Manganese Toxicity Upon Overexposure: a Decade in Review

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Abstract Exposure to manganese (Mn) causes clinical signs and symptoms resembling, but not identical to, Parkinson's disease. Since our last review on this subject in 2004, the past decade has been a thriving period in the history of Mn research. This report provides a comprehensive review on new knowledge gained in the Mn research field. Emerging data suggest that beyond traditionally recognized occupational manganism, Mn exposures and the ensuing toxicities occur in a variety of environmental settings, nutritional sources, contaminated foods, infant formulas, and water, soil, and air with natural or man-made contaminations. Upon fast absorption into the body via oral and inhalation exposures, Mn has a relatively short half-life in blood, yet fairly long half-lives in tissues. Recent data suggest Mn accumulates substantially in bone, with a half-life of about 8–9 years expected in human bones. Mn toxicity has been associated with dopaminergic dysfunction by recent neurochemical analyses and synchrotron X-ray fluorescent imaging studies. Evidence from humans indicates that individual factors such as age, gender, ethnicity, genetics, and pre-existing medical conditions can have profound impacts on Mn toxicities. In addition to body fluid-based biomarkers, new approaches in searching biomarkers of Mn exposure include Mn levels in toenails, noninvasive measurement of Mn in bone, and functional alteration assessments. Comments and recommendations are also provided with regard to the diagnosis of Mn intoxication and clinical intervention. Finally, several *hot* and promising research areas in the next decade are discussed.

Keywords Manganese · Biomarker · Toxicity · Environment · Parkinsonism

Abbreviations

Cu	Copper
DMT1	Divalent metal transporter-1
EDTA	Ethylenediaminetetraacetic acid
Fe	Iron
MMT	Methylcyclopentadienyl manganese tricarbonyl
Mn	Manganese
PAS	Para-aminosalicylic acid
PI	Pallidal index
XRF	X-ray fluorescence

Introduction

Manganese (Mn) is the 12th most abundant element on the earth [1]. As a transition metal, Mn exists in more than five valence states, with a majority as Mn^{2+} or Mn^{3+} [2]. In the environment, it is found mainly in its oxidized chemical forms, as MnO_2 or Mn_3O_4 [3]. Mn is essential to human health, acting as a co-factor in the active centers of various enzymes, and is required for normal development, maintenance of nerve and immune cell functions, and regulation of blood sugar and vitamins, among other functions [4–6]. Overexposure to this metal, however, can be toxic to many organ systems and across different life stages.

In 2004, we summarized the impact of Mn exposure on general human health [6]. At the time, a majority of evidence on Mn intoxication came from occupational settings, because

of high exposure levels. Over the past decade, much progress has been made in the Mn research field, from toxicokinetics to exposure assessment and from the mode of action to clinical therapeutic intervention. Recent studies from this and other laboratories have indicated that low-level occupational exposure, with air Mn concentrations at or below occupational standards, can also be detrimental. Neurochemical, neurobehavioral, and neuroendocrine changes may occur before structural damage occurs and are linked to pathogenic conditions [7–12]. In addition to the exposure level and duration, there are other unique factors, such as age, gender, ethnicity, genetics, location, and pre-existing medical conditions that may contribute to Mn toxicity.

This article seeks to provide a comprehensive review of the new insights into environmental Mn exposure gained in the last decade. The current understanding of Mn toxicokinetics and its distribution in the brain by using advanced synchrotron X-ray fluorescence imaging technique will be first introduced. The advantage and disadvantage of using bone Mn levels as a potential indicator for Mn body burden will be addressed. This will be followed by a general review of the updated knowledge on Mn systemic toxicities including effects on the brain, the liver, and the cardiovascular system. Finally, comments and recommendations will be made with regard to the diagnosis of Mn intoxication and clinical intervention.

Absorption, Distribution, and Elimination

The highest concentrations of Mn are present in the bone, liver, kidney, pancreas, and adrenal and pituitary glands [13]. The normal concentration of Mn in human tissues is 1 mg/kg in the bone [14], 1.04 mg/kg in the pancreas, and 0.98 mg/kg in the kidney cortex [13]. The normal blood Mn concentrations range from 4 to 15 μg/L in humans [15]. A recent survey among the general Chinese population suggests that women have a higher blood Mn level than men ($\sim$28.6 %) [16]. In the body, Mn is transported and regulated by several macromolecules (Table 1).

Chemical Species of Mn in Body Fluids

In the human body, Mn exists primarily in two oxidized states, i.e., Mn^{2+} and Mn^{3+}. Mn^{2+} species in the blood are bound to high-molecular-mass fractions, such as albumin and β-globulin as hydrated ions, and also in complexes with bicarbonate, citrate, and other low-molecular-mass species [32, 33]. Nearly 100 % of Mn^{3+} species are bound to transferrin (Tf), to form a more stable complex [34]. Mn molecules in tissues such as the liver, kidney, pancreas, bone, and brain exist primarily as Mn^{2+} [6].

