PENNSYLVANIA DEPARTMENT OF ENVIRONMENTAL PROTECTION

BUREAU OF CLEAN WATER

Development of the Human Health Criterion for Manganese

July 2021

Executive Summary

In October 2017, a law was passed in the Commonwealth ("Act 40") that directed the Environmental Quality Board (Board) to promulgate proposed regulations related to manganese. Act 40 has directed a modification to Pennsylvania's water quality standards (WQSs). As the existing Potable Water Supply use[1] criterion for manganese had not been comprehensively reevaluated since it was adopted as a statewide criterion in 1979 and as states have an obligation under Section 303(c)(1) of the Federal Clean Water Act (CWA) to periodically review and update, as appropriate, their WQSs to reflect current scientific knowledge and recommendations, the Department of Environmental Protection (DEP) evaluated the existing scientific data and information to ensure adequate criteria for manganese exist to protect all of this Commonwealth's water uses. On January 27, 2018, DEP published an advance notice of proposed rulemaking (ANPR) soliciting the information necessary to prepare the rulemaking documents required by law and support the Board's adoption of proposed regulations. The information received in response to the ANPR and recent scientific information relating to manganese were used to evaluate manganese water quality (WQ) criteria with respect to the protected water uses identified in this Commonwealth's WQSs regulation.

Following an evaluation of the available scientific data, in accordance with its regulations and policies, DEP developed a human health-based WQ criterion for manganese of 0.3 mg/L. DEP recommends that this criterion should apply in all surface waters (i.e., at the point of discharge) in accordance with DEP's Water Quality Toxics Management Strategy – Statement of Policy (25 Pa. Code Chapter 16) and regulations found at 25 Pa. Code Chapters 93 (relating to water quality standards) and 96 (relating to water quality standards implementation).

History of Regulation

Prior to 1971, the Sanitary Water Board (SWB) in the Department of Health had primary responsibility for maintaining the rules and regulations related to WQ criteria and standards in Pennsylvania. The Commonwealth has had a WQ criterion for manganese since Article 301 Water Quality Criteria was added to the SWB Rules and Regulations on June 28, 1967. The criterion contained in Article 301 of the SWB Rules and Regulations appeared as "k –Total Manganese – Not to exceed 1.0 mg/L". This criterion was originally applied as Specific Criteria in Section 7 of Article 301 for selected waterbodies,

[1] Potable Water Supply is described in 25 Pa. Code § 93.3 as "used by the public as defined by the Federal Safe Drinking Water Act, 42 U.S.C.A. § 300F, or by other water users that require a permit from the Department under the Pennsylvania Safe Drinking Water Act (35 P.S. §§ 721.1—721.18), or the act of June 24, 1939 (P.L. 842, No. 365) (32 P.S. §§ 631—641), after conventional treatment, for drinking, culinary and other domestic purposes, such as inclusion into foods, either directly or indirectly."

or segments, in the North Branch Susquehanna, Monongahela, Allegheny, and Ohio River basins. It was based primarily on testimony provided by the Wilkinsburg Joint Water Authority. In 1971, the SWB was abolished, and the authority and responsibilities of the SWB were transferred to the Pennsylvania Department of Environmental Resources (DER). Also, in 1971, the SWB Rules and Regulations, Article 301 Water Quality Criteria were replaced by the creation of 25 Pa. Code Chapter 93 Water Quality Standards, effective September 11, 1971 (1 Pa.B. 1804).

In 1979, manganese was adopted as a statewide Potable Water Supply use criterion, implemented at the point of discharge by being added to 25 Pa. Code § 93.7(d) (relating to specific water quality criteria), Table 4, as part of DER's first triennial review of WQSs[2]. The manganese criterion is currently found in § 93.7, Table 3, and Potable Water Supply use is identified as the *critical use*. As stated in § 93.7, the critical use is the designated or existing use the criteria are designed to protect, and more stringent site-specific criteria may be developed to protect other more sensitive, intervening uses. When the critical use is identified and applied statewide, the WQ criterion developed to protect the critical use should provide protection of all water uses, unless new information shows additional protection is needed. In accordance with the current regulations found at Chapter 93, the purpose of all Potable Water Supply use WQ criteria is to ensure that public water supply systems receive raw water at the point of water withdrawal that can achieve compliance with 25 Pa. Code Chapter 109 (relating to safe drinking water) utilizing only conventional treatment.

The only known rationale document for the existing statewide Potable Water Supply use criterion of 1.0 mg/L was prepared by Kenneth Schoener, a DER water supply engineer. DER's review of manganese in 1979 considered any new information available since 1967 including updated scientific literature, statewide WQ data, and the U.S. Environmental Protection Agency (EPA) WQ criteria recommendations. In EPA's rationale for its 1976 WQ recommendation for manganese, EPA indicated that manganese was not expected to be harmful to aquatic life or humans at levels expected to occur naturally in surface waters (that is, $\leq$1.0 mg/L). Mr. Schoener noted in the rationale that there were some discrepancies between the literature and the testimony provided in 1967. He subsequently followed up with the Wilkinsburg Joint Water Authority. In fact, EPA noted in its WQ criterion recommendation that "manganese is not removed in the conventional treatment of domestic waters." Despite these noted inconsistencies, DER continued to rely on the 1967 testimony as the basis for the 1.0 mg/L criterion. The 1979 rationale document explains the criterion was partially based on a 1967 testimony from Mr. Reginald Adams, an experienced water supply manager from the Wilkinsburg Joint Water Authority. Mr. Adams stated that an "average up-to-date water plant can probably handle soluble manganese concentrations without too much difficulty. A well-designed plant can handle 1.5 to 2 parts per million…". He further indicated that if the manganese content of the raw water is 1.0 mg/L, or less, addition of potassium permanganate ($KMnO_4$) to the coagulation-sedimentation area at a rate of 2 parts of $KMnO_4$ to 1 part of manganese will remove the manganese. Operators can simply add $KMnO_4$ until a "slight pink residual color appears in the sedimentation unit". This process was commonly used in western Pennsylvania, but it is considered a treatment process beyond "conventional treatment"[3]. DEP's

[2] Adopted by the Board on August 21, 1979, published in the *Pennsylvania Bulletin* on September 8, 1979 (9 Pa.B. 3051), effective October 8, 1979.

[3] The term "conventional treatment" is defined in § 93.1 as follows: "For the purpose of surface water protection of the Potable Water Supply use, coagulation, followed by filtration for the removal of solids, and disinfection for the control of pathogens to produce water for drinking and other human consumption."

historical records clearly indicate that the Potable Water Supply use criterion for manganese was adopted to protect the Potable Water Supply use and facilitate potable water supply treatment; it was not established to protect human health from toxic effects which, at the time, were assumed to be nonexistent.

The compliance point for several Potable Water Supply use criteria changed from the point of discharge to the point of any existing or planned surface Potable Water Supply withdrawal when § 93.5(e)[4] (relating to application of Potable Water Supply use criteria) was added in the 1985 triennial review[5]. Those Potable Water Supply use criteria included total dissolved solids (TDS), fluoride, phenolics (except those identified as priority pollutants) and nitrite plus nitrate.

DEP provided clarification to the manganese criterion in the 2000 Regulatory Basics Initiative (RBI) triennial review, which was published in the *Pennsylvania Bulletin* on November 18, 2000 (30 Pa.B. 6059), by adding a reference that the criterion be measured as total recoverable and based on Potable Water Supply use protection. The creation of 25 Pa. Code Chapter 96 also occurred during the RBI Triennial Review in 2000, which relocated the language in § 93.5(e) to § 96.3(d) (relating to water quality protection requirements). Subsequently, chloride and sulfate criteria were added to § 96.3(d) in 2002, as adopted by the Board on September 17, 2002, and published in the *Pennsylvania Bulletin* on December 14, 2002 (32 Pa.B. 6101). See Figure 1 for a summary of the regulatory changes to § 96.3(d).

Figure 1. Summary Table for § 96.3(d) Potable Water Supply exceptions.

Potable Water Supply Criteria including Manganese (Mn) & those listed in 96.3(d)	Year that the point of application was moved from the point of discharge to the point of Potable Water Supply withdrawal	Consistent with Primary Maximum Contaminant Level (MCL) values?	Primary MCL (Value in mg/L)	Consistent with Secondary MCL values?	Secondary MCL (Value in mg/L)	Not based on either primary or secondary MCL values
Chloride (Ch)	2002	--	--	yes	250	
Fluoride (F)	1985	no	4	yes	2	
Manganese (Mn)	--	--	--	no	0.05	yes
Nitrate (N)	1985	yes	10	--	--	
Nitrite (N)	1985	yes	1	--	--	
Phenolics (Phen)	1985	--	--	--	--	yes
Sulfate (Sul)	2002	--	--	yes	250	
Total Dissolved Solids (TDS)	1985	--	--	yes	500	

Since DEP's review of the current science on manganese indicates that manganese ingestion can lead to neurotoxic effects, its characteristics no longer align with those of the other Potable Water Supply use criteria included in § 93.7, which are: TDS, bacteria (Bac_2), color, phenolics, iron (Fe_2), fluoride,

[4] The language in § 96.3(d) was relocated from an earlier regulation, § 93.5(e), that is now a reserved section.

[5] Adopted by the Board on December 18, 1984, effective on February 16, 1985 as published in the *Pennsylvania Bulletin* (15 Pa.B. 544).

chloride, sulfate and nitrite plus nitrate. These substances are regulated in surface waters primarily because they cause organoleptic and esthetic issues at low levels. At the levels necessary to avoid these issues, these substances are generally known to be non-toxic to humans. It is important to note that the Potable Water Supply use criterion for total phenolics does not include those specific phenolic compounds that have been identified by EPA as priority pollutants (that is, toxic substances). Criteria for those specific phenolic compounds are found in § 93.8c, Table 5 (relating to human health and aquatic life criteria for toxic substances), and those criteria must currently be met in all surface waters in accordance with § 96.3(c).

Information relating to the implementation of WQSs can be found in 25 Pa. Code Chapter 96. Unlike other WQ criteria, compliance points differ for the various Potable Water Supply use criteria. Section 96.3(c) states (*emphasis* added) that "the water quality criteria described in Chapter 93, including the criteria in §§ 93.7 and 93.8(b) (relating to specific water quality criteria; and toxic substances) shall be achieved *in all surface waters* at least 99% of the time, unless otherwise specified in this title." Section 96.3(d) states (*emphasis* added) "as an exception to subsection (c), the water quality criteria for total dissolved solids, nitrite-nitrate nitrogen, phenolics, chloride, sulfate and fluoride established for the protection of potable water supply shall be met at least 99% of the time *at the point of all existing or planned surface potable water supply withdrawals* unless otherwise specified in this title."[6] Note that not all Potable Water Supply criteria are applied at the point of potable water supply withdrawal. Presently, there are four Potable Water Supply use parameters that must be met in all surface waters including manganese, color, coliform bacteria (Bac_2) and dissolved iron (Fe_2). In addition, it is important to note that all 122 of the human health criteria for toxic substances contained in § 93.8c, Table 5 are required to be met in all surface waters in accordance with § 96.3(c). This policy and expectation for compliance with toxic substances has been a longstanding policy of the Board and DEP.

Manganese Background

Natural and Anthropogenic Sources

Manganese (Mn) is a ubiquitous element that exists naturally at low levels in many types of rocks, soils, waterbodies and plants. Pure manganese is a silver-colored metal, but manganese does not exist as a free element in nature. It is typically found in a variety of salts and minerals often combined with iron (Fe).

While manganese can exist in multiple oxidation states, it is generally present in surface waters in only two oxidation states, Mn^{+2} and Mn^{+4}. The Mn^{+4} state is the insoluble manganese dioxide (MnO_2) and would be present in surface waters either as a suspended solid in the water column or as particles either on top of the benthic substrate or in the sediments. The Mn^{+2} is dissolved manganese. Manganese is very soluble in acid waters and is sparingly soluble in alkaline waters. Mn^{+2} slowly oxidizes to MnO_2 (Mn^{+4}) under most natural water conditions. However, it is important to recognize that the behavior of manganese in surface waters is complex, and many factors can influence the amount and forms of manganese present in a waterbody as well as the distribution of manganese downstream.

Surface water levels of manganese may increase either as a result of direct discharges of manganese to a waterbody or due to an alteration of the chemical composition of the surface waters through mobilization of existing manganese sinks (Kaushal, et. al., 2018 and 2021). Manganese appears to

[6] The language in § 96.3(d) was relocated from an earlier regulation, § 93.5(e), that is now a reserved section.

primarily enter surface waters of the Commonwealth as a result of anthropogenic activities including, but not limited to, DEP-permitted discharges of sewage, various types of discharges categorized as industrial waste, stormwater, other permitted discharges and non-permitted discharges such as those from abandoned mine lands (AMLs). Manganese also finds its way into surface waterbodies through the natural weathering of rocks and minerals present in the earth's crust which then enter the waterbody either via stormwater runoff or through groundwater base flow containing manganese. Groundwater in some areas of the Commonwealth is known to contain high levels of iron and manganese due to the underlying geology of those regions.

In addition to direct discharges and mobilization of terrestrial manganese sinks, atmospheric deposition may contribute to manganese in surface waters. Manganese particles can enter the air from steam electric generating stations, iron and steel manufacturing facilities, coke ovens, automobile emissions, and dust from mining operations. Lytle et al. (1994) noted that manganese is usually found in the subsoil layers and not in any significant level at the surface. Thus, high surface soil levels may indicate contamination from vehicle exhaust associated with the fuel additive, methylcyclopentadienyl manganese tricarbonyl (mmt®) (Lytle, et al., 1994).

