2. RELEVANCE TO PUBLIC HEALTH

2.1 BACKGROUND AND ENVIRONMENTAL EXPOSURES TO MANGANESE IN THE UNITED STATES

Manganese is a naturally occurring element and an essential nutrient. Comprising approximately 0.1% of the earth's crust, it is the twelfth most abundant element and the fifth most abundant metal. Manganese does not exist in nature as an elemental form, but is found mainly as oxides, carbonates, and silicates in over 100 minerals with pyrolusite (manganese dioxide) as the most common naturally-occurring form. As an essential nutrient, several enzyme systems have been reported to interact with or depend on manganese for their catalytic or regulatory function. As such, manganese is required for the formation of healthy cartilage and bone and the urea cycle; it aids in the maintenance of mitochondria and the production of glucose. It also plays a key role in wound-healing.

Manganese exists in both inorganic and organic forms. An essential ingredient in steel, inorganic manganese is also used in the production of dry-cell batteries, glass and fireworks, in chemical manufacturing, in the leather and textile industries and as a fertilizer. The inorganic pigment known as manganese violet (manganese ammonium pyrophosphate complex) has nearly ubiquitous use in cosmetics and is also found in certain paints. Organic forms of manganese are used as fungicides, fuel-oil additives, smoke inhibitors, an anti-knock additive in gasoline, and a medical imaging agent.

The average manganese soil concentrations in the United States is 40–900 mg/kg; the primary natural source of the manganese is the erosion of crustal rock. Its presence in soil results in vegetable and animal foods reliably containing varying amounts of the mineral. As an essential nutrient, manganese is added to certain foods and nutritional supplements. Vegetarians often have diets richer in manganese than those who select omnivorous diets.

The most important source of manganese in the atmosphere results from the air erosion of dusts or soils. The mean concentration of manganese in ambient air in the United States is 0.02 $\mu g/m^3$; however, ambient levels near industrial sources can range from 0.22 to 0.3 $\mu g/m^3$. Manganese is released into waterways mainly through the erosion of rocks and soils, mining activities, and industrial waste, or by the leaching of manganese from anthropogenic materials discarded in landfills or soil, such as dry-cell batteries. Surface waters in the United States contain a median manganese level of 16 µg/L, with 99^{th} percentile concentrations of 400–800 µg/L. Groundwater in the United States contains median

manganese levels of 5 to 150 µg/L, with the 99th percentile at 2,900 or 5,600 µg/L in rural or urban areas, respectively.

The general population is exposed to manganese through consumption of food and water, inhalation of air, and dermal contact with air, water, soil, and consumer products that contain manganese. The primary source of manganese intake is through diet. The Food and Nutrition Board (FNB) of the Institute of Medicine (IOM) has set adequate intake (AI) levels for manganese for humans. These levels are presented in Table 2-1.

The inhalation of air contaminated with particulate matter containing manganese is the primary source of excess manganese exposure for the general population in the United States. Populations living in close proximity to mining activities and industries using manganese may be exposed by inhalation to high levels of manganese in dust. Workers in these industries are especially vulnerable to exposure to manganese dust. Manganese concentrations in soil may be elevated when the soil is in close proximity to a mining source or industry using manganese and may therefore pose a risk of excess exposure to children who ingest contaminated soil. Manganese is ubiquitous in drinking water in the United States. Although certain water sources in the United States are contaminated with excess manganese, there is little risk of excessive exposure to manganese through ingestion of fish or shellfish emanating from contaminated waters, unless the manganese levels in the fish are extremely high and/or the fish are eaten as subsistence. Although many forms of manganese are water-soluble, there is little evidence that dermal contact with manganese results in significant absorption through the skin. Thus, dermal contact with manganese is not generally viewed as an important source of exposure to the population at large.

Excess exposure to manganese may be revealed by tests to detect heightened levels in body fluids as well as in hair samples. Normal ranges of manganese levels in body fluids are 4–15 µg/L in blood, 1–8 µg/L in urine, and 0.4–0.85 µg/L in serum. Excess manganese in the body characteristically accumulates in the brain region known as the basal ganglia. This accumulation can be revealed by magnetic resonance imaging (MRI) as a distinctive symmetrical high-signal lesion in the globus pallidus region of the basal ganglia on T1- but not T2-weighted MRI.

