.5(c).

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