In the cerebrospinal fluid, Mn^{2+} ions are bound to low-molecular-weight compounds, such as Mn citrate [34]. This form is thus thought to be transported by a citrate transporter [35]. More evidence, however, suggests that Mn^{2+} species are transported mainly by the divalent metal transporter DMT1 as the primary influx route to the brain, although the other transporting proteins such as ZIP8 are suggested to mediate Mn transport into the brain [4, 36]. Evidence in literature has also suggested that Mn^{2+} can enter the brain by store-operated calcium channels, but the extent of this route is much less than that of transporter-mediated transports [35]. The other Mn species entering the brain is Mn^{3+}, which is complexed with

Table 1 Proteins involving in maintaining Mn homeostasis

Name and abbreviation	Mn species bound	Function	Reference
Divalent metal transporter (DMT1/SLC11A2)	Mn (II)	Mn uptake	[17]
Transferrin (Tf)	Mn (III)	Mn uptake	[18]
Tf receptor (TfR)	Mn (III)	Mn uptake	[18, 19]
Citrate	Mn (II)	Mn uptake	[20]
ZIP8 (SLC39A8)	Mn (II)	Mn uptake	[21]
ZIP14 (SLC39A14)	Mn (II)	Mn uptake	[22]
Voltage regulated calcium channels	?	Mn uptake	[23]
Ionotropic glutamate receptor—calcium channels	?	Mn uptake	[24]
Store-operated calcium channels	Mn (II)	Mn uptake	[25]
SLC30A10	?	Mn efflux	[26]
Ferroportin (SLC40A1)	Mn (II)	Mn efflux	[27]
Metallothionein	?	storage protein	[28]
Iron regulatory protein-1 (IRP1)	Mn (II)	Mn can replace the 4th Fe in the 4Fe-4S Enzyme action center	[29]
Ceruloplasmin	Mn (II)	Potentially oxidizes Mn (II) to Mn (III)	[30]
Superoxide dismutase	Mn (II)	Oscillates between Mn (II) and Mn (III) species	[31]

Question mark indicates uncertainty

transferrin and via the transferrin receptor (TfR)-mediated process (Table 1) [19].

Absorption

The main route of Mn absorption is through the gastrointestinal tract, but the absorption also occurs in the lungs following inhalation exposure [1]. The intravenous injection of illegal narcotics containing Mn has recently provided a third route of exposure [37].

Inhalation exposure to airborne Mn is common among welders and smelters [38–40]. Inhaled Mn can bypass the liver to enter the blood stream; from there, it can enter the brain via the olfactory tract bypassing the blood-brain barrier [41, 42]. Studies in rats demonstrate that Mn is rapidly transported along the evolutionarily conserved olfactory pathway and is present within the olfactory bulb 8–48 h after exposure. It is believed that the trigeminal nerve may also play a role in delivering Mn from the nasal cavity to the brain [38, 42, 43].

Oral exposure is another common route of exposure. Mn is required in small quantities obtained through dietary intake. The average daily intake for many Western diets is between 2.3 and 8.8 mg [44], but this can be much higher. Consumption of food or water contaminated with high levels of Mn has toxic consequences [45]. For example, the water supply in Bangladesh is contaminated with Mn up to 2.0 mg/L [46], which is fourfold higher than the WHO standard for drinking water of 400 µg/L [47]. Studies among school children suggest that increased levels of Mn in the drinking water in Bangladesh area are inversely associated with students' achievement scores in mathematics [48]. High levels of Mn in drinking water in Canada have been found to lead to significantly higher levels of Mn in hair samples in school-age children. The increased hair Mn concentrations are significantly associated with increased hyperactive behaviors [49], impaired cognitive development [47], and a decrease in IQ points [50]. In Italy, school-age children living near a ferroalloy plant have been found to have significant impairment of motor coordination, hand dexterity, and odor identification after exposure to excess levels of Mn in soils [51]. It is alarming that the high Mn concentration in drinking water is not solely a public health issue unique to developing countries; approximately 5.2 % of the 2167 wells surveyed across the USA exceeded the health benchmark of 300 µg/L [52].

Another potential source of oral exposure is from consuming milk- or soy-based infant formulas, which contain high levels of Mn. The FDA sets a minimum nutritional requirement of 5 µg/100 kcal for the amount of Mn infant formulas must contain; yet, there is no maximum established. According to the Institute of Medicine's recommendation, infants can consume about 3 µg Mn/day for 0–6 months. Infants can drink up to a liter of formula a day. When formula is prepared according to the manufacturer's instructions, infants could consume from 32 to 51 µg of Mn per day, far exceeding the aforementioned recommendation. Soy-based formulas contain more Mn than cow-based formulas, and both contain much more Mn than does human breast milk [53]. Since only a small percentage of Mn is eliminated in human breast milk and because breastfed babies consume smaller volumes of milk than do bottle-fed babies at each feeding [54], feeding breast milk is considered much safer than feeding formulas to infants. It is also known that the concentrations of Mn in a mother's milk decrease as lactation progresses. Laboratory testing has shown that babies who drink formulas had higher concentrations of Mn in hair samples than those who were breastfed [55]. The higher level of dietary Mn intake has been suggested to be associated with the risk of developing the attention deficit hyperactivity disorder (ADHD) [56].

Recently, cases of Mn-induced Parkinsonism have been reported among intravenous ephedrone abusers in Estonia, Turkey, Eastern Europe, the Baltic States, and Canada [19, 57]. Mn is added to the drug cocktail as the oxidizing agent potassium permanganate; the final Mn concentration can be as high as 0.6 g/L. Multiple injections per day can result in doses ranging from 60 to 180 mg/day by intravenous administration. This amount far exceeds the 0.1 mg Mn/day recommended as an intravenous supplement. Continued uses can lead to elevated Mn concentrations in blood and urine, and patients have signs and symptoms such as impaired speech, cockwalk, bradykinesia, and ataxia [37]. Even after cessation of ephedrone use, some of the motor symptoms continue to progress [37, 57].