Unless otherwise impacted by anthropogenic activities, the World Health Organization (WHO) has stated that dissolved manganese concentrations in surface waters rarely exceed 1 mg/L and are usually less than 0.20 mg/L (WHO, 2004). An analysis of surface water samples collected across the Commonwealth generally supports this statement. DEP evaluated over 35,000 water quality samples for manganese collected in Pennsylvania. DEP collected over 21,000 of those samples between 2008 and 2018 from surface waters at Water Quality Network (WQN) stations, continuous instream monitoring (CIM) sites and other monitoring locations, such as surface waters in the vicinity of public water supply withdrawals. Sufficient data was available to calculate 641 yearly mean total manganese concentrations. Analysis of the data revealed that only 5% of the yearly mean total manganese concentrations exceeded the current Potable Water Supply use manganese criterion of 1.0 mg/L. DEP used its Water Quality Index (WQI) tool (Wertz and Shank, 2019) to assess the land use types of the sample locations (based on the calculated yearly mean total manganese concentrations) and scored them for land disturbance, which is a strong indicator of the presence of anthropogenic activity. This analysis was completed to distinguish between sample data representative of natural background conditions for manganese and sample data from waters impacted by anthropogenic activity. In accordance with § 93.1, *natural quality* is defined as "the water quality conditions that exist or that would reasonably be expected to exist in the absence of human related activity." DEP's analyses showed a very strong positive correlation between land disturbance (such as, the mining regions of Pennsylvania) and average manganese concentrations in surface waters, such that sample locations in areas with higher land disturbance measured higher average manganese concentrations. The natural quality of the Commonwealth's surface waters can generally be characterized by the overall mean total manganese concentration of the yearly mean total manganese data collected at locations with a WQI score of "Good", which is 0.037 mg/L. Thus, the available statewide data suggest that where anthropogenic activity is absent or limited, the natural manganese concentrations in the Commonwealth's surface waters are low and well below the manganese criterion recommendation developed by DEP as described in this rationale document.

In addition to being a naturally abundant element in rock and subsurface soils, manganese is commonly used in the manufacture of metal alloys (aluminum and stainless steels), dry cell batteries, U.S. coins, glass, matches, fireworks, micro-nutrient fertilizer additives, organic compounds used in paint driers,

textile bleaching, and leather tanning (EPA criteria, 1979; Santamaria, 2008). It is also used in the manufacture of fungicides, such as Maneb and Mancozeb (Mora et al., 2014; Bouabid et al., 2016). Wastewater discharges resulting from these industrial manufacturing processes may be more likely to contain measurable, and possibly significant, quantities of manganese. Furthermore, land application of manganese-containing pesticides could potentially result in the mobilization and discharge of manganese to waterbodies through discharges of stormwater runoff.

Discharges and Sources of Manganese in Pennsylvania

In Pennsylvania, historical coal mining activity has been and continues to be a significant contributor of manganese to waters of the Commonwealth. DEP's Bureau of Mining Programs (BMP) has identified approximately 706 active National Pollutant Discharge Elimination System (NPDES) mining permits containing manganese limits. It is unknown how many abandoned mine discharges, which do not require NPDES permits, may exist across the Commonwealth.

In addition to mining activities, a recent review of the Commonwealth's sewage and industrial waste NPDES discharge permits revealed that manganese is also present, or reasonably expected to be present, in the wastewater effluent of several non-mining sectors of the regulated community. These sectors include landfills, wastewater treatment plants (sewage and drinking water filter backwash plants) and power plants. Approximately 616 non-mining, individual NPDES permits contain permit conditions for manganese, and roughly 274 of those permits contain actual numeric effluent limits for manganese. These effluent limits are primarily WQ-based to ensure compliance with the Potable Water Supply use criterion for manganese of 1.0 mg/L, which is applicable in all surface waters. It is important to note that public water supply systems with NPDES permits to discharge filter backwash wastewater generally receive a more stringent technology-based limit (TBEL) of 1.0 mg/L applied at the end of the discharge pipe. This TBEL is not a regulatory requirement. It was established by DEP using its best professional judgement (BPJ). These permits account for approximately 78% of the Clean Water permits with numeric effluent limitations for manganese (214 of 274 permits). Permits containing manganese limits were identified across the state in each of the six DEP regions.

Human Health and Manganese

Physiological Need - Adequate Intake and Deficiency

Manganese is an essential micronutrient for plants and animals with Mn^{+2} and Mn^{+3} as the predominant oxidation states found in biological systems (Smith et al., 2017). The highest concentrations in the human body are found in the bone, liver, kidney, pancreas, adrenal glands and pituitary gland (O'Neal and Zheng, 2015). Within the body's cells, it is found primarily in mitochondrial superoxide dismutase (MnSOD). MnSOD is a vital enzyme that maintains the overall health of the body's cells through its potent antioxidant capacity. Rodent studies have demonstrated that complete knockout of this enzyme results in death shortly after birth (Holley et al., 2011). Beyond MnSOD, manganese is found in various metalloproteins especially glutamine synthetase in astrocytes, but it is also a cofactor for various enzymes that include hydrolases, kinases, decarboxylases and transferases (EPA IRIS). These manganese-based metalloproteins and enzymes play a critical role in the regulation of development, reproductive function, metabolism, blood clotting, digestion, bone growth, cell death and brain function (Agency for Toxic Substances and Disease Registry (ATSDR), 2012; Chen et al, 2015; Chung et al., 2015; Erikson et al., 2007; Smith et al., 2017; and Yoon et al., 2011).

Manganese deficiency can lead to bone malformation, skin lesions, hypocholesterolemia and seizures, but given the ubiquitous nature of manganese in the diet, deficiency is rarely observed except in susceptible individuals such as those with severely restricted diets or receiving total parenteral nutrition (TPN) formulated without manganese (Institute of Medicine (IOM), 2000; Crossgrove and Zheng, 2004; Hardy, 2009).

Adequate intake (AI) levels recommended by the National Academy of Medicine (formerly the IOM) vary by age group, gender and reproductive state (for women). The AI levels for infants are 0.003 mg/day of manganese for ages 0-6 months and 0.6 mg/day for ages 7-12 months. The AI levels for young children are 1.2 mg/day for ages 1-3 years and 1.5 mg/day for ages 4-8 years. The AI levels for older children and adolescents vary by age and gender. For boys, the AI levels are 1.9 mg/day for ages 9-13 and 2.2 mg/day for ages 14-18 years. For girls, the AI level is 1.6 mg/day for ages 9-18 years. The AI levels for adult males and non-pregnant, non-lactating females are 2.3 mg and 1.8 mg, respectively (IOM, 2000). In addition to the health issues noted above, low manganese levels have also been associated with specific disorders including Alzheimer's disease, amyotrophic lateral sclerosis (ALS), epilepsy, phenylketonuria, maple syrup urine disease and Perthes' disease (Cordova et al., 2013; Crossgrove et al., 2004; Finley and Davis, 1999). However, more research is needed to understand whether the observed low levels of manganese are present before (i.e., causal) or after the disease manifests.

Excessive Intake of Manganese - Effects of Elevated Manganese in the Human Body

As a micronutrient, only small quantities of manganese are necessary to achieve adequate health. As with many other heavy metals (i.e., lead, mercury), chronic exposure to levels of manganese beyond those necessary for good health may lead to adverse health effects including various irreversible neurological deficits in adults, children, infants, and the developing fetus.

Manganese is preferentially deposited in mitochondria-rich tissues such as the liver, pancreas and brain and has been shown to cross the placenta and the blood-brain barrier (BBB) (Bouabid et al., 2016; Lidsky et al., 2007; Chen et al., 2015; Aschner, 2000). Exposures to levels of manganese beyond those necessary for maintaining adequate health can lead to excess manganese in brain tissue resulting in a parkinsonian-like condition known as manganism. In 1837, James Couper became the first to describe this condition in a group of Scottish laborers working in the chemical industry (Menezes-Filho et al., 2009; Santamaria, 2008). Manganism is a neurodegenerative condition that results in extrapyramidal motor system dysfunction. It usually begins with neuropsychological symptoms that include aggressiveness, anxiety, headache, and decreased cognitive function. Upon very acute exposures to manganese or chronic exposures to elevated but non-acute levels of manganese, the condition will typically progress to changes in motor function which are characterized by a signature "cock-like" walk, dystonia, upright stance, difficulty walking backward and mild tremors (Aschner, 2000; Chen et al., 2015; Crossgrove et al., 2004). Depending upon the length and severity of the exposure, these neurological effects may result in permanent, irreversible damage to the brain. While the symptoms of manganism closely resemble Parkinson's disease, researchers have noted some distinct differences between these conditions (Bouabid et al., 2016). However, scientific research to establish the connections between manganese and Parkinson's disease, if any exist, is still ongoing.

Historically, public health policies were primarily concerned with addressing acute toxicities that resulted from occupational exposure of adults to various heavy metals and chemicals, and it is common for substances to initially be identified as toxic based on these acute exposure scenarios. However, past research on heavy metals and other toxic substances has demonstrated that chronic and subchronic toxic effects usually exist in addition to the acute effects. As scientists examine the effects of chronic and lower dose exposures to toxic substances and examine additional exposure pathways or specific subpopulations, other negative health effects are often identified. It is also not uncommon for different doses and exposure periods to result in different health effects, and it is typically only after much additional study has been completed, which evaluates the potential health effects at lower doses over extended periods of time, that scientists begin to understand whether or not safe levels of exposure exist for a particular substance. Unfortunately, significant amounts of time generally pass between the initial acute toxicity events and scientific understanding of the subtle and chronic impacts of a toxic substance on children and development. This extended period of study often results in considerable delays in removing the harmful exposure pathways. In fact, it took several decades of research and periodic reevaluation of the approved threshold level for lead for scientists to understand that there is no safe level of exposure for children (Lidsky et al., 2007).

The acute effects associated with high levels of manganese are widely known and well understood, but scientific understanding of the chronic, subclinical effects for manganese is currently evolving particularly with regards to children and neurodevelopment. A number of research studies evaluating the effects of chronic low-level exposures on children have been published over the past two decades. Preliminary data suggests that the period of fetal development through early childhood represents a sensitive time period, but more research is needed to determine possible exposure-related effects and what levels are considered safe in water. With respect to toxic substances and child development, one researcher noted that "consequences of low-level exposure are often subtle for an individual child and thus easily dismissed, but at the population level, such shifts in intellectual ability or behaviors can have a substantial impact" (Lanphear, 2015). The available research, including epidemiological data on children and animal toxicity studies, suggests that exposure to elevated manganese levels during critical periods of development may result in a variety of neurological and developmental deficits including symptoms consistent with attention-deficit, hyperactivity disorder (ADHD), short-term memory impairments, visual identification impairments, impaired performance on manual dexterity and rapidity tests, and a reduction in Intelligence Quotient (IQ) scores (Bouchard et al., 2007; Chung et al., 2015; Claus Henn et al., 2011; Grandjean and Landrigan, 2014; Haynes et al., 2015; Khan et al., 2011; Khan et al., 2012; Kim et al., 2009; Kullar et al., 2019; Menezes-Filho et al., 2009; Oulhote et al., 2014; Schullehner et al., 2020; Wasserman et al., 2006). In addition, Kim et. al. (2009) and Wasserman et al. (2011) examined the possibility that co-exposure to multiple neurotoxicants may have an additive effect on neurodevelopment. In these cases, Kim et al. (2009) assessed the intellectual function of school-aged children in Korea exposed to environmentally relevant levels of lead and manganese, and Wasserman et al. (2011) evaluated the effects of children exposed to elevated levels of arsenic and manganese. Furthermore, several recent prenatal and early-life studies on rats and mice generally corroborate these neurodevelopmental findings in children (Kern et al., 2010; Beaudin et al., 2013; Moreno et al., 2009).

Exposure Pathways and Homeostatic Control Mechanisms

There are two primary human exposure pathways for manganese – inhalation and oral exposure. Intravenous injection of illegal narcotics and TPN represent other possible routes of exposure.

It is known that inhalation of dusts containing small particles of manganese generally poses greater immediate toxicity risks and often results in significant acute and chronic neurotoxic effects. These increased neurotoxic effects occur because inhaled, ultrafine particles of manganese (that is, particles less than 10 microns in size) can bypass the body's normal homeostatic control mechanisms. The manganese particles contained in the fumes associated with many common occupational inhalation exposures, such as gas metal arc welding, are primarily, if not entirely, particles less than 10 microns in diameter (Zimmer et al., 2002; Sowards et al., 2010; Sen et al., 2011). The majority of manganese intoxication cases have been associated with occupational exposures involving inhalation of manganese particles (i.e., welders, miners, smelters, battery-manufacture workers, etc.) (Crossgrove et al., 2004). The increased level of toxicity associated with this exposure pathway is not unexpected since the ultrafine manganese particles have a direct pathway to the brain via the olfactory nerve (O'Neal and Zheng, 2015). The Elder et al. (2006) study found that the olfactory pathway efficiently transported ultrafine manganese particles into the central nervous system and referenced additional studies which showed manganese can be transported directly from the olfactory bulb to other brain regions such as the hypothalamus (Tjalve et al., 1995). Manganese can also be absorbed through the lungs. While manganese entering through the lungs and other thoracic tissues would be circulated through the blood and possibly pass through the liver before entering the brain, manganese entering the brain through the nasal cilia are transported directly along the olfactory nerve pathway and bypass the typical body control systems that limit absorption or retention associated with other inhalation, intravenous, or oral manganese exposures. The intestines and liver, which regulate manganese blood levels by reducing absorption from the digestive tract and by increasing excretion through the production of bile, are effectively bypassed when ultrafine particles of manganese are inhaled. Thus, the body will typically absorb most, if not all, of the inhaled manganese if the particles are $\leq$10 microns in diameter. Other possible environmental sources of inhalable manganese include power plant and automobile emissions.

While TPN without supplemental manganese can lead to manganese deficiency, it is also recognized that long-term TPN can lead to manganese toxicity in adults and children. (Erikson et al., 2007; Hardy, 2009). Similar to inhalation exposures, TPN via injection may bypass some, or all, of the body's normal homeostatic control mechanisms. TPN bypasses the intestines completely, and depending upon the site of injection, the TPN solution may or may not bypass the liver prior to being delivered to the brain.

In contrast to inhaled or injected manganese, the body of an individual in adequate health will tightly regulate the amount of ingested manganese that enters the circulatory system via intestinal absorption and the amount that circulates through the body via biliary excretion (Chen et al., 2015; Crossgrove et al., 2004; Erikson et al., 2007; O'Neal and Zheng, 2015; Schroeter et al., 2012; Yoon et al., 2011).

