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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, non-invasive 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

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

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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 (~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 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 sub-chronic 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 non-motor 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 preeclampsia [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]. 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 airborne 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.

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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

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•• Of major importance

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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

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

Permitting issue ends local firm's recycling of food scraps

Kevin Rector, krector@tribune.com

February 6, 2012, 1:44pm

A Woodbine company that had been processing food scraps into composted materials with commercial applications — a process lauded by state and local officials as the next great frontier in recycling — has ceased those operations after hearing concerns about pollution from the Maryland Department of the Environment.

The impact has been far reaching, causing a string of institutions and the Howard County government, which were all sending food scraps to the facility, to find other, out-of-state facilities to handle the material.

Recycled Green Industries, which is still processing yard waste at its Carroll County facility off Kabik Court, received a verbal request to stop its food waste operations from the department on Dec. 22 because it did not have correct permits or processes in place to handle food scraps, according to a department spokesman.

Food scraps present different environmental concerns than yard waste, the spokesman said.

Namely, food contains "nutrients and potential pathogens" not found in yard waste, and are harmful to the environment when washed into surface and ground water, said Jay Apperson, the spokesman, in an email.

The department followed its verbal request with a letter to the company Jan. 9 that outlined concerns and gave a 12-point plan for the company to mitigate problems and become properly permitted.

The letter said water samples taken by the department on or near the company's property "confirm that the operation is generating polluted leachate and storm water and is discharging pollutants without a permit in violation of state law."

The letter also said, "In addition to the nutrients and bacteria found through laboratory analysis of samples collected from the site, elevated levels of biochemical oxygen demand and low dissolved oxygen were also detected, indicating the presence of excessive organic pollutants in discharges from the site."

Current guidelines on composting practices in the state recommend composting operations be "containerized, or operated in a manner to prevent ground or surface water contamination."

According to Mike Toole, Recycling Green's business development manager, the company's food scrap operations, which began two years ago, were outside, and consisted of mixing the food scraps into large mounds of yard waste, at the ratio of one part food scraps per every 30 parts of yard waste.

After processing, the material was sold as a natural fertilizer. The company also creates mulches and other ground covers.

The company has always passed inspections by the environmental department's land management administration, and was unaware its composting process was not permitted correctly and did not meet requirements.

Officials of MDE's water management administration first visited the company's facility last summer, Toole said.

When told of the pollution concerns, the company "voluntarily ceased accepting food waste," he said.

Too costly to continue

Apperson said the company needs to obtain a permit that's in line with National Pollutant Discharge Elimination System protocols, as well as a state groundwater discharge permit and an air permit to run its concrete crusher.

The company may also need a mining permit, depending on the level of excavation intended for the property.

The department also spelled out steps the company would have to take to compost food materials, including installing a "low-permeability pad" or other surface, such as concrete, below the entire operation.

Until last week, Toole said the company was working to determine how to comply with the department's demands, but has since determined it'll be too costly to continue.

"We will have no choice but to abandon plans to re-engage in food waste recycling," he said.

Toole said he doesn't "understand what the difference is" between food scraps and the yard waste, and that composting shouldn't be lumped into the same category as waste disposal under state permitting.

Toole said the regulations are too complex and overbearing, and believes the company was already doing many things right.

"We're trying not to just open our arms and accept any and all food waste, by any stretch," he said.

Toole said that while losing the food scrap business did not have a major financial impact on the company, the company does see food scrap collection as having large potential moving forward — especially if more institutions and jurisdictions follow in the footsteps of its former food scrap clients like Howard County.

"We recognize the opportunity for growth in our business shows its greatest potential in food waste," Toole said.

Data from Howard County's pilot program show household waste dropped by about 25 percent among participating homes, county officials said.

Regulating confusion

Since Recycled Green had to stop accepting its scraps, Howard has had to divert thousands of pounds of materials collected through its program, which had been sending food scraps to the Woodbine facility since September, to a facility in Delaware, officials said.

Recycled Green's other food scrap clients — including the University of Maryland, College Park, the National Institutes of Health in Montgomery County, and American University and National Geographic in the District of Columbia — also had to find other facilities to deal with their scraps, Toole said.

Apperson said MDE is supportive of recycling food waste into compost — if it's done in the proper way.

But Toole is not the only one that thinks its regulations are confusing.

In fact, based on a bill introduced by Del. Heather Mizeur of Montgomery County and passed by the General Assembly last year, MDE is required this year to study composting in the state, and the laws and regulations that govern it, and report back to the General Assembly by Jan. 1, 2013.

Apperson said MDE is currently in the process of reviewing and updating its standards for composting.

Recycled Green Industries, LLC. From June through December of 2011, Recycled Green Industries accumulated food scraps and yard waste at its composting facility in Woodbine without controls in place to screen out inorganic refuse or to prevent pollution of ground and surface water, and without the required refuse disposal and discharge permits. MDE documented discharges of wastewater containing elevated levels of nutrients and bacteria from the facility. Recycled Green stopped accepting food waste in December 2011 and removed accumulated raw material and products containing food waste from the facility.

On March 5, 2013, MDE and Recycled Green entered into a settlement agreement and consent order to resolve violations of solid waste management, sediment pollution, and water pollution control. Under the consent order, the company agreed to perform a nature and extent of contamination study to determine the extent of groundwater and/or surface water pollution from its composting activities and to develop and implement a corrective measures plan to address any ongoing water pollution. Recycled Green will also submit a revised operations and maintenance plan, including procedures for screening incoming material and rejecting or properly disposing of materials that cannot be composted, for maintaining aerobic conditions in compost piles, and for ensuring that the facility meets the operational and product quality standards set by the Department of Agriculture. In addition, Recycled Green agreed

to pay civil penalties of $50,000; an additional penalty of $25,000 was held in abeyance pending completion of the required corrective action.

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Manganese neurotoxicity: new perspectives from behavioral, neuroimaging, and neuropathological studies in humans and non-human primates

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Manganese (Mn) is an essential metal and has important physiological functions for human health. However, exposure to excess levels of Mn in occupational settings or from environmental sources has been associated with a neurological syndrome comprising cognitive deficits, neuropsychological abnormalities and parkinsonism. Historically, studies on the effects of Mn in humans and experimental animals have been concerned with effects on the basal ganglia and the dopaminergic system as it relates to movement abnormalities. However, emerging studies are beginning to provide significant evidence of Mn effects on cortical structures and cognitive function at lower levels than previously recognized. This review advances new knowledge of putative mechanisms by which exposure to excess levels of Mn alters neurobiological systems and produces neurological deficits not only in the basal ganglia but also in the cerebral cortex. The emerging evidence suggests that working memory is significantly affected by chronic Mn exposure and this may be mediated by alterations in brain structures associated with the working memory network including the caudate nucleus in the striatum, frontal cortex and parietal cortex. Dysregulation of the dopaminergic system may play an important role in both the movement abnormalities as well as the neuropsychiatric and cognitive function deficits that have been described in humans and non-human primates exposed to Mn.

Keywords: manganese, neurotoxicity, Parkinson's disease, dopamine, motor function, cognitive function, working memory

INTRODUCTION

Manganese (Mn) is an essential trace metal that is required for a number of enzymes important for normal cellular functions (Aschner and Aschner, 2005). However, excess accumulation of Mn in the brain results in a neurological syndrome with cognitive, psychiatric and motor abnormalities (Pal et al., 1999; Olanow, 2004; Perl and Olanow, 2007; Guilarte, 2010). Following excess exposure to Mn, the highest concentrations of Mn in the brain occur in the basal ganglia, specifically in the globus pallidus, caudate/putamen, and substantia nigra (Dorman et al., 2006; Guilarte et al., 2006a). These same studies have shown that Mn also accumulates in other brain structures within the cerebral cortex and in white matter (Dorman et al., 2006; Guilarte et al., 2006a). The accumulation of Mn in the basal ganglia is likely to be responsible for a form of parkinsonism with overlapping, but distinct clinical features with those seen in idiopathic Parkinson's disease (PD) (see below). Recently, there has been a great deal of debate in the scientific literature regarding the possibility that Mn may have an etiological role in idiopathic PD or accelerate the expression of PD (Racette et al., 2001, 2005). From a different perspective during the last decade there is mounting experimental evidence that exposure to Mn, at lower doses than those needed to produce motor function deficits, has a significant effect on executive function and cognition (Klos et al., 2006; Schneider et al., 2006, 2009; Roels et al., 2013). In this review, I examine the available evidence from human and non-human primate studies on the impact of elevated Mn exposures and its effects on motor function and cognitive domains.

MANGANESE-INDUCED PARKINSONISM

The first description of Mn-induced parkinsonism goes back to 1837 when Couper provided the sequelae of workers employed in the grinding of Mn oxide ore (Couper, 1837). In more modern times, there has been a number of reports describing clinical expression of parkinsonism in occupationally exposed workers (Mena et al., 1967; Cook et al., 1974; Huang, 2007; also see studies in Perl and Olanow, 2007 and in Guilarte, 2010) with clear evidence that excess exposures to Mn produces motor function deficits in humans and non-human primates that resemble some aspects to those expressed in idiopathic PD (Perl and Olanow, 2007; Guilarte, 2010) as well as more subtle effects on motor function, specifically fine motor control depending upon the level of exposure (Perl and Olanow, 2007; Guilarte, 2010). However, there are clear differences between Mn-induced parkinsonism and idiopathic PD from a clinical perspective and in the underlying neuropathology (Perl and Olanow, 2007; Guilarte, 2010) (see next section).

The most compelling human evidence of Mn-induced parkinsonism in the last decade comes from a very unfortunate human experiment in which young drug users inject very high levels

of Mn from use of home-made psychostimulant preparations (ephedron, also called methcathinone) (de Bie et al., 2007; Meral et al., 2007; Sanotsky et al., 2007; Sikk et al., 2007, 2010, 2013; Selikhova et al., 2008; Stepens et al., 2008, 2010; Varlibas et al., 2008; Colosimo and Guidi, 2009; Yildirim et al., 2009; Iqbal et al., 2012). These cases of young drug users with clinical parkinsonism as a result of drug abuse are reminiscent of young addicts injecting 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and expressing clinical parkinsonism in the early 1980s (Langston et al., 1983). The ephedron home-made preparations is the result of using potassium permanganate to oxidize ephedrine or pseudoephedrine and it is injected with minimal purification; thus, users inject very high doses of Mn. These ephedron users exhibited clinical parkinsonism that is not responsive to L-dopa therapy (Sanotsky et al., 2007; Selikhova et al., 2008; Stepens et al., 2008; Colosimo and Guidi, 2009; Sikk et al., 2013). This clinical observation suggests that the underlying neurobiology associated with Mn-induced parkinsonism is different from the well-recognized loss of dopamine neurons in the substantia nigra pars compacta (SNpc) that is responsive to L-dopa therapy in idiopathic PD patients (Savitt et al., 2006) and in MPTP subjects (Forno et al., 1993; Forno, 1996) and MPTP exposed non-human primates (Nerastet et al., 1994).

The etiological role of Mn in producing the motor function deficits in these relatively young ephedron users can be confirmed by the extremely high levels of Mn measured in their blood (Selikhova et al., 2008; Stepens et al., 2008; Sikk et al., 2010, 2013) and the bilateral hyperintensive signal in the basal ganglia observed in T1-weighted magnetic resonance imaging (MRI) consistent with excess accumulation of Mn in the brain (Selikhova et al., 2008; Stepens et al., 2008; Sikk et al., 2010, 2013). Importantly, in Eastern European countries in which there is expression of Mn-induced parkinsonism in ephedron drug users, the synthesis of the ephedron uses potassium permanganate to oxidize ephedrin or pseudoephedrin. On the other hand, in the United States drug users make the same ephedrone preparation, however, they oxidize the ephedrine with chromate and there is no evidence of parkinsonism (Stepens et al., 2008). This provides compelling evidence that the culprit in the home-made ephedron preparations used in Eastern European countries is the high levels of Mn that are injected by these individuals.

MANGANESE-INDUCED PARKINSONISM: DEGENERATION OR DYSFUNCTION OF DOPAMINERGIC NEURONS?

HUMAN STUDIES

In the last decade there has been a great deal of debate in the scientific literature about the potential role of Mn on the etiology of idiopathic PD. Epidemiological studies indicate that long-term exposure (>20 years) to Mn is associated with idiopathic PD (Gorell et al., 1999). Studies in welders have suggested that Mn exposure precipitates an earlier expression of idiopathic PD (Racette et al., 2001, 2005). However, the studies in welders have been criticized from several perspectives (see Ravina et al., 2001; Guilarte, 2010) and a confounding problem in human studies is that workers occupationally exposed to Mn could have an underlying susceptibility to develop PD. Thus, it is difficult to know whether the Mn exposure is the etiological agent that induces idiopathic PD or whether there is a coincidental Mn exposure in individuals that are destined to express the disease. In an effort to examine the potential role of Mn in idiopathic PD, a recent study used [^{18}F]-Fluoro-L-Dopa Positron Emission Tomography ([^{18}F]-FDOPA PET) imaging on 20 asymptomatic welders (exposed to welding fumes containing Mn), 20 subjects with idiopathic PD and 20 normal controls (Criswell et al., 2011). [^{18}F]-FDOPA PET is a non-invasive neuroimaging method to assess presynaptic dopamine terminal activity *in vivo* and has been used in idiopathic PD patients as a marker of dopamine terminal integrity (Gallagher et al., 2011; Jaimini et al., 2013). Notably, [^{18}F]-FDOPA uptake is dramatically decreased with a distinct regional pattern in the caudate and putamen of idiopathic PD patients (Morrish et al., 1996; Nurmi et al., 2001; Hilker et al., 2005; Gallagher et al., 2011). An important aspect of the investigation by Criswell and colleagues is that welders were relatively young (mean age 45.2 years), apparently asymptomatic and in good health, thus reducing the possibility of expressing an underlying idiopathic PD etiology in order to minimize the likelihood of coincidental Mn exposure with idiopathic PD. The authors found that welders expressed significantly elevated levels of blood Mn and a higher pallidal index (the pallidal index is a measure of Mn accumulation on the globus pallidus as a ratio of the signal intensity in the globus pallidus over the intensity in the frontal white matter using T1-weighted MRI) than controls and subjects with idiopathic PD; thus confirming that the welders were actively exposed to Mn. Upon neurological examination, welders demonstrated a slightly elevated average United Parkinson's Disease Rating Scale-subscale 3 (UPDRS3) score relative to controls indicative of subtle effects of Mn on motor function while the idiopathic PD subjects had a much higher score consistent with their diagnosis. The results of the [^{18}F]-FDOPA-PET studies indicated that the welders had a small (10%) but significantly lower level of [^{18}F]-FDOPA uptake in the caudate nucleus relative to controls but no effect on the anterior or posterior putamen (Criswell et al., 2011). On the other hand, idiopathic PD patients expressed the expected pattern of [^{18}F]-FDOPA uptake deficits in the caudate and putamen relative to controls. That is, idiopathic PD subjects had marked reductions in [^{18}F]-FDOPA uptake in the putamen (~52% in the posterior putamen and 35% in the anterior putamen) with a smaller reduction in the caudate nucleus (~17%) (Criswell et al., 2011). This study showed that the pattern of the impairment in dopamine terminal function in welders actively exposed to Mn-containing welding fumes is not the same to that observed in idiopathic PD. It should also be noted that the interpretation of the decrease in [^{18}F]-FDOPA uptake in the caudate in the welders exposed to Mn should not necessarily be interpreted as representative of dopamine terminal degeneration as is the case in idiopathic PD. It is possible that Mn exposure could alter enzymes that are responsible for [^{18}F]-FDOPA metabolism and this possibility needs to be ruled out. Other types of PET studies should also be performed that would be more representative of dopamine terminal integrity and are less likely to be influenced by changes in dopamine metabolizing enzymes and/or changes in dopamine levels. For example, [^{11}C]-dihydrotetrabenazine (DTBZ) PET for vesicular monoamine trasnporter type-2 (VMAT-2) is more

likely to represent structural changes in dopamine terminals than [^{18}F]-FDOPA PET when it relates to studies with Mn.

The findings of Criswell et al. (2011) do suggests that the small but significant Mn-induced decrease in [^{18}F]-FDOPA uptake in the caudate nucleus may be associated with potential effects on cognitive domains since the caudate nucleus has extensive connections to cortical structures, especially to frontal cortical areas that are involved in executive function (see below). Consistent with this hypothesis, several studies in early idiopathic PD patients show that reductions in [^{18}F]-FDOPA uptake in the caudate nucleus are associated with deficits in working memory performance and executive function (Rinne et al., 2000; Jokinen et al., 2009, 2013), effects that were not associated with reduction in [^{18}F]-FDOPA uptake in the putamen.

This recent study using state-of-the-art PET instrumentation and analysis provides evidence of a relative lack of dopamine neuron terminal degeneration in welders expressing small increases on the UPDRS3 scale. Previous studies in smelter workers with clinical parkinsonism have reported normal [^{18}F]-FDOPA-PET in the striatum (Huang, 2007). Further, neuroimaging studies performed in the ephedrone users indicating normal levels of dopamine terminals, based on dopamine transporter (DAT) levels, in the striatum using SPECT imaging despite the fact that they express clinical parkinsonism (Selikhova et al., 2008; Colosimo and Guidi, 2009; Sikk et al., 2010, 2013; Iqbal et al., 2012). Thus, the most recent human studies with state-of-the-art neuroimaging methodologies indicate that there is a relative lack of dopamine neuron terminal degeneration in the caudate and putamen as a result of Mn exposure. These findings raise the important question, what is the underlying neurobiological deficit in dopaminergic neurons in Mn-induced parkinsonism?

NON-HUMAN PRIMATE STUDIES

During the last decade, our laboratory in collaboration with a multidisciplinary group of investigators has been studying the neurological consequences of chronic exposures to moderate levels of Mn (Guilarte et al., 2006a,b, 2008a,b; Burton and Guilarte, 2009; Burton et al., 2009; Verina et al., 2011, 2013; Schneider et al., 2006, 2009). These on-going studies use research naïve *Cynamolgos macaques* (5–6 years of age at the initiation of the study) in which there is extensive behavioral and neuroimaging assessment prior to (baseline) and at two different time points after initiation of Mn administration (Guilarte et al., 2006b, 2008a). After the animals have gone through the behavioral and neuroimaging protocols [the latter includes T1-weighted MRI (MRI), Magnetic Resonance Spectroscopy (MRS), PET and currently Diffusion Tensor Imaging (DTI)] *ex vivo* neurochemical and neuropathological confirmation of the PET findings as well as other neurochemical and neuropathological outcomes are performed. One of the neuroimaging studies performed is to assess DAT levels as a putative synaptic marker of dopamine terminal integrity in the caudate and putamen using [^{11}C]-methylphenidate PET. Another PET study uses a continuous infusion of [^{11}C]-raclopride (a D2-dopamine receptor ligand) with amphetamine challenge in order to measure both D2-dopamine receptor (D2R) levels and *in vivo* dopamine release (Laruelle, 2000; Zhou et al., 2006). Importantly, the imaging studies provide an internal control since each animal receives a "baseline" (prior to Mn exposure) imaging set (MRI/MRS/DTI/PET). In addition, to the Mn-exposed animals, an "imaged-control" group was used. This group of animals goes through the same imaging protocol, but they do not receive Mn. A second "naïve controls" group was also used for the neuropathological endpoints and this group of animals does not receive Mn exposure nor does it go through the imaging protocol.

The results of our PET studies demonstrate that chronic exposure to moderate levels of Mn does not produce the loss of dopamine terminals, i.e., there was a lack of dopamine terminal degeneration in the caudate and putamen based on [^{11}C]-methylphenidate PET for DAT under our experimental Mn dose and exposure conditions (Guilarte et al., 2006b, 2008a). On the other hand, we found a highly significant effect of Mn on dopamine terminal dysfunction since there was a marked (~60% from baseline) and progressive decrease of *in vivo* dopamine release in the striatum of Mn-exposed animals measured by PET (Guilarte et al., 2006b, 2008a). This effect was not observed in the "imaged-control" group. Therefore, the impairment of *in vivo* dopamine release was the direct result of the Mn administration (Guilarte et al., 2008a).

One potential explanation for the impairment of *in vivo* dopamine release measured by PET in the Mn-exposed animals is that Mn produces a decrease in the synthesis of dopamine, thus resulting in lower levels of synaptic (vesicular) dopamine available for release. To answer this questions, a number of *ex vivo* neurochemical studies were performed in the caudate and putamen of the same animals in which PET studies were performed. The results show that when all control groups were combined (imaged-controls and naïve controls) and used as a referent group, there were no significant differences on the levels of dopamine and metabolites in the caudate and there was only an effect of Mn on dopamine levels in the putamen when compared to the naïve controls only (Guilarte et al., 2008a). A similar effect was observed for DAT and vesicular dopamine transporter-2 (VMAT-2) in the caudate and putamen. Lastly, there was no effect of Mn-exposure on DAT or tyrosine hydroxylase (TH) immunostaining in the caudate and putamen. In summary, the non-human primate studies performed under highly controlled experimental and Mn dosing conditions indicate that exposure to moderate levels of Mn does not result in dopamine neuron degeneration as in idiopathic PD but it produces significant dopamine neuron dysfunction. We have proposed that the subtle fine motor control deficits observed in these animals is the result of a dopamine release deficit (Guilarte et al., 2006b, 2008a; Guilarte, 2010). Our non-human primate findings are consistent with the most recent neuroimaging studies in humans indicating a lack of dopamine neuron terminal degeneration in subjects with clinical parkinsonism resulting from ephedrone use (Selikhova et al., 2008; Colosimo and Guidi, 2009; Sikk et al., 2010; Iqbal et al., 2012).

While the current review does not include rodent studies, there is recent evidence in the literature that rodents exposed to Mn also have impairment in dopamine release with no change in total tissue dopamine levels, dopamine neuron terminals in the striatum, or TH-positive dopaminergic cell bodies in the SNpc

(Vidal et al., 2005; Peneder et al., 2011). Combined these studies provide evidence that Mn-induced parkinsonism may be the result of the inability of dopamine neuron terminals to release dopamine rather than a decrease of dopamine synthesis in intact terminals and/or the loss of dopamine as a result of terminal degeneration. These findings provide a logical explanation to the evidence that Mn-induced parkinsonism is not responsive to L-dopa therapy (Lu et al., 1994; Sanotsky et al., 2007; Selikhova et al., 2008; Stepens et al., 2008; Colosimo and Guidi, 2009; Sikk et al., 2013) as is idiopathic PD since in Mn-induced parkinsonism there is no apparent loss of dopamine terminal or dopamine levels in the striatum. Our findings in non-human primates that Mn impairs dopamine release needs to be confirmed in humans exposed to Mn. Collectively, our PET findings implicate a novel mechanism by which dopamine neuron dysfunction, that is, the inability to release dopamine, rather than a degenerative process can result in clinical parkinsonism as a result of Mn exposure.