Distribution

Once Mn enters the circulation from either the small intestine or lung, it accumulates mainly in the liver (1.2–1.3 mg/kg), brain (0.15–0.46 mg/kg), and bone (1 mg/kg up to 43 %) [13, 14, 58, 59]. Mn is detectable in the cerebrospinal fluid before it is detectable in the brain parenchyma, suggesting that it is transported through the choroid plexus [60].

The brain is the target organ of Mn toxicity. In human subjects exposed to Mn in the work place, magnetic resonance imaging (MRI) studies have established higher levels of Mn accumulation in the globus pallidus than in other brain structures [9, 61]. Rapid advancement in synchrotron X-ray fluorescence (XRF) imaging technique has made it possible to illustrate the Mn distribution pattern in the brain. In rat brains, Mn accumulates with the highest concentration in the globus pallidus, followed by the substantia nigra pars compacta, thalamus, caudate putamen, axon bundles, and cortex [62]. While the hippocampus does not accumulate more Mn than other regions in control animals, Mn exposure in fact increases hippocampal Mn to the same level as those in the substantia nigra pars compacta and thalamus. Thus, it appears that the hippocampus has an equal susceptibility to Mn toxicity. Moreover,

the XRF data show that Mn tends to accumulate in brain regions that also have a high iron (Fe) concentration [63].

Mn concentrations are thought to be greater in astrocytes than in neurons [57]. However, the XRF data from single cells show a diffuse Mn distribution pattern within cells of the hippocampus CA3, which are likely neurons. Since only 30 % of astrocytes are saturated after Mn exposure, it seems unlikely that astrocytes serve as the primary target of Mn accumulation in the rodent model [63].

In addition to the brain and liver, Mn accumulates extensively in the human bone under normal physiological conditions [64]. By examining the human bone collected during autopsy, it is estimated that bone contains about 40 % of the total body burden of Mn [65]. Our recent study in rats has shown that after subchronic oral exposure to Mn, Mn accumulates in the femur, tibia, humerus, and parietal bone with accumulation reaching steady-state concentrations after 6 weeks of dose administration [66••].

Mn is intracellularly distributed in red blood cells due to the presence of transferrin receptor and DMT1 in this cell type [6, 61]. Inside of the cell, Mn acts on the mitochondria and disrupts energy production [67–69]. But mitochondria may not be the major intracellular organelles where Mn ions accumulate. Morello and colleagues used electron spectroscopy imaging and demonstrated that the highest concentrations of Mn were present in the heterochromatin and the nucleolus, followed by a lower concentration of Mn in the cytoplasm, with the lowest levels in the mitochondria. After chronic Mn exposure, the highest levels of Mn were observed in the mitochondria [70].

In a comparative in vitro study utilizing choroidal epithelial Z310 cells, rat brain endothelial RBE4 cells, and dopaminergic N27 and PC12 cell lines, cells were fractionated to separate the nuclei and mitochondria. After Mn exposure, the highest levels of accumulation were found in the PC12 and N27 neuronal cell types compared with the non-neuronal brain barrier Z310 and RBE4 cell types. Most Mn was present within the nuclei, which was true for all four cell lines; only limited accumulation was observed in the mitochondria (<0.5 %) and microsomes (<2.5 %) [71]. Nonetheless, the profound Mn toxicity on mitochondrial function should not be underestimated.

Elimination

The primary route of Mn elimination is via the fecal hepatobiliary excretion with limited urinary excretion [72]. Some Mn-containing molecules such as Mn-DPDP and Mn nanoparticles show different elimination patterns from the metal Mn [73–75]. Mn is also eliminated in milk as mentioned above. However, this route of elimination does not constitute a major route of Mn excretion. Similarly, very low levels of Mn are excreted in sweat [76].

In the brain parenchyma, Mn rapidly accumulates in the brain structures such as the superior and inferior colliculi, amygdala, stria terminalis, hippocampus, and globus pallidus. The half-lives of Mn in these tissues are about 5–7 days, with the longest retention in the periaqueductal gray, amygdala, and entorhinal cortex [77]. The elimination rate from brain tissue is expected to be slower than from either liver or kidney. In the rat, the half-lives of 16 brain regions are between 52 and 74 days [6].

In a recent study in rats, we administered Mn by oral gavage at 50 mg/kg for 10 weeks. It was interesting to observe that by the fourth week of dose administration, Mn in blood reached the steady-state concentration, which was maintained for the duration of the study. Mn concentrations in the cerebrospinal fluid, however, continued to increase even at the eight week. It is possible that a slow elimination of Mn from the cerebrospinal fluid may contribute to the high level of Mn in the brain [66••]. It is also possible that a redistribution of Mn from the bone compartment to the central nervous system may account, at least partially, for the high level of Mn in the cerebrospinal fluid. By studying the elimination rate constant and half-lives, our data revealed that the half-lives of Mn in various rat bones were between 77 and 690 days with an average of 143 days for the whole skeleton [66••]. A comparative study between human and rat estimates that every 16.7 days of a rat's life is equivalent to one human year [78]. By using this figure, the range of Mn half-lives in the rat skeleton is estimated approximately 4.6–41.3 years in humans with an average half-life of 8.6 years for humans [66••].