By far, the major route of manganese exposure for most individuals is through the oral pathway (that is, dietary sources). In addition to food and beverages (that is, tea, juices, soft drinks, etc.), individuals may also consume manganese via surface water and groundwater sources. Dietary sources and amounts vary greatly with average intake for adults ranging between 2 and 9 mg/day. Significant dietary sources of manganese include nuts, whole grains, legumes and rice. Moderate to high amounts can also be found in tea, green leafy vegetables, egg yolks, chocolate, seeds, and some fruits (Aschner, 2000; Chen et al., 2015; Finley and Davis, 1999). Thus, manganese levels at the higher range are more likely to be encountered with vegetarian (plant-based) diets. According to the EPA's Integrated Risk Information System (IRIS) database assessment and 2003 Health Effects Support Document for Manganese, studies have suggested that absorption rates may differ between drinking water and food sources due to

differences in bioavailability and the fasting state of the individual. For example, while a vegetarian diet can provide in excess of 9 mg/day of manganese, much of the manganese present is not bioavailable. It is important to understand that the plant-based diet contains many substances that bind to other substances within the food matrix. Dietary fiber, tannins, oxalates, and phytates are known to bind with mineral ions and significantly reduce their bioavailability (EPA IRIS). In addition, many manganese-rich foods are likely to contain a wide variety of other minerals in addition to manganese, and the mineral transport mechanisms within the membranes of intestinal cells may have a greater affinity for those minerals, such as iron, thus limiting the absorption of manganese. Unlike for other heavy metals, the oral exposure pathway is generally not expected to result in toxic levels of manganese within the body due to the dietary limitations on bioavailability and the tight homeostatic control mechanisms mentioned above. Proper functioning of these homeostatic control mechanisms generally ensures that manganese levels remain within the appropriate range necessary for good health. Compared to the inhalation route which results in nearly 100% absorption of ultrafine particles, absorption of manganese from the diet averages only 3-5% (ATSDR, 2012; Smith et al., 2017). Smith et al. (2017) also noted that the biological half-life of manganese in the body is on the order of weeks to months. Thus, changes in absorption or elimination efficiency can increase the body's burden of manganese.

It is important to recognize that manganese exposures and homeostatic control mechanisms may be functionally different in early life stages, including the neonate and infant. Less is understood about the nutritional and developmental needs, dietary exposures to manganese and ability to absorb or eliminate manganese for this age group. While additional research in this area would be beneficial, the available literature suggests that there are significant differences in these factors that may result in young children, spanning the period of birth through infancy, absorbing and retaining more manganese than older children and adults (Aschner and Aschner, 2005; ASTDR, 2012; Claus-Henn et al., 2010; Ljung and Vahter, 2007; Menezes-Filho et al., 2009; O'Neal and Zheng, 2015; Yoon et al., 2011; Neal and Guilarte, 2013; Scher et al., 2021).

Factors influencing Manganese levels in the body

Although diet is not generally expected to lead to elevated manganese levels, the blood and tissue manganese levels within specific individuals of the population are highly variable and influenced by a number of factors including oxidation state of the manganese, liver function, gender/mineral status, fasting state, genetic/epigenetic mutations and age. Furthermore, with a potentially narrow range between inadequate and excess intake and such low oral absorption rates in adults (typically less than 5%), a small variation in absorption or elimination of manganese could substantially change the overall body burden of manganese (Smith et al., 2017).

Different oxidation states of manganese are absorbed by different cell membrane transport proteins and pathways. The divalent metals transporter-1 (DMT-1) shuttles primarily divalent manganese while the transferrin (Tf)/transferrin receptor (TfR) system is responsible for transporting trivalent manganese (Chen et al., 2015). Trivalent manganese (Mn^{+3}) has a high affinity for the Tf system. On the other hand, divalent manganese (Mn^{+2}) may be transported across cell membranes through a variety of transporters other than DMT-1 including the zinc transporters (ZIP8 and ZIP14), the citrate transporter, the choline transporter, the dopamine transporter (DAT), and calcium (Ca) channels (Chen et al., 2015). The divalent oxidation state is one of two oxidation states typically found in surface waters.

As already discussed, the liver plays an important role in maintaining manganese homeostasis within the body. Liver impairment has a profound effect on manganese levels in the blood. If excess manganese has been absorbed, biliary excretion is the major pathway for elimination (Crossgrove et al., 2004). Thus, any form of liver impairment (i.e., cirrhosis, hepatitis, fatty liver disease, biliary atresia, neonatal cholestasis, etc.) may reduce the amount of manganese that the liver is capable of removing from the blood, leading to increased blood plasma levels and neurotoxicity.

Individuals with iron-deficiency anemia are also at risk for increased manganese levels in the body because iron-deficiency increases the expression of cell membrane transport systems typically used by both minerals (Chen et al., 2015; Erikson et al, 2007). Manganese is structurally and biochemically similar to iron in numerous ways (Smith et al., 2017). Both metals are transition elements, carry similar valence charge under physiological conditions (2+ and 3+), strongly bind Tf (Fe and Mn^{3+}) and preferentially accumulate in the mitochondria of cells (Aschner, 2000). Not surprisingly, females of childbearing age have been shown to absorb more manganese than males. Iron-deficiency anemia is prevalent among this group (Bouchard et al., 2007; O'Neal and Zheng, 2015; Oulhote et al., 2014).

Studies have also shown that genetic and epigenetic mutations can cause the body's cells to retain manganese and are associated with an inherited type of manganese-induced Parkinsonism as well as negative effects on child neurodevelopment. In Chen et al. (2015), the major cell uptake and efflux mechanisms for manganese were evaluated. Proper functioning of both influx and efflux mechanisms are critical to regulating cellular levels of essential metals. Genetic alterations associated with these mechanisms are known to lead to heritable disorders, including Wilson's disease and Menke's disease, and may lead to other disorders, such as Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Alzheimer's disease. A recent study by Aydemir et al. (2020) demonstrated that an intestine-specific deletion of metal transporter ZIP14 (SLC39A14) caused brain manganese overload and locomotor dysfunction, thus revealing the importance of intestinal ZIP14 as a route of manganese excretion. Refer to the section on "Scientific Literature and Data Related to the Human Health Effects of Manganese" for more detailed discussion on this topic.

As discussed by EPA in the Health Effects Support Document for Manganese (2003), fetuses, neonates and infants are known to retain greater amounts of manganese than adults due to several unique features of these life stages (Brown and Foos, 2008; O'Neal and Zheng, 2015; Yoon et al., 2011). First, this age group appears to lack a fully-developed excretory pathway via the liver (Aschner and Aschner, 2005; ASTDR, 2012; Claus-Henn et al., 2010; Ljung and Vahter, 2007; Menezes-Filho et al., 2009; O'Neal and Zheng, 2015; Yoon et al., 2011; Scher et al., 2021). Second, there is evidence that neonate and infant digestive systems may absorb more manganese than adults (Aschner and Aschner, 2005; ASTDR, 2012; Claus-Henn et al., 2010; Ljung and Vahter, 2007; Menezes-Filho et al., 2009; O'Neal and Zheng, 2015; Yoon et al., 2011; Neal and Guilarte, 2013; Scher et al., 2021). The increased absorption may be related to increased expression of the DMT-1 protein at the cell surface due to the need for large amounts of iron during early development. Third, formula-fed infants consume more water per unit of body weight. This difference is at a maximum in the first month and decreases with increasing age. Infants consuming at the 95^{th} percentile of intake ingest 8 times more water on a ml/kg basis than a 70 kg adult (Brown and Foos, 2008). Fourth, there is evidence of increased permeability across the BBB and retention of manganese in infant tissues (Mena et al., 1974). To date, this tendency has been attributed to an immaturity of the BBB mechanisms. However, some research has suggested that the uptake of manganese may be due to increased expression of metals transporters, such as DMT-1,

allowing for greater uptake of minerals required for normal development (Neal and Guilarte, 2013; Yoon et al., 2011).

Guidelines for Manganese

Health-based and Aesthetic Guidelines

EPA first mentions manganese in its "Water Quality Criteria 1972" book, also known as the "Blue Book". At that time, EPA established a recommendation that soluble manganese not exceed 0.05 mg/L in public water sources. In EPA's 1976 Quality Criteria for Water book, known as the "Red Book", EPA retained the 0.05 mg/L criterion to protect domestic water supply and added a 0.1 mg/L "organism only" criterion for protection of consumers of marine mollusks. These criteria remained unchanged in EPA's Quality Criteria for Water 1986, known as the "Gold Book". According to these EPA documents, public water systems with conventional treatment should be able to partially sequester manganese with special treatment, but manganese is not removed by conventional filtration. Complaints of laundry staining and objectionable tastes are common when manganese levels exceed 0.150 mg/L and low concentrations of Fe may increase these effects. In 1963, McKee and Wolf summarized the available toxicity data on freshwater aquatic life. Tolerance values ranged from 1.5 mg/L to over 1,000 mg/L. According to the EPA Red Book (1976), background surface water levels of manganese were not expected to exceed 1 mg/L. As background levels were not expected to exceed 1.0 mg/L, manganese was not considered to be a problem in freshwater waterbodies. It is unclear whether this data included consideration of impacts of manganese on freshwater mollusks. With respect to marine mollusks, manganese was found to bioaccumulate in the edible portions with bioaccumulation factors (BAFs) as high as 12,000. As such, EPA established the 0.1 mg/L "organism only" criterion. At concentrations of slightly less than 1.0 mg/L to a few milligrams per liter, manganese may be toxic to plants from irrigation water applied to soils with pH values lower than 6.0 (EPA Red Book, 1976).

Elevated manganese levels in groundwater and surface water tend to be limited to specific regions within the country. In addition, the funding and staff available to develop national water quality criteria recommendations are limited, so EPA, much like states, is likely to focus its limited resources on developing criteria recommendations for the highest priority pollutants. These observations may partly explain why EPA does not currently have a national water quality criterion recommendation for manganese for the protection of human health or aquatic life.

EPA's IRIS database provides human health assessment information on chemical substances following a comprehensive review of toxicity data as outlined in the *IRIS assessment development process*. An oral reference dose (RfD) is based on the assumption that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in units of mg/kg-day. In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. The RfD for manganese is for the total oral (dietary) intake of manganese and was published in IRIS in November 1995. In its recommendation, EPA specified that a modifying factor of 3 be applied if the RfD is used for assessments involving nondietary exposures (that is, soils or water). EPA provided four primary reasons for the recommendation.

First, fasting individuals have been shown to absorb more manganese from drinking water than non-fasting individuals (Ruoff, 1995; EPA, 2003).

Second, a study by Kondakis et. al. (1989) raised concerns about possible adverse health effects associated with a lifetime consumption of drinking water containing approximately 2 mg/L of manganese.

Third, formula fed infants have been found to have a much higher concentration of manganese in hair samples versus breast fed infants. Not only does infant formula contain higher amounts of manganese than breast milk, the valence form of the manganese in formula may increase the rate and/or amount of manganese absorbed. Studies have shown that the levels of manganese in learning-disabled children were significantly increased in comparison with that of non-disabled children. Although no causal relationship was determined, EPA stated that further research was needed. There is evidence that the infant digestive tract absorbs more manganese than adults and that infants are less able to excrete it. More recently, ATSDR (2012) referenced animal studies that showed increased absorption of manganese in young. In addition, the Minnesota Department of Health (MDH) (Scher, 2021) evaluated the manganese content of a variety of infant formulas and found the measured amount of manganese was 1.3 to 5 times more than the labeled amount.

Manganese levels in infant formula have been shown to contain as much as 75 times more manganese per liter than human breastmilk not including any additional manganese from the water used in the mixture (Brown and Foos, 2008; Ljung et al., 2007). Breastmilk manganese content can range between 1.8 and 27.5 µg/L and has been shown to vary with the stage of lactation (Erikson et al., 2007; Ballard and Morrow, 2013). Higher levels of manganese occur during the initial weeks of breastfeeding and gradually decrease over the first several months. Levels at this later time generally average around 3 µg/L (Erikson et al., 2007). Concentrations in infant formula, however, can range dramatically from 33 µg/L to well over 300 µg/L (EPA IRIS 1995). Soy-based formulas have been shown to contain the highest levels of manganese with a typical level between 200-300 µg/L. In 2018, MDH evaluated the manganese content of various infant formulas and found that the measured amount of manganese in formula was 1.3 to 5 times greater than the labeled amount (Scher et al., 2021). While the Food and Drug Administration (FDA) has set a minimum level of manganese in infant formula (~34 µg/L), no maximum level has been established. If manganese is present in the drinking water used to prepare the infant formula, the manganese content will be further increased.

Unlike the natural decline of manganese levels observed in breastmilk over time, infant exposure to the manganese levels in formula will remain fairly constant until weaned, which generally occurs on or after one year of age in accordance with the recommendations of the American Academy of Pediatrics (AAP). It is notable that human breastmilk manganese is also in a different oxidation state than infant formula. Human milk manganese is in the trivalent oxidation state whereas infant formula manganese is in the divalent oxidation state (Erikson et al., 2007). As discussed previously, trivalent manganese selectively binds with the transferrin receptor system, but divalent manganese can enter cells using a variety of transport mechanisms including DMT-1, ZIP8, ZIP14, DAT, choline transporter and calcium channels. Differences in manganese retention have been observed and may be partially attributed to the transport mechanisms that allow for manganese uptake across the gastrointestinal tract. Studies have found that formula-fed infants consume, absorb, and retain more manganese per day than breastfed infants (ATSDR, 2012; Brown and Foos, 2008). ATSDR (2012) noted that more manganese was

absorbed by infants consuming formula even though the manganese absorption rates between cow- and soy-based milk sources were slightly less than breastmilk, 80-90% and 70%, respectively. This is likely due to the fact that cow- and soy-based formulas contain substantially higher amounts of manganese when compared to breastmilk.