EFFECTS OF MANGANESE EXPOSURE ON NEUROPSYCHIATRIC SYMPTOMS AND COGNITIVE FUNCTION

The clinical expression of Mn-induced neurotoxicity in humans has been described as a continuum with different stages with distinct clinical manifestations (Mergler et al., 1999). Humans exposed to Mn express changes in sleep patterns and mood with uncontrollable laughter and crying, euphoria, aggressiveness, hallucinations and psychosis (Donaldson, 1987). An acute effect of Mn intoxication has been described as a clinical condition with symptoms reminiscent of schizophrenia and amphetamine-induced psychosis (Donaldson, 1987; Perl and Olanow, 2007). Although the current knowledge on the psychiatric aspects of chronic Mn exposure are limited, recent studies indicate that humans with increased exposure to Mn (Bowler et al., 2003, 2006, 2007a,b; Josephs et al., 2005; Park et al., 2009) and from medical conditions that results in increased Mn accumulation in the brain (Mirowitz et al., 1991; Klos et al., 2006) express impairments in attention and learning and memory function suggestive of frontal lobe and subcortical dysfunction. Studies have shown that workers occupationally exposed to Mn have a higher incidence of neuropsychiatric symptoms than referents (Bouchard et al., 2007) and elevated levels of Mn markedly increase neuropsychiatric symptoms associated with alcohol abuse (Sassine et al., 2002). An increasing number of reports also indicate effects on working memory (Bowler et al., 2003, 2006, 2007a,b; Klos et al., 2006) and poor cognitive performance (Mergler and Baldwin, 1997; Santos-Burgoa et al., 2001; Bowler et al., 2003, 2007a,b; Klos et al., 2006). Importantly, the effects of Mn on working memory points to deficits in frontal lobe function, a brain region known to be involved in neuropsychiatric illnesses such as schizophrenia (Goldman-Rakic, 1999; Abi-Dargham et al., 2002). A growing number of reports in children with elevated exposures to Mn indicate below average performance in verbal and visual memory tests (Woolf et al., 2002; Wright et al., 2006) and intellectual function (Wasserman et al., 2006; Claus Henn et al., 2010; Bouchard et al., 2011; Menezes-Filho et al., 2011; Khan et al., 2012). Children followed from birth through the early years have cord blood Mn concentrations that were negatively correlated with scores on attention, non-verbal memory and hand skills (Takser et al., 2003). Despite these studies, basic knowledge on mechanism(s) by which Mn produces psychiatric symptoms and cognitive impairment is lacking. Therefore, a great deal can be learned not only from Mn effects on basal ganglia function but also from effects on cognitive domains associated with the frontal cortex and other cortical and subcortical structures.

THE CEREBRAL CORTEX—A NOVEL TARGET OF MANGANESE NEUROTOXICITY

There is a paucity of knowledge on the neuropathological consequences of excess Mn accumulation in cortical regions and specifically in the frontal cortex. This is based in part on the fact that: (1) most studies on Mn-induced neurochemical and neuropathological changes have been focused on basal ganglia structures due to its association with movement abnormalities and parkinsonism, and (2) Mn accumulates to a high degree in the basal ganglia. Besides the suggestion from neuropsychological and cognitive tests of frontal cortex involvement in Mn-induced neurological dysfunction, a review of the literature brings to light a lack of neuropathological studies in which Mn effects on the cerebral cortex have been performed. It is only recently when neuroimaging studies have interrogated cortical regions to examine their susceptibility to Mn-induced neurotoxicity. In this context, our recent studies in non-human primates have reported proton MRS metabolite changes in Mn-exposed animals (Guilarte et al., 2006a). This includes a decrease in N-acetylaspartate (NAA) to creatine (Cr) ratio (NAA/Cr) in the parietal cortex with a nearly significant decrease ($p = 0.055$) in frontal white matter (Guilarte et al., 2006a). A decrease in the NAA/Cr ratio is representative of neuronal dysfunction and/or neuronal loss (Clark, 1998; Block et al., 2002). Since this original publication, two human studies have described effects of Mn on brain metabolites in the cerebral cortex. Chang et al. (2009) have shown that cognitive decline in welders was associated with a decrease in myoinositol/creatine (mI/tCr) ratio in the anterior cingulate cortex indicative of glial involvement. More recently, another MRS study in smelters showed a small but significant decrease in NAA/tCr ratio in the frontal cortex that was strongly correlated with cumulative Mn exposure (Dydak et al., 2011). Therefore, there is emerging evidence that exposure to Mn results in altered levels of brain metabolites in the cerebral cortex that reflect neuronal loss or dysfunction and glial cell activation. The only other evidence describing cortical involvement with brain Mn accumulation is a case report of an individual exhibiting progressive dementia, and extrapyramidal syndrome with an elevated Mn body burden (Banta and Markesbery, 1977). Brain biopsy and examination of cortical tissue revealed numerous neuritic plaques and neurofibrillary tangles in the right frontal lobe typical of Alzheimer's disease (AD) (Banta and Markesbery, 1977).

NEUROPATHOLOGICAL CHANGES IN THE FRONTAL CORTEX OF MN-EXPOSED NON-HUMAN PRIMATES

Previous reports from our on-going studies on the neurological effects of Mn in non-human primates have provided compelling evidence of Mn-induced pathology in the frontal cortex of young, research naïve animals (Guilarte et al., 2008b; Verina

et al., 2013). Using microarray technology in frontal cortex tissue from Mn-exposed and control animals, we found significant alterations in genes with biological functions associated with: (1) cholesterol metabolism and transport, (2) axonal/vesicular transport, (3) inflammation and the immune response, (4) cell cycle regulation and DNA repair, (5) and proteasome function and protein folding and turnover. The most highly upregulated gene was β-amyloid precursor-like protein 1 (APLP1), a member of the amyloid precursor protein (APP) family associated with AD (Guilarte et al., 2008b). The increase in APLP1 gene expression was confirmed at the protein level using immunohistochemistry. We also found diffused β-amyloid plaques (6E10 antibody immunohistochemistry) in the frontal cortex from Mn-exposed animals that were not observed in age-matched controls. These findings were unexpected as these were young adolescent animals and normally non-human primates do not express β-amyloid diffuse plaques at an early age, although there is evidence of diffused β-amyloid plaques in aged (>20 years of age) non-diseased monkeys (Kimura et al., 2003, 2005). Examination of frontal lobe tissue also provided evidence of cortical and subjacent white matter degeneration based on silver staining. In the gray matter, histological staining provided evidence of neurons with a significant degree of intracytoplasmic vacuolization. In some of the animals, we observed neurons with hypertropic nuclei, a condition that has been associated with the early stages of AD (Iacono et al., 2008, 2009). Histological assessment of the frontal cortex also showed cells with apoptotic stigmata and astrocytosis in both the gray and white matter. More recently, we have reported evidence of α-synuclein aggregation in the frontal cortex gray and white matter from the same Mn-exposed animals (Verina et al., 2013). As noted earlier, these Mn-exposed animals expressed a near significant ($p = 0.05$) decrease in NAA/Cr ratio in the frontal cortex white matter (Guilarte et al., 2006a) consistent with the observation of white matter degeneration in post-mortem brain tissue. Therefore, our studies provided the first evidence of significant pathology in the frontal and parietal cortex of non-human primates exposed to Mn.

Recent human studies also support neurodegenerative changes resulting from Mn exposure in frontal cortex white matter. Stepens et al. (2010) report that individuals injecting ephedron-containing Mn express white matter abnormalities based on DTI. The authors describe evidence of diffuse white matter changes reflected by reductions in fractional anisotropy (FA) in the ephedron users. They also find effects specific to white matter underlying the right ventral premotor cortex and the medial prefrontal cortex. The authors indicate that the clinical features of these ephedron users point to a disorder of higher-level motor programming and that the pattern of motor function deficits resemble executive function deficits similar to those displayed by patients with prefrontal cortex lesions (Stepens et al., 2010). Another human study examining white matter ultrastructural integrity in welders also reveal white matter changes measured by DTI (Kim et al., 2011). They show that FA was significantly reduced in the corpus callosum and frontal white matter of welders. The FA values in these white matter regions was significantly associated with blood Mn levels and pallidal index. Importantly, the degree of FA disruption was associated with impaired attention, lower working memory and deficits in executive function tests (Kim et al., 2011).

These findings provided strong evidence that the frontal cortex gray matter and subjacent white matter are vulnerable, but previously unrecognized targets for Mn-induced neurotoxicity despite the fact that Mn accumulates in cortical structures at significantly lower concentrations than in the basal ganglia. These observations suggest that the neurotoxicological effects of Mn are not solely based on the degree to which Mn accumulates in different brain regions but they are also based on the vulnerability of a specific brain region to Mn-induced neurotoxicity. The emerging evidence in humans and non-human primates suggest that future studies on subjects with environmental and occupational exposures to Mn or in patients with medical conditions in which excess brain Mn accumulation occurs should be tested for neuropsychiatric symptoms and cognitive function deficits.

EFFECTS OF MANGANESE EXPOSURE ON WORKING MEMORY

In the previous section evidence is provided that exposure to elevated levels of Mn results in detrimental effects on cortical structures, specifically the frontal and parietal cortex. Recent human and non-human primates studies suggest that a resulting effect of Mn-induced neuropathology in the frontal cortex is working memory deficits. Chang et al. (2010) report that welders with chronic Mn exposure express increased brain activity measured by functional MRI in working memory networks during the 2-back verbal working memory task. They interpret these findings as the welders requiring more neural resources in working memory networks to compensate for subtle deficits in working memory. In another study, Wasserman et al. (2011) found significant associations between Mn levels in drinking water and reductions in Perceptual Reasoning and Working Memory scores.

Our non-human primate studies were the first to provide initial evidence of Mn effects on working memory under highly controlled experimental conditions (Schneider et al., 2006, 2009). We showed that chronic Mn exposure resulted in deficits in non-spatial and spatial working memory. Non-spatial working memory assessed by delayed matching to sample performance appeared to be more affected than spatial working memory using a variable delayed response task (Schneider et al., 2009). In general, the human and non-human primate studies provide substantial evidence for impairments of cognitive domains that are mediated by the frontal cortex. Further, the non-human primate findings also implicate brain metabolite changes in the parietal cortex, a brain region that is important for working memory performance and plays an important role in integrating sensory information and visuo-spatial processing (Constantinidis and Wang, 2004; Seger, 2006; Linden, 2007).

CAN DOPAMINE NEURON DYSFUNCTION IN THE STRIATUM AND/OR FRONTAL CORTEX EXPLAIN THE WORKING MEMORY DEFICITS OBSERVED IN Mn EXPOSED NON-HUMAN PRIMATES?

Working memory is closely associated with frontal cortex function (Constantinidis and Wang, 2004; Linden, 2007) and dopamine neurotransmission in the striatum (Rinne et al., 2000;

Sawamoto et al., 2008; Jokinen et al., 2009) and the frontal cortex (Brozoski et al., 1979; Rotaru et al., 2007). The dopamine cell bodies located in the SNpc project to the caudate and putamen and this nigrostriatal system is involved in motor control. In addition, there are direct mesolimbic dopaminergic projections from the ventral tegmental area to the frontal cortex (Bjorklund and Dunnett, 2007). The caudate nucleus receives dopaminergic input from the SNpc and it can influence frontal cortex function via well-defined frontostriatal circuits (Alexander et al., 1986; Seger, 2006). Human and non-human primates studies show that the dorsolateral prefrontal cortex (DLPFC) is an important region for the execution of working memory tasks with reciprocal connections to other cortical structures such as the parietal, temporal and cingulate cortex and these combined participate in a cortical network related to working memory (Kubota and Niki, 1971; Petrides et al., 1993; Berman et al., 1995; Cohen et al., 1997).

Lesions or dysfunction of the caudate nucleus has been reported to produce impairment in the delayed response tasks that assesses working memory (Levy et al., 1997; White, 2009). Relevant to our own studies, Mn-exposed animals have impairments of both spatial and non-spatial working memory (Schneider et al., 2009) and they also express a significant impairment of *in vivo* dopamine release in the striatum (Guilarte et al., 2008a). Further, welders exposed to Mn express an early deficit in dopamine neuron function specific to the caudate nucleus and not the putamen (Criswell et al., 2011). These findings suggest that dopamine neuron dysfunction via impairment of dopamine release in the striatum and specifically in the caudate may be associated with the working memory deficits expressed in Mn-exposed non-human primates and in humans. Other studies have shown that the levels of NAA in the DLPFC predict the activation of cortical regions involved in the execution of working memory tasks such as the frontal, parietal and temporal cortices and this network has been found to be affected in mental disorders such as schizophrenia (Bertolino et al., 2000; Castner et al., 2004). Postmortem studies in the frontal cortex of Mn-exposed non-human primates have found a significant degree of neuronal degeneration with diffused β-amyloid plaques and α-synuclein aggregation (Guilarte et al., 2008b; Verina et al., 2013) implicating a potentially important role of this neuropathology in the working memory deficits observed in Mn-exposed non-human primates (Schneider et al., 2009). Imaging studies in welders exposed to Mn support a Mn-induced neuronal cell death or dysfunction in the frontal cortex based on decreased NAA/tCr ratio (Dydak et al., 2011), an effect that was associated with cumulative Mn exposure. Combined these studies provide evidence that several brain regions (i.e., the caudate nucleus, the frontal cortex and the parietal cortex) within the working memory network appear to have substantial neuropathology and/or dysfunction as a result of chronic exposure to Mn.

Experimental animal and human studies have shown that dopamine is a key neurotransmitter in the regulation of working memory in the frontal cortex and caudate nucleus (Levy et al., 1997; Aalto et al., 2005; Cools et al., 2008; Landau et al., 2009; Backman et al., 2011; Cools and D'Esposito, 2011). Microdialysis studies have shown that working memory tasks induce the release of dopamine in the prefrontal cortex of monkeys (Watanabe et al., 1997) and rats (Phillips et al., 2004) and there is increased blood flow to prefrontal and parietal cortex in humans performing working memory tasks (Bertolino et al., 2000; Cabeza and Nyberg, 2000). Other studies have shown that D1-dopamine receptor (D1R) antagonists can impair working memory (Sawaguchi and Goldman-Rakic, 1991) while low doses of D1R agonists can improve working memory (Arnsten et al., 1994). Contrary to using low doses of dopamine receptor agonists, high doses of D1R agonists also impair working memory performance, an effect that is abrogated by pretreatment with a D1R antagonist (Zahrt et al., 1997; Goldman-Rakic et al., 2000). These findings suggest that either low levels or excessive levels of D1R dopamine receptor stimulation can have a negative impact on working memory performance (Goldman-Rakic et al., 2000; Cools and D'Esposito, 2011). Based on this literature, it is likely that the impairment of *in vivo* dopamine release measured in the striatum of Mn-exposed animals may be responsible for their impairment in working memory (see Guilarte et al., 2008a; Schneider et al., 2009). Alternatively, it is possible that chronic Mn exposure may also alter *in vivo* dopamine release in the frontal cortex, and along with deficits of dopamine release in the caudate nucleus may precipitate deficits on working memory performance.

ANALYSIS OF *In vivo* DOPAMINE RELEASE IN THE FRONTAL CORTEX: PET IMAGING WITH [^{11}C]-FLB 457

While the displacement of D2R specific PET ligands such as [^{11}C]-raclopride by an acute amphetamine challenge has been validated and used extensively to measure *in vivo* dopamine release in the striatum (Laruelle, 2000). The use of this methodology is just emerging for the cerebral cortex (Narendran et al., 2009, 2011a,b, 2013). Since dopamine innervation to cortical structures is significantly lower than to the striatum, that is, dopamine terminals and dopamine receptor levels are much lower in the frontal cortex than in the caudate/putamen, *in vivo* dopamine release PET in cortical structures is a much more difficult task to perform. However, the development and use of high affinity D2R-PET ligands such as [^{11}C]-FLB 457 ($Kd = 0.06$ nM) and [^{18}F]-fallypride ($Kd = 0.14$ nM) have made such studies possible. Several publications have now described the reliability of using [^{11}C]-FLB 457 to measure *in vivo* dopamine release in the cerebral cortex of humans and non-human primates (Narendran et al., 2009, 2011a,b, 2013). Further, a recent study has shown that the degree of [^{11}C]-FLB 457 binding potential reduction measured by PET was directly associated with the amount of extracellular dopamine release induced by the acute amphetamine administration (Narendran et al., 2013). In summary, the ability to measure *in vivo* dopamine release in the cerebral cortex using PET is an extremely valuable approach to understand the molecular basis of the working memory impairments observed in humans and non-human primates exposed to Mn. We are currently performing these types of studies in our Mn-exposed animals in order to make associations between *in vivo* dopamine in cortical regions and working memory performance.

SUMMARY

In the last decade there has been significant progress using state-of-the-art neuroimaging and behavioral methodologies that have opened up a new understanding of Mn neurotoxicology. While historically the focus of Mn neurotoxicity has been associated with parkinsonism as a result of the high levels of exposure that occurred in the mining and processing of Mn ore and in other occupational settings, the last decade has brought about compelling experimental evidence that at lower cumulative doses of Mn that are likely to occur from occupational and environmental exposures, other non-motor neurological effects appear to be more prevalent and these seem to be associated with cognitive function deficits. The later may be the result of Mn producing brain chemistry and structural changes in cortical regions, and the frontal and parietal cortex appear to be sensitive targets. Lastly, because of its relevance to motor and cognitive domains, it is possible that dysfunction of the dopaminergic system could be a common mechanism by which Mn could have an impact on both cognitive and motor function deficits observed in humans and non-human primates.

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REVIEW Open Access

Measuring the impact of manganese exposure on children's neurodevelopment: advances and research gaps in biomarker-based approaches

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Abstract

Background: Children's exposure to manganese (Mn) is a public health concern and consistent policy guidelines for safe levels of Mn exposure is lacking. The complexity of establishing exposure thresholds for Mn partially relates to its dual role as an essential micronutrient with low levels required for good health, but also as a neurotoxin at high levels. Questions exist about the age-related susceptibility to excess Mn, particularly for children, and how best to measure chronic exposures. To address this concern we conducted a systematic review of studies examining children's exposure to Mn and neurodevelopmental outcomes focused on selection of biomarker-based and environmental measurements of Mn exposure to identify the scientific advances and research gaps.

Methods: PubMed and EMBASE databases were searched through March 2016 for studies that were published in English, used a biomarker-based or environmental measurement of Mn exposure, and measured at least one neurological outcome for children aged 0–18 years. Ultimately, thirty-six papers from 13 countries were selected. Study designs were cross-sectional (24), prospective cohorts (9), and case control (3). Neurodevelopmental outcomes were first assessed for Mn exposure in infants (6 papers), toddlers or preschoolers (3 papers) and school-age children (27 papers).

Results: Studies of school-aged children most frequently measured Intelligence Quotient (IQ) scores using Mn biomarkers of hair or blood. Higher hair concentrations of Mn were consistently associated with lower IQ scores while studies of blood biomarkers and IQ scores had inconsistent findings. Studies of infants and toddlers most frequently measured mental and psychomotor development; findings were inconsistent across biomarkers of Mn (hair, cord blood, tooth enamel, maternal or child blood and dentin).
Although few studies measured environmental sources of Mn, hair biomarkers were associated with Mn in drinking water and infant formula. Only one paper quantified the associations between environmental sources of Mn and blood concentrations.

Conclusion: Hair-Mn was the more consistent and valid biomarker of Mn exposure in school-aged children. Accurate measurement of children's exposure to Mn is crucial for addressing these knowledge gaps in future studies. However, research on biomarkers feasible for fetuses and infants is urgently needed given their unique vulnerability to excessive Mn.

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Keywords: Manganese, Exposure biomarkers, Exposure measurement, Children's neurodevelopment

Abbreviations: ADHD, Attention deficit hyperactivity disorder; As, Arsenic; B-Mn, Blood biomarker of manganese; B-Pb, Blood biomarker of lead; CBC, Children's behavioral checklist; Cd, Cadmium; CHAMCOS, Center for Health Assessment of Mothers and Children of Salinas; CI, Confidence interval; cm, Centimeter; Cu, Copper; Fe, Iron; FeS, Iron sulfide; g, Gram; GM, Geometric mean; H.A., Health advisory; Hair-Fe, Hair biomarker of iron; Hair-Mn, Hair biomarker of manganese; Hair-Pb, Hair biomarker of lead; HD, Hyperkinetic disorder; Hg, Mercury; I.Q., Intelligence quotient; L, Liter; m3, Cubic meter; MDI, Mental Development Index, Bayley Scales of Infant Development; Mg, Magnesium; mg, Milligram; Mn, Manganese; NS, A statistically nonsignificant association; Pb, Lead; PDI, Psychomotor Development Index, Bayley Scales of Infant Development; PM10, Particulate matter with a diameter of 10 micrometers or less; PM2.5, Particulate matter less than 2.5 microns in width; SD, Standard deviation; SES, Socioeconomic status; WASI, Wechsler Abbreviated Intelligence Test; Water-Mn, Water as an environmental measure of manganese; WISC, Wechsler Intelligence Scale for Children; Zn, Zinc

Background

Neurodevelopmental disabilities exact a significant toll on children. The global burden of attention deficit hyperactivity disorder (ADHD)/hyperkinetic disorder (HD) was estimated at 5.3 % in 2006 with acknowledgement of the limitations of pooled national estimates [1]. Recent prevalence estimates from the United States (U.S.) identify 4.9 million (8 %) children are learning disabled and another 5.9 million (9.5 %) have attention deficit disorder [2]. The number of U.S. children diagnosed with learning and behavioral problems has increased with time. From 1998–2000 through 2007–2009, the prevalence of ADHD among children aged 5–17 years grew from 6.9 % to 9.0 % [3]. Smaller, subclinical decrements in brain function are more common than diagnosed disorders and such conditions may decrease children's academic success, disturb behavior, and diminish quality of life [4]. These conditions are associated with a growing list of potential neurotoxicants including manganese (Mn). As with most divalent metals (e.g., iron, lead and cadmium), excessive environmental exposure to Mn adversely affects the brain function in adult humans and pre-clinical (animal) models of maternal-fetal dyads. The health implications for fetuses and infants are a concern given the propensity for Mn accumulation in tissue is higher during development [5], and their rapidly developing brain may be at risk of injury at lower levels of Mn exposure, relative to older children and adults [6, 7]. However, the potential adverse effects of excessive levels of Mn on the infant brain are poorly understood. Manganese is an essential micronutrient that plays a critical role in normal growth and development, particularly for brain development [8]. Humans need Mn in their daily diet because it is required for normal amino acid, lipid, protein, and carbohydrate metabolism [6]. Mn deficiencies are considered rare because Mn is present in numerous commonly consumed food items such as seafood, nuts, spinach, and tea. However, overexposure to Mn is also detrimental to health. Accumulation of Mn in the brain results in neurotoxic effects. Neurons in their early developmental stage are especially sensitive to the neurotoxic effects of Mn [9]. Animal studies demonstrate that Mn uptake by the brain is higher in the pre-weaning period, relative to later ages. Exposure to excess Mn in the prenatal and postnatal periods leads to tissue Mn deposition in the striatum and hippocampus [5, 7], brain regions that are important for cognitive function. Increased startle, hyperactivity, and learning and memory deficits are the functional consequences of exposure to excess Mn during development in rats [7, 10]. Some of these effects are long-term and persist into adulthood, despite the cessation of exposure to excess Mn [11]. Mn neurotoxicity is greater with combined prenatal and postnatal exposures than with exposure limited to either prenatal or postnatal period, and is mediated by altered neurotransmission, neuronal apoptosis and mismigration, excitotoxicity and oxidative stress [5]. In addition, Mn may indirectly affect brain function by altering tissue homeostasis of other divalent metals that are important for normal neurodevelopment, such as iron, by altering the expression of transporters that are common to all divalent metals [12].