Human Exposure to Mn

The primary source of clinically identified Mn intoxication is due to occupational exposure. Neurotoxicity due to inhalation exposure to airborne Mn has been reported in miners in Mn dioxide mines [79], workers in dry-cell battery factories [80], smelters [7, 8, 39, 61, 81], and steel manufacturing workers or welders [82–86]. Our own studies on 3200 welders in 142 factories in the metropolitan area of Beijing reveal a significant correlation between airborne Mn level and manganism among welders with an estimated exposure dosages (calculated by the weight of welding rods) of 5–20 kg (containing 0.3–6 % Mn) per working day per person [6, 87].

There are many environmental sources of Mn, which include eroded rocks, soils, and decomposed plants. Human activities expose individuals to additional sources containing Mn, including the fungicides, maneb and mancozeb, medical imaging contrast agents, and water purification agents. Additionally, several countries including the USA, Canada, Argentina, Australia, Bulgaria, France, Russia, New Zealand, China, and the European Union have approved use of the fuel additive methylcyclopentadienyl manganese

tricarbonyl (MMT) [1, 34]. Combustion of gasoline containing MMT releases Mn phosphates, sulfates, and oxides into the air, especially where there is high traffic density releasing particles within the respirable size range [1, 88]. Mn-containing emissions contaminate soil, dust, and plants near roadways, which introduces additional Mn to the environment [89]. Recent projections of MMT use indicate the average person's Mn absorption may increase by several percent. It should be noted that this is an estimated average level of exposure; therefore, some people may be exposed more substantially than others [88].

Ultimately, Mn from these various sources ends up in the water supply. As Mn filters down through the soil, it is reduced to the more soluble Mn^{2+} form where it can easily make its way into the ground and surface waters. Ground water has the highest concentration of Mn, but surface water and water near mining operations contain high levels of Mn as well [1].

Manganese-Induced Toxicities

Mn-Induced Neurotoxicity

Cumulative evidence has established that Mn exposure induces signs and symptoms similar but not identical to Parkinson's disease [39, 57, 90–93]. A study on six manganism patients who were occupationally exposed to Mn as welders or smelters in Guangxi, China, suggested that Mn exposure led to clinical manifestations of Parkinsonian syndromes with considerable variations. One patient who had a classic presynaptic syndrome and responded to L-DOPA was clearly Mn intoxicated. Moreover, a case with a 25-year Mn exposure showed a syndrome of Parkinsonism at an early age with MRI abnormalities bilaterally in the globus pallidus [92, 93]. Thus, these observations support an overlap in syndromes between Mn-induced movement disorder and Parkinson's disease [90–93].

While the linkage between manganism and Parkinson's disease is noteworthy, animal studies suggest that dopaminergic neurons in the substantia nigra and their terminals in the striatum, which are selectively lesioned in Parkinson's disease, remain intact after Mn intoxication [5]. Thus, changes in neurotransmission, rather than a massive dopamine neuronal cell loss, likely underlie behavioral observations.

Reports of Mn exposure altering neurotransmitter and metabolite levels have been published in literature [94, 95•]. To investigate the changes in dopamine, dopamine metabolites, such as 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and GABA, in rat brain after Mn exposure, we exposed rats subchronically with intraperitoneal injections of 15 mg Mn/kg for 4 weeks. Data showed a significantly increased dopamine level in the striatum; this increase was accompanied by increased levels of DOPAC and HVA in the same region. Interestingly, the HVA level was also increased in the substantia nigra and hippocampus, indicating an increased dopamine turnover in the substantia nigra, which is the pathogenic region in Parkinson's disease. In the same animals, a significant increase of GABA in the hippocampus was also evident, although no structural abnormalities appeared to be identifiable in the striatum, substantia nigra, or hippocampus in response to the low-level subchronic Mn exposure [96]. In agreement with our report, Vorhees and colleagues recently showed that Mn exposure increased striatal dopamine and HVA concentrations compared to controls. They also observed an increased norepinephrine in the striatum and increased dopamine, NE, and serotonin levels in the hippocampus. By utilizing various ages of animals, these investigators reported that Mn exposure altered monoamines as a function of age [97].

In a human study utilizing magnetic resonance imaging and spectroscopy (MRI/S) to investigate changes in neurochemistry of smelting workers, increases in GABA and decreases in myo-inositol were seen in the thalamus. Changes in thalamic GABA were associated with reduced fine motor performance as assessed by the Purdue Pegboard test [98].

Recent investigation of Mn neurotoxicity has also extended to the field of adult neurogenesis, which takes place in two critical niche areas in the brain, i.e., the subventricular zone and the subgranular zone. Application of the synchrotron X-ray fluorescent imaging technique to study brain distribution of copper (Cu) and Fe with or without Mn exposure has led to an unexpected discovery that Cu accumulated in the subventricular zone extraordinarily higher than in any other brain regions [99]. Further in vivo studies revealed that subchronic Mn exposure in rats greatly increased the cell proliferation in the subventricular zone and the associated rostral migratory stream, but significantly reduced the Cu levels in the subventricular zone [100•]. These observations raise interesting questions as to what is the role of Cu in adult neurogenesis and how Mn, by interacting with Cu for its transport, intracellular storage, and trafficking, may alter the normal neuronal repair process, which may contribute to nonmotor symptoms in Mn-induced Parkinsonian disorder.