Furthermore, manganese has been shown to readily cross the BBB in infants. A study by Mena et. al. (1974) found the rate of penetration in animal experiments to be 4 times higher in infants than in adults. These considerations, in addition to the likelihood that any adverse neurological effects of manganese associated with early exposure are likely to be irreversible and not manifested for many years after exposure, warrant caution when establishing safe levels of manganese in water until more definitive data are available (EPA IRIS 2017).

EPA completed an updated review of the scientific data and literature available on manganese as published in the 2003 "Health Effects Support Document for Manganese" (EPA-822-R-03-003). This updated review continues to support the use of the modifying factor of 3 for nondietary exposure pathways.

EPA's Drinking Water Health Advisory Program, sponsored by the Health and Ecological Criteria Division of the Office of Science and Technology (OST), Office of Water (OW), provides information on the health and organoleptic (e.g., taste, odor, color) effects of contaminants in drinking water. A health advisory level (HAL) is not an enforceable standard, but rather provides technical guidance to assist Federal, State and local officials when emergency spills or contamination situations occur. The current HAL for manganese was issued in 2004. The recommendation was based partly on the ATSDR's final Toxicological Profile for Manganese (ATSDR, 2000) and the IOM Dietary Reference Intakes for Manganese (IOM, 2000). HALs are generally determined for one-day, ten-day and lifetime exposure if adequate data are available that identify a sensitive noncarcinogenic end point of toxicity. There was no suitable information to develop a one-day HAL for manganese. The ten-day HAL of 1 mg/L for a child is recommended as a conservative estimate for a 1-day exposure for both children and adults. The ten-day HAL for a 10-kg child is 1 mg/L. The lifetime HAL for adults and children is 0.3 mg/L and was calculated using the recommendations in IRIS and the updated 2003 Health Effects Support Document for Manganese. For infants younger than 6 months, the lifetime HAL of 0.3 mg/L is also recommended for acute exposures (ten-day, one-day) due to similar concerns identified by EPA in establishing the oral RfD for manganese (EPA manganese HAL). Currently, the Federal Safe Drinking Water Act (SDWA) regulations only regulate manganese as a secondary contaminant. Under Federal regulations, secondary maximum contaminant levels (SMCLs) are considered non-enforceable federal guidelines for contaminants that may cause cosmetic or aesthetic effects. However, SMCLs are enforceable standards in the Commonwealth of Pennsylvania, and they are regulated under 25 Pa. Code Chapter 109. The SMCL for manganese in Pennsylvania is 0.05 mg/L and is based on the Federal SDWA standard.

MDH evaluated the science on manganese in 2012 and developed an RfD based on the Kern et al. (2010) study. This RfD was used to develop Risk Assessment Advice (RAA) of 100 µg/L (or 0.1 mg/L) and used tiered guidance based on age instead of MDH's typical duration-specific guidance. In 2017, MDH re-evaluated the available information and updated their risk assessment methodology, which resulted in no change to the existing RAA. In 2018, the tiered guidance methodology was removed and the guidance value was converted from RAA of 100/300 µg/L to a short-term health-based guidance value (HBGV) of 100 µg/L (or 0.1 mg/L) to protect bottle-fed infants less than one year of age from

exposure to manganese through drinking water. For older children and adults, MDH supports the EPA lifetime HAL of 0.3 mg/L. In 2020, MDH incorporated updated intake rates (US EPA 2019), which did not result in any changes to the guidance values. MDH reviewed many of the same toxicological studies as DEP.

In 2019, Health Canada reviewed the science on manganese and updated its "Guidelines for Canadian Drinking Water Quality" for manganese. Health Canada developed a health-based valued of 0.12 mg/L to protect infants and neonates. Many of the same scientific studies reviewed by DEP during the development of the manganese criterion recommendation were evaluated by Health Canada. As with MDH, Health Canada used the lowest observed adverse effect level (LOAEL) from the Kern et al. (2010, 2011) and Beaudin et al. (2013) animal studies in their risk assessment. The agency noted:

> "The Kern and Beaudin studies were chosen as a basis for the current risk assessment because of their thoroughness in assessing neurodevelopmental endpoints (observed neurobehavioral effects are supported with corresponding neurochemical findings) in early life that are consistent with the findings reported in epidemiological studies (Bouchard et al., 2011; Khan et al., 2011; Roels et al., 2012; Oulhote et al., 2014). These studies identified a LOAEL of 25 mg Mn/kg body weight per day for various neurological endpoints in rats. In addition to demonstrating that exposure to manganese in early life can result in behavioral and sensorimotor effects, these studies provided mechanistic support by demonstrating corresponding neurostructural and neurochemical changes. Further, Kern et al. (2011) and Beaudin et al. (2013) demonstrated the ability of manganese exposure in early life to result in effects that persist into adulthood, after levels of manganese in the brain have returned to normal. Despite their above-mentioned strengths, it should be noted (1) that the key studies chosen do not reflect the lowest LOAELs reported in the literature for neurological effects following oral exposure to manganese, and (2) that benchmark dose analysis was not possible because only two doses were tested."

In addition to Health Canada, WHO recently evaluated the science on manganese in drinking water and published a draft update to the "WHO Guidelines for Drinking-Water Quality" in December 2020 for public review and comment. As with MDH and Health Canada, WHO also relied on the Kern et al. (2010, 2011) and Beaudin et al. (2013, 2017) studies in calculating the RfD used in the development of their drinking water guideline. Utilizing an RfD of 0.025 mg/kg body weight per day, a relative source contribution (RSC) of 0.5 and exposure inputs specific to infants (5 kg body weight and 0.75 L water per day), WHO drafted a health-based guideline for manganese of 0.08 mg/L to protect bottle-fed infants.

While the recent evaluations by MDH, Health Canada and WHO were used to develop drinking water guidelines for manganese, the scientific data and literature used by these organizations is nearly identical to that used by DEP in its water quality criterion recommendation. If these peer-reviewed toxicological studies and data were acceptable for use by these notable health organizations, they should be acceptable for use for other health-focused efforts, such as DEP's water quality criterion for manganese for the protection of human health.

Technology-based Guidelines

Effluent limitation guidelines (ELGs) are national, technology-based wastewater discharge regulations that are developed by EPA on an industry-by-industry basis. DEP received comments from the mining industry during the public comment period of the ANPR regarding ELGs. The mining sector has pointed to the federal ELGs found at 40 CFR 434, which place limitations on the amount of manganese that can be legally discharged in mining effluent. Pennsylvania's mining regulations found at 25 Pa. Code §§ 87.102, 88.92, and 89.52 mirror these federal limitations. Both the state and federal mining regulations effectively limit discharges of manganese to 2.0 mg/L as a 30-day average, 4.0 mg/L as a daily maximum and 5.0 mg/L as an instantaneous maximum. The mining sector contends that moving the application of the Potable Water Supply use criterion to the point of potable water supply system withdrawal would not result in harmful levels of manganese in waters of the Commonwealth at the point of discharge because the federal ELGs effectively prevent mining companies from discharging at such levels. DEP recognizes that this industry has these additional regulations that would limit the amount of manganese in their discharges if the Commonwealth's Potable Water Supply use manganese criterion would be applied at the point of potable water supply system withdrawal. However, the other industrial sectors identified earlier in this rationale document do not have federal ELGs in place to restrict the discharge of manganese to waters of this Commonwealth. Therefore, the mining ELGs and regulations do not adequately address control of manganese at the point of discharge for any industrial sector other than mining. Conversely, WQ criteria are applicable to, and are necessary to prevent pollution from, all types of activities associated with and discharges to surface waters of the Commonwealth. These criteria are also used by DEP in the assessment of waterbodies and for other permit and non-permit related activities. Furthermore, DEP does not agree with the mining industry's contention that manganese levels of 4 mg/L and 5 mg/L are not harmful to aquatic life and other protected water uses. In fact, these levels are known to be toxic to some aquatic life and may also negatively impact livestock watering and irrigation.

WQ criteria are developed by DEP to protect all existing and designated water uses, and their application is not restricted to any one particular group or activity. DEP must follow appropriate federal and state statutes and regulations when developing WQ criteria. Under section 303(c)(1) of the CWA, DEP is also required to review and update its WQSs periodically, but at least once every 3 years. Therefore, DEP must develop the necessary WQ criteria to protect Pennsylvania's water uses as defined in 25 Pa. Code § 93.3 (relating to protected water uses) based on the best available scientific information and recommended guidelines, as appropriate.

Scientific Literature and Data Related to the Human Health Effects of Manganese

DEP has reviewed the scientific literature on the human health effects of manganese, which is a metal that will behave similarly to other heavy metals at levels beyond those necessary to maintain adequate health. The available research suggests that a narrow dose range exists between inadequate and excess intake and that small variations in the body's absorption and handling of manganese could substantially change the body's burden of manganese resulting in negative health outcomes (Smith et al., 2017). Since the last review of manganese completed by both DEP (1979) and EPA (2003), many peer-review scientific research studies have been published that examine the effects of manganese exposure on the developing fetus, infants and children. Although research is ongoing, the summary that follows highlights some of the current knowledge on the health effects of manganese.

<u>Epidemiology Studies</u>

In 2006, Grandjean and Landrigan reviewed the scientific literature and identified five industrial chemicals as neurodevelopmental toxicants: lead (Pb), methylmercury, PCBs, arsenic (As) and toluene. Since that time, epidemiological studies have documented at least six additional neurotoxicants: manganese, fluoride, chlorpyrifos, dichlorodiphenyltrichloroethane, tetrachloroethylene, and polybrominated diphenyl ethers (PBDEs) (Grandjean and Landrigan, 2014). Lidsky et al. (2007) and Grandjean and Landrigan (2014) recognized that the risks of industrial chemicals to brain development has historically required decades of research to identify and understand the subclinical neurotoxic effects since the initial discovery of toxicity often begins with poisoning and episodes of high-dose exposure. In addition, the full effects of early damage may not become apparent until school age or beyond due to the normal sequence of developmental stages (Grandjean and Landrigan, 2014). Efforts to control and restrict developmental neurotoxicity are hampered by the lack of data required by law on developmental neurotoxicity for chemicals. The authors noted that while scientific understanding of the effects of early manganese exposure is currently limited, the recent research on well-documented neurotoxicants such as lead and methylmercury has generated new insights into the consequences of early exposure to heavy metals.

Between 2007 and 2011, Chung et. al. (2015) recruited 232 mother-infant pairs from the Mothers and Children's Environmental Health study (MOCEH) in South Korea to evaluate the relationship between neurodevelopment and maternal blood manganese level without a specific source of occupational or environmental exposure (Chung et al., 2015). Chung et al. (2015) evaluated a number of possible confounding factors including maternal age/height/weight, maternal and paternal education level, marital status at enrollment, and family income. Participants were asked to provide information about their entire food intake during the 24 hours before they were interviewed. Interviews occurred multiple times: at the time of recruitment into the study, at the visit for delivery and at each infant follow-up visit. Data collected before delivery included any exposure through passive smoking at home, parents' physical condition, medical records, and family history of diseases. Information on birth outcome was recorded (date of delivery, mode of delivery, birth weight and height, gestational age, head circumference, parity, and infant's sex). Information was also collected on variables that could affect infant growth. This study does recognize the potential for several confounding variables including the following factors: high education level of the mothers which may not reflect the general population; single blood manganese measurement of the newborn at delivery; lack of quantification of dietary sources of manganese; and insufficient maternal blood manganese data to complete certain analyses. Despite these limitations, the results of the study suggest an association between maternal blood manganese at delivery and neurodevelopmental scores of infants at 6 months of age. Similar to the observations made by Haynes et al. (2015), the association between manganese and neurodevelopmental scores followed an inverted U-shaped curve after adjustment for potential confounding factors and indicates that increasing levels of manganese are beneficial to neurodevelopment up to a certain level beyond which negative effects on neurodevelopment are observed.

Bouchard et. al. (2007) conducted a pilot study of 46 Canadian children (boys and girls ages 6-15 years) to assess differences in children's exposure to public well water from two wells with different manganese concentration. Manganese levels in Well 1 had increased from 230 to 610 µg/L over the period from 1996-2005 with a mean value of 500 $\pm$ 129 µg/L. Well 2 was drilled in 1999 and had stable manganese levels that averaged 160 µg/L. Most families drank bottled water due to the bad taste associated with elevated manganese in the public water supply. However, the well water was used in

cooking and to prepare soups and concentrated fruit juices. Well water was also consumed by study subjects, some on a daily basis, while at school since the public water supplier also provided the tap water in those buildings. Thus, all of the study subjects had some level of exposure to manganese in drinking water. Manganese body burden was determined by measuring the manganese content of hair samples. The authors determined that elevated levels of manganese in hair was associated with increased hyperactivity and oppositional behaviors in the classroom after adjusting for income, age and sex. Girls had significantly higher hair manganese levels than boys. The group was ethnically homogeneous, had an economic level above provincial average and most had a biparental family structure. The authors identified a number of potential confounders that could not be ruled out and stressed that additional follow-up evaluation of the study subjects was warranted. While it is not capable of being used to establish a reference dose, this study is part of the collective research and information on manganese to provide support for neurotoxicity as a critical endpoint.

Following the 2007 pilot study, Bouchard et. al. (2011) conducted a cross-sectional study on 362 children (ages 6-13) living in southern Quebec. Researchers examined the effects of manganese intake from diet and drinking water on intellectual impairment. The results showed that children exposed to higher concentrations of manganese in tap water had lower IQ scores after adjustment for socioeconomic status and other metals present in the water. The study also showed that manganese intake from water ingestion, but not from the diet, was significantly associated with elevated hair manganese. This finding suggests that the body's normal homeostatic control mechanisms may not respond to drinking water manganese in the same manner as dietary manganese and may not prevent increased body burden (Bouchard et al., 2011). As noted with Bouchard et al. (2007), additional research is warranted due to potential confounding factors, but the study provides support for neurotoxicity as a critical endpoint.