In adult humans, excess Mn may result in anxiety, learning and memory deficits, and motor impairment [13, 14]. Inhalation of Mn is a long-standing concern for workers in the ferromanganese, iron and steel mining, welding and battery assembly industries that contain extremely high levels of Mn (>1–5 milligrams Mn/meter,^{3} or mg Mn/m^3) [6]. Community exposures to Mn also exist and include air contaminants from industrial activities [15], residential proximity to hazardous waste [16] or ingestion of water with naturally occurring Mn [17, 18]. Mn inhalation may bypass the biliary excretion mechanism and enter the brain through facilitated diffusion and active transport across the blood-brain barrier [19], or be passively transported from the olfactory bulb to the cerebral cortex [20], Mn has been considered to be less toxic when ingested than inhaled because adult humans regulate Mn absorption in the gastrointestinal tract and usually excrete excess Mn taken orally [21]. However,

infants' regulatory system is immature thus the risk of tissue Mn accumulation is greater for fetuses and infants [6, 22] raising questions about a World Health Organization's (WHO) [23] decision to suspend guidelines addressing Mn concentrations in water [24].

The former WHO drinking water guideline of 400 micrograms/L for Mn was withdrawn in 2011 as unnecessary with an assertion that this health-based level was well above Mn concentrations normally found in drinking water [23]. However, Frisbie and colleagues report that over 50 countries have drinking water or potential drinking-water supplies that contain a Mn concentration greater than 400 micrograms /L and argue that protective policy guidance is needed [24]. The US Environmental Protection Agency (EPA) provides Health Advisory (HA) values for unregulated contaminants that may cause non-cancerous health effects. EPA has identified that a lifetime HA at 0.3 mg/L Mn in water is not expected to cause adverse neurological effects [25]. While age-specific exposure limits are not available, for infants younger than 6 months, the lifetime HA of 0.3 mg/L Mn in water is recommended for acute exposures of 10 days, given concerns for differences in Mn content in human milk and formula and the possibility of a higher absorption and lower excretion in young infants [25].

A rapidly growing body of literature reveals the complexity of the association between exposure to Mn and children's adverse neurodevelopmental outcomes given a child's age, developmental and nutritional status (e.g., hemoglobin levels). However, the levels, timing and duration of exposure at which these outcomes may occur, and the potential effect of various routes of exposure to Mn (e.g., drinking water, dietary practices and contaminated air or soil), are not well established. Furthermore, the mechanisms of Mn toxicity are poorly understood and are complicated by interactions with other toxic metals such as lead (Pb) [26–28] and arsenic (As) [16, 29, 30] and limited and inconsistent evidence of gender-specific neurological effects (generally greater effects in girls [31, 32], but also found in boys) [33]. Accurate measurement of children's exposure to Mn is critical to address these knowledge gaps in future studies. Our paper examines the evidence for the association of Mn exposures to children's neurodevelopmental outcomes, focused on the contribution of biomarkers and environmental measures for elucidating the exposure-outcome relationship.

Methods

We identified studies using PubMed and EMBASE search engines in March of 2016. The searches were conducted by combining the results from a search on 'manganese' combined with the results from a strategy that used the concept of neurological outcomes including the following keywords: 'neurobehavioral manifestations' or 'intelligence' or 'child behavior' or 'child development' or 'psychomotor performance' or 'neuropsychological tests' or 'psychomotor disorders' or 'cognition' or 'intelligence test' or 'intelligence quotient.' The inclusion criteria were that the article was published in English and reported a study that measured both Mn exposure and a neurological outcome in humans aged 0–18 years. Any study that met the selection criteria, regardless of the publication date, was included in an initial phase of review. Measurements of Mn exposure varied including biomarkers and environmental sources; both types of exposure measurements were included. While various neurological outcomes were assessed, no limits were placed on the types of neurological outcomes examined.

Results

The searches returned 132 unique references. Fifty-six papers were outside the scope of this review because they were published in languages other than English, were review articles or meeting abstracts, had animal subjects, or did not include both a measure of Mn exposure and a neurological outcome. Abstracts were reviewed for the remaining 76 articles; ultimately 36 papers met all selection criteria and were included in this paper (Table 1).

Thirty six studies were conducted in thirteen countries investigating populations from the U.S. (six papers), Bangladesh and Mexico (five papers each), Brazil and South Korea (four papers each), Canada (three papers), Italy and China (two papers each), and France, Sweden, Taiwan, the United Kingdom and Uruguay (one paper each). Study designs were primarily cross-sectional ($N = 24$), and less frequently, prospective cohorts ($N = 9$), and case control ($N = 3$), although Collipp et al. [34] augmented the primary cross-sectional study with a secondary case control study (which is not included in the count of study designs). Sample sizes ranged from 16 (cases only) to 1,588 with approximately 7,639 children in total (except for children classified as controls in the case-control studies). Ten studies enrolled newborns. The ages at which neurodevelopmental outcomes were *first* assessed in relation to Mn exposure included infants (6 papers), toddlers or preschoolers (3 papers) and school-age children (27 papers). Exposure was more frequently measured with biomarkers (33 papers) than environmental samples (13 papers), (Table 1).

Neurodevelopmental outcomes

Studies examining the potential for the adverse impact of Mn on neurological outcomes most frequently assessed measures of IQ [15–18, 26, 30–32, 35–41], infant and toddler development [27, 28, 42–46], motor skills [33, 39, 47–49], attention deficit and hyperactivity disorder [39–41, 50, 51], attention [36, 43, 52, 53]

Table 1 Summary of study characteristics

Study Author Date of Publication	Country	Study design	Study Population	Sample size	Environ-mental Mn Measure	Biomarker Measure	Neurodevel
Barlow et al. (1983) [62]	United Kingdom	Case control	Children ≤ 16 years	68 exposed (65 controls)	None measured	Hair	Diagnosis o physicians, social worke
Collipp et al. (1983) [34]	Long Island, New York, US	Cross-sectional	Infants and children ≤ 4 years	70	Infant formula	Hair	No health o
		Case control	Learning disabled children and controls 7–10 years	16 learning disabled children; (44 controls)		Hair	Learning dis (parent and interview, ar
Takser et al. (2003) [43]	Paris, France	Prospective	Mother-infant pairs followed until 6 years	247 mother- infant pairs, 100 after 6 years)	None measured	Hair, cord blood, placenta	Attention, n skills, genera (Brunet-Lézi at 9 months Cognitive In
Wasserman et al. (2006) [18]	Araihazar Bangladesh	Cross sectional	Children 9.5–10.5 years	142	Well water	Blood	IQ (Wechsle Children, WI
Wright et al. (2006) [16]	Miami, OK, US	Cross sectional	Children 11–13 years	31	Not measured, but location coexisted with a Superfund site (Pb, Zn, Mn, Cd)	Hair	IQ (Wechsle Intelligence Assessment receptive sc of Language Verbal Learn Assessment of story mer of Memory a Depression I Rating Inven
Bouchard et al. (2007) [17]	Québec, Canada	Cross sectional	Children 6–15 years	46	Well water	Hair	Hyperactivity cognitive pr (Revised Cor Rating Scale
Ericson et al. (2007) [52]	United States	Prospective	NICHD Study of Early Child Care and Youth Development who shed a tooth	27	None measured	Tooth enamel	Behavioral di Toy Task), su Continuous F impulsive err Test), and tot and attentior Checklist)
Kim et al. (2009) [26]	Seoul, Seongnam, Ulsan, and Yeoncheon, South Korea	Cross sectional	Children 8–11 years	261	None measured	Blood	IQ including arrangement Educational I Intelligence S
		Prospective		448	None measured	Blood	

Table 1 Summary of study characteristics *(Continued)*

Claus Henn et al. (2010) [44]	Mexico City, Mexico		Children enrolled at or before birth and followed through age 3				Mental Deve Psychomoto (Bayley Scale
Riojas- Rodriguez et al. (2010) [31]	Hidalgo, Mexico	Cross sectional	Children 7–11 years	79 (93 controls)	None measured	Hair, blood	IQ (WISC-Rev
Bouchard et al. (2011) [32]	Québec, Canada	Cross sectional	Children 6–13 years	362	Water, diet	Hair	IQ (WASI)
Hernández-Bonilla et al. (2011) [48]	Hidalgo, Mexico	Cross sectional	Children 7–11 years	100 exposed (95 controls)	Prior studies show airborne Mn levels (median 0.10 g/m^3) exceed 2006 US EPA Reference Concentration (0.05 μg/m^3)	Hair, blood	Motor functi tapping, anc
Khan et al. (2011) [29]	Araihazar Bangladesh	Cross sectional	Children 8–11 years	201	Water	Blood	Child behavi externalizing (TRF Achenb Based Asses
Menezes-Filho et al. (2011) [35]	Salvador, Brazil	Cross sectional	Children 6–12 years	83	None measured	Hair, blood	IQ (WISC - III
Parvez et al. (2011) [49]	Araihazar Bangladesh	Cross sectional	Children 8–11 years	303	Drinking water	Blood, toenails	Motor functi including to coordinatior and hands, I agility
Khan et al. (2012) [58]	Araihazar Bangladesh	Cross sectional	Children 8–11 years	840	Water	None measured	Academic ac exams in ma
Wasserman et al. (2011) [30]	Araihazar Bangladesh	Cross sectional	Children ages 8–11 years	299	Well water	Blood	IQ, (WISC-IV) perceptual r processing s
Claus Henn et al. (2012) [27]	Mexico City, Mexico	Prospective	Children enrolled prenatally; followed to 36 months	455	None measured	Blood	Bayley Scale and PDI)
Lucchini, Zoni, et al. (2012) [41]	Valamonica and Garda Lake, Italy	Cross sectional	Children 11–14 years	299	PM10, soil	Hair, blood, urine	IQ (WISC-III) performance Wells' Adole Form)
Lucchini, Guazzetti et al. (2012) [33]	Valamonica and Garda Lake, Italy	Cross sectional	Children 11–14 years	54 exposed (157 control)	PM10, soil, tap water, diet	Hair, Blood	Motor coorc including ha and tremor
Bhang et al. (2013) [40]	South Korea	Cross-sectional	Children 8–11 years	1005	None measured	Blood	IQ (WASI), A Color-Word ADHD Ratin Evaluation S

Table 1 Summary of study characteristics *(Continued)*

							and the Dia Children-IV
Torres- Agustín et al. (2013) [54]	Hidalgo, Mexico	Cross sectional	Children 7–11 years	79 (95 control)	PM10, soil	Hair, Blood	Memory and Verbal Learn curve and le recall, recog memory spa
Lin et al. (2013) [28]	Taipei, Taiwan	Prospective	Mother-Infant pairs in the Taiwan Birth Panel	230 (pairs)	None measured	Cord blood	Developmer Inventory fo cognitive, la motor, socia developmer
Carvalho et al. (2014) [36]	Simões-Filho district, Bahia, Brazil	Cross sectional	Children 7–12 years	70	None measured; participants lived near a ferromanganese alloy plant	Hair	IQ (WISC-III), sustained at (Wisconsin C sustained at
Menezes- Filho et al. (2014) [53]	Salvador, Bahia, Brazil	Cross sectional	Children 7–12 years	70	None measured, but airborne exposure from residential proximity to ferromanganese plant	Hair, blood	Internalizing attention pr Checklist)
Oulhote et al. (2014) [47]	Quebec, Canada	Cross sectional	Children 6–13 years	375	Tap water, water consumption	Hair	Memory and Learning Tes Continuous Digit Span, S tapping
Rink et al. (2014) [46]	Montevideo, Uruguay	Cross sectional	14–45 months old	60	None measured	Hair	Bayley Scale cognitive, la abilities
Yang, et al. (2014) [57]	Shangai, China	Prospective	Mother-infant pairs	933	None measured	Cord blood	Neonatal Bel
Chung et al. (2015) [42]	Seoul, Ulsan and Cheonan, South Korea	Prospective	Maternal - infant pairs recruited prenatally	232 mother- infant pairs assessed at 6 months postpartum and followed for 3 years	None reported	Maternal blood	Bayley Scale
do Nascimento et al. (2015) [37]	Rio Grande do Sul, Brazil	Cross sectional	Children 6–12 years	69	Tap water	Hair, blood	Nonverbal IC Matrices)
Gunier et al. (2015) [45]	Salinas Valley, California, US	Prospective	Children recruited from prenatal cohort; followed to 7 years	197 (prenatal) 193 (postnatal)	Residential proximity to agricultural use of Mn-containing fungicides and 'take home'exposures	Teeth (pre- and postnatal dentin from incisors)	Cognitive ab coordination Developmen
Haynes et al. (2015) [15]	Marietta, Ohio, US	Cross sectional	Children 7–9 years	404	None reported; PM2.5 associated with residential	Hair, blood	IQ (WISC-IV), processing s verbal comp

Table 1 Summary of study characteristics *(Continued)*

					proximity to a ferromanganese refinery		
Mora et al. (2015) [39]	Salinas Valley, California, US	Prospective	Children enrolled prenatally provided shed teeth starting at 7–9 years, followed to 10.5 years	248 (prenatal) 244 (postnatal)	Residential proximity to agricultural use of Mn-containing fungicides	Teeth (pre- and postnatal dentin from incisors)	Behavior incl and hyperac System for C Deficit Hype Manual of M accuracy anc Continuous I Cognition ar (WISC-IV), ve reasoning, w speed and fu verbal memc Designs) verl abilities (CAV Motor incluc including fin age 7, and s Motor Batter
Ode et al. (2015) [50]	Malmö, Sweden	Case control	Children born 1987 to 2000 diagnosed with ADHD 5–17 years; matched controls	166 (case-control pairs)	None measured	Cord serum	ADHD diagn Manual of M
Shin et al. (2015) [51]	Seoul, South Korea	Case control	Children, 6–16 years, ADHD cases referred post-diagnosis	40 cases (43 controls)	None measured	Hair	ADHD diagn DSMMD- IV, Disorders an Lifetime Vers
Sun et al. (2015) [38]	Jiangsu, China	Cross sectional	Children, 8–12 years with natural environmental lead exposure	446	Mean community Pb concentrations in surface soil: 27.7 mg/kg, $^{-1}$ and undetected levels in outdoor air (<0.0035 mg/ m^{-3}	Blood	IQ (Combine modified in

memory [15, 16, 30, 31, 36, 39, 43, 54] and behavioral problems [16, 17, 29, 39, 40, 52, 53].

IQ was most frequently studied among children ages 7–14 years [15, 16, 18, 26, 30–32, 35, 36, 38–41] with the Wechsler Intelligence Scale for Children (WISC), consistent with its design for children ages 6 to 16 years and 11 months, using both full-scale IQ (global intelligence) and specific (verbal or performance) scores [55]. Study findings varied across study designs. Lower full-scale IQ scores were associated with increased concentrations of Mn in six studies investigating IQ as the only neurodevelopmental outcome [15, 26, 30, 32, 35, 37] and in three additional studies evaluating several neurological outcomes [16, 31, 36]. However, six studies did not report a significant association between IQ and Mn including studies only measuring IQ [18, 31, 35, 38] and one measuring additional outcomes [41]. In contrast to the former studies, Mora et al. examined several neurological outcomes and reported a positive association between postnatal Mn concentrations and IQ only for boys [39].

Motor function was measured in children 7 to 14 years and measures included grooved pegboard (a manipulative dexterity test), finger tapping [33, 39, 46, 47], the Santa Ana test which assesses manual dexterity and motor coordination [47, 48], the Bruininks – Oseretsky test which evaluates gross and fine motor functioning [49], the Aiming Pursuit test of hand dexterity [33] and subtests of motor coordination from the Luria Nebraska Battery [33]. Findings varied by study design and measures of outcomes. Mora et al. found higher concentrations of prenatal and postnatal Mn was associated with improved motor outcomes, but only in boys [39]. Oulhote et al. reported a significant association between intake of water-Mn and poorer motor function [47]. Hernández-Bonilla et al. reported a subtle, negative association of Mn with specific areas of motor speed and coordination [48]. Lucchini et al. reported higher Mn levels associated with poorer motor coordination and hand dexterity, and increased tremor intensity [33]. In contrast to the preceding studies, Parvez et al. did not find associations between Mn and motor function [49].

Among toddlers and infants ages 1 to 42 months the Bayley Scales of Infant Development (BSID-II) [56] were most frequently used to measure mental and psychomotor development [27, 42, 44, 46, 47]. A significant, inverted U-shaped association between Mn and development scores was reported in two studies. Chung et al. reported a dose-response relationship with both lower and higher concentrations of Mn associated with poorer Mental Development Index (MDI) and Psychomotor Development Index (PDI) scores in 6 month old infants [42]. Claus Henn et al. found an association between concurrent MDI scores at 12 (but not 24) months of age, but no association for PDI scores at either time period [44]. Two additional studies reported significant interactions of Mn and development scores by sex. A significant interaction of postnatal Mn exposures and poorer 6 month MDI and PDI scores and sex was reported by Gunier et al.; a significant inverse, linear relationship was seen only for girls [45]. A significant, positive interaction between postnatal Mn and sex was also seen at 24 months, but only for boys who had better MDI scores [45]. Rink et al. also reported a positive association between Mn and MDI scores only in boys, on average 29 months of age [46].

Biomarkers

Studies generally used biomarkers of children's hair or blood to assess Mn, but a few investigators measured fetal cord blood or serum, maternal blood or children's enamel or dentin from shed teeth; one study measured urine, (Tables 2, 3 and 4). Hair-Mn was the biomarker most consistently associated with a range of neurodevelopmental deficits. Higher levels of hair-Mn in school-aged children were significantly, and inversely associated with IQ scores [15, 16, 31, 32, 35–37], learning [16, 54], memory [16, 36, 54], perceptual reasoning [15] and positively related with greater hyperactive and oppositional behavior [17, 34].

IQ was the most frequently identified neurodevelopmental deficit associated with hair-Mn. Lower IQ scores were associated with increased concentrations of hair-Mn in four studies investigating IQ as the only neurocognitive outcome [15, 32, 35, 37], but IQ was also determined to be the only significant association with hair-Mn in a fifth study which measured several neurological outcomes [31]. Only one study found no significant association between IQ and Mn concentrations in hair [41]. In this study the mean Mn concentration in the hair was low, perhaps because Mn exposure in this study was from historical ferroalloy emissions.

Estimates of the effect size of hair-Mn on the average, full scale IQ scores of children, (mean age 9 years), were reported by Bouchard and colleagues to decline slightly (from 106 to 104) with hair-Mn values less than 1.5 micrograms/g, but significantly so for IQ scores of 101 with mean hair-Mn values of 3.2 micrograms/g, suggestive of biological significance [32]. Evidence of a U-shaped relationship with both high and low concentrations of hair-Mn associated with lower full scale IQ scores in children, on average, 8 years old were reported by Haynes et al. [15], suggestive of Mn as both a neurotoxicant and a micronutrient. Study findings revealed a significant, negative association between the highest quartile versus middle two quartiles of hair-Mn (ß -3.66; 95 % CI: -6.9, -0.43) and full scale IQ [15].

Blood-Mn levels were associated with a neurodevelopmental outcome in nine of sixteen papers reviewed.