Kikuchihara and colleagues further confirmed that oral Mn exposure resulted in reduced numbers of local Pvalb (+) GABAergic interneurons in the other neurogenic niche, the subgranular zone of the dentate gyrus in the hippocampus of mice [101]. Similar to the data published by our group, Kikuchihara's group also observed a reduced Cu level in the subgranular zone after Mn exposure, although differences between these two studies in animal species, exposure route, and duration are evident. Since Mn exposure results in reduced Cu levels in both neurogenic niches, these two independent studies may suggest a similar molecular mechanism underlying Mn neuropathology.

Mn-Induced Cardiovascular Toxicity

Despite a lack of epidemiological evidence, animal and human evidences support the view that Mn exposure significantly alters cardiovascular function. Intravenous injection of Mn at a high dose (5–10 mg Mn/kg) caused a decreased heart rate and blood pressure and increased P-R and QRS intervals [102]. In perfused rat hearts, an MRI contrast agent Mn-DPDP had similar but reduced effects on cardiac function as compared with Mn^{2+} [103]. Limited data from human populations are available, but it somewhat contradicts the data from animal studies. As opposed to the decreased blood pressure and heart rate observed in animal studies, smelters showed significantly faster heart rates than control subjects. Additionally, while animal studies showed increased P-R intervals, the reverse was true for the smelters, although the QRS and T waves were wider and elevated in both male and female smelters compared to controls [68].

Overexposure to the MRI enhancer Mn-DPDP causes flushed face and the head and ears feeling hot. Postural hypotension has also been observed in Mn-DPDP-overdosed patients [68]. Even when cardiac function is not significantly altered, the mean diastolic blood pressure can be significantly lower, while and diastolic hypotension can be significantly higher, in Mn-exposed workers as compared to control subjects. Workers with the highest level of exposure to Mn exhibit the lowest systolic blood pressure [68].

Despite differences in the levels of exposure between human and animal studies, it appears that Mn exposure inhibits myocardial contraction, dilates blood vessels, and induces hypotension, suggesting that Mn exposure has a significant effect on cardiac function. The exact mechanism of cardiac toxicity remains unknown; it has been shown that Mn has a direct effect on mitochondrial function resulting in a reduced myocardial contraction, and causes vasodilation, leading to a decreased blood pressure following acute exposure [68]. However, the research evidence on whether and how chronic low-level Mn exposure causes cardiovascular toxicities from both human and animal studies remains sparse. Future work to evaluate these effects is well warranted.

Mn Exposure and Infant Mortality

Increased Mn levels in water sources have been linked to increased infant mortality. An analysis of groundwater concentrations in North Carolina reveals that infant mortality increases by a factor of 2 per 1000 live births for every log increase in groundwater Mn concentration [104]. Hafeman et al. also report an increased mortality in the first year of life in infants in Bangladesh exposed to Mn concentrations at or above the WHO's standard of 400 μg Mn/L compared to unexposed infants [105].

Mn Toxicity and Liver Function

Since the original report by Klaassen in 1976 describing the hepatobiliary excretion of Mn from the liver [72], not much work has been done to describe Mn-induced hepatotoxicity. The liver is a known storage organ for Mn; the highest Mn uptake occurs in the liver, only second to brain uptake [36]. Hepatic Mn accumulation in mice intravenously injected with Mn nanoparticles persisted significantly longer than other highly perfused tissues such as kidney and spleen; however, no histopathological damage was observed [75].

Hepatobiliary excretion of Mn represents a primary route of Mn clearance from the body, accounting for 80 % of Mn elimination. Thus, severe liver damage, owing to various chronic liver diseases, can result in an excessive accumulation of Mn in brain with ensuing signs and symptoms clinically called Mn hepatic encephalopathy [106]. With weakened liver function, there is also an increased risk of neurodegeneration with continued Mn exposure [107]. In those patients with chronic hepatic encephalopathy, liver transplant has proven to be effective in reducing brain Mn concentrations. When patients were re-examined 5 months after transplant, the T1-weighted MRI signals in the basal ganglia were absent [106]. These data suggest that the normal liver function is essential to maintain homeostasis of Mn in the body, including the CNS.

Mn Toxicity and Individual Susceptibility

There are many factors that may predispose one individual to Mn toxicity over another. These individual factors include age, gender, ethnicity, genetics, and pre-existing medical conditions, such as chronic liver disease.

Age is a common factor which may influence an individual's susceptibility to Mn toxicity. Very young animals as well as humans have increased intestinal Mn absorption [97] and also have increased accumulations of Mn in the CNS [108], due to increased permeability of neuronal barriers to Mn [34]. The young also have a reduced biliary excretion capacity [56]. The 2011–2012 National Health and Nutrition Examination Survey (NHANES), a study of US residents, found higher Mn levels in the younger population, with the highest levels in 1-year-old infants [109]. These age-related factors can increase the risk of neurotoxicity following exposure.

Alternatively, the very old are a population of special concern, because of the large number of people who develop idiopathic Parkinsonism. Brain regions such as the globus pallidus, substantia nigra, and striatum are involved in both Mn neurotoxicity and Parkinsonism; thus, it is possible that the elderly may have a subclinical pathology and could be "pushed over the edge" by increased doses of Mn [110]. For example, in one of our occupational exposure studies, we found that smelters without clinical symptoms performed significantly worse on the Purdue Pegboard test, which is a

measure of fine motor coordination, than control subjects. The scores got worse with age, which was not unexpected as fine motor coordination declines with age. However, Mn exposure appears to exacerbate this decline [8].