Oulhote et. al. (2014) conducted an additional assessment of the Bouchard cohort to determine possible associations between manganese in water and behavioral impairments (i.e., issues with memory, attention, motor function and hyperactive behaviors.) Water samples were collected from each home at the beginning of the study, and a subset of homes were sampled four times (once per season) over one year to examine any seasonal variability in manganese levels that might exist. Sample results indicated very little variability. Oulhote et al. (2014) did evaluate a number of potential confounding factors including maternal education and intelligence; family income; maternal tobacco and alcohol consumption during pregnancy; and tap water lead concentrations. Total manganese intake, including dietary intake, and the home environment were previously evaluated in Bouchard et al. (2011). While the authors could not rule out additional unmeasured confounding factors, they noted their findings were unlikely to be explained by anthropogenic contaminants because the manganese contamination in the well water was known to result from natural processes associated with the bedrock geology of the region. In addition, the study area lacked industrial sources of manganese emission, and the gasoline additive, mmt®, had not been used in Canada since 2004. Bolte et al. (2004) showed atmospheric manganese concentrations in the rural areas of Quebec were 10 times lower than EPA's inhalation reference concentration of 0.05 $\mu g/m^3$. Although the cross-sectional design of the study limits the ability to draw strong causal inferences, the results suggest that higher levels of manganese exposure are associated with poorer performance of memory, attention and motor functions, but not hyperactivity, in children.

Haynes et. al. (2015) assessed the impact of manganese on neurocognition in a cohort of school-age children (age 7-9) residing in communities near Marietta, Ohio, which is home to the longest operating ferromanganese refinery in North America. Mothers of selected children must have resided in the area during their pregnancies. The authors evaluated potential confounding factors in the home by using the Parenting Relationship Questionnaire (Reynolds and Kamphaus, 2004), which evaluates attachment, communication, parenting confidence, discipline practices, involvement, school satisfaction and relational frustration. Results showed that both high and low levels of manganese may be associated with cognitive impairment. High or low levels of manganese in the body may result from disease (both), nutritional deficiency (low) or exposure to excess levels of manganese in a person's environment (high), including from air, drinking water, diet, supplements, etc. In addition, unlike the difference in IQ scores between the high blood manganese group and the average group, the authors noted that the difference between the average group and those with the lowest blood manganese levels was not statistically significant. A measurement and evaluation of serum cotinine levels was included in the study since it could be a potential confounding factor. Cotinine is the predominant metabolite of nicotine and is used as a biomarker for exposure to tobacco smoke. In addition to cotinine, the authors measured and evaluated the serum levels of lead, a known neurotoxicant. As noted by the authors, "inclusion of multiple neurotoxicants in this study provided a robust analysis between manganese exposure and intellectual function in children because we were able to adjust for potential confounding by lead and environmental tobacco smoke." While this study did not specifically evaluate manganese exposure in drinking water, it does provide information that supports a link between manganese exposure and impacts on neurodevelopment.

Khan et. al. (2011) assessed the effects of manganese on a community in Bangladesh. The authors examined the effects of manganese exposure through drinking water, but also attempted to evaluate the combined effects of exposure to manganese plus other neurotoxicants in drinking water such as arsenic. As part of the study, basic home environment information was collected during a home interview that included characteristics of the home (roof, wall, and floor materials), paternal and maternal education, paternal occupation, access to television or radio, and maternal intelligence. This study is related to additional similar studies published by Wasserman et al. (2006, 2011). The participants in these studies came from a larger cohort study of adults in the region, knowns as the Health Effects of Arsenic Longitudinal Study (HEALS which used the same HEALS cohort). Children (ages 8-11) were designated into one of four groups: a) high arsenic, high manganese; b) high arsenic, low manganese; c) low arsenic, high manganese; d) low arsenic, low manganese. Each group contained approximately 75 children. Significant associations were found between manganese (water) and test scores for both externalizing and internalizing behaviors. Manganese was significantly more strongly related to externalizing behavior problems. Interestingly, arsenic was not associated with either externalizing or internalizing behavior problems (Khan et al., 2011). Khan et al. (2011) noted possible confounding factors including teacher bias and the inability to establish "geographic generalizability". This inability, however, was not due to areas with lower water manganese being excluded due to distance from the study region. The study was conducted in rural Araihazar, Bangladesh, which is relatively well-developed. Thus, the authors stated "the study population may represent only comparable communities with similar sociodemographic characteristics. Our findings may not be generalizable to children living in urban communities." The authors' primary purpose for the study was to draw attention to the elevated manganese levels that are naturally occurring in the groundwater of that region. While the digging of deeper wells has greatly reduced arsenic exposure in some areas, the authors noted that the manganese levels in these deeper wells may still exceed established drinking water guidelines for

manganese. This study adds to the collection of data supporting a link between manganese and neurodevelopment.

Wasserman, et. al. (2006) examined associations between drinking water manganese (WMn) and intellectual function in 142 children (ages 9.5-10.5) from Araihazar, Bangladesh. Wasserman, et al. (2011) evaluated possible synergistic effects of simultaneous exposure to arsenic and manganese in well water. As with the Khan et al. (2011) study, the participants in these studies came from a larger cohort study of adults in the region, knowns as the HEALS cohort. As part of that study, detailed information on smoking of tobacco products was collected and recorded. Nutritional and dietary information was also collected for the HEALS cohort via surveys. While not specifically discussed by Wasserman et al. (2006, 2011), the HEALS study data could have been available to the authors, but it is unclear whether it was considered by the authors in their selection of participants or evaluation of the manganese study data. The mean manganese concentrations in the drinking water of the 2006 and 2011 studies were 795 µg/L and 527 µg/L, respectively. After adjusting for sociodemographic factors, drinking water manganese was associated with significantly reduced Full-Scale, Performance, and Verbal raw scores in a dose-dependent fashion. Blood arsenic levels were also associated with negative effects. The authors noted that while they did not observe statistically-significant interactive associations with intellectual function, only two children in the study drank from wells with very high levels of both arsenic and manganese (Wasserman et al., 2011).

Cellular Studies

Smith et al. (2017) examined the role of manganese in intracellular functions, such as in cell structure and mitochondrial antioxidant systems, and the consequences of excess intracellular manganese. Since manganese is similar to several other important metals in the body, it has the ability to displace those other metals in critical enzymes, alter metals transport, and induce oxidative stress, which can disrupt numerous processes within the cell. Several research groups have shown in model systems that exposure to excess manganese results in various cytotoxic events and signals apoptosis (that is, programmed cell death). Organelles involved in manganese-induced apoptosis include mitochondria and endoplasmic reticulum.

Genetic/Epigenetic Studies

Chen et al. (2015) examined the four efflux transporters for manganese that were known at the time of the study. Of those mechanisms, loss of function mutations in *SLC30A10* were known to cause a hereditary manganese-induced parkinsonian syndrome (Chen et al., 2015). In addition, genetic mutations affecting DMT-1 expression appeared to increase susceptibility to several diseases, including Parkinson's disease. More research is needed to understand the effects of the other manganese transport mechanisms on various health outcomes. Additional research on *SLC30A10* by Wahlberg et al. (2018a) demonstrated that specific genotypes were associated with negative neurodevelopmental outcomes in children. The blood and dentin (teeth) of children with the affected genotypes contained increased levels of manganese compared to non-affected children, and the affected children demonstrated lower performance for certain IQ subtests, decreased motor function, and increased scores for behavioral problems (Wahlberg et al., 2018b). The authors also evaluated an influx transporter, known as *SLC39A8*, and observed similar effects. However, Wahlberg et al. (2018a) noted additional research on *SLC39A8* was warranted due to potential confounding factors. In a related publication by Broberg et al.

(2019), additional analyses by the authors suggested that sex-related differences may exist and that girls affected by the genetic mutations experience greater negative intellectual effects than boys.

In concert with genetic research, emerging epigenetic research has identified manganese as a likely modifier of epigenetic regulation. Epigenetics describes the heritable changes in gene expression that occur without mutations to the DNA sequence. In other words, the genetic code (DNA) doesn't change, but how the body's cells read and translate DNA into functional processes does change. A number of studies have been published recently evaluating the effects of manganese on epigenetic regulation, specifically DNA methylation.

This emerging research in epigenetics indicates that the risk of development and progression of many human diseases depends upon epigenetic modifications triggered by environmental cues during sensitive early life stages (Vaiserman, 2015). Human studies by Maccani et al. (2015) and Appleton et al. (2017) found that prenatal exposure to increased levels of manganese and other neurotoxic metals changed DNA methylation patterns in the placenta. Studies by Qiao et al. (2015), Miranda-Morales et al. (2017), and Tarale et al. (2016) have examined the role of epigenetics in manganese-induced neurotoxicity and Parkinson's disease.

Maccani et al. (2015) studied specific changes in DNA methylation patterns in the fetal placenta that were associated with manganese exposure (as measured by manganese in infant toenail samples). The authors identified 713 CpG loci that were associated with manganese exposure and input those genes into the Database for Annotation, Visualization and Integrated Discovery (DAVID) functional annotation tool (https://david.ncifcrf.gov). Their analysis found that many of the 713 affected genes are involved in neurodevelopment and neurogenesis. Further, many of the genes are associated with the development of or risk for various neurological disorders, including autism, ADHD, Tourette's syndrome, schizophrenia and Alzheimer's disease (Maccani et al., 2015). The authors of Maccani et al. (2015) stated "these results suggest that in utero manganese exposure may result in potentially harmful disruption to normal placental and fetal growth and development, which is important considering existing links between placental methylation patterns and fetal growth (Wilhelm-Benartzi et al., 2012; Banister et al., 2011) and neurobehavior (Bromer et al., 2013; Lesseur et al., 2014)." While this study requires additional work to validate the results, it continues to support the link between manganese exposure and negative impacts on neurodevelopment.

Appleton et al. (2017) considered the role of the placenta in the development of children's hypothalamic-pituitary-adrenal (HPA) axis, including the regulation of cortisol exposure to the fetus, through the actions of the glucocorticoid receptor (*NR3C1*) and downstream targets of its regulation. Studies by Monk et al. (2016), Bromer et al. (2013), and Stroud et al. (2014) linked variations in the DNA methylation pattern of the *NR3C1* promoter region to fetal and newborn neurobehavioral phenotypes. Appleton et al. (2017) examined whether prenatal exposure to neurotoxic metals, including manganese, would be associated with *NR3C1* methylation and whether any associations observed differed by infant sex (males vs. females). The study examined 222 mothers and infants from Providence, Rhode Island. Metals concentrations in infants, including manganese, were determined from toenail samples. After controlling for biological and social confounding factors, the authors found that exposure to higher levels of manganese contributed to the methylation of placental *NR3C1* in females but not males. However, they noted the sex-specific differences may not have been statistically significant. Several potential limitations of the study were also noted including a lack of data on the

sources of the metal exposure (that is, dietary, water, home environmental or other) and the lack of a standard biomarker for metals, including manganese.

Qiao et al. (2015) evaluated the literature on Parkinson's disease and manganese-induced neurotoxicity and noted that these conditions share common genetic features and associations. The authors stated that:

> "Manganese causes oxidative stress in primary cultures of astrocytes, leading to mitochondrial dysfunction and energy insufficiency…In addition, manganese has been reported to disturb dopamine metabolism via direct oxidation of monoamine oxidase activity in brain mitochondria (Shih 2004)." Qiao et al. (2015) also report that "environmental factors, biological and chemical, have long-lasting phenotypic effects without apparent underlying genetic change through epigenetic modifications. In other words, environmental factors may change the gene expression directly or indirectly through epigenetic alterations such as DNA methylation or histone modifications. These epigenetic changes in the development stages due to prenatal exposure to the environmental factors including manganese may contribute to the abnormal phenotype including neurodegeneration. It has been reported that epigenetic gene regulation may contribute to manganese-induced neurogenesis in mouse offspring after maternal exposure to manganese. Sustained promoter hypermethylation of Mid1, Atp1a3, and Nr2f1 and transient hypermethylation in Pvalb and consequent down regulation of these genes were found in mouse offspring after maternal exposure to manganese (Wang et al., 2013)".

Miranda-Morales et al. (2017) reviewed data from a number of published studies, including Masliah et al. (2013), which examined DNA methylation patterns between blood and brain tissue samples obtained from Parkinson's disease patients and age-matched healthy subjects to determine if such patterns were consistent between the two types of tissue and whether patterns were significantly different between diseased and healthy subjects. The comparison showed that both blood and brain tissues exhibited highly similar DNA methylation patterns, and analysis of DNA methylation profiles of blood from Parkinson's disease patients clearly distinguished Parkinson's disease patients from healthy subjects or those with other diseases. The study authors noted that:

> "Epigenetic regulation of biological processes is known to be essential during embryonic development, early brain programming, neurogenesis and brain plasticity (Yao et al., 2016). Therefore, it is not surprising that epigenetic deregulation can be critical for the onset of various neurodegenerative diseases, such as Parkinson's disease (Ammal Kaidery et al., 2013)…Twin studies determined that in each human, age-dependent aggregation of distinct epigenetic changes, termed 'epigenetic drift', is thought to be influenced predominantly by environmental factors (Fraga et al., 2005; Tan Q. et al., 2016)…Taken together, changes in DNA methylation patterns and their effects on chromatin and gene expression appear to add increasingly to our understanding of age-related diseases, including Parkinson's disease."

Regarding factors that may influence DNA methylation and Parkinson's disease, Miranda-Morales et al. (2017) also stated that "gene activity of PARK2 and PINK1 was altered via DNA hypermethylation in dopaminergic human neuroblastoma SH-SY5Y cells upon manganese exposure (Tarale et al., 2016).

Furthermore, mice exposed to $MnCl_2$ showed DNA hypo- and hypermethylation of different loci in substantia nigra (Yang et al., 2016)."

In addition to Qiao et al. (2015), Tarale et al. (2016) also evaluated the available, peer-reviewed literature as it relates to the role of epigenetics in the development of Parkinson's disease and manganese-induced neurotoxicity. The authors identified several potential ways that epigenetic modifications may influence the deregulation of cellular processes leading to neurodegenerative diseases, but stated that additional research is needed to investigate whether molecular pathways that are responsible for manganese-induced dopaminergic cell death in humans are similar to those reported in scientific studies using in vivo/in vitro cell models (Tarale et al., 2016). The identification of definitive epigenetic pathways may be used to understand disease progression and develop therapeutics in relevance to manganese exposure.