Table 2 Summary of results from studies examining manganese concentrations in hair (Hair-Mn or H-Mn)

Study	Children's Ages and Mean Mn Level (μg/g), (SD)	Association with Environmental Mn	Association with Neurodevelopment	Other metals' mean concentrations in hair (μg/g), (SD)
Barlow et al. (1983) [62]	<16 years Hyperactive: 0.84 (0.64) Control: 0.68 (0.45)	None measured	Hyperactivity was more prevalent in hyperactive children (mean age: 7.6 years) but at lower levels of statistical significance (90 % confidence) using bivariate analyses.	Lower levels of zinc (Zn) were associated with hyperactive children; 83.4 (32.3) compared to controls: 99.1 (54.3), 95 % confidence using bivariate analysis. Other metals were nonsignificant in association with the outcome including: (Cadmium (Cd), Copper (Cu), Iron (Fe), Lead (Pb), and Magnesium (Mg).
Collipp et al. (1983) [34]	*Ages 7–10 years* Learning- disabled:0.43 Control: 0.27	None measured	Significantly higher hair-Mn levels from learning disabled children, 7–10 years old, compared to children without the condition.	Not applicable
	Age 4-months Breastfed: 0.33 Formula fed: 0.685	Significantly greater hair-Mn in formula-fed infants.	None applicable	Not applicable
Takser et al. (2003) [43]	Newborns to 6 years 0.75[1]	None measured	No association was found between hair-Mn post-childbirth and general psychomotor developmental indices at 9 months and a general cognitive index at 3 and 6 years in models adjusted for maternal age and education, smoking, labor duration, children's sex and cord blood lead levels and other confounders.	Not applicable
Wright et al. (2006) [16]	11–13 years 0.47	None measured	Lower full-scale IQ, verbal learning and memory scores were associated with higher concentrations of hair-Mn from children, on average 12.6 years old, in analyses adjusted for maternal education, child sex and concentrations of lead PbH.	Higher arsenic (As) levels, particularly in combination with higher Mn levels, associated with lower IQ, verbal learning, and memory scores. No associations found with Cd levels.
Bouchard et al. (2007) [17]	6–15 years 5.1 (4.3)	Greater MnH concentrations from children who drank well water with higher Mn-water.	Greater hyperactive and oppositional classroom behavior was associated with higher hair-Mn from children, on average, 11 years old, in analyses adjusted for age, sex and income. No interaction between hair-Mn and child sex.	Not applicable
Hernández-Bonilla et al. (2011) [48]	7–11 years 12 (exposed) 0.57 (nonexposed)	Respiratory Mn exposures were associated with residential proximity to Mn mines, but specific measures were not reported.	Hair-Mn was not associated with neuromotor outcomes (grooved pegboard, finger tapping repetition and Santa Anna test in children, on average, 9 years old in analyses adjusted for Pb in blood, hemoglobin, sex, age and maternal education.	Not applicable
Menezes-Filho et al. (2011) [35]	6–12 years 5.83[1] (11.5) Hair-Mn levels were 6 times higher than those in the general Brazilian population (mean 0.47 μg/g, range 0.89–2.15 μg/g)	None measured, but Mn exposures were from residential proximity to Mn alloy production plant.	Lower full scale and verbal IQ scores in children, on average, 8.8 years old, in analyses adjusted for maternal education and nutritional status. A ten-fold increase of hair-Mn was associated with a 6.7 - point loss in Verbal IQ score.	Children with iron deficiency had higher hair-Mn (15.94 ± 19.68 μg/g; $p = 0.06$) compared to those with FeS in normal range (8.69 ± 8.23 Mn/g).
Riojas-Rodriguez et al. (2010) [31]	7–11 years Exposed: 12.13 Unexposed: 0.57	Median airborne concentration of Mn in PM10 of exposed (0.13 μg/m^3) versus unexposed (0.02 μg/m^3) communities, but personal exposures were not reported.	Lower full scale, verbal and performance IQ scores in children, on average, 9 years old, in analyses adjusted for blood-Pb, hemoglobin, age, sex and nutritional status. Sex	Not applicable

Table 2 Summary of results from studies examining manganese concentrations in hair (Hair-Mn or H-Mn) *(Continued)*

			significantly modified the association with the strongest inverse association in young girls. There was little evidence of an association in boys.	
Bouchard et al. (2011) [32]	6–13 years Median: 0.7	Hair-Mn levels were associated with higher Mn in water (Water-Mn) (mean: 98 μg/L, GM:[1]20 μg/L), but not in diet.	Lower full-scale IQ scores were associated with increased hair-Mn concentrations in children, on average, 9 years old, in analyses adjusted for maternal intelligence and education, income, sex and age of children, Fe concentrations in water and other confounders. A 10-fold increase in water-Mn was associated with a decrease of 2.4 IQ points (95 % CI:-3.9 to -0.9, $p < 0.01$) adjusting for maternal intelligence and other confounders. Sex stratification showed a slightly higher impact of hair-Mn for girls' full-scale IQ, but the interaction term was nonsignificant. Water-Mn was more strongly associated with performance than verbal IQ.	Not applicable
Lucchini, et al. (2012) [33]	11–14 years 0.16 Median	Significant differences for Mn concentrations in soil (soil-Mn) and air (air-Mn) by proximity to industrial sites with historical Mn emissions, but not for tap water, diet or hair. Impairment of motor coordination, hand dexterity and odor identification was associated with median concentrations of soil-Mn in exposed (897 ppm) versus reference (409 ppm) communities.	Tremor intensity in dominant hand was positively associated with hair-Mn in children, on average, 12.9 years old, in analyses adjusted for age, gender, SES, family size, parity order, parents' education, smoking habits and soil concentrations of Pb and other metals. Boys had increased tremor intensity relative to girls.	Not applicable
Lucchini, et al. (2012) [41]	11–14 years 0.17	No association between concentrations of hair-Mn with soil- or air-Mn	No association between hair-Mn concentrations with full-scale, verbal or performance IQ, or behavioral and attention deficit hyperactivity scores for children, on average, 12.9 years old, with Mn exposure modeled as a main effect or an interactive term with blood-Pb in analyses adjusted for age, gender, family size, SES, area of residence, hemoglobin, ferritin and confounders.	
Torres-Agustín et al. (2013) [54]	7–11 years Exposed: 14.2 Unexposed: 0.73	Greater hair-Mn concentrations in children in exposed group. Mn concentrations in outdoor air from Mn mining and ranged from the median: 0.08, μg/m^3 in the exposed location compared to the median: 0.02, μg/m^3 in the control location.	Lower long-term memory and learning scores were associated with increased hair-Mn in children, on average, 9 years old, in analyses adjusted for children's sex, blood-Pb, age, hemoglobin and maternal education. The negative association was stronger for girls.	Not applicable
Rink et al. (2014) [46]	14–45 months 0.98 (0.74)	None measured	Lower scores in cognitive and expressive language tests in children, on average, 28.8 months old, but only in unadjusted models. Boys had a significantly positive association between hair-Mn concentrations and receptive languages scores in analyses adjusted for hair-Pb concentrations, child hemoglobin and age, paternal	Not applicable

Table 2 Summary of results from studies examining manganese concentrations in hair (Hair-Mn or H-Mn) *(Continued)*

			education, maternal IQ, SES, and other confounders.	
Carvalho et al. (2014) [36]	7–12 years 14.6 (11.8)	None were measured, but Mn exposure was related to residential location and air emissions from an iron-Mn alloy plant.	Lower full-scale IQ, and lower scores on Vocabulary, Block Design, and Digit Span tests were associated with increased hair-Mn for children, on average, 9.4 years old, in analyses adjusted for maternal education and children's age. Each 1 μg/g increase in hair-Mn was associated with a decrease of approximately 1 full-scale IQ point and lower test scores for executive function, strategic visual formation and verbal working memory. No significant sex differences for hair-Mn concentrations.	
Menezes-Filho et al. (2014) [53]	7–12 years Boys: 15.3 (9.9) Girls: 13.9 (13.4)	None were measured, but Mn exposure was related to residential location and air emissions from an iron-Mn alloy plant.	Externalizing behaviors and attention problems on the Child Behavior Checklist (CBC) for girls was significantly associated with higher hair-Mn. No significant association was found between CBC scores for boys in sex-stratified models adjusted for age (with boys) or maternal IQ (with girls).	
Oulhote et al. (2014) [47]	6–13 years Boys: 0.75 Girls: 0.80	Greater water-Mn (mean: 99 μg/L; GM: 20 μg/L) & hair.	Mn exposure was associated with significant decrements in memory (hair and water) and attention (hair), and motor function (water) adjusted for maternal education and nonverbal intelligence, tobacco consumption, child sex, age and other confounders. Estimates of associations by sex were similar.	
do Nascimento et al. (2015) [37]	6–12 years Rural: 2.07 (2.6) Urban: 0.45 (0.2)	Greater Mn in drinking water (mean: 20 μg/L) rural sites and (mean: 1.0 μg/L) urban sites) associated with greater Mn levels in hair.	Lower (nonverbal) IQ scores were associated with hair-Mn and water-Mn concentrations for children, on average, 8.5 years old using models adjusted for age, gender and parental education.	Additional, similarly specified models were tested for the association of Pb, Cr, As, Hg, and Fe in hair on cognitive outcomes. Only hair-Fe showed a significant and inverse association with outcomes.
Haynes et al. (2015) [15]	7–9 years 0.42[1] (0.002)	Air-Mn associated with home proximity to ferromanganese refinery.	Lower full-scale IQ and perceptual reasoning scores were associated with hair-Mn for children, 7–9 years old in analyses adjusting for blood-Pb, blood-Mn, serum creatinine, community of residence, child sex, parents' IQ, education and parenting confidence. A U-shaped association was observed as children with hair-Mn concentrations > 747 μg/g had significantly lower IQ than children with hair-Mn concentrations between 207.2–747 μg/g (ß -3.66, 95 % CI: -6.9, -0.43). Children with hair-Mn levels < 207 had lower, but nonsignificant associations with full scale IQ than those with concentrations between 207.2–747 μg/g.	Not applicable
Shin et al. (2015) [51]	6–16 years Case: 0.31 (0.46) Control: 0.22 (0.10)	None measured	No association between hair-Mn and ADHD was found in children on, average, 9.7 years old when analysis was adjusted for confounders of age, sex and full-scale IQ.	Not applicable

[1] Geometric Mean used

Most studies reporting associations between blood-Mn and neurological outcomes measured several outcomes. However, seven studies only examined IQ as the primary outcome and the findings were inconsistent. Four investigations did not find an association between IQ and blood-Mn [18, 31, 35, 38], but studies by Haynes et al. [15], Kim et al. [26], Wasserman et al. [30], showed a significant, inverse association between blood-Mn and IQ scores for children, on average, 8–9 years of age with mean concentrations of blood-Mn at 9.7 micrograms/L, 14.3 micrograms/L and 14.8 micrograms/L, respectively. Evidence of an inverse, U-shaped association between low and high levels of blood-Mn and low IQ scores was seen in three studies with children [15, 42, 44], two of which used the same outcome measure. Claus Henn et al. reported a significant association between concurrent MDI scores and blood- Mn in 12 month old infants comparing the middle three Mn quintiles with the lowest Mn quintile (ß -3.3, 95%CI: -6.0, -0.7) and the highest Mn quintile (ß -2.8, 95%CI: -5.5, -0.2) [44]. Chung and colleagues also reported a significant, inverse U-shaped association between maternal blood-Mn with infant PDI scores at 6 months. Increasing maternal blood-Mn levels up to 24–28 micrograms/L were positively associated with PDI scores while higher blood-Mn concentrations were associated with decreased PDI scores suggesting adverse effects of both low (<20 micrograms/L) and (high ≥ 30 micrograms/L) maternal blood-Mn levels [42].

Evidence for the usefulness of other Mn biomarkers included three papers that reported significant associations between Mn in cord blood or serum and early life neurodevelopment indicative of the importance of prenatal Mn exposure. Takser et al. reported an inverse association between cord blood-Mn at birth (Geometric Mean: 38.5 micrograms/L) and attention and non-verbal memory in three year olds and a significant, negative association with hand skills, significantly poorer scores in boys [43]. Lin et al. found cord blood-Mn (mean 50.7 micrograms/L; SD: 16.7 micrograms/L) and blood-lead (13.0 micrograms/L; SD: 7.51 micrograms/L) levels above the 75th percentile had a significant association with overall (ß -7.03; SE = 2.56; $p = 0.009$), cognitive (ß -8.19, SE = 3.17; $p = 0.012$) and language scores (ß -6.81, SE = 2.73, $p = 0.013$) [28]. Yang et al. found that a high cord serum-Mn (≥75th percentile, median: 4.0 micrograms/L) was associated with significantly lower scores on a Neonatal Behavioral Neurological Assessment (NBBA) at 3 days of age [57]. An interactive, protective effect was seen with prenatal selenium (Se); as the Mn/Se ratio increased, NBNA scores decreased while high levels of Se had a protective effect in the high Mn group (Mn ≥ 9.1 micrograms/L; Se ≥ 63.1 micrograms/L).

Teeth-Mn levels were analyzed in three studies suggestive of their potential value as biomarkers of early life exposures providing insight on the timing of Mn exposure and developmental windows of susceptibility. Ericson et al. measured tooth enamel in shed molars and found significant associations between Mn levels in enamel formed during the first 20 weeks of gestation and increased childhood behavioral inhibition at 36 months [52]. Studies from the Center for the Health Assessment of Mothers and Children of Salinas (CHAMCOS) birth cohort provided findings on the timing of early life Mn exposures. Gunier et al. reported small decreases in mental and motor development among 6 month old infants in association with prenatal dentin-Mn concentrations, but only for girls whose mothers had lower hemoglobin levels [45]. Additionally, a two-fold increase of postnatal dentin-Mn, reflecting exposures from birth to 2.5 months, was associated with a small, but significant decrease for infants' mental development scores at 6 and 12 months. A significant interaction between postnatal dentin-Mn concentrations and sex for MDI (-1.5 points; 95 % CI: -2.4, -0.6) and PDI (-1.8 points; 95 % CI: -3.3, -0.3) scores at 6 months was reported, but only for girls; it was no longer evident by 24 months. Mora et al. reported increased Mn levels in pre-and postnatal dentin adversely associated with behavior problems in school aged children [39]. In contrast, the authors also reported positive effects of pre- and postnatal dentin-Mn specific to boys including better cognition, memory and motor function.

Environmental sources of manganese

Levels of Mn in environmental sources were less frequently quantified than biomarkers of Mn. Collipp et al. found higher levels of hair-Mn in infants fed formula relative to breastfed infants [34]. Since the study's publication, levels of Mn in infant formula have declined. This study was one of the first published papers to show an association between ingestion of dietary Mn (formula) and hair biomarkers. However, it is unclear if water containing Mn was used to reconstitute the formula which may have influenced levels of Mn in hair.

Findings supporting the exposure – outcome relationship between Mn concentrations in water, hair and child neurodevelopment were reported in three papers. Bouchard et al. reported higher levels of hair-Mn in children whose well water had higher Mn levels [32], and higher levels of Mn in water and hair were significantly associated with lower IQ scores. A 10-fold increase of Mn intake from water consumption was associated with a decrease of 2.5 IQ points (95 % CI:-3.9, -0.9; $p < 0.01$) among 9 year olds [32]. Oulhote et al. reported higher concentrations of Mn in hair and water were associated with poorer scores on memory, attention and motor function from the same population [47]. Average Mn water levels in this study were lower than the earlier

Table 3 Summary of results from studies examining manganese concentrations in blood (blood-Mn)

Study	Mean Mn Level (μg/L),(SD)	Association with Environmental Mn	Association with Neurodevelopment	Association of other metals' mean (SD) concentrations in blood, with the outcome
Wasserman et al. (2006) [18]	10 year olds 12.8 (3.2)	No association was found with water-Mn (Mean: 795 μg/L).	No association was found between blood-Mn concentrations and overall, verbal and performance IQ scores in adjusted analyses, but water-Mn was associated with lower full-scale, performance and verbal IQ raw scores in a dose-dependent fashion.	Blood-Mn was not significantly correlated with blood-Pb or blood-As. When all three blood metals were included in analyses only mean blood-Pb concentrations,12 μg/dL (3.7) were associated with IQ scores.
Kim et al. (2009) [26]	8–11 years olds 14.3 (3.8)	None measured	Lower overall and verbal (but not performance) IQ scores were associated with blood-Mn in analyses adjusted for maternal age, parental education and smoking, SES, child gender and age and other confounders.	Blood-Pb concentrations of 1.73 μg/dL (0.8) were associated with IQ scores in adjusted analyses with evidence of an additive interaction with blood-Mn. Effect modification was suggested as IQ scores of children with blood-Mn > 14 μg/L were significantly associated with blood-Pb whereas scores for children with blood-Mn < 14 μg/L were not.
Claus Henn et al. (2010) [44]	12 month: 24.3 (4.5) 24 month: 21.1 (6.2)	None measured	Blood-Mn had an inverse, U-shaped association with a concurrent measure of the Mental Development Index (MDI) scores at 12 months of age. Declines of 3.4 and 2.8 MDI points for the lowest and highest quintiles of blood-Mn relative to the middle three quintiles, correspond to declines of 0.37 and 0.31 SD units in the MDI. This association declined by 24 months and was nonsignificant in adjusted analyses including blood-Pb, sex, maternal IQ and education, hemoglobin and gestational age. No association was found with the PDI score.	Blood-Pb (cord, 12 and 24 month) concentrations were positively associated with 24 month blood-Mn concentrations. Indices of iron status (hemoglobin, ferritin) were inversely associated with Mn at 12 and 24 months of age.
Riojas-Rodriguez et al.(2010) [31]	7–11 year olds 9.7[a] (Exposed) .2 (Control)	24-h median Mn in PM10 for the exposed (0.13 μg/m^3) and control (0.02 μg/m^3) communities	Exposed children showed nonsignificant, inverse associations of blood-Mn with lower full scale, verbal and performance IQ scores compared to controls. Analyses were adjusted for age, sex, hemoglobin, maternal education, blood-Pb. Differences by sex were nonsignificant.	Blood-Pb was higher in control (7.96 μg/dL) versus Mn-exposed (3.37 μg/dL) children and was correlated with blood-Mn (r - 0.24) in the population. It was not significantly associated with IQ outcomes.
Hernández-Bonilla et al. (2011) [48]	7–11 year olds 9.5 (Exposed) 8.0 (Control)	Prior studies show airborne Mn levels (median 0.10 μg/m^3) exceed EPA 1999 Reference Concentrations (0.05 μg/m^3).	Blood-Mn was inversely associated with poorer finger tapping in analyses adjusted for age, sex, maternal education, hemoglobin and blood-Pb. Other motor function measures (grooved pegboard and Santa Anna test scores) were not significantly associated with blood-Mn. Sex differences for blood-Mn were nonsignificant.	Blood-Pb concentrations were higher in the Mn control (median: 8 μg/dL) versus the Mn exposed (median: 3.3 μg/dL) children. The associations with the outcomes were not reported.
Kahn et al. (2011) [29]	8–11 year olds 15.1 (3.9)	Non-significant association of blood-Mn with water-Mn (mean: 900 μg/L).	No association was found between blood-Mn and externalizing (attention problems and aggression) and internalizing (anxiety) behaviors and a total behavioral score in analyses adjusted for water-As, water-Mn, urinary creatin-adjusted As and blood-As, sex, maternal education and other variables.	There was no statistical association between biomarkers of As (blood or urine) with blood-Mn.
Menzes-Filho et al. (2011) [35]	6–12 year olds 8.2 (3.6)	None measured; Mn exposure was due to home proximity to Mn alloy production.	Blood-Mn concentrations were not associated with IQ scores in analyses	Blood-Pb was above 2 μg/dL for 51 % (n = 36) and there was no

Table 3 Summary of results from studies examining manganese concentrations in blood (blood-Mn) *(Continued)*

			adjusted for blood-Pb or low serum iron levels.	association with blood-Mn or serum-Fe (mean: 55.6 μg/dL).
Parvez et al. (2011) [49]	8–11 year olds 17.7 (3.7)	Water-Mn (mean: 725.5 μg/L). Children with higher water Mn (>500 μg/L) did not have higher levels of blood-Mn (14.5 vs. 15.0 μg/L; $p < 0.05$).	No significant associations were found between blood-Mn and motor function measures (fine manual control, manual and body coordination, strength and agility).	Blood-Mn correlated slightly with blood-As (mean: 4.8 μg/L; SD: 3.2; r = 0.12; $p = 0.02$) and moderately with blood-Se (mean: 104.9 μg/L; SD:17.2; r = -0.33, $p < 0.0001$). There was a significant, inverse association between As exposure measures (blood, water, urinary and nails) and overall motor function, and a significant association between blood-Se and manual coordination in adjusted analyses. No significant association was found between blood-Pb and motor function.
Wasserman et al. (2011) [30]	8–11 year olds 14.78 (3.7)	Water-Mn (mean: 725.54) and blood-Mn did not vary predictably across groups with high and low levels of water-Mn.	Higher blood-Mn was associated with lower perceptual reasoning and working memory scores in analyses adjusted for maternal intelligence and age, children's time in school, plasma ferritin, blood-As and other variables. Significant associations were not found for full scale IQ, verbal comprehension or processing speed scores.	Increased concentrations of blood-As (mean: 4.81 μg/L; SD: 3.22) were significantly associated with lower verbal comprehension in adjusted analyses. However, Mn by As interactions were not significant in adjusted models predicting IQ.
Claus Henn et al. (2012) [27]	12 months: 24.7 (5.9) 24 months: 21.5 (7.4)	None measured	A synergistic interaction between lead and Mn for mental and psychomotor development scores was found at 12 (but not 24) months; greater lead toxicity with higher Mn levels in analyses adjusted for sex, hemoglobin, gestational age, maternal education and IQ. There were no significant sex differences in blood-Mn.	Concentrations of blood-Pb at 12 (mean:5.1 μg/dL; SD: 2.6) and 24 months (mean: 4.8 μg/dL; SD: 2.5).
Lucchini et al. (2012) [33]	11–14 year olds 11.11 μg/dL	Mn was measured in air PM10 airborne particles (median: 31.4 ng/m^3 vs. 24.7 ng/m^3) and soil (median:897 ppm vs. 409 ng/m^3) in impacted compared to control areas, and water (below LD at 1 μg/L) and diet (median 2.66 mg/day) with no differences by locations. Soil-Mn was significantly, inversely associated with performance on the olfactory test.	Tremor intensity, dominant hand, was significantly and positively associated with blood-Mn in adjusted models (including parental smoking and alcohol use, and Mn in soil, air and hair). Sex differences were found with boys having lower increased tremor intensity.	Blood-Pb concentrations in the Mn exposed (mean: 1.72 μg/dL) and control (mean: 1.6 μg/dL) communities were very low.
Lucchini et al. (2012) [41]	11–14 year olds 11.11 μg/dL	Mn was measured in soil (median: 529.12 ppm), air: (median: 29.37 μg/m^3), water, and diet	Mn was not associated with IQ (full scale, verbal and performance) or behavioral (hyperactivity, attention deficit) scores in adjusted analyses.	Blood-Pb concentrations averaged 1.71 μg/dL and were adversely associated with cognitive measures in adjusted analyses declining about 2.4 IQ points with a two-fold increase of blood-Pb. A bench-mark level of blood-Pb was associated with loss of 1 IQ point at 0.19 μg/dL and a lower 95%CI of 0.11 μg/dL. No interaction of Pb and Mn was observed.
Torres-Agustín et al. (2013) [54]	7–11 year olds Exposed: 9.5[b] Unexposed: 8.0	Air sampling (PM10) conducted and Mn concentrations in outdoor air from Mn mining significantly higher for exposed (Outdoor median: 0.08 mg/m^3) versus comparison group (Outdoor median: 0.08 mg/m^3) Significantly greater blood-Mn concentrations in exposed than comparison children.	No significant associations between blood-Mn and verbal learning or memory in adjusted analyses.	Blood-Pb concentrations were significantly higher in the comparison group (8.0 μg/dL) than the Mn exposed group (3.3 μg/dL) and included in multivariate models of Mn exposure.
Bhang et al. (2013) [40]	8–11 year olds 14.42 (4.1)	None measured	Excess blood-Mn was associated with lower scores in thinking, reading, calculation, and learning scores and higher cognitive inhibition test scores	Analyses were adjusted for blood-Pb and cotinine.