Gender is another common factor which may influence an individual's susceptibility to Mn toxicity. The 2011–2012 NHANES study of US residents reported significantly higher blood Mn levels in women of all ethnicities than men. The authors suggest metabolic differences in the regulation of Mn between men and women may underlie the difference [109]. A recent study among the Chinese general population also indicates that women's blood Mn levels are about 29 % higher than men's [16], consistent with reports in the literature that Korean and Italian women's Mn levels are 25 % higher [111, 112] and Canadian women have about 23 % higher levels [113] than the respective men's population.

Gender may also be a contributing factor to developing cardiovascular toxicity after Mn exposure. In a study of male and female smelters exposed to Mn, female smelters had significantly shorter P-R intervals compared to controls, and there was no difference in males. QRS and T waves were also significantly different for female smelters [68]. Ethnicity could potentially be a factor that could influence susceptibility to Mn toxicity. In the 2011–2012 NHANES, the Asian population tended to accumulate significantly more Mn than either non-Hispanic Caucasians or non-Hispanic Black individuals [109].

Individuals with pre-existing neurological disease may be at special risk of developing Mn toxicity, because of the potential for combined insults. Persons with iron deficiency are of special concern, because animal evidence indicates that gastrointestinal absorption of manganese is enhanced by iron deficiency [110].

While pregnancy is not a pre-existing condition, it is a condition during which the susceptibility to Mn toxicity may be increased. Again, the 2011–2012 NHANES demonstrates that pregnant women accumulate higher levels of Mn than do other persons [109]. In a recent study of maternal blood Mn levels and neurodevelopment of infants at 6 months of age, researchers discovered significant associations between the mother blood Mn levels and their children's scores on mental and psychomotor developmental indexes. Interestingly, both high and low Mn blood levels were associated with lower scores [114]. Maternal blood Mn levels have also been shown to be associated with inhibited enzyme activity of newborn erythrocyte Ca pump at both low and high levels of maternal Mn [115]. A study conducted among pregnant women from Paris suggests that environmental exposure to Mn may increase the risk of preeclampsia. Mn cord blood concentrations in that study were significantly higher in women with pre-eclampsia [116].

From a mechanistic point of view, SLC30A10, a solute carrier (family 30 and member 10), has been suggested to regulate Mn export from the cells. This protein is highly expressed in the liver with a higher specificity for Mn than Zn. Genetic alterations in the SLC30A10 enzyme have recently been discovered. An autosomal-recessive mutation in this transport protein leads to an inherited Mn hypermanganesemia [26, 57] and results in a pleomorphic phenotype, including dystonia and adult-onset Parkinsonism [117].

Diagnosis and Clinical Intervention

Biomarkers of Mn Exposure

In Mn occupational exposures, the symptoms often develop quickly because the exposure levels are relatively high. In comparison, symptoms resulting from environmental exposures may be much more subtle and thus difficult to detect because they develop slowly, over a lifetime. Thus, it is crucial to detect these changes with a reliable biomarker in order to prevent the irreversible damage or the loss of function resulting from Mn toxicity. The biomarkers associated with monitoring Mn exposure in animal and human studies are summarized in Table 2.

Blood and urine are the most commonly used biological matrices for biomonitoring. However, the poor relationships between Mn concentrations in blood and urine and the external exposure levels make it very difficult to determine the internal exposure [120, 128••]. For example, the half-life of Mn in blood is less than 2 h [129]. Plasma Mn concentrations measured during the dosing phase of a chronic Mn exposure study began to decline after 2 weeks, although Mn exposure was still ongoing [66••]. Mn can be detected in human saliva samples. Our human study on Mn-exposed welders found that changes of saliva Mn concentrations mirrored those of serum Mn levels. But, because of a fairly large variation in saliva Mn levels, the authors did not recommend to use saliva Mn to assess Mn exposure [119]. Because more than 95 % of Mn is eliminated in bile to feces, urine Mn levels are expected to be very low [65]. For these reasons, we do not recommend using Mn levels in blood, urine, or saliva as the biomarkers of Mn exposure.

Attempts to identify additional non-invasive biomarkers have concluded that using hair and nail samples may be a possibility. In our own studies [7, 8], we collected hair and nail samples from smelters and control subjects. The data showed such a vast variation to the degree that we believe it would be misleading to report these data. A thorough, yet rapid process must be developed in order to eliminate the external contamination before hair and nail samples can be used in research. Regardless, studies of residents living near a ferromanganese refinery in Brazil have shown that significant correlations exist between hair and fingernail Mn levels and the performance on neuropsychological tests [130].