These emerging studies on genetics/epigenetics and exposure to manganese may eventually be able to help explain the neurobehavioral observations described in animal toxicity studies like Kern et al. (2010), Beaudin et al. (2013) and Moreno et al. (2009), including sex-related differences.

Animal Toxicity Studies

As discussed previously, several recent animal toxicity studies, including those by Kern et al. (2010), Beaudin et al. (2013, 2017) and Moreno et al. (2009), have been used by health organizations to develop health-based guidelines for manganese. In addition, a study by Dearth et al. (2014) evaluated the role of early life exposure to manganese in mammary gland development and hyperplasia (that is, the enlargement of an organ caused by an increase in the rate of cell production, often associated with the early stages of cancer development) in female rats.

Kern et. al. (2010) conducted experiments on neonate Sprague-Dawley rats to better understand the relationship between early, pre-weaning manganese exposure and neurobehavioral deficits. The pre- and early post-weaning period coincides with the development of dopaminergic pathways in specific brain regions that are instrumental in the regulation of executive function behaviors involving learning, memory and attention (Kern et al., 2010).

The authors stated that the following about the exposure doses used in the study (0, 25, and 50 mg Mn/kg/day over postnatal day (PND) 1-21):

> "These oral manganese exposure levels increased manganese intake by ~350 and ~700-fold over levels consumed by rats from lactation alone, which approximates the relative ~300 to ~500-fold increases in manganese exposure suffered by infants and young children exposed to manganese contaminated water or soy-based formulas (or both) compared to manganese ingestion from human breast milk. Human breast milk contains ~6 ug Mn/L, yielding normal infant intake rates of ~0.6 ug Mn/kg/day, based on infant daily milk consumption rates of ~0.8 L/day for an 8-kg 6-9 month old infant (Arcus-Arth et al., 2005; Dewey et al., 1991; Dorner et al., 1989; Stastny et al., 1984). By comparison rat milk manganese levels are ~200-300 ug Mn/L (Dorman et al., 2005; Keen et al., 1981), and pre-weaning rats consume an average of 260 mL/kg/day over PND 1-21 (Godbole et al., 1981; Yoon and Barton, 2008). Thus, pre-weanling control rats consume

> ~70 ug Mn/kg/day, which is ~100 times higher than normal human infant manganese intake from breast milk." (Kern et al., 2010).

As the authors explain, the doses are higher because normal manganese intake for rats is naturally higher than normal manganese intake for humans. These doses were intentionally selected to mimic the expected human exposures as described above.

Regarding the nutritional requirements of rats, the National Academies of Science (NAS), Engineering and Medicine (National Research Council) published dietary requirement information for experimental animals. The "Nutrient Requirements of Laboratory Animals, Fourth Revised Edition" (1995) identifies a recommendation of 10 mg Mn/kg diet for normal rat growth and 25 mg Mn/kg diet for reproduction. In comparison, the IRIS reference dose for a 70-kg adult is 0.14 mg/kg/day based on a dietary No Observed Adverse Effect Level (NOAEL) of 10 mg/day. The publication also notes that "postnatal growth of rats is unaffected by dietary manganese intakes as high as 1,000 to 2,000 mg/kg diet, provided dietary iron is adequate." Thus, rats and mice appear to have much higher normal dietary requirements for manganese and/or are less sensitive to dietary manganese than humans particularly in early life stages (as noted above). The Kern et al. (2010) study used commercially available rodent chow containing 118 mg/kg of manganese and supplemented the diet with manganese-laden drinking water at doses designed to mimic human exposures to manganese in drinking water. The NAS publication also makes the following statement regarding dietary vs. drinking water manganese and toxicity: "Although the concentrations of dietary manganese needed for overt toxicity are quite high [in excess of 3,500 mg/kg], weanling rats given water containing 55 ug Mn/mL for 3 weeks were reported to have reduced rates of brain RNA and protein synthesis (Magour et al., 1983)."

Kern et al. (2010) assessed rat responses in an open arena, elevated plus maze and 8-arm radial maze. The authors found that pre-weaning exposure to manganese caused rats to travel greater distances in the open arena and spend more time in the center zone of the arena when compared to the control rats, but early exposure did not affect the response to the elevated plus maze. In the discussion, the authors explain:

> "the elevated plus maze and the open arena are both considered screening tests for emotional reactivity (Ducottet and Belzung, 2005), but there is a fundamental difference between the two paradigms. The open area introduces a novel environment with stressors of a wide-open unfamiliar space, as well as isolation from cage mates. Normally, animals show a preference for thigmotaxic (wall touching) behavior in response to these stress cues, but in the absence of normal inhibition of exploratory behavior in this novel environment, animals will more readily venture into the center of the enclosure (Prut and Belzung, 2003), as we observed here. The elevated plus maze also presents a novel environment and isolation from cage mates, but includes additional stress factors in the elevated open arms that are absent of thigmotaxic cues and introduce a potentially harmful situation (Carobrez and Bertoglio, 2005)."

With respect to the differences in tests, Kern et al. (2010) goes on to state the following:

> "Disinhibition of exploratory behavior in the open arena, but appropriate innate fear response in the elevated plus maze may suggest differential susceptibilities of dopamine

systems controlling these behaviors to early manganese exposure. Inhibitory control of exploratory behavior is governed in part by dopamine release in the accumbens and prefrontal cortex (Arnsten and Goldman-Rakic, 1998; Bandyopadhyay et al., 2005; Grace, 2000), but innate fear conditions, such as those presented by the elevated plus maze, elicit dopamine release in relatively primitive structures such as the amygdale and bypass prefrontal cortex influence, resulting in greater autonomic control of behavioral responses (Arnsten, 2000; Corcoran and Quirk, 2007; LeDoux, 2000; LeDoux, 2003). This may suggest that behavioral tests that rely only on innate or conditioned fear responses to possible injury, such as shock avoidance, may not be as sensitive for detecting effects of manganese exposure.

Behavioral disinhibition, observed as increased center zone activity in the open arena, was associated with decreased levels of D1 receptors and DAT in the nucleus accumbens and dorsal striatum, and increased D2 receptors in the prefrontal cortex of manganese-exposed animals. It is possible that these effects on dopamine-related proteins resulted in dysregulation of dopaminergic control over suppression of outward exploratory behavior in the open arena, leading to increased center zone activity. The dopamine system normally functions in the prefrontal cortex and nucleus accumbens to modulate neuronal activity to elicit appropriate behavioral responses to relevant stimuli, such as novel stressful environment, and for suppression of neuronal activity that might otherwise lead to contextually inappropriate behavioral responses (Arnsten and Goldman-Rakic, 1998; Arnsten, 2006; Russell, 2003). Alteration of the levels/functions of these dopamine-related proteins in manganese-exposed animals may have led to impairment of proper inhibitory control of contextually appropriate behavior. The lack of a manganese effect in the elevated plus maze, and the observation that manganese had no effect on dopamine receptors or DAT levels in the olfactory tubercle, both support the suggestion that early manganese exposure targets specific dopaminergic nuclei, while sparing others.

The pre- and early post-weaning period coincides with the development of dopaminergic pathways in brain regions such as the prefrontal cortex, nucleus accumbens, and dorsal striatum that are instrumental in the regulation of executive function behaviors involving learning, memory, and attention (Arnsten, 2006; Broaddus and Bennett, 1990a, b; Goto and Grace, 2005; Leo et al., 2003; Packard and Knowlton, 2002). The dopaminergic system is also a sensitive target of manganese exposure, based on studies in adult animals and humans (Donaldson, 1985; Eriksson et al., 1992; Guilarte et al., 2006; Huang et al., 2003; Kessler et al., 2003; Newland et al., 1989; Normandin and Hazell, 2002) and on recent studies in pre- or early post-weaning rodents (Calabresi et al., 2001; Dorman et al., 2000; McDougall, 2008; Reichel et al., 2006; Tran et al., 2002a, b).

Pre-weaning oral manganese exposure also led to significant learning deficits in the 8-arm radial maze, as evidenced by the significantly greater number of learning errors, and the significant delay or failure of manganese-exposed animals to achieve the learning criterion [$\leq$ 4 errors over 3 consecutive session days]. These deficits may reflect lasting effects of early manganese exposure, since they were measured at a time (PND 33-46) when brain manganese levels had declined to near-control levels [being 15% and 27% higher than controls]…An animal's normal initial response in the radial maze utilizes

declarative, short-term, working memory when an environmental cue is associated with reinforcement such as a food bait reward (Packard and Knowlton, 2002). The stimulus-response associations develop and strengthen with repeated presentation of the reinforcement for long-term, reference memory applications (Packard and White, 1990; White and McDonald, 2002). Thus, the significantly greater number of reference errors and borderline greater number of working errors committed by manganese-exposed animals evidences deficits in both short and long-term learning abilities. Notably, these deficits were most pronounced during the active learning (acquisition) phase of the radial maze test period, and were not evident in the 'performance' phase of maze testing where manganese-exposed animals did not differ significantly from controls.

These radial maze learning deficits are consistent with the significant changes in levels of D1, D2, and DAT measured in manganese-exposed animals on PND 24. In addition to regulating reactivity to external stimuli, the ascending dopamine system is involved in the integration of external stimuli necessary for goal-directed learning (Arnsten, 2006; Goldman-Rakic et al., 2000; Grace, 2000; Grace et al., 2007; Seamans et al., 2001; Williams and Goldman-Rakic 1995; Williams and Goldman-Rakic, 1998). An intact dopaminergic cortico-striato-thalamo-cortical loop is essential for proper evaluation of external stimuli in goal-directed behaviors, and is the main interface for the dopaminergic system's influence on behavior (Carr et al., 1999; Pattij et al., 2007). Thus, the altered D1, D2, and DAT protein levels observed here may be an underlying contributor to the significant learning deficits in manganese-exposed animals, and together suggest an impaired ability to regulate reactivity, establish appropriate contextual associations with environmental cues, and process and establish stimulus-reward associations required in learning the maze (Haber et al., 2000; Johansen and Sagvolden, 2004). The significantly increased use of stereotypic response strategy by manganese-exposed animals in the 8-arm radial maze is further evidence of disrupted learning behavior….

In summary, pre-weaning Mn exposure produced deficits in behavioral inhibition, and spatial and associative learning that were associated with significant alterations in dopamine receptors and DAT levels in selected brain regions. These results, together with animal studies showing that Mn targets the dopaminergic system (Chen et al., 2006; Donaldson, 1985; Eriksson et al., 1992; Guilarte et al., 2006; Newland et al., 1989; Newland, 1999; Yamada et al., 1986), and epidemiologic studies in children showing associations of cognitive deficits and ADHD-like behaviors with elevated Mn exposure (Bouchard et al., 2007; Collipp et al., 1983; Ericson et al., 2007; Wasserman et al., 2006; Wright et al., 2006), support the notion that early elevated Mn exposure produces behavioral deficits by targeting dopaminergic pathways of executive function. This suggestion is consistent with animal model studies linking disruption of the dopaminergic system to ADHD-like behavioral deficits in executive function (Giedd et al., 2001; Oades et al., 2005; Schrimsher et al., 2002; Swanson et al., 1998), and with recent human studies reporting altered DAT binding in striatum, sustantia nigra, and ventral tegmentum in adults and children with ADHD (Jucaite et al., 2005; Larisch et al., 2006; Madras et al., 2005; Spencer et al., 2007). Together, these results support a need for further animal model and human studies to establish the causal relationship between early Mn exposure

> and persistent cognitive and ADHD-like deficits, and the mechanistic basis of these effects."

Beaudin et. al. (2013) evaluated fine sensorimotor dysfunction in 55 adult Long-Evans rats following either pre-weaning or lifelong manganese exposure using objective measurements that are directly relevant to the types of motor outcomes studied in pediatric manganese research. As previously described for Kern et al. (2010), the drinking water manganese doses used in the study (0 mg/kg/day, 25 mg/kg/day and 50 mg/kg/day) were set at the selected levels in order to mimic equivalent human exposure values. The results of Beaudin et al. (2013) showed:

> "that early life manganese exposure restricted to the pre-weaning period produced selective long-lasting impairment in reaching skills in adults, and that lifelong manganese exposure produced wider-spread deficits in both reaching and grasping skills. Early (pre-weaning) exposure at the highest dose (50 mg Mn/kg/day) lead to deficits in forelimb sensorimotor function in the adults approximately 3 months after their last oral manganese dose, when blood and brain manganese levels had long since returned to background levels. The authors note "these long-lasting deficits suggest permanent or irreversible damage to the basal ganglia systems of the adult rat brain as a result of early life manganese exposure, consistent with evidence from our prior studies showing that adult (postnatal day 100) rats exposed to the same levels of pre-weaning manganese early in life exhibited increased expression of dopamine D2 receptors and activated astrocytes in frontal – subcortical neuronal circuits."

> "Lifelong oral exposure to manganese produced widespread impairment in skilled motor performance that was apparent across multiple staircase test outcomes in adult rats."

No effect was observed in the early life exposure group receiving 25 mg Mn/kg/day, but the authors did observe significant effects on behavior in the lifelong exposure group receiving 25 mg Mn/kg/day. In contrast, behavior was selectively affected in the early life group receiving 50 mg Mn/kg/day and those effects continued to be observed in the lifelong group receiving 50 mg Mn/kg/day. The lifelong group also consumed fewer food pellets. The authors concluded, "overall the continuous exposure to 50 mg Mn/kg/day in drinking water caused little *additional* impairment in skilled motor behavior beyond that produced by early life exposure at the same dose." Additional research to examine the reasoning behind these observed effects would be helpful, but this research supports the link between manganese and developmental neurotoxicity. Beaudin et al. (2013) was funded by a grant from the National Institutes of Health.