Table 3 Summary of results from studies examining manganese concentrations in blood (blood-Mn) *(Continued)*

			in analyses adjusted for maternal and child age and IQ, child sex, and age, cotinine, blood-Pb and other variables. Lower blood-Mn was associated with lower cognitive inhibition scores.	
Chung et al. (2015) [42]	Maternal, pre- delivery, 30.1 ± 3.5 years; 22.5 (6.5)	Not measured	Inverted U-shaped dose- response curve with lower psychomotor development scores in infants at 6 months with both low and high levels of Mn. Adjusted mean PDI (but not MDI) scores differed significantly across Mn concentration groups. No differences in effects by sex were observed.	None reported.
do Nascimento et al. (2015) [37]	6–12 year olds Rural: 16.0 (4.2) Urban: 19.0 (4.3)	Water-Mn concentrations differed significantly between rural (mean: 0.20 μg/L) and urban (mean:1.0 μg/L) children; associations with blood-Mn were not reported.	No significant associations found for blood-Mn and nonverbal IQ in analyses adjusting for age, parents education and child sex.	No associations were found between metals in blood and serum (Pb, Cr, As, Hg and Fe) and nonverbal IQ.
Haynes et al. (2015) [15]	7–9 year olds 9.67 (1.27)[c] [2]	Mn exposure resulted from residential proximity to ferromanganese refinery although measurements relative to the blood-Mn and the cognitive outcomes were not reported.	Blood-Mn was significantly associated with lower full scale IQ, perceptual reasoning, lower processing speed scores in analyses adjusted for hair-Mn, serum cotinine, blood-Pb, and community residence. Full scale IQ scores among children in the highest quartile of blood-Mn (>11.2 μg/L) were significantly lower than scores in children with blood-Mn between 8.2 μg/L to 11.2 μg/L (-3.51 points; 95 % CI:-6.64, -0.38). Children with the lowest quartile of blood-Mn (<8.2 μg/L) also had lower full scale IQ scores than children in the reference group although findings were nonsignificant (-2.14 points; 95%CI: -5.37, 1.09). The perceptual reasoning and processing speed scores had the strongest negative associations with blood-Mn.	Correlations between biomarkers found statistically significant included: blood-Mn and serum ferratin (mean: 34.4 ng/mL; r - 0.19, $p < 0.01$), blood-Mn and blood-Pb (mean: 0.82 μg/dL; r - 0.13, $p = 0.02$), and serum cotinine (0.08 μg/L) and blood-Pb ($r = 0.34$, $p < 0.0001$). Blood-Pb was significantly associated with processing speed, but not full scale IQ or other subscales. Cotinine was significantly associated with full scale IQ, perceptual reasoning, working memory and verbal comprehension.
Sun et al. (2015) [38].	8–12 year olds 16.2 μg/L	Not measured	Blood-Mn was not significantly associated with IQ, but it was associated with urinary retinol binding protein (RBP) which was associated with blood-Mn.	Blood-Pb (GM: 33.7 μg/L) was significantly, inversely associated with IQ.

[a] Geometric Means are given for exposed and control groups
[b] Median values for BMn
[c] Geometric Mean (GM) and Standard Deviation (GSD)

study (20 micrograms/L. vs 300 micrograms/L.). do Nascimento et al. also reported higher levels of Mn in hair and household tap water were associated with poorer IQ scores in children 6–12 years [37].

Only one study reported blood levels of Mn associated with both a measured environmental source and neurodevelopmental outcomes. Lucchini et al. reported levels of Mn in blood and hair were both positively associated with tremor intensity in the dominant hand; the authors also found a borderline association between soil-Mn and tremor intensity [33]. Comparisons between the exposed and reference communities revealed average concentrations of Mn in soil (958 ppm versus 427 ppm), respectively. The authors describe metals in soil as good indicators of general environmental insult given their stability over time in the environment reflecting both background soil deposition and cumulative inputs from atmospheric deposition of historical industrial emissions.

Two additional studies reported higher levels of Mn in both the hair and the blood of children who lived near an industrial source of Mn [15, 31]. Haynes et al. reported low and high Mn levels in blood and hair were associated with lower full IQ and subscale scores, with significant negative associations between the highest

Table 4 Summary of results from studies examining manganese in teeth

Study Author and Publication Date	Sample and Mean Mn Level (μg/L), (SD)	Association with Environmental Mn	Association with Neurodevelopment	Association with Metals
Ericson et al. (2007) [52]	Children from a maternal prenatal cohort that provided shed molars at 11–13 years. Mn concentrations from teeth enamel were measured but values not reported.	None measured	Prenatal Mn levels, representing exposures from the 20th gestational week were positively associated with behavioral outcomes: higher levels of disinhibition (36 months), impulsivity (4.5 years), externalizing and internalizing problems (1st and 3rd grades) and disruptive behaviors (3rd grade). No differences on standardized tests of cognitive ability or achievement. Analyses were adjusted for mothers' education, family income and child ethnicity. Postnatal Mn levels, representing exposures from gestational weeks 62–64, only correlated with teachers' reports of externalizing behaviors (1st and 3rd grades).	No association between pre- and postnatal Mn ($r = 0.13$, NS [1]), Mn and Pb (prenatal $r = 0.09$, NS; postnatal $r = -.08$, NS). A significant association was seen with prenatal Mn and Fe ($r = 0.74$, $p < 0.001$) but not postnatal Mn ($r = -.06$, NS).
Gunier et al. (2015) [45]	Children from a maternal prenatal cohort provided shed teeth starting at age 7. Mn from dentin of deciduous teeth [2] Prenatal: 0.51 (0.19) Postnatal: 0.20 (0.23).	Not reported, but related to residential proximity or use of agricultural fungicides with Mn.	Prenatal Mn levels were not associated with MDI or PDI at 6, 12 or 24 months and no interactions by sex. A two-fold increase in postnatal dentin- Mn levels was associated with small, significant decreases in MDI at 6 and 12 months (but NS at 24 months). Postnatal dentin-Mn levels were inversely related (but NS) with PDI scores at 6 months, but not 12 or 24 months. Effect modification by sex was reported with significant interactions between prenatal Mn and maternal hemoglobin (HGB) in girls at 6 months.	Girls whose mothers had lower prenatal hemoglobin (HGB, <11.6 g/dL) had a decrease of 10.5 points (95%CI: -16.2, -4.8; $n = 38$) on the MDI and 11.6 points (95%CI: 19.3, -3.9) on the PDI per two-fold increase in prenatal Mn at 6 months. No interactions with blood-Mn and blood-Pb observed or any relationships with neurodevelopment at 24 months.
Mora et al. (2015) [39]	Children from two integrated prenatal cohort samples provided teeth at 7–9 years. Mn from dentin of deciduous teeth: Prenatal: 0.46 (1.48); Postnatal: 0.14 (2.47)	None reported, but exposure related to agricultural exposures to Mn-containing fungicides.	Behavior: No significant associations for prenatal Mn and behavioral outcomes in children ages 7, 9 or 10.5 years. Higher postnatal Mn was significantly associated with maternal reports of hyperactive, internalizing and externalizing behaviors for children aged 7 years, but not at older ages. Cognition: Neither prenatal nor postnatal Mn was consistently and significantly associated with cognitive outcomes. A sex effect was shown only for boys with a positive, significant relationship between postnatal Mn and cognitive scores (full scale, verbal comprehension, and perceptual reasoning IQ) at ages 7 and 10.5 years, and working memory IQ at 7 years. Memory: Higher prenatal dentin Mn levels associated with significantly better memory scores for children ages 9 and 10.5 and in sex stratified analyses. Postnatal Mn levels were not associated with memory scores	Higher prenatal Mn levels were associated (NS) with poorer visual spatial memory outcomes at 9 years and poorer cognitive scores at 7 and 10. 5 years in children with higher Pb levels (≥0.8 μg/dL).

Table 4 Summary of results from studies examining manganese in teeth *(Continued)*

	in analyses of all children. Sex-stratified analyses revealed higher Mn significantly associated with better memory scores at 9 and 10. 5 years in boys. Motor function: No consistent, significant associations of prenatal Mn with motor function for all children. Sex-stratified analyses showed higher dentin Mn levels significantly associated with better motor function only in boys (finger tap Z-score at 7 years), Luria-Nebraska Motor Scale at 10.5 years). Postnatal Mn levels showed no consistent, significant associations for all children, but sex effects show higher dentin Mn levels associated with significantly better motor function scores only in boys at 7 years.

[a] NS refers to a statistical association that is not significant
[b] Geometric Means and Standard Deviations

versus middle two quartiles of blood-Mn (ß -3.51; 95 % CI: -6.64, -0.38) and hair-Mn (ß -3.66; 95 % CI: -6.9, -0.43) and full scale IQ in children ages 7–8 years [15]. Riojas-Rodriguez et al. found hair-Mn was inversely associated with verbal IQ (ß -0.29; 95%CI: -0.51, -0.08), performance IQ (ß -0.08; 95%CI: -0.32, -0.16), and total IQ (ß -0.20; 95%CI: -0.42, 0.02), in children ages 7–11 years [31]. The authors reported the 24 h median Mn in PM10 in exposed communities (0.13 micrograms/m^3) was higher than the exposed communities (0.02 micrograms/m^3).

Finally, Kahn et al. reported an inverse association between Mn in drinking water and children's annual test scores in mathematics [58]. Levels of Mn in water above 400 micrograms/L (the former WHO standard) was associated with a 6.4 percentage score loss (95 % CI = 0.5, 12.3) in test scores. This study did not test any Mn biomarkers, but a prior paper showed a lack of association between blood-Mn and water-Mn [29].

Discussion

A growing body of literature has examined the association of increased levels of Mn with neurodevelopmental effects in children from across the world. The evidence is most consistent in studies reporting decrements in IQ scores among primary school-aged children exposed to excessive levels of Mn. However, the inconsistency of findings in other studies reflects, in part, the considerable variation in study design including the source of Mn (water, air, or soil), exposure pathway (ingestion or inhalation), biomarkers measured (blood, hair, teeth, urine), study population (age, sex, and developmental and nutritional status) and neurological outcomes examined (IQ, motor skills, infant or early childhood development).

A recent pilot study tested the use of fMRI to reveal specific brain changes associated with Mn exposure. The findings revealed long-term exposure to Mn in the first stage of life can decrease olfactory function. There was also evidence that Mn exposure can adversely affect the functionality of the limbic system which the authors describe as suggestive of an alteration of the brain network in addressing emotional responses [59]. While scientifically promising, this approach may be less feasible for large, population studies of infants and young children given the expense and potential resistance of parents to having their children scanned for research in the absence of disease. However, with further testing in larger samples this approach could complement the use of biomarkers in studies of Mn exposure.

While relatively few studies investigated Mn exposures with biomarkers and neurodevelopment outcomes in infants, those studies using prospective study designs provided compelling evidence of the adverse effect of Mn. Biomakers of Mn using cord blood or serum provided a temporal association between fetal Mn exposures and later outcomes including cognitive and language development scores in 2 year olds [28], attention and nonverbal memory and hand skills in 3 year olds [43], and behavioral neurological development in newborns [57].

Measurement of Mn deposits in shed teeth provided insights more precise than those of cord blood or serum into the timing of early life exposures. While the CHAMACOS study is a large and comprehensive study of potential neurodevelopmental effects from pre-and postnatal dentin-Mn exposure in school-aged children [39, 45], the findings raise questions as the direction of the effects observed with higher levels pre- and postnatal Mn included both adverse effects with behavioral outcomes and positive effects with better memory abilities

[39] inconsistent with other studies of school age children reporting higher Mn levels associated with poorer memory [4, 16, 54] and cognitive outcomes [15, 16, 31, 32, 35–37]. These authors posit the inconsistent findings may be due to differences in the exposure matrix used to quantify Mn levels or Mn exposure pathways or possibly that the levels of Mn in their sample could be within the range at which Mn acts as a beneficial nutrient rather a than a neurotoxicant suggesting a need for additional research [39].

Based on the studies reviewed here, hair-Mn was the most frequently examined biomarker, and it was consistently associated with lower child IQ scores suggesting hair may be the most consistent and valid biomarker for Mn to date for children in population studies. While blood-Mn was associated with a range of neurodevelopmental outcomes, the findings across studies were inconsistent.

Bouchard and colleagues acknowledged the lack of consensus on an optimal biomarker of exposure to Mn and blood-Mn levels can vary widely in the short-term and likely does not reflect long-term exposure [32]. Oulhote et al. reported that blood and urine are poor measures of Mn exposure [47].

In contrast, hair-Mn is posited by these investigators as a more consistent and valid biomarker of Mn. Bouchard et al. reported that hair-Mn will reflect the metal uptake averaged over the duration of the follicle formation although the mechanism of Mn uptake into hair is not well understood [32]. Hair typically grows 1 cm per month thereby providing an exposure estimate of 1–6 months [15]. Lucchini et al.'s preliminary analysis of hair biomarkers of Mn suggests it may be a better measure of integrated exposure and body burden over the prolonged period of hair growth, relative to biomarkers of blood or urine Mn, due to its rapid homeostatic control [33]. However, variability in hair-Mn concentrations may be related to various factors including difference in exposure, pharmacokinetics, hair pigmentation and issues of sample collection and cleaning [15]. Hair analysis for Mn requires rigorous cleaning procedures to minimize contribution of external Mn contamination without comprising endogenously incorporated Mn [33, 60].

Interpretation of Mn levels in hair must be carefully evaluated because Mn levels may be higher in some hair types than others (i.e., in darker hair), and because dye, bleach or other topical treatment may either contaminate hair or effect Mn incorporation into its structure [61, 62], although topical hair treatment is less relevant for studies of children. Additionally, in a pilot study in progress we have found some infants lack sufficient hair to analyze.

The literature also lacks sufficient analyses of the connections between the environmental source, the internal dose and the associated neurodevelopmental and cognitive outcomes. Studies reported findings supporting the exposure-outcome relationship between Mn concentrations in water, hair and adverse outcomes in child neurodevelopment [32, 37, 47]. In contrast, investigators who collected data on well water-Mn, blood-Mn and neurological outcomes failed to demonstrate an association between Mn concentrations in water and blood [18, 29, 30]. However, a statistically significant and dose-dependent association between water-Mn concentrations and IQ scores (Full Scale, Performance and Verbal) was reported [18]. This result is important as it provides strong evidence that ingestion of drinking water is a major source of environmental Mn potentially related to adverse neurodevelopment.

Few studies provided evidence of the association of environmental sources, biomarkers of Mn and neurodevelopment outcomes. Torres-Agustín et al. reported significantly higher Mn in blood and hair in an exposed (versus control) group with respiratory exposure to fine particulate matter of 2.5 microns or less in width, although only hair-Mn was significantly associated with poorer neurological outcomes [54]. This is important, though they did not report how increases in air Mn content affected Mn biomarker levels. However, Lucchini et al. reported evidence of both blood-Mn and hair-Mn being associated with increased tremor intensity in the dominant hand, and a borderline association between soil-Mn and tremor intensity. These authors report that the soil-Mn reflects past or cumulative exposures [33]. Future studies also need to quantify the association between environmental sources and selected biomarkers.

Finally, it is important that the continuum of exposure is carefully measured given the possibility of an inverted U-shaped association between Mn exposure and children's health, neurodevelopment and cognitive outcomes. Ultimately, if public health programs are to provide prevention guidance for specific exposure sources such as drinking water, PM10 and soil regarding over-exposure to Mn, the threshold of beneficial Mn exposure must also be identified to ensure children receive the optimal benefit and the safe limit relative to their age and duration of exposure.

Conclusion

With evidence mounting for the negative impact of Mn on children, research is needed to address the gaps in the literature that would help elucidate safe levels of Mn exposure for fetuses, infants and children. There is a particular need for a consistent measurement approach to biomarkers of Mn, as well as for environmental exposure sources and neurological outcomes, to make research findings comparable across studies. Additionally, feasibility issues are important when selecting biomarkers of exposure. The most promising Mn biomarker to date for the study of children

is hair, but hair collection is not feasible for all infants and cleaning exogenous contamination of hair requires particular attention to evidence-based procedures. While cord blood appears an effective biomarker for measuring fetal exposure, it is logistically challenging and expensive to collect if study participants give birth at multiple hospitals. The use of teeth as a biomarker of Mn is intriguing, but it requires a minimum of 8 years from enrollment of pregnant women before children start to shed teeth that can be analyzed for Mn concentrations. The scientific and practical challenges of selecting the best biomarkers of Mn in children suggests the need for novel applications of additional biomarkers of chronic exposure to Mn. to help inform the science and ultimately determine public health prevention policies particularly for fetuses and infants given their heightened vulnerability to excessive Mn.

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Authors' contributions
DC conducted the original literature search, critically reviewed the human studies, drafted the paper and developed the tables. PM conceived of the paper, critically reviewed the human studies, revised drafts and supervised DC's work. RR drafted manuscript sections on the preclinical studies on the effects of excess Mn exposure on neurodevelopment and the pathways involved and reviewed all drafts of the paper. LH critically reviewed studies assessing Mn intake via diet, water and infant feeding practices and reviewed all drafts of the paper. MG helped conceptualize the paper and reviewed all drafts of the paper. IS helped conceptualize the paper, reviewed all drafts of the paper and oversaw review of the scientific content. All authors read and approved the final manuscript.

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Manganese Health Risk

- Recently published research identifies exposure to manganese via drinking water causes adverse health effects such as neurological disorders similar to Parkinson's disease
- Neuro-developmental disabilities including autism, attention deficit, hyperactivity, dyslexia and other cognitive impairments
- Epidemiological studies document manganese as a developmental neuro-toxicant
- Maternal manganese levels are associated with low birth weight

Groundwater Contamination

- Four distinct sites
  - New York State Department of Environmental Conservation (NYDEC) verified surface and groundwater manganese contamination from more than 12 mulch and natural vegetative composting facilities
  - Bassler Forest Recycling Products site in Howard County, Maryland is identified with groundwater metals contamination

Groundwater Contamination

- Oregon State Engineers Office and Oregon Department of Environmental Quality published a research paper titled "Groundwater Pollution by Wood Waste Disposal"- identified Manganese groundwater contamination

- Connecticut Department of Energy and Environmental Pollution, Remediation Division Chief Bill Warzecha confirmed wood waste leachate as causing significant manganese groundwater contamination

New York Environmental Investigation Report

- New York State Department of Environmental Conservation (NYSDEC)
- NY State Department of Health
- Suffolk County Department of Health Services
  - Horseblock Road Investigation, Yaphank, NY (July 2013)

New York State Investigation Conclusion

- "This data in conjunction with the data from the current investigation suggests that compost/vegetative organic waste site operations can cause an elevation of manganese concentrations in groundwater."

New York State Response

- Residents using drinking water wells were connected to municipal water supply due to exposure to high levels of manganese
- Tens of millions of dollars was spent to remediate, retrofit facilities, and promulgated new regulations for operations and to limit the amount and type materials allowed at wood waste recycling facilities

Bassler Forest Recycling Products (FRP)

- Howard County Natural Yard Waste Composting Facility
  - Accepted wood waste to naturally decompose through compost processes in static and windrow piles
  - Located west of Clarksville, MD, 1.7-miles east of the proposed Dayton mulch/compost and soil screening facility with the same geologic setting "Wissahickon Schist"
  - Seven wells continue to monitor groundwater quality since at least 2007

Bassler FRP Groundwater Contamination

Contaminant	Max Conc. (µg/L)	Average Conc. (µg/L)	MCL/RSL (µg/L)	Number of Exceedances
Lead	77	44	15	19
Thallium	13	2.2*	2	10
Antimony	34	21.1	6	3
Cadmium	12	11.6	5	3
Arsenic	11	9.2*	10	3
Manganese	13,000	1960	320	56
Iron	52,000	31,000	11,000	12

Five of the seven metals noted have maximum contaminant levels (MCLs) regulated by the Safe Drinking Water Act that are legally enforceable in public water supply systems

RSLs are risk based calculations that set concentration limits

*Calculated using ½-U qualifier concentration

Oregon Environmental Investigation

- Groundwater Pollution by Wood Waste Disposal

- Investigation identified:
  - Wood waste leachate-yielded high concentrations of volatile organic acids
  - Leachate was oxygen demanding and created a reducing environment
  - High concentrations of Manganese were identified in the groundwater to 106,000 μg/L

Oregon Environmental Investigation

- Investigation Conclusion:
  - The reducing environment disassociated manganese from the substratum significantly increasing manganese in the groundwater
  - These environmental factors degraded groundwater to non-potable quality

Oregon Environmental Investigation Response

- Response:
  - City of Turner extended community water supply to the affected home owners

Connecticut Department of Energy and Environmental Protection

- Remediation Division Chief Bill Warzecha
  Tel: 860-424-3776

- Confirmed significant environmental contamination associated with organic leachate
  - Confirmed the process by leachate creating reducing environment
  - Currently gathering data for distribution

Manganese

Manganese (µg/L)	FDA Bottled Water Limit	EPA Regional Screening Level (May 2013)	Connecticut Drinking Water Action Level	ATSDR 1-Day Child Health Advisory	Max Conc. (µg/L)
New York	50	320		1,000	43,000
Bassler (MD)	50	320		1,000	13,000*
Oregon (City of Turner)	50	320		1,000	106,000
Connecticut	50	320	500	1,000	

*Manganese background average for Clarksville West- 20 µg/l

Sources of pollution rich in organic matter such as wood compost can increase the release of manganese and other metals from soil and bedrock into groundwater.

Connecticut Factsheet

Connecticut Department of Public Health maintains a factsheet titled "Manganese in Drinking Water."

- Set a drinking water action level for manganese at 500 μg/L to ensure the protection against manganese toxicity
- "Exposure to high concentrations of manganese over the course of years has been associated with toxicity to the nervous system, producing a syndrome that resembles Parkinsonism."

Leaching Mechanism

Natural wood waste recycling/composting operations allow ground up natural vegetation to compost in large windrows over long time periods. The piles are wetted to help eliminate spontaneous combustion. The water used in wetting operations including rain creates an organic discharge that infiltrates the porous ground surface.