2.2 SUMMARY OF HEALTH EFFECTS

Although low levels of manganese intake are necessary for human health, exposures to high manganese levels are toxic. Reports of adverse effects resulting from manganese exposure in humans are associated

Table 2-1. Adequate Intake (AI) for Manganese

Life stage	Age	Males (mg/day)	Females (mg/day)
Infants	0–6 Months	0.003	0.003
Infants	7–12 Months	0.6	0.6
Children	1–3 Years	1.2	1.2
Children	4–8 Years	1.5	1.5
Children	9–13 Years	1.9	1.6
Adolescents	14–18 Years	2.2	1.6
Adults	19 Years and older	2.3	1.8
Pregnancy	All ages	—	2.0
Lactation	All ages	—	2.6

Source: FNB/IOM 2001

primarily with inhalation in occupational settings. Inhaled manganese is often transported directly to the brain before it is metabolized by the liver. The symptoms of manganese toxicity may appear slowly over months and years. Manganese toxicity can result in a permanent neurological disorder known as manganism with symptoms that include tremors, difficulty walking, and facial muscle spasms. These symptoms are often preceded by other lesser symptoms, including irritability, aggressiveness, and hallucinations. Some studies suggest that manganese inhalation can also result in adverse cognitive effects, including difficulty with concentration and memory problems. Although the workplace is the most common source of excess inhalation of manganese, frequent inhalation of fumes from welding activities in the home can produce a risk of excess manganese exposure leading to neurological symptoms. Environmental exposures to airborne manganese have been associated with similar preclinical neurological effects and mood effects as are seen in occupational studies. Acute or intermediate exposure to excess manganese also affects the respiratory system. Inhalation exposure to high concentrations of manganese dusts (specifically manganese dioxide [MnO_2] and manganese tetroxide [Mn_3O_4]) can cause an inflammatory response in the lung, which, over time, can result in impaired lung function. Lung toxicity is manifested as an increased susceptibility to infections such as bronchitis and can result in manganic pneumonia. Pneumonia has also been observed following acute inhalation exposures to particulates containing other metals. Thus, this effect might be characteristic of inhalable particulate matter and might not depend solely on the manganese content of the particle.

A number of reports indicate that oral exposure to manganese, especially from contaminated water sources, can produce significant health effects. These effects have been most prominently observed in children and are similar to those observed from inhalation exposure. An actual threshold level at which manganese exposure produces neurological effects in humans has not been established. However, children consuming the same concentration of manganese in water as adults are ultimately exposed to a higher mg/kg-body weight ratio of manganese than adults (as a consequence of the lower body weight of children as well as their higher daily consumption volume and greater retention of manganese). Children are also potentially more sensitive to manganese toxicity than adults. A study conducted in infant monkeys suggests that soy-based infant formula, which contains a naturally higher concentration of manganese than human or cow's milk, may produce mild effects on neurological development, although such effects have not been documented in humans. While many of the studies reporting oral effects of excess manganese have limitations that preclude firm conclusions about the potential for adverse effects, these studies collectively suggest that ingestion of water and/or foodstuffs containing increased concentrations of manganese may result in adverse neurological effects.

There is indirect evidence that reproductive outcomes might be affected (decreased libido, impotence, and sexual dysfunction have been observed in manganese-exposed men). The available studies on the effect that manganese has on fertility (as measured by birthrate) is inconclusive. Two studies in men occupationally exposed to manganese show adverse effects on reproductive parameters: one found increased sexual dysfunction and the other found reduced sperm quality, but neither measured birthrate in wives of affected workers. Impaired sexual function in men may be one of the earliest clinical manifestations of manganese toxicity, but no dose-response information is currently available, so it is not possible to define a threshold for this effect. There is a lack of information regarding effects in women since most data are derived from studies of male workers. Developmental data in humans exposed to manganese by inhalation are limited and consist mostly of reports of adverse pulmonary effects from inhaling airborne manganese dust and adverse neurological effects in offspring following ingestion exposure. Animal studies indicate that manganese is a developmental toxin when administered orally and intravenously, but inhalation data concerning these effects are scarce and not definitive. Some studies in children suggest that routine exposures to high levels of manganese from contaminated drinking water may ultimately impair intellectual performance and behavior.

The few available inhalation and oral studies in humans and animals indicate that inorganic manganese exposure does not cause significant injury to the heart, stomach, blood, muscle, bone, liver, kidney, skin, or eyes. However, if manganese is in the (VII) oxidation state (as in potassium permanganate), then ingestion may lead to severe corrosion at the point of contact. Studies in pigs have revealed a potential for adverse coronary effects from excess manganese exposure.

There is no evidence that manganese causes cancer in humans. Although no firm conclusions can be drawn from the mixed results in animal studies, there are little data to suggest that inorganic manganese is carcinogenic. The IRIS has provided manganese with a weight-of-evidence classification D—not classifiable as to human carcinogenicity.