Table 2 Possible biomarkers of Mn exposure

Potential biomarkers	Measured by	Interpretation	Usefulness in epidemiological studies	Reference(s)
Blood (whole blood)	ICP-MS; AAS	Most commonly studied; reflects recent exposure; large variation	Limited	[15, 118]
Blood (plasma)	AAS	Short half-life may miss periods of peak exposure; large variation	No	[118]
Blood (serum)	AAS	Low concentration; large variation	No	[119]
Plasma Mn/Fe ratio	AAS	Good correlation to neurobehavioral changes; limited data	Possible	[7, 8]
Erythrocyte Mn/Fe ratio		Same as above	Possible	[7, 8]
Mn citrate		Difficult to measure; never tested in humans	unknown	[20]
Urine		No association between Mn inhalation and urinary Mn levels	No	[120, 121]
Saliva	ICP-MS	Partly changes in response to airborne Mn concentrations; large variation	No	[119]
Hair	ICP-MS	Susceptible to external contamination; cleaning methods may affect accuracy of measurement	Limited	[122–124]
Nails	ICP-MS	Correlated with brain Mn levels; large variation; external contamination issue	Possible	[121, 125]
Teeth (dentin)	ICP-MS	Characterizes prenatal and early postnatal Mn exposure; incorporated directly into developing dentin	Limited	[126]
Teeth (enamel)	IMS	Predicts exposure	Limited	[127]
Bone	AAS; NAA	Reflects body burden; technical possible	Yes	[66••]
Cerebrospinal fluid	AAS	Correlated with brain and bone Mn levels	Possible	[66••]
Breast milk			No	
Sweat			No	

ICP-MS inductively coupled plasma mass spectroscopy, *AAS* atomic absorption spectroscopy, *NAA* neutron activated analysis, *IMS* ion mass spectrometry

Grashow and colleagues have recently suggested using toenail Mn concentration as a biomarker of occupational welding fume exposure [131]; their study, however, did not relate the toenail Mn level to any biological outcomes.

In a study of Mn-exposed smelters, Mn concentrations in plasma and erythrocytes were found to increase with a corresponding decrease of Fe concentrations in plasma and erythrocytes [7, 8]. Since Mn concentrations reflect the environmental exposure and Fe concentrations reflect a biological response to Mn exposure, combining both parameters by dividing the Mn concentration by the Fe concentration (i.e., MnC/FeC) would enlarge the difference between groups and therefore increase the sensitivity. This thought process led to the development of a concept of Mn/Fe ratio in plasma (pMIR) or erythrocytes (eMIR) [7]. Because there is a significant correlation between pMIR and eMIR to airborne Mn concentration, both pMIR and eMIR appear to be good candidates as the biomarkers for Mn exposure assessment. Nonetheless, the same study also showed a better correlation between eMIR and low- or high-exposure outcomes [7]. The utility of pMIR in environmental exposure assessment requires more rigorous testing. Additionally, as Mn citrate in blood rapidly enters brain, elevated levels of plasma or serum Mn citrate may be a biomarker of elevated risk of Mn-dependent neurological disorders in occupational health [34].

A relatively long half-life (about 8–9 years in human) of Mn in the skeletal system (see above) renders bone Mn concentration an ideal indicator to assess the body burden of Mn.

The technical challenge has always been the development of equipment with appropriate sensitivity for such a purpose. The good news is that such a technology has now become a reality. In recently published manuscripts, Nie and colleagues have optimized and verified a neutron activation-based analysis (NAA) technique for non-invasive, real-time quantification of Mn concentrations in the bone. The equipment, at this writing, is compact enough to be transportable to the sites for testing human workers and subjects. The method is sensitive and can quantify Mn concentrations as low as 0.5 ppm of Mn in bone [14, 132] and recently even lower to 0.3 ppm (personal communication).

Another non-invasive technique that can be used to analyze Mn exposure in vivo is magnetic resonance imaging (MRI). Mn accumulation in the brain can be visualized as an increased T1-weighted *hyper-intense* MRI signal. By dividing the signal observed in the globus pallidus by the signal observed in the white matter in the frontal cortex and multiplying by 100, a pallidal index (PI) can be calculated to quantify Mn intensity. The PI has been proven to be a reliable marker for Mn exposure [9, 61]. Workers with more than 5 years' experience showed nearly 100 % occurrence of enhanced PI, suggesting that the PI is specific for Mn exposure even when no clinical symptoms are evident [61]. One downside for using MRI is that it is only good for recent exposures. In human studies of smelters or intravenous ephedrone users, the signal in the globus pallidus almost completely disappears 5–6 months after cessation of exposure [37, 61].

Magnetic resonance spectroscopy (MRS) is another useful technique to quantify neurochemical markers associated with Mn exposure [61]. Quantitation of GABA, glutamate, total creatine (tCr), and N-acetyl-aspartate (NAA)/tCr values, along with other macromolecules, has been made available by MRS. In the thalamus and basal ganglia of Mn-exposed smelters, levels of GABA were nearly doubled, whereas the mean air-borne Mn level was only 0.18 mg/m^3, which is below the occupational standard. This may indicate an early metabolic or pathological change associated with low-level Mn exposure, and MRS appears capable of detecting these biochemical changes before the full-blown symptoms become evident [9].

For animal researchers, recent advancement in the synchrotron X-ray fluorescent (XFR) imaging technique allows to visualize the concentration and distribution pattern of multiple metals in the brain. The technique can now reach the resolution down to the single-cell level [63].

Clinical Intervention

The foremost therapeutic strategy in treatment of Mn toxicity is to remove the patient from the source of the Mn exposure. If the intoxication is life threatening, the procedures to relieve the critical signs and symptoms should first be employed. For a thorough treatment, chelation therapies can help reduce the body burden of Mn, but such treatments may not be able to improve symptoms. Another possible therapy includes Fe supplementation.