The discharge water is high in organic content (carbohydrates, organic acids, lignin, humic material, carboxylic, hydroxides and amino acids). When the high organic discharge water infiltrates the ground, multiple geochemical reactions occur that mobilize the existing metals from the soil structure

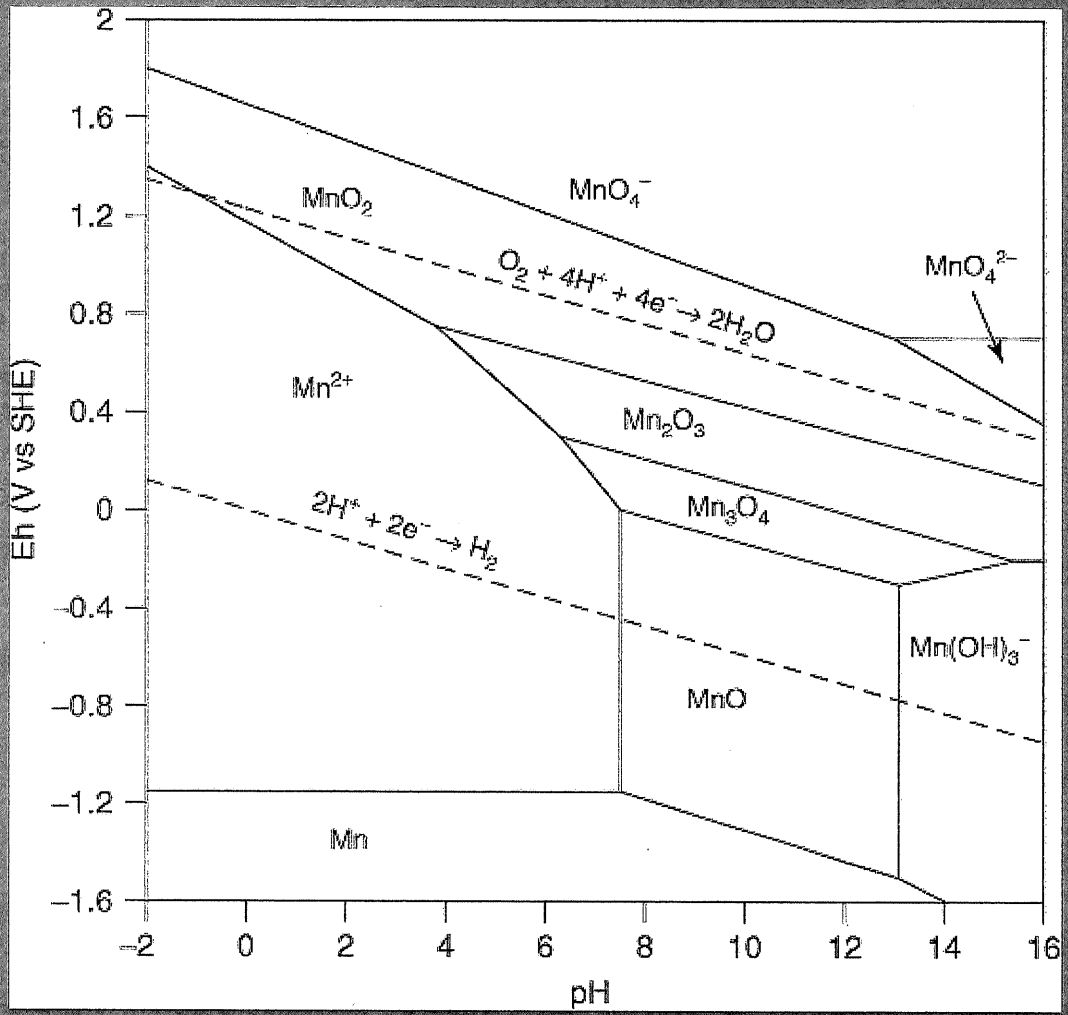
- Creates a negative Oxidation Reduction Potential environment
- Creates a low pH environment
- Water soluble complexes form
- Colloidal transport

Negative ORP

- Organic material, high in chemical and biological oxygen demand, create a low Eh / negative Oxidation Reduction Potential (ORP) or reducing environment
- Negative oxygen reducing potential allows the manganese (cations) to be electron acceptors
- Metal oxides reduce, allowing the cations to become mobile in a low valence, soluble ionic form

$$Mn^{(4+)}O_2 + C_xH_y \longrightarrow Mn^{(0)} + CO_2 + H_2O$$

Manganese Eh-pH Diagram



Low pH Environment

- Organic acids reduce the pH and allow the H^+ ions to replace cations in soil structure releasing metals in ionic form
- Metals phase stability of manganese begins leaching at a pH of 6
- As water reaches a lower pH, a wider variety of metals are liberated and migrate

Water Soluble Complexes

- Organics form water-soluble complexes with the metals that are less reactive with the soil structure and become mobile.

Colloidal Flow

- Flushing of metals through the soil to the groundwater table occurs as colloidal particles

Manganese Health Risk

- Recently published research identifies exposure to manganese via drinking water causes adverse health effects such as neurological disorders similar to Parkinson's disease
- Neuro-developmental disabilities including autism, attention deficit, hyperactivity, dyslexia and other cognitive impairments
- Epidemiological studies document manganese as a developmental neuro-toxicant
- Maternal manganese levels are associated with low birth weight

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COUNTY OF SUFFOLK

STEVEN BELLONE
SUFFOLK COUNTY EXECUTIVE

DEPARTMENT OF HEALTH SERVICES

JAMES L. TOMARKEN, MD, MPH, MBA, MSW
Commissioner

January 27, 2016

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Dear Ms. Gallagher:

Attached is a Suffolk County Department of Health Services (SCDHS) report summarizing additional groundwater sampling conducted in the vicinity of vegetative organic waste management facilities (VOWM). This "Investigation of the Impacts to Groundwater Quality from Compost/Vegetative Organic Waste Management Facilities in Suffolk County" was conducted in follow up to a prior SCDHS groundwater investigation in the vicinity of the Great Gardens/Long Island Compost facility in Yaphank, NY, results of which were released by the New York State Department of Environmental Conservation (NYSDEC) in a 2013 report titled; *Horseblock Road Investigation, Yaphank NY.*

SCDHS initiated this additional study to investigate whether groundwater impacts similar to those observed in the Horseblock Road investigation would be observed downgradient of other VOWM sites. The attached report provides the results of groundwater samples taken downgradient of eleven VOWM sites between July of 2011 and October 2014.

The results of this groundwater sampling effort confirm the prior observation of elevated metals, primarily manganese, and atypical elevated concentrations of radiological parameters, in groundwater downgradient of VOWM facilities. Based on these findings, the attached report provides specific recommendations to address these groundwater concerns, including revisions to NYSDEC Solid Waste Management regulations.

SCDHS would like to acknowledge our appreciation to the Region 1 Office of the New York State Department of Environmental Conservation for their assistance, and the New York State Department of Health (NYSDOH) Wadsworth Laboratory for performing a subset of the radiological analyses of the groundwater samples.

Sincerely,

James L Tomarken

James L. Tomarken, MD, MPH, MBA, MSW
Commissioner

JLT/srg

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Cynthia Costello, MS, MPH, CHP, Chief Environmental Radiation/Radon Section, NYSDOH
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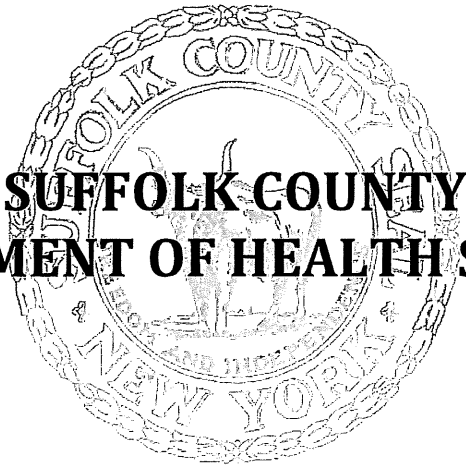
Investigation of the Impacts to Groundwater Quality from Compost/Vegetative Organic Waste Management Facilities in Suffolk County



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DEPARTMENT OF HEALTH SERVICES

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January 22, 2016

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List of Figures and Tables

Executive Summary

The Suffolk County Department of Health Services (SCDHS) Office of Water Resources investigated impacts to groundwater at eleven current or former vegetative organic waste management (VOWM) sites located throughout Suffolk County. These investigations were prompted after samples collected from a residential drinking water well, and subsequently installed monitoring wells, located downgradient of the Long Island Compost/Great Gardens facility in Yaphank indicated several contaminants at concentrations in excess of New York State drinking water maximum contaminant levels (MCLs) and New York State Department of Environmental Conservation (NYSDEC) groundwater standards/guidance values. This report summarizes the data from 233 groundwater and two surface water samples that were collected from 30 temporary profile wells and six permanent monitoring wells installed by the SCDHS primarily downgradient of VOWM related sites. The general investigation approach used in this study is consistent with other landuse impact studies the SCDHS has performed in the past.

Samples were collected from July of 2011 through October of 2014. Elevated metals concentrations were the primary impact observed to the groundwater downgradient of the sites investigated. Elevated metals concentrations were observed in monitoring wells downgradient of 10 sites, and in four private wells downgradient of one site. The primary constituent that exceeded groundwater and drinking water standards most frequently, and at the highest concentrations, was manganese. Other metals such as antimony, arsenic, beryllium, cadmium, chromium, cobalt, germanium, molybdenum, thallium, titanium and vanadium exhibited detection rates that were at least two times that of typical Suffolk County shallow private wells. Additionally, the number of radiological detections (gross alpha and gross beta) was higher than what is typically observed in native Suffolk County groundwater. Relatively low concentrations of pesticides were reported at a majority of the sites, but due to past and current farming activities at many of the sites, these impacts cannot be exclusively attributable to VOWM activities. The pesticide dichlorvos was reported at two sites that have no apparent history of farming, and therefore its presence could be attributable to the VOWM activity. Additionally, low concentrations of pharmaceuticals, personal care products and wastewater related contaminants (PPCPWRCs) were consistently detected downgradient of the sites, and in some instances may be attributable to the VOWM activity at the sites.

The potential for the existence of private wells downgradient of the investigation sites was evaluated. Private well sampling surveys were performed at three of the sites. Site #1 was the only site that has private wells downgradient which exhibited degraded water quality consistent with VOWM related groundwater impacts. This information has been forwarded to the NYSDEC. The location of public water supply wellfields in the vicinity of each investigation site was also evaluated. Three of the eleven sites have public water supply wellfields located in the downgradient

groundwater flow direction. Two of the sites are located greater than 100 years of groundwater travel time to the wellfields, and the third site is located outside the wellfield's groundwater contributing area, therefore no public wellfields have been identified as being imminently threatened by the groundwater impacts observed in this study.

The data collected indicates that water quality downgradient of the vegetative organic waste management facilities studied exhibited impacts. Further evaluation indicates that groundwater impacts are attributable to VOWM activities at eight of the sites, and impacts were indeterminate at three sites. The water quality data shows similar impacts to the groundwater quality that was previously observed in the SCDHS data collected at the Great Gardens/Long Island Compost facility in Yaphank NY, and documented in the report entitled *Horseblock Road Investigation, Yaphank NY* issued by the New York State Department of Environmental Conservation. Most notably, an increase in metals concentrations, particularly manganese, and increased detections of radiological parameters (gross alpha and gross beta) were observed downgradient of both the Great Gardens/Horseblock Road Facility and the sites evaluated in this study. The groundwater impacts observed downgradient of the Great Gardens/Horseblock Road Facility do not appear to be unique to this facility. Similar groundwater impacts have now been observed at many compost/vegetative organic waste facilities throughout Suffolk County and appear to be related to the compost/vegetative waste operations taking place at these sites.

Based upon the study's findings and conclusions, the following recommendations are made:

- The NYSDEC should ensure that mechanisms are in place and that operating practices at VOWM facilities prevent detrimental impacts to groundwater and surface water quality.

- NYSDEC Part 360 Solid Waste Management Regulations governing VOWM facilities should be revised to protect against impacts to groundwater and surface water quality. Until this is accomplished, prior to the issuance of any new VOWM permits/registrations, the NYSDEC should evaluate, and take measures to ensure that any potential impacts to public/private wells, and/or surface water bodies located hydraulically downgradient of these facilities are mitigated.

- NYSDEC Part 360 Solid Waste Management Regulations should be expanded to include facilities that process vegetative organic type materials which currently do not fall under the purview of current regulations.

- The NYSDEC should further investigate the detection of parameters typically related to septic waste (e.g., pharmaceuticals, personal care products, wastewater related

contaminants, etc.) observed downgradient and within surface water run-off related to vegetative organic wastes.

- The NYSDEC should investigate the mechanisms that cause elevated concentrations of gross alpha/gross beta, metals, inorganic parameters and detections of pharmaceuticals and personal care products downgradient of compost/vegetative organic waste management sites.

- The Suffolk County Department of Health Services should continue to identify areas where private wells may be used downgradient of VOWM sites, and conduct private well sampling surveys as appropriate. The NYSDEC should provide an alternative water supply or filtration to owners whose on-site water sources are determined to have been impacted from VOWM operations.

- New or current facilities that are permitted or registered for vegetative organic waste operations should be required by the NYSDEC to assess the quality of the groundwater migrating from the site.

Summary of Findings

Site #	Site Name	Location	Impacted Groundwater from VOWM Activity Observed	Comments
1	Fifth Avenue	Speonk	Yes	Significant impacts observed in the on-site and 3 downgradient private wells.
2	Moriches-Riverhead Rd Farm	Eastport	Yes	Significant groundwater impacts observed in 2 of 3 monitoring wells.
3	Papermill Rd Facility	Manorville	Yes	Significant impacts observed in all 3 monitoring wells. Groundwater impacts from historical site use (landfill, septic sludge lagoons) also observed.
4	Exit 69 LIE Ramp	Manorville	Yes	Significant groundwater impacts observed in the groundwater profile well. Contaminants typically associated with septic waste observed in a pool of run-off water.
5	South Street Farm	Manorville	Indeterminate	Although slight groundwater impacts were observed, no definitive conclusions can be drawn due to the significant distance from the compost windrows to the monitoring wells.
6	Moriches-Yaphank Rd Farm	Manorville	Indeterminate	Although slight groundwater impacts were observed, no definitive conclusions can be drawn most likely due to the site not having any significant VOWM activity for 5 years prior to groundwater sampling.
7	East Main Street	Yaphank	Yes	Significant groundwater impacts observed in 4 of 5 monitoring wells.
8	LIE North Service Rd Farm	Yaphank	Indeterminate	Additional wells need to be installed further to the east in order to appropriately assess potential impacts from vegetative organic wastes. The significant distance from potential sources to well locations could be a confounding factor.
9	Islip Town Compost Facility	Ronkonkoma	Yes	Significant groundwater impacts observed in both the monitoring wells installed at this site.
10	Conklin St. Site	Farmingdale	Yes	Moderate groundwater impacts observed in 1 of 3 monitoring wells.
11	Peconic Ave Site	Medford	Yes	Significant groundwater impacts observed in 3 of 5 downgradient monitoring wells.

Background

In order to investigate the source of impacts to a private well located on Horseblock Road in Yaphank, in 2009, the Suffolk County Department of Health Services (SCDHS) initiated a groundwater investigation in the vicinity of the Great Gardens/Long Island Compost facility in Yaphank, N.Y. This groundwater investigation consisted of the installation and sampling of groundwater monitoring wells. The results of this investigation are included in a report entitled *Horseblock Road Investigation, Yaphank NY* and was released by the New York State Department of Environmental Conservation (NYSDEC) in July of 2013. This report concluded that the Great Gardens/Long Island Compost Facility was the source of the exceedances of groundwater standards for manganese, iron, thallium, gross alpha, gross beta, radium, chloride and ammonia.

The present study was undertaken to evaluate the groundwater quality downgradient of other vegetative organic waste management (VOWM) sites (e.g., storing of land clearing debris, composting, mulching, etc.) to determine if impacts similar to those documented at the Great Gardens/Long Island Compost facility were occurring. This study was performed in conjunction with the NYSDEC and the New York State Department of Health (NYSDOH). The NYSDEC primarily assisted in obtaining access for the SCDHS to install groundwater monitoring wells at the Town of Islip Compost Facility, and Brookhaven Town's Papermill Road Composting Facility, and also coordinating a subset of radiological analyses performed by the NYSDOH Wadsworth Laboratory.

Approach to Investigations

The investigations consisted of the installation of between one and five temporary profile monitoring wells at 10 of the sites, and six permanent monitoring wells at one site, for a total of 36 wells. These wells were located hydraulically downgradient of the site with respect to the direction of regional groundwater flow. Wells were installed to depths ranging from 65 feet to 135 feet deep, with a well screen five feet in length. Each of the temporary profile wells were initially sampled at the deepest level and then pulled up every ten feet and sampled again. This process was repeated until the top of the water table was reached. This procedure resulted in the collection of five to nine samples in each well, producing in an analytical profile of the groundwater from the top of the water table down to the depth at which the well was drilled. A total of 233 groundwater samples were collected. Samples were collected beginning in July of 2011 and continued through October of 2014. At two locations, surface water samples were collected and analyzed.

It should be noted that, except for Site #11, temporary profile wells were only installed in the general downgradient groundwater flow direction. The general approach used in this investigation is consistent with other landuse impact studies the SCDHS has performed in the past.

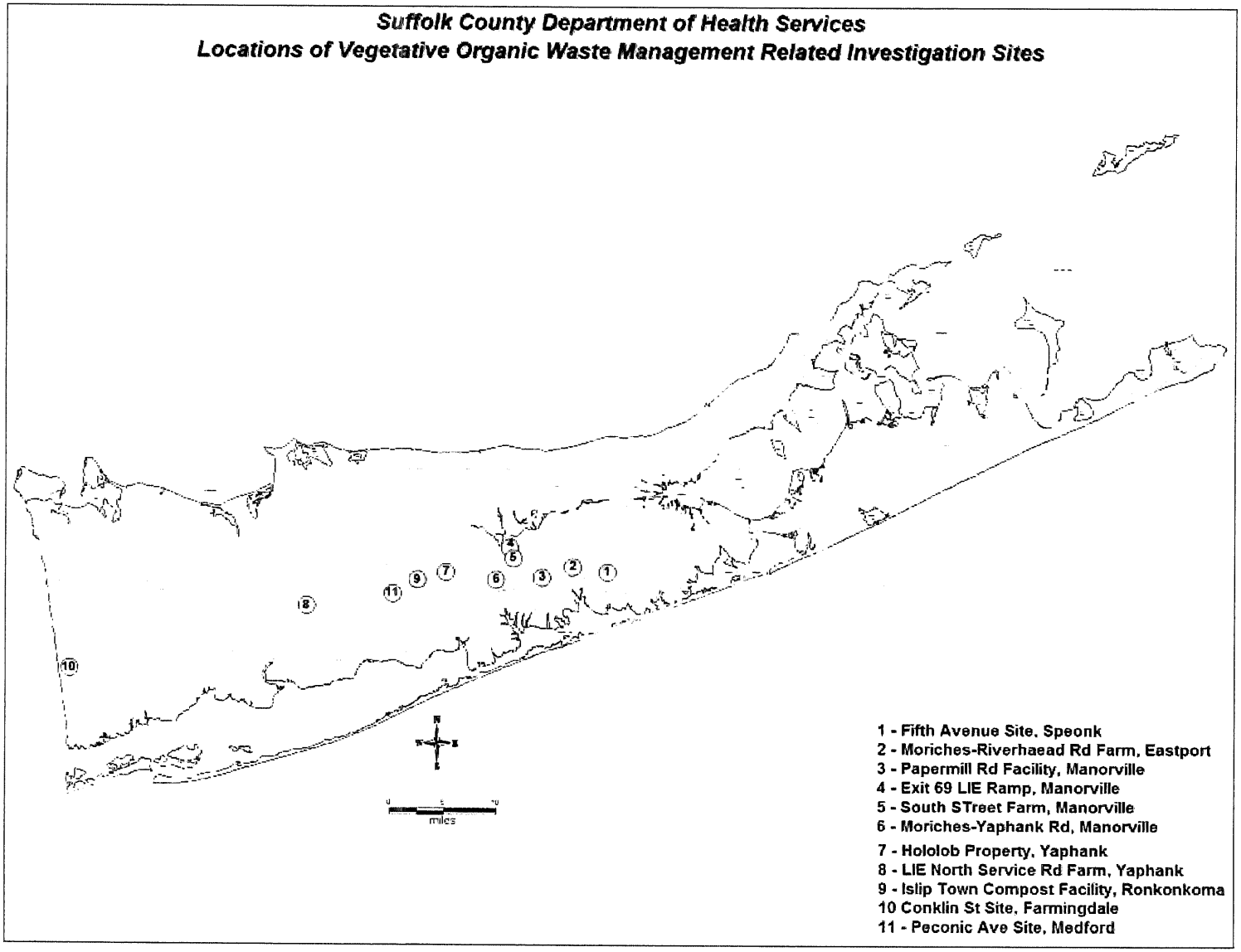
Sites

Table 1 lists the sites investigated for this study. Sites were selected either from information obtained from the NYSDEC, or from the review of landuses using aerial photographs. One important factor that had to be considered prior to an inclusion of a site in this study was appropriate access for the installation of groundwater monitoring wells in the downgradient groundwater flow direction from the site. The subsequent sections provide a description of the investigative activities performed at each of the sites and the findings.

Table 1 - List of Study Sites

Site #	Site Name	Location
1	Fifth Avenue	Speonk
2	Moriches-Riverhead Rd Farm	Eastport
3	Papermill Rd Facility	Manorville
4	Exit 69 LIE Ramp	Manorville
5	South Street Farm	Manorville
6	Moriches-Yaphank Rd Farm	Manorville
7	East Main Street	Yaphank
8	LIE North Service Rd Farm	Yaphank
9	Islip Town Compost Facility	Ronkonkoma
10	Conklin St. Site	Farmingdale
11	Peconic Ave Site	Medford

Figure 1 –Study Site Locations



Site #1
Fifth Avenue
Speonk, NY

Site Description

The site is located on a nine acre tax lot along Fifth Avenue in Speonk. Review of historical aerial photography (Appendix A) indicates that approximately half the site was cleared in 1947, and by 1969-70 the entire site was cleared and being used for the storage of vehicles. This site use appears to be consistent through 1999. The 2001 photograph shows the first indication of possible vegetative organic waste material on the site, primarily on the northern half of the property. All the subsequent aerial photographs (2004 – 2013) indicate significant VOWM activity across most of the site. The site is regulated by NYSDEC as a Part 360 Registered Facility, and is authorized to process unaltered wood. Another NYSDEC registered yard waste composting facility (Long Island Compost Farm #30) is located in the vicinity, to the northwest of this site (Figure 2).

SCDHS Monitoring Wells

The SCDHS installed 3 temporary profile monitoring wells in the vicinity of this site. The locations of these wells were based upon a south-southwest regional groundwater flow direction. Subsequent to the installation and sampling of these wells, additional site-specific groundwater flow direction information became available from the NYSDEC BB&S Lumber Superfund site, located just to the west of the facility (Figure 2). This site specific groundwater flow information indicated a slight variation from the regional groundwater flow direction, suggesting a more south-southeast groundwater flow direction. A consequence of the slight shift in groundwater flow direction is that the three temporary profile wells do not appear to be located downgradient of the target site. Therefore, the results from the three profile wells are not indicative of the water quality downgradient of this facility, and cannot be used to assess potential impacts of the site related activity on groundwater quality.

In each of the three wells, six levels were sampled resulting in the collection of 18 distinct groundwater samples. None of the parameters tested exceed their respective drinking water maximum contaminant levels (MCLs), guidance values or groundwater standards. However, as discussed above, information obtained subsequent to the installation of these wells indicate that they were not optimally located downgradient of the facility, and the results cannot be used to assess impacts to water quality from the operations from this facility.