It should be noted that individuals with cirrhosis of the liver, as well as children with a congenital venous anomaly known as a portosystemic shunt, may be at heightened risk of health deficits from exposure to dietary and environmental sources of manganese. Manganese is ordinarily eliminated from the body through bile, but cirrhosis and portosystemic shunts impair the normal functioning of the liver and thus limit the ability of the body to excrete manganese, which then can accumulate in the blood and, eventually, the brain.

A more detailed discussion of the critical targets of manganese toxicity (i.e., the nervous system, respiratory system, reproductive system, and development) follows.

Neurological Effects. There is clear evidence from studies of humans exposed to manganese dusts in mines and factories that inhalation of high levels of manganese can lead to a series of serious and ultimately disabling neurological effects in humans. This disease, termed manganism, typically begins with feelings of weakness and lethargy. As the disease progresses, a number of other neurological signs may become manifest. Although not all individuals develop identical signs, the most common are a slow and clumsy gait, speech disturbances, a masklike face, and tremors. The neurological symptoms may improve when exposure ceases; however, in most cases, the symptoms are found to persist for many years post-exposure. In addition, a syndrome of psychological disturbances (hallucination, psychosis) frequently emerges, although such symptoms are sometimes absent. As the disease progresses, patients develop severe muscle tension and rigidity and may be completely and permanently disabled. Workplace inhalation exposure levels producing overt symptoms of manganism have been on the order of 2–22 mg manganese/m^3. While manganese neurotoxicity has clinical similarities to Parkinson's disease, it can be clinically distinguished from Parkinson's. Manganism patients present a hypokinesia and tremor that is different from Parkinson's patients. In addition, manganism patients sometimes have psychiatric disturbances early in the disease, a propensity to fall backward when pushed, less frequent resting tremor, more frequent dystonia, a "cock-walk", and a failure to respond to dopaminomimetics.

Subclinical neurological effects have been observed in numerous studies of workers exposed to manganese dusts at lower exposure levels than those associated with symptoms of overt manganism. These effects include decreased performance on neurobehavioral tests; significantly poorer eye-hand coordination, hand steadiness, and reaction time; poorer postural stability; and lower levels of cognitive flexibility. Manganese air concentrations producing these effects in chronically exposed workers range from about 0.07 to 0.97 mg manganese/m^3.

Studies in communities surrounding manganese industries have also reported associations between manganese exposure and subclinical neurological effects in adults and children. In a study of men and women living close to a manganese alloy production plant, a blood manganese level-age interaction was observed, with the poorest performance on neurological tests occurring among those >50 years old who had the highest blood manganese levels. Additional studies of environmentally exposed adults reported attention impairments, poorer postural stability, and subclinical motor impairments at environmental air exposures >0.1 μg manganese/m^3; however, other potential sources of environmental exposure were not

accounted for. In several studies of children, associations have been reported between manganese concentrations in blood or hair and motor impairment and deficits in neurodevelopment and intellectual functions.

There is also an accumulating body of evidence suggesting that exposure to excess levels of manganese in drinking water (≥0.2 mg/L) may lead to neurological deficits in children, including poor school performance, impaired cognitive function, abnormal performance on neurobehavioral tests, and increased oppositional behavior and hyperactivity. Several cases of apparent manganism in both children and adults have been reported where exposures to high levels of manganese in drinking water were implicated as the probable cause. The symptoms in these case reports are similar to those in individuals with high levels of exposure in manganese mining operations. Taken together, these studies provide added weight to the evidence for the neurotoxic potential of excessive manganese in children, but one or more of the following uncertainties preclude the characterization of causal and dose-response relationships between the observed effects and manganese exposure: (1) whether or not the observed effects were solely due to excess manganese alone or could have been influenced by other drinking water or dietary components; (2) the lack of quantitative information about manganese levels from different environmental sources (food, water, and air); and (3) the small sample sizes.

Respiratory Effects. Inhalation exposure to manganese dusts often leads to an inflammatory response in the lungs of both humans and animals. This generally leads to an increased incidence of cough and bronchitis and can lead to mild-to-moderate injury of lung tissue along with minor decreases in lung function. In addition, susceptibility to infectious lung disease may be increased, leading to increased pneumonitis and pneumonia in some manganese-exposed worker populations. These effects have been reported primarily in workers exposed to fairly high concentrations of manganese dusts in the workplace, although there are some data that indicate that, in populations living and attending school near ferromanganese factories, there was an increased prevalence of respiratory effects. The risk of lung injury in people exposed to the levels of manganese typically found in the general environment is expected to be quite low. However, exposure to manganese-containing dusts from factories, mining operations, automobile exhaust, or other sources may be of concern. It should be noted that these effects on the lung are not unique to manganese-containing dusts but are produced by a variety of inhalable particulate matter. On this basis, it seems most appropriate to evaluate the risk of inflammatory effects on the lung in terms of total suspended particulate matter (TSP) or particulate matter <10 μm in diameter (PM_{10}), as well as the concentration of manganese in the air. Studies involving controlled inhalation exposures in humans or animals to methylcyclopentadienyl manganese tricarbonyl (MMT), a gasoline

additive that improves combustion efficiency, are not available because the compound breaks down readily in light to form inorganic manganese compounds. Rats exposed to high concentrations of car exhaust containing oxidation products from MMT-containing fuel exhibited labored breathing.