Chelation of free Mn with intravenous ethylenediaminetetraacetic acid (EDTA) has been shown to increase Mn excretion in urine and decrease Mn concentrations in blood, but chelation does not significantly improve patients' clinical symptoms [6, 39]. A recent report by Tuschl et al. demonstrates that two patients with inherited hypermanganesemia who received EDTA chelation had a significantly increased urinary excretion of Mn. Whole blood Mn levels and the MRI signals in the globus pallidus were also reduced [57]. In vitro studies have documented that EDTA can effectively block toxic effects of Mn on mitochondrial oxygen consumption when added either before or after Mn exposure [132]. Thus, for the purpose of reducing Mn in the blood compartment in the initial emergency phase, EDTA has a therapeutic benefit. However, EDTA molecules are highly water soluble and poorly pass across the blood-brain barrier. The low brain bioavailability of EDTA limits its effectiveness in treatment of Mn intoxication [39].

Para-aminosalicylic acid (PAS) is an FDA-approved drug used for the treatment of tuberculosis. Studies mainly in Chinese patients show the promising effectiveness in treating severe Mn intoxication with promising prognosis [39]. Animal studies further verify its chelating effect in removing Mn from the body [133]. As a hard Lewis acid, Mn^{3+} can form a stable complex with hard donor atoms such as oxygen donors in PAS structure, while the Mn^{2+} cation prefers relatively softer donors such as nitrogen, which is also present in PAS structure. Thus, it is possible that PAS may form stable complexes with both Mn^{2+} and Mn^3 species and remove them from where they are stored. Moreover, the salicylate structure in PAS, which has a proven anti-inflammatory effect, may contribute to the therapeutic prognosis of PAS in treatment of manganism [39, 134]. Our recent studies also demonstrated that the parent PAS was found predominantly in blood and in choroid plexus tissues, whereas its metabolite N-acetyl-para-aminosalicylic acid (AcPAS) was found in the brain parenchyma, cerebrospinal fluid, choroid plexus, and capillary fractions [135]. Both PAS and AcPAS were transported in the brain by the multidrug resistance-associated protein 1 (MRP1), a member of the superfamily of ATP-binding cassette (ABC) transporters. However, the removal or efflux of PAS from brain parenchyma into the blood was mediated by the multidrug resistance protein 1 (MDR1), also called P-glycoprotein [136].

One additional therapy includes Fe supplementation. In a pilot study with a sample size of one, Tuschl et al. showed that Fe supplementation, in addition to chelation therapy, led to a marked improvement of neurological symptoms, whereas the chelation therapy alone did little to improve symptoms. The authors proposed that supplementing with Fe may help reduce blood Mn levels and lower Mn body burden [57].

Conclusions

The past decade is a thriving period in the history of Mn research. The total volume of publications related to *manganese toxicity* by a PubMed search in the last 11 years is 1619 (from our last published review on 1 April 2004 to this writing on 5 April 2015), which far exceeds the cumulative numbers of 1199 published papers on Mn toxicity for the past 167 years ever since Couper [79] reported on the first case of manganism in 1837 (~ to 31 March 2004). On a more fundamental level, the essence of what we consider to be a Mn exposure has undergone a significant change, from traditionally recognized occupational manganism to low-level Mn exposures in a variety of environmental settings, nutritional sources, contaminated foods, infant formulas, and water, soil, and air with natural or man-made contaminations. Cumulative evidence on Mn toxicities and the vast public interest in this metal speak volumes of its public health importance, calling for a thorough understanding of its risk, the mechanism of its harm, some forms of effective clinical interventions, and any applicable strategy for prevention. Thus, we predict that the research on Mn toxicity, or its nutritional benefit for that matter, is far from finished and will become even more productive in the coming decade. Several key developing areas are summarized below.

First, individual factors such as age, gender, and ethnicity can influence an individual's susceptibility to Mn toxicity. Children's susceptibility to Mn toxicity is of utmost concern as children accumulate higher levels of Mn and eliminate less Mn than adults. The toxic exposures tend to impact academic performance and biochemical processes. More research is deemed necessary in this area.

Second, Mn neurotoxicities, once the signs and symptoms appear, are usually irreversible and actually continue to progress, despite removal from the exposure scene. A long existing challenge in the Mn research has always been the search for an effective biomarker that is clinically useful for diagnosis or early diagnosis of Mn intoxication. Understandably, without such biomarkers, however wishful one would be, the risk assessment remains a futile task. Currently, several approaches, such as using Mn/Fe ratio, toenails, and hair, appear to be promising; yet, many of these and other approaches remain in their infancy, and more needs to be done.

Third, the recent progress in theory and technical development has made it possible for non-invasive assessment of bone Mn in humans. This approach is likely to generate innovative information not only for risk assessment but also for nutritional monitoring of Mn levels in children as well as adults. It is possible, and even likely, that Mn stored in bone may be released slowly over time and thus serves as an internal source of Mn exposure. Topics such as Mn and bone, its causes and consequences, interactions with other metals, and biochemical mechanism of its transport and storage, along with pertinent technical innovation, will become a hot area in Mn research.

Finally, in mechanistic investigation, recent observations of the disruptive effect of Mn on adult neurogenesis in both the subventricular zone and subgranular zone have identified a new direction in Mn toxicological research. Understanding how environmental exposures to toxic metals impact the proliferation, differentiation, and migration of neural stem/progenitor cells in the adult brain for neural repair and functional integrity should have profound implications not only for studying Mn neurotoxicity but also for a better grasp of other neurodegenerative diseases such as Parkinson's disease or Alzheimer's disease.

Compliance with Ethics Guidelines

Conflict of Interest The authors declare that they have no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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