Figure 2 - Site #1 & Vicinity – Fifth Ave, Speonk

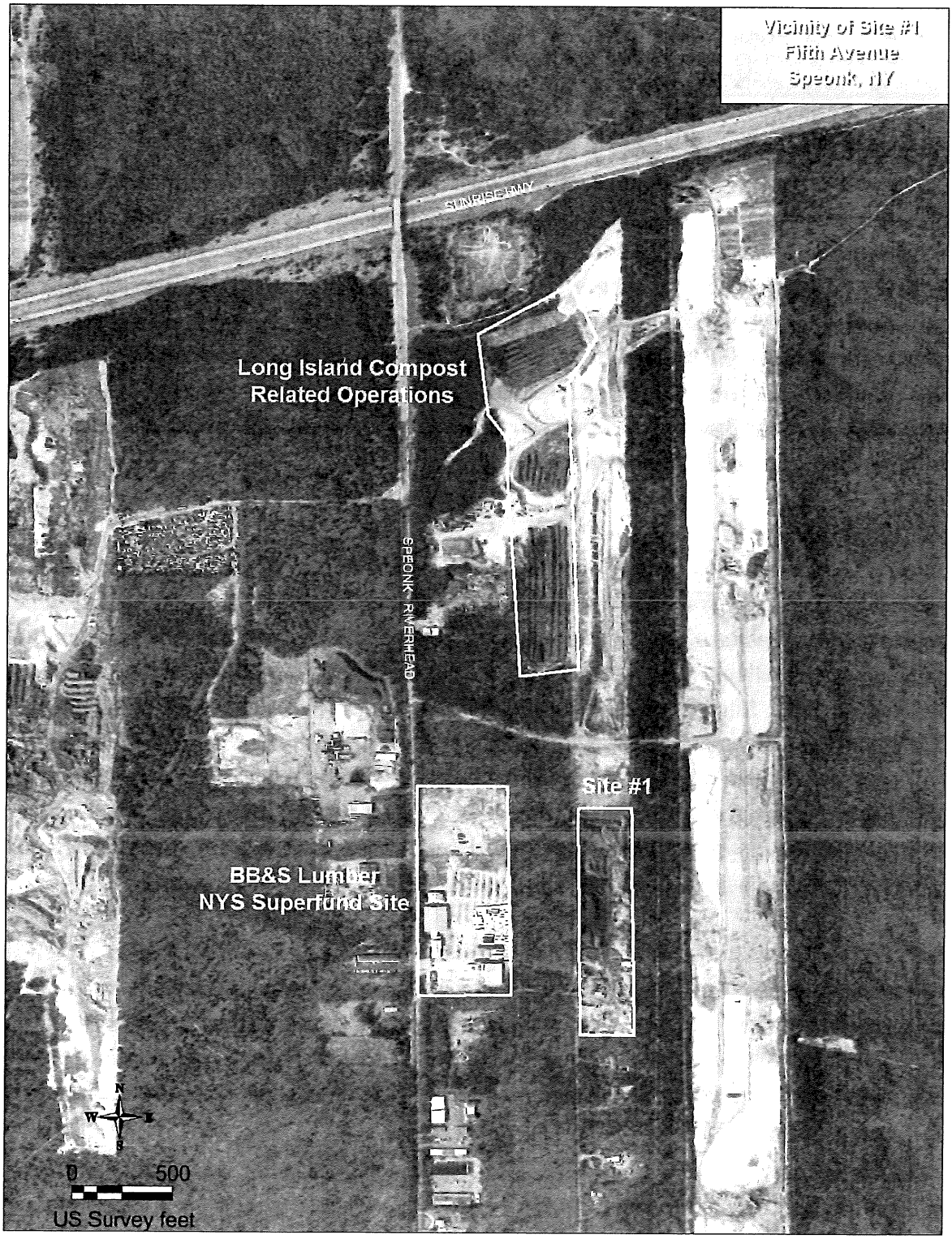
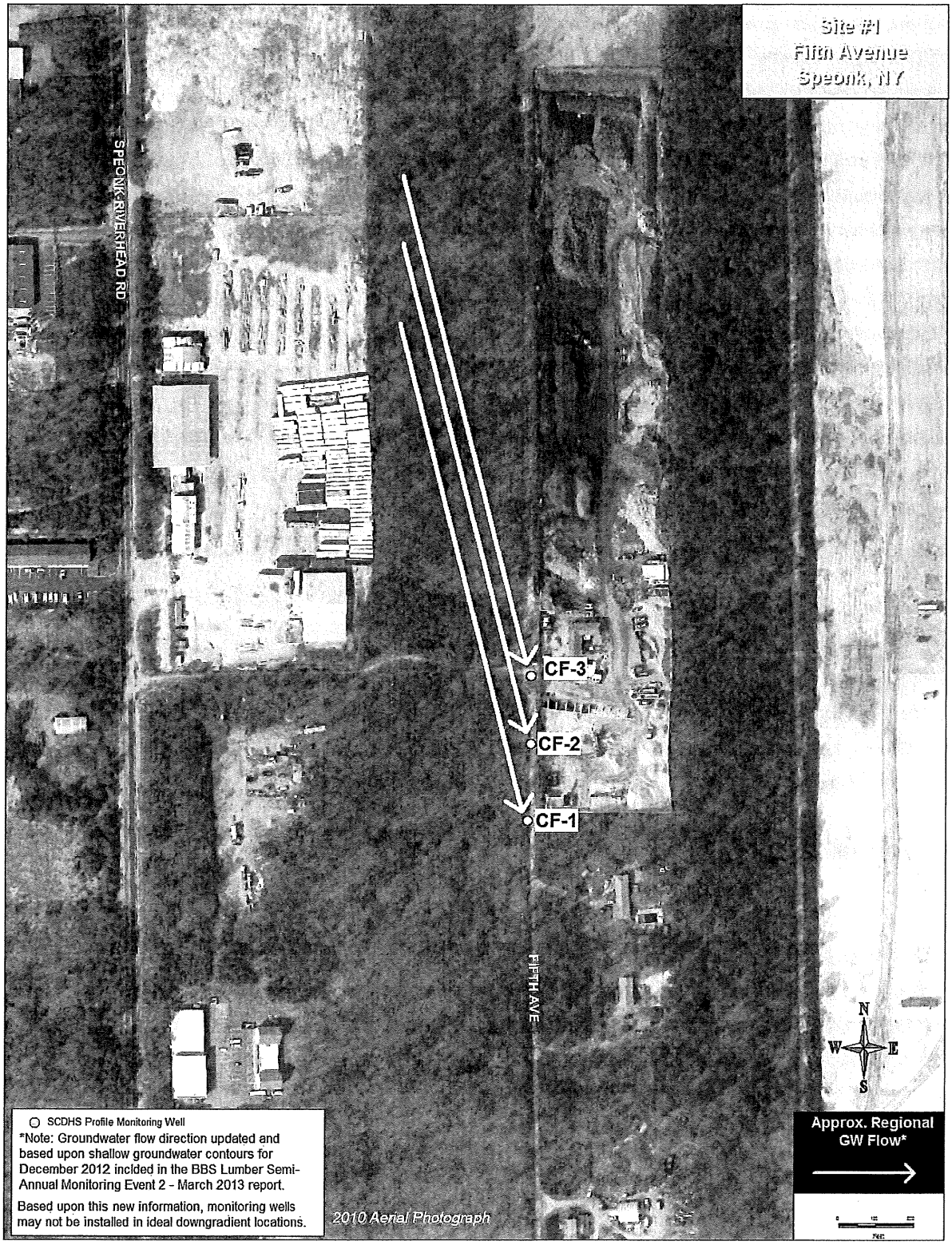


Figure 3 - Site #1 – Fifth Ave, Speonk Well Locations



Private Wells

Ten properties in the vicinity of this facility are located in the general downgradient direction from the site and are served by private wells (including the facility itself). Due to the proximity of this facility to the NYSDEC BB&S Lumber Superfund Site, the SCDHS and NYSDEC have historically conducted a number of private well sampling surveys in the area. Samples have been collected on some of these properties as early as 1999. A review of the data (SCDHS & NYSDEC) indicates that the quality of the water in four private wells are exhibiting impacts consistent with those from groundwater impacted at other vegetative organic waste management sites within Suffolk County. Recent sampling in all four of these private wells shows a general increasing trend in metal concentrations when compared with the older samples. Metals such as barium, manganese and potassium, which were also found at elevated concentrations downgradient of the Great Gardens/Long Island Compost Facility in Yaphank, exhibited particularly significant increases in these wells (e.g., in one well the 1999 manganese concentration was 8.8 parts per billion (ppb), by 2013 it had increased to 1,070 ppb). Since the older private well samples had relatively low concentration of these metals, it appears likely that more recent landuse activity upgradient of these wells has caused the degradation of the water quality in this area. The following analytes have been detected in these private wells at concentrations exceeding a drinking water and/or groundwater standard:

Manganese	**Zinc**
Copper	**Iron**

Public Wellfields

The nearest public supply wellfield is approximately 0.75 miles from the site and is not located downgradient of the site. Any impacts to groundwater quality as results of this site's operations would not be expected to affect the water quality of this wellfield.

Summary of Significant Analytical Results

Metals

As noted above, there was an increasing trend in the concentration of manganese, zinc, copper and iron in four of the private wells located downgradient of the site (e.g., in one well the 1999 manganese concentration was 8.8 parts per billion (ppb), by 2013 it had increased to 1,070 ppb). Other metals such as barium and potassium also showed increasing trends.

Discussion

The three groundwater monitoring wells installed at this site were subsequently found to be located side gradient of the site rather than downgradient, and therefore the results from these wells cannot be used to assess impacts to groundwater quality occurring from operations at this site. However,

since these wells are not located downgradient of this site, the information can be used to provide information on the general background water quality that may be expected in this area. Review of the private well data indicates that at least 4 private wells appear to have been impacted by VOWM related activities.

Wells Impacted by VOWM Activity

There were no profile wells that were affected; however, at least 4 private wells appear to be impacted in connection with VOWM related activities.

Table 2
Summary of Detected Analytes
Monitoring Wells Installed in the Vicinity of Site #1
Speonk, NY

Well Information			Parameters					Metals											
Well ID	Screen Interval (ft) (depth below grade)	Sample Date	Depth To Water (Feet)	Dissolved Oxygen(mg/L)	Temperature (Celsius)	pH	Conductivity (uS)	Aluminum (ppb)	Barium (ppb)	Cobalt (ppb)	Manganese (ppb)	Molybdenum (ppb)	Nickel (ppb)	Lead (ppb)	Strontium (ppb)	Magnesium (ppm)	Sodium (ppm)	Calcium (ppm)	Potassium (ppm)
DEC TOGS 1.1.1 Guidance Values			-	-	-	-	-	-	-	-	-	-	-	-	-	35	-	-	-
DEC Part 703 Class GA Groundwater Standards			-	-	-	-	-	-	1,000	-	300	-	100	25	-	-	20	-	-
DOH Drinking Water Standards Subpart 5-1			-	-	-	-	-	-	2,000	-	300	-	100	15***	-	-	-	-	-
CF-1	50-55	1/31/2012	41	6.24	11.8	5.4	74	28	17	<1	15	<1	<0.5	<1	64	2.9	5.6	2.7	0.9
	60-65	1/31/2012	41	6.44	11.7	5.6	43	12	8	<1	3	<1	<0.5	<1	24	1.3	3.8	1.4	0.5
	70-75	1/31/2012	41	6.52	11.7	5.7	49	7	9	<1	1	<1	<0.5	<1	19	1.5	4.2	1.7	0.6
	80-85	1/4/2012	41	8.75	10.2	6.12	62	<5	9	<1	<1	<1	<0.5	<1	17	1.9	5.3	1.5	0.5
	90-95	1/4/2012	41	9.93	10.2	6.2	48	<5	7	<1	<1	<1	<0.5	<1	12	1.2	4.2	0.9	0.4
	100-105	1/4/2012	41	9.36	9.2	6.1	61	<5	8	<1	<1	<1	<0.5	<1	16	1.7	4.7	1.5	0.4
CF-2	50-55	2/6/2012	41.65	5.99	12.9	6.71	69	19	18	2	39	<1	<0.5	<1	60	1.7	5.7	1.8	0.7
	60-65	2/6/2012	41.65	6.27	13.3	6.78	61	6	12	<1	2	<1	<0.5	<1	34	1.7	4.5	1.7	0.6
	70-75	2/6/2012	41.65	5.98	13	6.84	58	<5	11	<1	<1	<1	<0.5	<1	26	1.5	4.2	2.7	0.6
	80-85	2/6/2012	41.65	6.45	13	6.8	69	5	12	<1	<1	<1	<0.5	<1	23	2.2	5.6	1.7	0.6
	90-95	2/6/2012	41.65	7.04	13.4	6.98	50	15	7	<1	<1	<1	<0.5	<1	14	1.4	4.1	1	4
	100-105	2/6/2012	41.65	6.78	NA	7.32	60	<5	7	2	<1	<1	<0.5	1	17	1.6	4.4	1.3	0.4
CF-3	50-55	2/15/2012	41.6	6.71	12.5	6.55	77	32	2.1	<1	90	<1	1.1	<1	55	1.7	6.1	2.6	0.8
	60-65	2/15/2012	41.6	7.79	12.2	6.78	65	25	12	<1	4	<1	<0.5	<1	36	1.8	4.6	2.4	0.6
	70-75	2/15/2012	41.6	7.54	11.4	7.17	74	8	14	<1	2	<1	0.5	<1	31	2	5.3	2.5	0.7
	80-85	2/14/2012	41.6	7.08	11.8	8.71	175	18	15	<1	1	2	0.6	<1	26	2.7	5.7	2.1	0.7
	90-95	2/14/2012	41.6	8.41	11.6	7.55	53	<5	7	<1	<1	<1	<0.5	<1	15	1.5	4.2	1.1	0.4
	100-105	2/14/2012	41.6	8.43	11.4	9.93	69	<5	9	<1	<1	<1	<0.5	<1	22	1.9	4.9	1.8	0.4

Notes:
NA = Sample collected, analyte not reported
NS = No Sample Collected
"<" = less than, indicating no detection
ppb = part per billion
ppm = part per million
uS = micro siemens
☐ indicates concentration exceeds a standard or guidance value

Table 2
Summary of Detected Analytes
Monitoring Wells Installed in the Vicinity of Site #1
Speonk, NY

Well Information			Radiologicals (pCi/L)												Standard Inorganics				VOCs (ppb)
			SCDHS PEHL			NYSDOH Wadsworth													
Well ID	Screen Interval (ft) (depth below grade)	Sample Date	Gross Alpha	Gross Beta	Adjusted Gross Beta*(AGB)	Gross Alpha	Gross Beta	Ruthenium 106	Cesium 137	Zirconium 95	Potassium 40	Actinium 228	Radium 224	Radium 226	Chloride (ppm)	Sulfate (ppm)	Nitrate (ppm)	Total Alkalinity(mg CaCO3/L)	Chloroform (ppb)
DEC TOGS 1.1.1 Guidance Values			-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-
DEC Part 703 Class GA Groundwater Standards			15^	1,000^^	-	15^	1,000	-	-	-	-	-	-	-	250	250	10	-	7
DOH Drinking Water Standards Subpart 5-1			15	-	50**	15	-	-	-	-	-	-	-	5^^^	250	250	10	-	80
CF-1	50-55	1/31/2012	<1	6.9±0.7	6.7±0.7	<0.25	3.1 ±0.8	<2.9	<0.3	<0.78	<2.5	<1	NA	NA	10	11	<0.5	NA	1.3
	60-65	1/31/2012	<1	4.9±0.7	4.5±0.7	<0.18	0.8 ±0.7	<2.3	<0.23	<0.66	<2.1	<0.84	NA	NA	7	5	<0.5	NA	0.7
	70-75	1/31/2012	<1	5.0±0.7	4.5±0.7	<0.18	<0.8	<2.6	<0.24	<0.96	<2.1	<0.79	NA	NA	7	6	<0.5	NA	1.3
	80-85	1/4/2012	<1	<1	<1	<0.18	<0.8	<2.5	<0.24	<0.87	<1.9	<0.81	NA	NA	7	7	<0.5	8	0.7
	90-95	1/4/2012	<1	<1	<1	<0.17	<0.8	<3.1	<0.32	<1.2	<2.8	<1.1	NA	NA	6	6	<0.5	4	1.2
	100-105	1/4/2012	<1	<1	<1	<0.31	<0.8	<2.9	<0.31	<1.2	0.4 ±0.29	<1	NA	NA	7	7	<0.5	9	1.4
CF-2	50-55	2/6/2012	<1	3.1±0.2	2.5±0.2	<0.35	3 ±0.7	<2.4	<0.24	<0.8	<2.1	NA	NA	NA	9	6	<0.5	NA	0.8
	60-65	2/6/2012	<1	<1	<1	<0.37	1.4 ±0.6	<2.4	<0.24	<0.8	<2	NA	NA	NA	8	5	<0.5	NA	0.7
	70-75	2/6/2012	<1	<1	<1	<0.24	<1	<2.2	<0.25	<0.63	0.8 ±0.73	<0.64	NA	NA	7	6	<0.5	NA	1.2
	80-85	2/6/2012	<1	<1	<1	<0.25	<1	<2.7	<0.26	<0.7	<2.5	<0.82	NA	NA	8	6	<0.5	NA	1.4
	90-95	2/6/2012	<1	<1	<1	<0.22	<0.7	<2.2	<0.27	<0.57	<2.1	<0.82	NA	NA	5	6	0.5	NA	0.9
	100-105	2/6/2012	<1	<1	<1	<0.22	<0.7	<3.3	<2.8	<1.1	<2.4	1.3 ±0.8	NA	NA	5	7	0.6	NA	<0.5
CF-3	50-55	2/15/2012	<1	3.1+/-0.2	2.4±0.2	<0.2	3 ±0.8	<2.2	<0.27	<0.74	3.5 ±1.7	NA	NA	NA	11	5	<0.5	11	0.7
	60-65	2/15/2012	<1	1.4+/-0.1	0.9±0.1	<0.2	1.5 ±0.7	<2.4	<0.27	<0.73	1.9 ±1.2	NA	NA	NA	8	7	<0.5	7	0.9
	70-75	2/15/2012	<1	<1	<1	<0.28	<0.8	<2.5	<0.25	<0.64	<2.2	NA	0.88 ±0.76	NA	9	6	<0.5	10	1
	80-85	2/14/2012	<1	<1	<1	0.55 ±0.43	<0.8	<2.8	<0.32	<0.88	0.5 ±0.46	NA	NA	NA	8	6	<0.5	NA	1
	90-95	2/14/2012	<1	<1	<1	<0.22	<0.6	<2.6	<0.26	<0.66	3.5 ±1.9	NA	NA	NA	6	5	0.9	NA	0.9
	100-105	2/14/2012	<1	<1	<1	<0.27	<0.6	<3.1	<0.3	<0.87	<2.6	NA	NA	NA	6	7	0.8	NA	<0.5

Notes:
NA = Sample collected, analyte not reported
NS = No Sample Collected
"<" = less than, indicating no detection
ppb = part per billion
ppm = part per million
pCi = picocurie

^ = excluding radon and uranium
^^ = excluding strobtium-90 and alpha emitters
^^^ = MCL is for combined Radium 226 + Radium 228
* AGB = gross beta - 0.82* potassium conc. in mg/l
**AGB has a guidance activity value of 50 pCi/l that is used for screening under Subpart 5-1 of the NYS Sanitary Code
☐ indicates concentration exceeds a standard or guidance value

Site #2
Moriches-Riverhead Road Farm
Eastport NY

Site Description

The site is located on the south-west corner of Moriches-Riverhead Road and Port Jefferson-Westhampton Road, in Eastport. It consists of two tax parcels totaling 27 acres in size. Review of aerial photography (Appendix B) shows that the site was vacant in 1947, and although some structures appear on the northeast portion of the site in the 1984 photo, the majority of the land was still vacant. This is consistent on the 1994 and 1996 photos. In 1999, the first compost windrows appear on the site, parallel to the site's northwestern boundary. With the exception of 2001, these windrows are consistent up to and including the 2006 aerial photo. Several additional, smaller windrows appear on the site's northern and southern boundary in 2003 and only on the northern boundary in 2004. No windrows appear on the 2007 photo, and the 2010 and 2013 photos do not indicate any evidence of compost windrows on the site. This site is regulated by the NYSDEC as "Long Island Compost Farm #18", and is authorized to accept yard waste for composting.

SCDHS Monitoring Wells

The SCDHS installed three temporary profile monitoring wells (RC-1, RC-2 and RC-3) in the vicinity of this site, on Moriches-Riverhead Road, south of Eastport Manor Road. Figure 4 shows the location of the profile wells on the 2010 aerial photograph, and Figure 5 shows the well locations relative to the historic windrow locations on the 2006 aerial photograph. The locations of these wells were based upon a south-southwest regional groundwater flow direction, and were sited to assess past and/or current impacts from vegetative organic waste activity occurring on the parcels located south of Eastport Manor Road. All three wells were installed to a depth of 95 feet below grade (fbg), and sampled at 10 foot intervals as they were retracted. Five levels were sampled from RC-1, with the uppermost located at the 50 to 55 foot interval, whereas six levels were sampled in both RC-2 and RC-3, with the uppermost level located at the 40-45 foot interval, yielding a total of 17 groundwater samples collected and analyzed from this site. The following analytes were detected in the indicated monitoring wells at concentrations exceeding drinking water and/or groundwater standards:

Manganese (RC-2, RC-3)	**Sodium (RC-1, RC-2, RC-3)**
Magnesium (RC-2)	**Nitrate (RC-3)**

Table 3 contains a summary of the results of the analytes detected.