Beaudin et al. (2017) further evaluated behavior, focused attention tasks and selective attention tasks in 155 Long-Evans rats following oral exposure to manganese. As in Beaudin et al. (2013), the rats were exposed to 0 mg/kg/day, 25 mg/kg/day, or 50 mg/kg/day of manganese. Exposure occurred from either PND 1-21 or PND 1 until the end of the study (~PND 192). The results of the study demonstrated that early postnatal manganese exposure can cause lasting attentional dysfunction in a rodent model of childhood manganese exposure that is similar to the dysfunctions displayed by ADHD children. Exposure did not affect impulse control and was consistent predominately with the ADHD-inattentive subtype.

Moreno et. al (2009) investigated whether exposure to manganese in early life alters susceptibility to manganese during aging. C57B1/6 mice were exposed to manganese by gavage as juveniles, adults or juveniles and again as adults. Moreno et. al. examined metal accumulation in multiple brain regions and serum as well as catecholamine and monoamine neurotransmitter levels and neurobehavioral parameters. Regarding the behavioral findings, the authors noted that the observation of higher manganese levels in the control mice than those in the treatment groups for the juvenile life stage may have been due to either stress resulting from juvenile gavage or to experimental variation between the two study groups. As this study was the first to report such findings, either possibility could not be ruled out and additional studies would be needed to confirm the findings. Nonetheless, this study provides important information on the neurobehavioral effects of ingested manganese. The study showed that the period of development in mice spanning weaning to early adulthood represents a critical window of sensitivity and that male mice are more severely affected than females. Furthermore, the study found that pre-exposed adult mice were not only more sensitive to manganese toxicity than naïve mice not exposed early in life, but pre-exposure also resulted in greater effects on both dopaminergic and serotonergic neurochemical parameters in the brain.

Dearth et al. (2014) studied the role of manganese in mammary gland development and hyperplasia in female rats. The study abstract states the following information:

> "Evidence suggests that environmental substances regulating estrogenic pathways during puberty may be detrimental to the developing mammary gland (MG). Manganese is a trace mineral required for normal physiological processes. Prepubertal exposure to manganese induces precocious puberty in rats, an event associated with early elevations in puberty-related hormones, including estradiol (E2). However, until now the effect of manganese-induced precocious MG development has not been determined. Therefore, we assessed the ability of prepubertal manganese exposure to advance normal MG development and alter E2 driven pathways involved in tumorigenesis. Sprague Dawley female rats were gavaged daily with either 10 mg/kg manganese chloride (MnCl2) or saline (control) from [PND] 12 through PND 30. Blood and MGs were collected on PNDs 30 and 120. Compared to controls, serum E2 levels on PND 30 were elevated ($p < 0.001$). Levels of manganese (ppm) were not elevated in these MGs. Manganese-treated animals (40%) exhibited reactive stroma and intra-luminal focal hyperplasia in hemotoxylin and eosin stained MGs at PND 120. Furthermore, manganese exposure resulted in elevated protein expression levels of estrogen receptor α, activator protein 2α, phosphorylated (p)-Akt, and p53 in MGs on PND 120, but not on PND 30. Collectively, these data show that exposure to a supplemental dose of manganese causes accelerated pubertal MG growth which can progress to adult hyperplasia; thus, providing evidence that early life manganese exposure may increase susceptibility to breast cancer."

Evaluation of Available Recommendations and Scientific Data

DEP has reviewed and considered the available scientific data and recommendations in accordance with 25 Pa. Code Chapter 16. Water Quality Toxics Management Strategy – Statement of Policy and 25 Pa. Code Chapter 93. Human health criteria are based on one of two approaches – either threshold level or non-threshold level toxic effects (carcinogens). DEP guidelines for the development of threshold level toxic effect human health-based criteria are found specifically at 25 Pa. Code §16.32 (relating to

threshold level toxic effects). When no criteria have been developed by EPA for a substance identified or expected in a discharge, DEP will develop criteria following EPA's standard toxicological procedures outlined in the Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health (EPA-822-B-00-004, October 2000) as amended and updated (25 Pa. Code §16.32(c)(2)). As further stated in §16.32(d), the sources DEP uses to obtain relevant risk assessment values for protection for threshold level toxic effects to human health as are follows:

(1) Verified references doses, listed in the EPA agency-wide supported data system known as IRIS and other EPA approved data sources referred through IRIS
(2) Maximum Contaminant Level Goals (MCLGs)
(3) The EPA's CWA § 304(a) health criteria listed under the National Toxics Rule in 40 CFR 131.36 (57 FR 80848, December 22, 1992) (relating to toxics criteria for those States not complying with CWA section 303(c)(2)(B)), as amended and updated and other final criteria published by the EPA and the Great Lakes Initiative Clearinghouse.
(4) Teratology and other data that have been peer-reviewed may provide information for criteria development.

In accordance with this policy, DEP uses the verified reference dose for manganese listed in EPA's IRIS database unless more recently published, peer-reviewed studies are available which provide sufficient information for DEP to develop an updated reference dose. At this time, DEP has reviewed the available, peer-reviewed scientific data and literature and is not proposing to develop an updated reference dose. However, the available data continue to support neurodevelopment as a critical endpoint and the application of the modifying factor of 3 to the IRIS oral RfD for manganese.

Development of Manganese Criteria

Criteria for the protection of Human Health from Toxic Substances

As described above, DEP develops human health-based criteria in accordance with its Water Quality Toxics Management Strategy – Statement of Policy. Human health criteria development considers various exposure pathways including exposures from drinking water and fish consumption and may include exposures from inhalation or dermal absorption. The inclusion of multiple exposure pathways and the toxicity risk of the substance make development of human-health based criteria different than Potable Water Supply criteria. Some of the Commonwealth's existing Potable Water Supply criteria are based on SDWA primary MCLs or SMCLs, and many are related to aesthetic qualities of the water (i.e., taste and odor). MCLs and SMCLs are not developed using the same risk assessment factors required by DEP's regulations for the development of WQS, and SMCLs are not based on concerns related to toxicity.

Development of a Human Health Criterion based on IRIS

The EPA developed an oral RfD for manganese and published it in the IRIS database in 1995. Central nervous system effects were identified as the non-threshold critical health effect. EPA re-evaluated the science on manganese in 2003 and continues to recommend the use of the IRIS oral RfD with a modifying factor of 3. As discussed throughout this rationale, the research on the chronic and subchronic effects of manganese is advancing, and it continues to support the need for an RfD for manganese. DEP

did not develop a new approach, or RfD, to develop its human health-based manganese criteria. DEP used EPA's existing IRIS RfD for manganese with the recommended application of a modifying factor of 3. DEP's criterion recommendation reflects the best available science and data and is in accordance with DEP's Water Quality Toxics Management Strategy – Statement of Policy. As the science and knowledge on manganese toxicity progresses, DEP will continue to review and evaluate manganese exposure recommendations and will revise the manganese criterion, as appropriate, through DEP's required and ongoing WQSs review process.

Although recent research by Dearth et al. (2014) suggests a possible link between early life exposure to manganese and breast cancer in adult females, manganese is currently not identified as a carcinogen by EPA, and there are currently no published cancer risk level (CRL) values available. Therefore, the manganese WQ criterion has been developed following the threshold level approach. The applicable RfD in IRIS is for the total daily oral intake of manganese, which includes drinking water and dietary sources. However, the NOAEL study data which informed the RfD value were obtained solely from dietary studies. Based on this information and as previously discussed, EPA recommends that an assessment of other exposures (including soils or drinking water) should include a modifying factor of 3. DEP agrees with this recommendation and has applied a modifying factor 3 to the 1995 IRIS RfD in its calculation of the criterion. The published RfD assumes an uncertainty factor (UF) of 1 and a modifying factor (MF) of 1.

Calculation of the RfD in IRIS

RfD = (NOAEL) ÷ (UF) or (MF)

= 0.14 mg/kg-day ÷ 1

= 0.14 mg/kg-day

Calculation of the modified RfD

In order to assess manganese exposure from water consumption, DEP followed the EPA recommendation to apply an MF of 3 to the RfD.

RfD_{DW} = (0.14 mg/kg-day ÷ 3)

= 0.05 mg/kg-day

In accordance with the 2000 EPA Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health using the 2015 updated exposure input values (body weight, drinking water intake, and fish consumption) and Pennsylvania's Chapter 93 guidelines, DEP derived the following human health criterion for manganese. Manganese is currently not known to significantly bioaccumulate in fish; therefore, a bioaccumulation factor of 1 has been assumed. While it has been observed in marine mollusks (EPA Red Book), it is not known if significant bioaccumulation occurs in freshwater mussels. Bioaccumulation factors (BAFs) for manganese may be adjusted in the future if peer-reviewed, published research shows that bioaccumulation is occurring in freshwater fish or mussels.

$AWQC_{Mn}$ = RfD x RSC x (BW ÷ [DWI + (FI x BAF)])

Where:

RfD = 0.05 mg/kg-day
Relative Source Contribution (RSC) = 0.2
Body Weight (BW) = 80 kg
Drinking Water Intake (DWI) = 2.4 L
Fish Intake (FI) = 0.022 kg/day
Bioaccumulation factor (BAF) = 1

$AWQC_{Mn}$ = 0.05 mg/kg-day x 0.2 x (80 ÷ [2.4 + (0.022 kg/day x 1)])

$AWQC_{Mn}$ = **0.3 mg/L**

Conclusion

DEP has calculated a threshold level toxic effect human health-based criterion for manganese of 0.3 mg/L. Since this criterion is not limited to the protection of the Potable Water Supply use or to addressing aesthetic concerns, DEP recommends that it apply in all surface water (i.e., at the point of discharge). WQ-based effluent limits (WQBELs) for manganese will be developed using the design flow conditions for threshold human health criteria contained in 25 Pa. Code § 96.4, Table 1. In addition, DEP recommends that the human health water quality criterion for manganese shall be achieved in all surface waters at least 99% of the time as specified in 25 Pa. Code § 96.3(c).

References

Agency for Toxic Substances and Disease Registry (ATSDR). (2012). Toxicological Profile for Manganese.

Appleton, A.A., et al. (2017). "Prenatal exposure to neurotoxic metals is associated with increased placental glucocorticoid receptor DNA methylation". Epigenetics. 12(8): 607-615.

Aschner, M. (2000). "Manganese: Brain Transports and Emerging Research Needs". Environmental Health Perspectives. 108(3): 429-432.

Aschner, J. L. and M. Aschner. (2005). "Nutritional aspects of manganese homeostasis". Molecular Aspects of Medicine. 26: 353-362.

Aydemir, T.B., et al. (2020). "Intestine-specific deletion of metal transporter *Zip14*(*Slc39a14*) causes brain manganese overload and locomotor defects of manganism". American Journal of Physiology: Gastrointestinal and Liver Physiology. 318: G673-G681.

Beaudin, S.A., S. Nisam, and D.R. Smith. (2013). "Early life versus lifelong oral manganese exposure differently impairs skilled forelimb performance in adult rats." Neurotoxicology and Teratology. 38: 36-45.

Beaudin, S.A., et al. (2017). "Early Postnatal Manganese Exposure Causes Lasting Impairment of Selective and Focused Attention and Arousal Regulation in Adult Rats". Environmental Health Perspectives. 125(2): 230-237.

Bock, N.A., et al. (2008). "Cerebrospinal Fluid to Brain Transport of Manganese in a Non-Human Primate Revealed by MRI". Brain Research. 1198:160-170.

Bouabid, S., et al. (2016). "Manganese Neurotoxicity: Behavioral Disorders Associated with Dysfunctions in the Basal Ganglia and Neurochemical Transmission." Journal of Neurochemistry. 136: 677-691.

Bouchard, M.F., et al. (2007). "Hair manganese and hyperactive behaviors: pilot studies of school-age children exposed through tap water." Environmental Health Perspectives. 119(1): 138-143.

Bouchard, M.F., et al. (2011). "Intellectual Impairment in School-age Children Exposed to Manganese from Drinking Water." Environmental Health Perspectives. 119(1): 138-143.

Bravi, F., et al. (2016). "Impact of maternal nutrition on breast-milk composition: a systematic review". American Journal of Clinical Nutrition. 104: 646-662.

Brna, P., et al. (2011). "Manganese Toxicity in a Child With Iron Deficiency and Polycythemia". Journal of Child Neurology. 26(7): 891-894.

Broberg, K., et al. (2019). “Manganese transporter genetics and sex modify the association between environmental manganese exposure and neurobehavioral outcomes in children”. Environment International. 130:104908.

Bromer, C., et al. (2013). “Genetic and Epigenetic Variation of the Glucocorticoid Receptor (*NR3C1*) in Placenta and Infant Neurobehavior”. Developmental Psychobiology. 55(7): 673-683.

Brown, M.T. and B. Foos. (2009). “Assessing Children’s Exposures and Risks to Drinking Water Contaminants: A Manganese Case Study.” Human and Ecological Risk Assessment. 15: 923-947.

Chen, P., et. al. (2015). “Manganese homeostasis in the nervous system”. Journal of Neurochemistry. 134: 601-610.

Chung, S.E., et al. (2015). “Maternal Blood Manganese and Early Neurodevelopment: The Mothers and Children’s Environmental Health (MOCEH) Study.” Environmental Health Perspectives. 123: 717-722.

Claus Henn, B., et al. (2010). “Early Postnatal Blood Manganese Levels and Children’s Neurodevelopment.” Epidemiology. 21(4): 433-439.

Cordova, F.M., et al. (2013). “Manganese-exposed developing rats display motor deficits and striatal oxidative stress that are reversed by Trolox.” Archives of Toxicology. 87: 1231-1244.

Crossgrove, J. and W. Zheng. (2004). “Manganese toxicity upon overexposure.” NMR in Biomedicine. 17(8): 544-553.

Dearth, R.K. et al. (2014). “Prepubertal exposure to elevated manganese results in estradiol regulated mammary gland ductal differentiation and hyperplasia in female rats”. Experimental Biology and Medicine (Maywood). 239(7): 871-882.

Elder, A., et al. (2006). “Translocation of Inhaled Ultrafine Manganese Oxide Particles to the Central Nervous System.” Environmental Health Perspectives. 114(8):1172-1178.

Erikson, K.M., et al. (2007). “Manganese Neurotoxicity.” Pharmacology and Therapeutics. 113(2): 369-377.