Figure 4 - Site #2 Well Locations -2010 Aerial Photograph

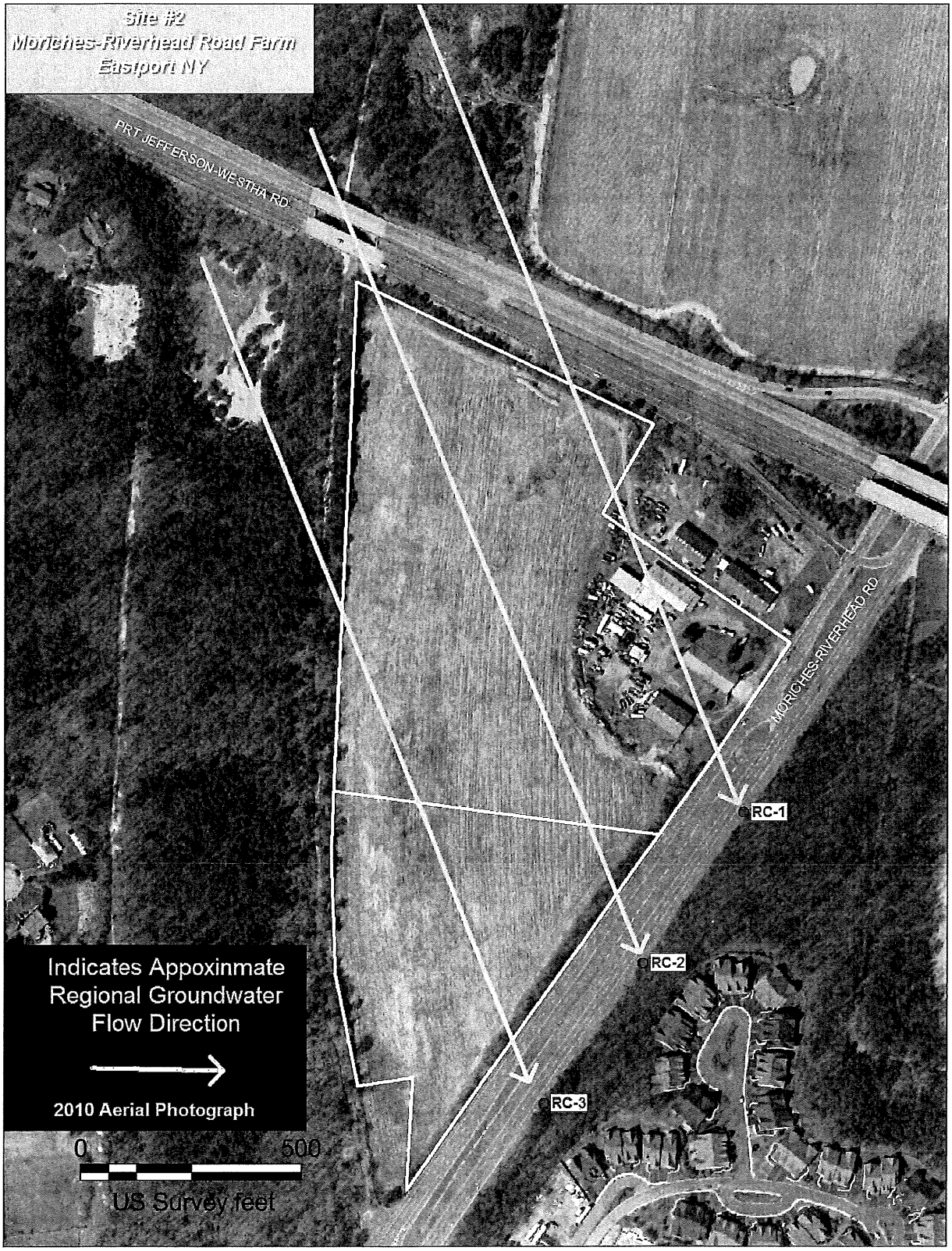


Figure 5– Site #2 Well Locations - 2006 Aerial Photograph

Private Wells

Five potential private wells were initially identified in the vicinity of this site. Subsequently, all five locations were confirmed to be served by public water.

Public Wellfields

The nearest public supply wellfield is approximately 1.1 miles from the site and is not located downgradient of the site. Any impacts to groundwater quality as results of this site's operations would not be expected to affect the water quality of this wellfield.

Summary of Significant Analytical Results

Metals

Of the three monitoring wells, RC-3 exhibited the most degraded water quality with manganese concentrations of 2,730 ppb, which is over nine times the NYS drinking water standard of 300 ppb. The sodium concentration exceeded the groundwater standard (20 ppm) in profile level 80-85 fbg (20.1 ppm). Other analytes were also detected in RC-3 at elevated concentrations, but their concentrations either did not exceed a drinking water standard, or no standard currently has been established. These include aluminum (up to 892 ppb), barium (up to 872 ppb), beryllium (up to 1.4 ppb), thallium (0.4 ppb), and potassium (up to 55.7 ppm).

Manganese concentrations in RC-2 also were elevated and exceeded standards in three profile levels (50-55 fbg, 60-65 fbg and 70-75 fbg) , with the highest concentration detected at 1,970 ppb in the 60-65 fbg profile level. Sodium concentrations were elevated, exceeding the groundwater standard (20 ppm) in four levels in both RC-1 (maximum 87.7 ppm) and RC-2 (maximum 70.4 ppm). The groundwater standard for magnesium (35 ppm) was exceeded in well RC-2 in the 50-55 fbg profile level (461 ppm), and for thallium (0.5 ppb) in RC-2 (0.6 ppb) and RC-3 (0.6 ppb) each at the 60-65 fbg profile level.

Radionuclides

Gross alpha concentrations, although not exceeding the drinking water standard, were elevated in RC-3 at concentrations above what is typically observed in Suffolk County groundwater (Table 16), the highest concentration (8.9 pCi/l) was in the 80-85 fbg profile level.

Other Notable Results

The drinking water and groundwater standards for nitrate (10 ppm) were exceeded in six of the eight profile levels of well RC-3 (up to 17.9 ppm). Ammonia was detected below the groundwater standard in the two deepest profile levels of well RC-3 (80-85 fbg and 90-95 fbg) at 0.76 ppm and 1.58 ppm respectively. All three wells had detections of the pesticide metolachlor and/or a

metolachlor metabolite. The pesticides simazine, atrazine and two atrazine metabolites were detected in low concentrations in well RC-3, as was the pesticide degredate 2,6-dichlorbenzamide.

Discussion

Review of historic aerial photographs of this site (Appendix B) indicates that the western portion of the site was used for VOWM activities for approximately eight years (1999 – 2006). VOWM activities are not evident in aerial photographs taken within the last seven years. Water quality data from the three monitoring wells installed hydraulically downgradient of this site indicate the western-most well (RC-3) exhibited the most degraded water quality, and the eastern well (RC-1) was the least impacted. The degraded water quality, particularly in well RC-3, is consistent with water quality impacts observed downgradient of the Great Gardens/Long Island Compost facility in Yaphank that were determined to be a result of VOWM activities.

Figure 5 is an aerial photograph of the site from 2006 that shows the site VOWM activity, the SCDHS monitoring wells, and the approximate direction of the regional groundwater flow direction in relation to each of the monitoring wells. This figure illustrates that water quality in well RC-3 appears to have been most influenced from the VOWM activity on this site. It also shows that water quality in well RC-2 may have been slightly influenced by the northern extent of VOWM activity, and water quality in well RC-1 does not appear to incur any influence from the VOWM activity. The extent of potential VOWM influence on each well's water quality, with respect to groundwater flow direction, appears to coincide with the severity of water quality degradation observed in each well (e.g., the more potential influence from VOWM activity, the more degraded the water quality).

Wells Impacted by VOWM Activity

Two of the three profile wells (RC-2 and RC-3) that were installed appear to have been impacted from past VOWM activity that occurred at this site.

Table 3
Summary of Detected Analytes
Monitoring Wells Installed in the Vicinity of Site #2
Eastport, NY

Well Information			Parameters					Metals												
Well ID	Screen Interval (ft)(depth below grade)	Sample Date	Depth To Water (Feet)	Dissolved Oxygen (mg/L)	Temperature (Celsius)	pH	Conductivity (µS)	Aluminum (ppb)	Barium (ppb)	Beryllium (ppb)	Chromium (ppb)	Manganese (ppb)	Nickel (ppb)	Strontium (ppb)	Thallium (ppb)	Titanium (ppb)	Magnesium (ppm)	Sodium (ppm)	Calcium (ppm)	Potassium (ppm)
DEC TOGS 1.1.1 Guidance Values			-	-	-	-	-	-	-	3	-	-	-	-	0.5	-	35	-	-	-
DEC Part 703 Class GA Groundwater Standards			-	-	-	-	-	-	1,000	-	50	300	100	-	-	-	-	20	-	-
DOH Drinking Water Standards Subpart 5-1			-	-	-	-	-	-	2,000	4	100	300	100	-	2	-	-	-	-	-
RC-1	50-55	2/21/2012	41.93	NS	NS	5.6	335	35	11	<1	<1	47	1.2	101	<0.3	<1	2.3	42.7	7.8	3.5
	60-65	2/21/2012	41.93	NS	NS	5.7	467	16	124	<1	<1	81	1.2	132	<0.3	<1	2.3	68.9	5.9	3.9
	70-75	2/21/2012	41.93	NS	NS	5.7	480	15	166	<1	<1	70	0.7	124	<0.3	<1	3.3	65.4	8.2	4.6
	80-85	2/21/2012	41.93	NS	NS	5.9	648	10	166	<1	<1	24	1	104	<0.3	<1	6.1	87.7	8	3.5
	90-95	2/21/2012	41.93	NS	NS	6.4	118	<5	8	<1	<1	3	<0.5	16	<0.3	<1	1.3	15.5	2	0.6
RC-2	40.45	3/6/2012	38.74	6.57	14.3	6.5	482	29	67	<1	<1	128	1.6	101	<0.3	2	3	70.4	11.7	3.6
	50-55	3/6/2012	38.74	9.09	14.1	5.7	205	49	291	<1	<1	461	1.5	131	<0.3	<1	461	10.3	7.8	9.9
	60-65	2/28/2012	38.65	5.77	13.5	5.7	206	29	158	<1	<1	1,560	1.8	64	0.6	<1	3.8	18.2	4.2	6.5
	70-75	2/28/2012	38.65	6.47	12.8	6.2	208	<5	48	<1	<1	1,970	<0.5	14	<0.3	<1	0.6	28.7	1.6	5.2
	80-85	2/28/2012	38.65	6.29	12.7	6.4	218	6	42	<1	<1	155	<0.5	23	<0.3	<1	1.6	29.5	1.9	4.3
	90-95	2/28/2012	38.65	5.18	12.6	6.4	215	<5	66	<1	<1	64	0.6	38	<0.3	<1	2.9	22.3	3.8	6.8
RC-3	40-45	3/20/2012	35.69	2.64	16.3	5.3	253	280	107	0.5	2	111	1.5	23	<0.3	<1	6.3	10.3	20	5.2
	50-55	3/20/2012	35.69	2.27	15.6	4.8	342	892	50	1.4	3	677	2.6	31	<0.3	<1	6.2	10.4	20.4	24.6
	60-65	3/20/2012	35.69	0.65	15.2	5.1	352	546	66	0.7	2	549	1.7	12	0.6	<1	5.8	9.1	9.3	46.7
	70-75	3/6/2012	35.69	3.4	14.1	5.3	425	636	63	0.6	<1	793	2.1	<2	0.4	<1	7.5	12.4	8.4	55.7
	80-85	3/6/2012	35.69	1.07	14.4	5.6	348	167	461	<0.3	<1	2,650	1.2	34	<0.3	<1	4	20.1	8.5	28
	90-95	3/6/2012	35.69	11.49	14.5	5.9	375	37	872	<0.3	3	2,730	6.3	44	<0.3	<1	5	18.2	11.1	30.5

Notes: NA = Sample collected, analyte not reported
NS = No Sample Collected
"<" = less than, indicating no detection
uS = micro siemens

ppm = part per million
ppb = part per billion
☐ indicates concentration exceeds a standard or guidance value

Table 3
Summary of Detected Analytes
Monitoring Wells Installed in the Vicinity of Site #2
Eastport, NY

Well Information			Radiologicals (pCi/L)												Standard Inorganics						VOCs (ppb)	
			SCDHS PEHL			NYSDOH Wadsworth																
Well ID	Screen Interval (ft) (depth below grade)	Sample Date	Gross Alpha	Gross Beta	Adjusted Gross Beta* (AGB)	Gross Alpha	Gross Beta	Ruthenium 106	Cesium 137	Zirconium 95	Potassium 40	Actinium 228	Radium 224	Radium 226	Chloride (ppm)	Sulfate (ppm)	Nitrate (ppm)	Ammonia (ppm)	Total Alkalinity(mg CaCO3/L)	Perchlorate (ppb)	Chloroform (ppb)	Methyl-tertiary-butil-ether
DEC TOGS 1.1.1 Guidance Values			-	-	-	-	-	-	-	-	-	-	-	3	-	-	-	-	-	-	-	10
DEC Part 703 Class GA Groundwater Standards			15^	1,000^^	-	-	1,000	-	-	-	-	-	-	-	250	250	10	2	-	-	7	-
DOH Drinking Water Standards Subpart 5-1			15	-	50**	15	-	-	-	-	-	-	-	5^^^	250	250	10	-	-	18	80	10
RC-1	50-55	2/21/2012	<1	3.6±0.2	<1	<0.6	3.3 ±0.8	<3	<0.27	<0.93	<2.3	NA	NA	NA	84	8	3.1	<0.5	5	1	<0.5	<0.5
	60-65	2/21/2012	1.2±0.6	6.4±0.6	3.2±0.6	<0.7	3.5 ±0.8	<3.1	<0.31	<0.94	1.4 ±1.2	NA	NA	NA	123	7	3.8	<0.5	6	<0.2	<0.5	<0.5
	70-75	2/21/2012	1.7±0.4	3.7±0.2	<1	1.8 ±1.3	4.7 ±0.9	<2.4	<0.25	<0.78	3.8 ±2.9	NA	NA	NA	129	<15	2.4	<0.5	5	1.2	<0.5	<0.5
	80-85	2/21/2012	1.1±0.7	5.0±0.6	2.13±0.6	<1.1	2.3 ±1.1	<3	<0.29	<0.84	<2.3	NA	NA	NA	180	<10	2.3	<0.5	7	0.7	<0.5	<0.5
	90-95	2/21/2012	<1	<1	<1	<0.3	<0.7	<2.7	<0.25	<1.4	<2.3	NA	NA	NA	24	<5	1.2	<0.5	8	0.2	0.7	<0.5
RC-2	40.45	3/6/2012	<1	4.1±0.2	1.1±0.2	<0.94	4.9 ±0.9	<2.6	<0.31	<0.6	5.9 ±4.9	NA	1.68 ±0.71	NA	102	20	4.7	<0.5	24	0.5	<0.5	<0.5
	50-55	3/6/2012	1.4±0.4	13.2±0.3	4.9±0.3	1.3 ±0.7	10 ±1.2	<2.3	<0.25	<0.6	9.9 ±2.8	NA	NA	1.5 ±1.2	24	16	6.7	<0.5	5	0.5	<0.5	<0.5
	60-65	2/28/2012	<1	7.8±0.2	2.5±0.2	0.5 ±0.5	6.2 ±0.9	<2.7	<0.32	<0.8	2.7 ±2.2	NA	NA	NA	39	10	4	<0.5	6	0.4	<0.5	0.6
	70-75	2/28/2012	<1	4.2±0.2	<1	<0.3	4.3 ±0.8	<2.4	<0.26	<0.76	3.7 ±2.6	NA	NA	NA	40	8	2.7	<0.5	13	0.4	<0.5	0.9
	80-85	2/28/2012	<1	3.0±0.2	<1	<0.3	2.6 ±0.7	<2.7	<0.32	<0.96	4.5 ±3.2	NA	NA	NA	52	5	<0.5	<0.5	11	0.2	<0.5	1
	90-95	2/28/2012	<1	6.0±0.2	<1	<0.3	4.4 ±0.8	<2.8	<0.27	<0.98	5.2 ±3.4	NA	NA	NA	54	<5	<0.5	<0.5	9	<0.2	0.6	<0.5
RC-3	40-45	3/20/2012	1.4±0.3	7.3±0.2	3.0±0.2	2.3 ±1	5.8 ±1	<3	<0.28	<1.2	5 ±2.7	NA	NA	NA	19	41	8.9	<0.5	5	0.2	<0.5	<0.5
	50-55	3/20/2012	3.0±0.3	26.7±0.6	5.8±0.6	2 ±1	22.9 ±2	<2.4	<0.26	<0.69	23 ±5.2	1.7 ±1.5	NA	NA	19	66	11.5	<0.5	1	0.4	<0.5	<0.5
	60-65	3/20/2012	6.0±0.5	49.7±1.1	10.7±1.1	4.1 ±1.3	43.3 ±3.2	<3.2	<0.34	<1.1	39 ±7.1	2.2 ±1.3	0.95 ±0.62	NA	17	73	9.6	<0.5	3	0.7	<0.5	<0.5
	70-75	3/6/2012	5.5±0.4	53.9±1.0	7.3±1.0	3.5 ±1.3	51 ±3.6	<2.8	<0.31	<0.79	61 ±9	2.4 ±1.5	NA	NA	21	76	14	<0.5	5	0.8	<0.5	<0.5
	80-85	3/6/2012	8.9±0.4	28.7±0.6	5.0±0.6	4.3 ±1.4	27 ±2.2	<3.1	<0.31	<0.74	27 ±7.2	2.4 ±1.6	NA	NA	46	16	14.5	0.76	3	0.7	<0.5	<0.5
	90-95	3/6/2012	7.8±0.4	30.4±0.6	5.0±0.6	5.7 ±1.6	29 ±2.4	<3.3	<0.25	<0.58	31 ±5.5	2.5 ±1.2	0.98 ±0.69	NA	40	17	17.9	1.58	9	1.1	<0.5	<0.5

Notes:
NA = Sample collected, analyte not reported
NS = No Sample Collected
"<" = less than, indicating no detection
ppb = part per billion
ppm = part per million
pCi = picocurie

^ = excluding radon and uranium
^^ = excluding strobtium-90 and alpha emitters
^^^ = MCL is for combined Radium 226 + Radium 228
* AGB = gross beta - 0.82* potassium conc. in mg/l
**AGB has a guidance activity value of 50 pCi/l that is used for screening under Subpart 5-1 of the NYS Sanitary Code
☐ indicates concentration exceeds a standard or guidance value

Table 3
Summary of Detected Analytes
Monitoring Wells Installed in the Vicinity of Site #2
Eastport, NY

Well Information			Herb Mets (ppb)								Semi-Volatile Organic	
Well ID	Screen Interval (ft) (depth below grade)	Sample Date	Bisphenol A	Deisoprpylatrazine	Desethylatrazine	2,6-Dichlorobenzamide	Imidacloprid	Metolachlor	Metolachlor OA	Metolachlor ESA	Atrazine (ppb)	Simazine (ppb)
DEC TOGS 1.1.1 Guidance Values			-	-	-	-	-	-	50	50	7.5	0.5
DEC Part 703 Class GA Groundwater Standards			-	-	-	-	-	10	-	-	7.5	0.5
DOH Drinking Water Standards Subpart 5-1			50	50	50	50	50	50	50	50	3	4
RC-1	50-55	2/21/2012	<0.2	<0.2	<0.4	<0.5	0.3	<0.2	Trace	0.5	<0.1	<0.07
	60-65	2/21/2012	<0.2	<0.2	<0.4	<0.5	<0.2	Trace	0.4	0.6	<0.1	<0.07
	70-75	2/21/2012	<0.2	<0.2	<0.4	<0.5	<0.2	Trace	Trace	0.3	<0.1	<0.07
	80-85	2/21/2012	<0.2	<0.2	<0.4	<0.5	<0.2	Trace	Trace	0.3	<0.1	<0.07
	90-95	2/21/2012	<0.2	<0.2	<0.4	<0.5	<0.2	<0.2	<0.3	<0.3	<0.1	<0.07
RC-2	40.45	3/6/2012	<0.2	<0.2	<0.4	<0.5	<0.2	<0.2	Trace	0.3	<0.1	<0.07
	50-55	3/6/2012	<0.2	<0.2	<0.4	<0.5	<0.2	<0.2	0.3	0.4	<0.1	<0.07
	60-65	2/28/2012	Trace	<0.2	<0.4	<0.5	<0.2	<0.2	0.5	0.4	<0.1	<0.07
	70-75	2/28/2012	<0.2	<0.2	<0.4	<0.5	<0.2	<0.2	0.3	0.3	<0.1	<0.07
	80-85	2/28/2012	<0.2	<0.2	<0.4	<0.5	<0.2	<0.2	<0.3	Trace	<0.1	<0.07
	90-95	2/28/2012	<0.2	<0.2	<0.4	<0.5	<0.2	<0.2	<0.3	Trace	<0.1	<0.07
RC-3	40-45	3/20/2012	<0.2	0.2	Trace	<0.5	<0.2	<0.2	<0.3	0.3	0.4	<0.07
	50-55	3/20/2012	<0.2	Trace	Trace	<0.5	<0.2	<0.2	<0.3	Trace	0.2	<0.07
	60-65	3/20/2012	<0.2	Trace	Trace	<0.5	<0.2	<0.2	<0.3	0.3	<0.1	<0.07
	70-75	3/6/2012	<0.2	<0.2	<0.4	<0.5	<0.2	<0.2	<0.3	Trace	Trace	0.1
	80-85	3/6/2012	<0.2	<0.2	<0.4	Trace	<0.2	<0.2	<0.3	Trace	Trace	0.1
	90-95	3/6/2012	<0.2	<0.2	<0.4	<0.6	<0.2	<0.2	<0.3	Trace	<0.1	0.2

Notes: NA = Sample collected, analyte not reported
NS = No Sample Collected
"<" = less than, indicating no detection
ppm = part per million
ppb = part per billion
☐ indicates concentration exceeds a standard or guidance value

Site #3
Papermill Road Facility
Manorville NY

Site Description

The site is located in Manorville, at the northern end of Papermill Road and approximately 1,000 feet north of Jamaica Avenue, and is comprised of three tax parcels totaling approximately 33 acres. The Town of Brookhaven has owned and operated the Papermill Road Compost Facility (PRCF) site since the mid-1950's. The site has had a variety of waste disposal and waste treatment uses throughout the years, including landfilling and the disposal of septic and municipal sanitary waste sludges. Historical aerial photographs (Appendix C) indicate that the site was undeveloped in 1947, and by 1962 the center of the site was cleared and actively being used. The first compost windrows appear on the site in the 1994 aerial photograph, and these windrows are consistently present on all subsequent photos, up to and including the 2013 photograph. Currently, the site is regulated by the NYSDEC as a Part 360 permitted yard waste composting facility.

SCDHS Monitoring Wells

The SCDHS installed three temporary profile monitoring wells (CB-1, CB-2 and CB-3) south of the facility, on Chapman Blvd (Figure 6). The locations of these wells were based upon a south-southwest regional groundwater flow direction, and were sited to assess past and/or current impacts from vegetative organic waste activity occurring on the site. All three wells were installed to a depth of 115 fbg, and sampled at 10 foot intervals as they were retracted. Eight levels were sampled from CB-2, with the uppermost located at the 40 to 45 foot interval, whereas seven levels were sampled in both CB-1 and CB-3, with the uppermost level located at the 50-55 foot interval, yielding a total of 22 groundwater samples collected and analyzed from this site. The following analytes have been detected in these monitoring wells at concentrations exceeding the drinking water standard:

Arsenic	**(CB-3, Pond)**	**Sodium**	**(CB-1)**
Manganese	**(CB-1, CB-2, CB-3)**	**Gross Alpha**	**(CB-3)**
Thallium	**(CB-1, CB-2)**	**Gross Beta**	**(CB-3)**
Iron	**(CB-1, CB-2, CB-3, Pond)**	**Ammonia**	**(CB-1, CB-2, CB-3)**
		Chlorobenzene	**(CB-1, CB-2)**

Table 4 contains a summary of the results of the analytes detected.