Finley, J.W. and C.D. Davis. (1999). “Manganese deficiency and toxicity: Are high or low dietary amounts of manganese cause for concern?”. BioFactors. 10(1): 15-24.

Fordahl, S., et al. (2012). “Waterborne manganese exposure alters plasma, brain and liver metabolites accompanied by changes in stereotypic behaviors.” Neurotoxicology and Teratology. 34(1): 27-36.

Frisbie, S.H., et al. (2012). “World Health Organization Discontinues Its Drinking-Water Guideline for Manganese.” Environmental Health Perspectives. 120(6): 775-778.

Grandjean P., and P.J. Landrigan. (2006). “Developmental neurotoxicity of industrial chemicals”. Lancet. 368: 2167-78.

Grandjean, P., and P.J. Landrigan (2014). "Neurobehavioral effects of development toxicity." Lancet Neurology. 13: 330-38.

Hardy, G. (2009). "Manganese in Parenteral Nutrition: Who, When, and Why Should We Supplement?". Gastroenterology. 137: S29-S35.

Haynes, E.N., et al. (2015). "Manganese Exposure and Neurocognitive Outcomes in Rural School-Age Children: The Communities Actively Researching Exposure Study (Ohio, USA)." Environmental Health Perspectives. 123(10): 1066-1071.

Health Canada. (2019). "Guidelines for Canadian Drinking Water Quality: Guideline Technical Document – Manganese". Water and Air Quality Bureau, Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, Canada. (Catalogue No. H144-39/2017E-PDF).

Holley, A.K., et al. (2011). "Manganese Superoxide Dismutase: Guardian of the Powerhouse." International Journal of Molecular Sciences. 12: 7114-7162.

Institute of Medicine. (2000). *Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc*. Washington D.C.

Jenkitkasemwong, S., et al. (2018). "SLC39A14 Deficiency alters manganese homeostasis and excretion resulting in brain manganese accumulation and motor deficits in mice". Proceedings of the National Academy of Sciences. 115(20): E1769-E1778.

Kern, C., G. Stanwood and D.R. Smith (2010). "Pre-weaning manganese exposure causes hyperactivity disinhibition, and spatial learning and memory deficits associated with altered dopamine receptor and transporter levels." Synapse. 64(5): 363-378.

Khan, K., et al. (2011). "Manganese Exposure from Drinking Water and Children's Classroom Behavior in Bangladesh." Environmental Health Perspectives. 119(10): 1501-1506.

Khan, K., et al. (2012). "Manganese Exposure from Drinking Water and Children's Academic Achievement." Neurotoxicology. 33(1): 91-97.

Kim, Y., et al. (2009). "Co-exposure to environmental lead and manganese affects the intelligence of school-aged children." Neurotoxicology. 30: 564-571.

Kondakis, X.G., N. Makris, M. Leotsinidis, M. Prinou and T. Papapetropoulos. 1989. Possible health effects of high manganese concentration in drinking water. Arch. Environ. Health. 44(3): 175-178.

Kullar, S.S., et al. (2019). "A benchmark concentration analysis for manganese in drinking water and IQ deficits in children". Environmental International. 130: 104889.

Kumar, K.K., et al. (2014). “Cellular manganese content is developmentally regulated in human dopaminergic neurons”. Scientific Reports. 4: 6801.

Kaushal, S., et. al. (2018). “Watershed ‘chemical cocktails’: forming novel elemental combinations in Anthropocene fresh waters”. Biogeochemistry. 141:281-305.

Kaushal, S., et. al. (2021). “Freshwater salinization syndrome: from emerging global problem to managing risks”. Biogeochemistry. 154: 255-292.

Kwakye, G.F, et al. (2015). “Manganese-Induced Parkinsonism and Parkinson’s Disease: Shared and Distinguishable Features”. International Journal of Environmental Research and Public Health. 12: 7519-7540.

Lanphear, B.P., et al. (2015). “The Impact of Toxins on the Developing Brain.” Annual Review of Public Health. 36: 211-30.

Leyva-Illades, D., et al. (2014). “SLC30A10 Is a Cell Surface-Localized Manganese Efflux Transporter, and Parkinsonism-Causing Mutations Block Its Intracellular Trafficking and Efflux Activity”. Journal of Neuroscience. 34(42): 14079-14095.

Lidsky, T. I., Heaney, A. T., Schneider, J. S., & Rosen, J. F. (2007). Neurodevelopmental effects of childhood exposure to heavy metals: Lessons from pediatric lead poisoning. In M. M. M. Mazzocco & J. L. Ross (Eds.), *Neurogenetic developmental disorders: Variations in the manifestation in childhood* (pp. 335–363). Cambridge, MA: MIT Press.

Ljung, K. and M. Vahter. (2007). “Time to Re-evaluate the Guideline Value for Manganese in Drinking Water?” Environmental Health Perspectives. 115(11): 1533-1538.

Lytle C.M., et. al. (1994). Manganese accumulation in roadside soil and plants. Naturwissenschaften. 81:509–510.

Maccani, J.Z.J., et al. (2015). “DNA Methylation Changes in the Placenta Are Associated With Fetal Manganese Exposure”. Reproductive Toxicology. 57: 43-49

Mena, I. (1974). The role of manganese in human disease. Ann. Clin. Lab. Sci. 4(6): 487-491.

Menezes-Filho, J.A., et al. (2011). “Elevated manganese and cognitive performance in school-aged children and their mothers”. Environmental Research. 111(1):156-163.

Miranda-Morales, E., et al. (2017). “Implications of DNA Methylation in Parkinson’s Disease”. Frontiers in Molecular Neuroscience. 10:225.

Monk, C., et al. (2016). “Distress During Pregnancy: Epigenetic Regulation of Placenta Glucocorticoid-Related Genes and Fetal Neurobehavior”. American Journal of Psychiatry. 173(7): 705-713.

Mora, A.M., et al. (2014). “Blood and Hair Manganese Concentrations in Pregnant Women from the Infants’ Environmental Health Study (ISA) in Costa Rica. Environmental Science & Technology. 48: 3467-3476.

Moreno, J.A., et al. (2009). “Neurobehavioral Function in School-Age Children Exposed to Manganese in Drinking Water”. Toxicological Sciences. 112(2):394-404.

Neal, A.P. and T.R. Guilarte. (2013). “Mechanisms of lead and manganese neurotoxicity”. Toxicology Research. 2: 99-114.

O’Neal, S.L and W. Zheng (2015). “Manganese Toxicity Upon Overexposure: a Decade in Review”. Current Environmental Health Reports. 2(3):315-328.

Oulhote, Y., et al. (2014). “Age-Dependent Susceptibility to Manganese-Induced Neurological Dysfunction”. Environmental Health Perspectives. 122(12):1343-1350.

Oulhote, Y. et al. (2014). “Sex- and age-differences in blood manganese levels in the U.S. general population: national health and nutrition examination survey 2011-2012”. Environmental Health. 13(87)

Qiao, Y., et al. (2015). “Epigenetics Involvement in Parkinson’s Disease and Manganese-Induced Neurotoxicity”. Journal of Clinical Epigenetics. 1(1): 1-7.

Sahni, V., et al. (2007). “Case Report: A Metabolic Disorder Presenting as Pediatric Manganism”. Environmental Health Perspectives. 115(12): 1776-1779.

Santamaria, A.B. (2008). “Manganese exposure, essentiality & toxicity”. Indian Journal of Medical Research. 128:484-500.

Scher, D.P, H.M. Goeden and K.S. Klos. (2021). “Potential for Manganese-Induced Neurologic Harm to Formula-Fed Infants: A Risk Assessment of Total Oral Exposure”. Environmental Health Perspectives. 129(4): 047011-1–047011-13.

Schroeter, J.D., et al. (2011). “Analysis of Manganese Tracer Kinetics and Target Tissue Dosimetry in Monkeys and Humans with Multi-Route Physiologically Based Pharmacokinetic Models”. Toxicological Sciences. 120(2):481-498.

Schroeter, J.D., et al. (2012). “Application of a Multi-Route Physiologically Based Pharmacokinetic Model for Manganese to Evaluate Dose-Dependent Neurological Effects in Monkeys”. Toxicological Sciences. 129(2):432-446.

Schullehner, J., et al. (2020). “Exposure to Manganese in Drinking Water during Childhood and Association with Attention-Deficit Hyperactivity Disorder: A Nationwide Cohort Study”. Environmental Health Perspectives. 128(9): 097004-1—097004-10.

Sen, S., et al. (2011). “Manganese Accumulation in the Olfactory Bulbs and Other Brain Regions of “Asymptomatic” Welders”. Toxicological Sciences. 121(1):160-167.

Shih, J., et al. (2018). "Association between peripheral manganese levels and attention-deficit/hyperactivity disorder: a preliminary meta-analysis". Neuropsychiatric Disease and Treatment. 14: 1831-1842.

Signes-Pastor, A.J., et al. (2019). "Toenail manganese as biomarker of drinking water exposure: a reliability study from a U.S. pregnancy cohort". Journal of Exposure Science and Environmental Epidemiology. 29(5): 648-654.

Smith, M.R., et. al. (2017). "Redox dynamics of manganese as a mitochondrial life-death switch". Biochemical and Biophysical Research Communications. 482(3):388-398.

Sowards, J.W., et al. (2010). "Characterization of Welding Fume from SMAW Electrodes – Part II". Welding Journal. 89:82S-90S.

Streifel, K., et. al. (2013). "Manganese inhibits ATP-induced calcium entry through the transient receptor potential channel TRPC3 in astrocytes". Neurotoxicology. 34:160-166.

Stroud, L.R., et al. (2014). "Maternal smoking during pregnancy and infant stress response: Test of a prenatal programming hypothesis". Psychoneuroendocrinology. 48: 29-40.

Tarale, Prashant, et al. (2016). "Potential Role of Epigenetic Mechanism in Manganese Induced Neurotoxicity". BioMed Research International. 2016: 2548792.

Tjalve, H., C. Mejare, and K. Borg-Neczak. (1995). "Uptake and Transport of Manganese in Primary and Secondary Olfactory Neurones in Pike." Pharmacology and Toxicology. 77(1):23-31.

USEPA (U.S. Environmental Protection Agency). 2000. *Methodology for Deriving Ambient Water Quality Criteria for the Protection of Human Health*. EPA 882-B-00-004. U.S. Environmental Protection Agency, Office of Water, Office of Science and Technology, Washington, DC. https://www.epa.gov/wqc/human-health-water-quality-criteria

USEPA (U.S. Environmental Protection Agency). *Integrated Risk Information System Chemical Assessment Summary for Manganese (CASRN 7439-96-5)*. U.S. EPA Office of Research and Development, National Center for Environmental Assessment, Integrated Risk Information System Program. Washington, D.C. Accessed November 2016.

USEPA (U.S. Environmental Protection Agency). 2003. *Health Effects Support Document for Manganese*. EPA-822-R-03-003. EPA Office of Water; Health and Ecological Criteria Division. Washington, D.C.

USEPA (U.S. Environmental Protection Agency). *Water Quality Criteria 1972 ("Blue Book")*. EPA-R3-73-033. National Academy of Sciences. Washington, D.C.

USEPA (U.S. Environmental Protection Agency). *Quality Criteria for Water 1976 ("Red Book")*. EPA 440-9-76-023. EPA Office of Water Planning and Standards. Washington, D.C.

USEPA (U.S. Environmental Protection Agency). *Quality Criteria for Water 1986 ("Gold Book")*. EPA 440/5-86-001. EPA Office of Water Regulations and Standards. Washington, D.C.

USEPA (U.S. Environmental Protection Agency). *Drinking Water Health Advisory for Manganese*. EPA-822-R-04-003. EPA Office of Water; Health and Ecological Criteria Division. Washington, D.C.

Vaiserman, A. (2015). "Epidemiologic evidence for association between adverse environmental exposures in early life and epigenetic variation: a potential link to disease susceptibility?". Clinical Epigenetics. 7: 96.

Valcke, M., et al. (2018). "Deriving a Drinking Water Guideline for a Non-Carcinogenic Contaminant: The Case of Manganese". International Journal of Environmental Research and Public Health. 15(6): 1293.

Wahlberg., K.E., et al. (2018a). "Polymorphisms in Manganese Transporters SLC30A10 and SLC39A8 are Associated With Children's Neurodevelopment by Influencing Manganese Homeostasis". Frontiers in Genetics. 9 (664).

Wahlberg, K. E., et al. (2018b). "Polymorphisms in manganese transporters show developmental stage and sex specific associations with manganese concentrations in primary teeth". Neurotoxicology. 64: 103-109.

Wasserman, G.A., et al. (2006). "Water Manganese Exposure and Children's Intellectual Function in Araihazar, Bangladesh". Environmental Health Perspectives. 114(1):124-129.

Wasserman, G.A., et al. (2011). "Arsenic and manganese exposure and children's intellectual function". Neurotoxicology. 32(4):450-457.

Weber, S., et al. (2002). "Effects of Manganese (Mn) on the Developing Rat Brain: Oxidative-Stress Related Endpoints". NeuroToxicology. 23: 169-175.

Wertz, T.A. and M.K. Shank. (2019). Land use from water quality: development of a water quality index across Pennsylvania streams. Ecosphere. 10(11).

Woolfe, A., et al. (2002). "A Child with Chronic Manganese Exposure from Drinking Water". Environmental Health Perspectives. 110(6): 613-616.

World Health Organization (WHO). (2004). Manganese and Its Compounds: Environmental Aspects.

World Health Organization (WHO). (2020). Draft Manganese in Drinking-Water.

Yoon, M., et al. (2011). "Physiologically Based Pharmacokinetic Modeling of Fetal and Neonatal Manganese Exposure in Humans: Describing Manganese Homeostasis during Development". Toxicological Sciences. 122(2): 297-316.

Zimmer, A.T., P.A. Baron and P. Biswas. (2002). “The influence of operating parameters on number-weighted aerosol size distribution generated from a gas metal arc welding process”. Journal of Aerosol Science. 33(3):519-531.