Reproductive Effects. Impotence and loss of libido are common symptoms in male workers afflicted with clinically identifiable signs of manganism. These symptoms could lead to reduced reproductive success in men. Impaired fertility (measured as a decreased number of children/married couple) has been observed in male workers exposed for 1–19 years to manganese dust (0.97 mg/m^3) at levels that did not produce frank manganism. This suggests that impaired sexual function in men may be one of the earliest clinical manifestations of manganese toxicity, but no dose-response information is available; therefore, it is not possible to define a threshold for this effect. Evidence obtained in laboratory mammals indicates that exposure to high levels of manganese may adversely effect sperm quality, produce decreased testicular weights, and impair development of the male reproductive tract.

No direct effect of manganese toxicity has been observed on fertility in women. Although many studies in laboratory mammals have attempted to detect effects of manganese on female fertility, only one study demonstrated the possibility that excess manganese exposure outside of pregnancy may impair future fertility (decreased number of offspring).

Developmental Effects. There is evidence to suggest that children exposed to high levels of manganese from environmental sources (airborne, drinking water, dietary) may develop a variety of adverse developmental effects, particularly neurological effects (as discussed above). Many studies suggest that children exposed to particularly high levels of manganese over a long period of time (months or years) will eventually develop one or more symptoms, including general cognitive impairment, diminished memory, attention deficit, motor impairments, aggressiveness, and/or hyperactivity. However, it is not clear from any of these studies whether other factors, perhaps environmental or genetic, are responsible for these changes in the presence of manganese, or whether manganese alone can produce these effects.

A potentially serious developmental effect of manganese was suggested by the results of a study where high infant mortality in a Bangladesh community was reported in conjunction with the presence of a local drinking water supply containing high levels of manganese (concentration up to 8.31 mg/L). Infants exposed to levels of manganese equal to or greater than those recommended by the World Health Organization (WHO) were at the highest risk of mortality prior to 1 year of age. The nature of this

epidemiological study, with nutritional deficits in the population anticipated but not documented, prevents a determination that manganese alone was responsible for the high rate of infant mortality.

Developmental studies involving the use of laboratory animals have detected subtle changes in growth; (e.g., diminished body weight, in animals provided with relatively high doses of manganese). These changes have been observed both when the animals were exposed while *in utero* or postpartum when the animals have already been born.

2.3 MINIMAL RISK LEVELS (MRLs)

Estimates of exposure levels posing minimal risk to humans (MRLs) have been made for manganese. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

Although methods have been established to derive these levels (Barnes and Dourson 1988; EPA 1990), uncertainties are associated with these techniques. Furthermore, ATSDR acknowledges additional uncertainties inherent in the application of the procedures to derive less than lifetime MRLs. As an example, acute inhalation MRLs may not be protective for health effects that are delayed in development or are acquired following repeated acute insults, such as hypersensitivity reactions, asthma, or chronic bronchitis. As these kinds of health effects data become available and methods to assess levels of significant human exposure improve, these MRLs will be revised.

A User's Guide has been provided at the end of this profile (see Appendix B). This guide should aid in the interpretation of the tables and figures for Levels of Significant Exposure and the MRLs.

Inhalation MRLs for Inorganic Manganese

Acute and Intermediate Inhalation Exposure. MRL values were not derived for acute- or intermediate-duration inhalation exposures to manganese. The available data on the toxicity of inhaled manganese

were considered inadequate for derivation of acute- or intermediate-duration inhalation MRLs. Data are lacking on whether exposure to inhaled manganese across these durations has any significant adverse effects on numerous end points including reports on developmental and reproductive effects.

Reports of human exposure at acute and intermediate durations (i.e., 15–364 days) indicate adverse respiratory and neurological effects, but these reports consist of anecdotal case studies and lack quantitative exposure values.

A few animal studies for these durations also evaluated respiratory effects in rodents and monkeys and reported no-observed-adverse-effect levels (NOAELs). Inhalation of particulate manganese compounds such as manganese dioxide or manganese tetroxide leads to an inflammatory response in the lungs of animals, although inhalation of $MnCl_2$ did not cause lung inflammation in rabbits (Camner et al. 1985). Several acute- and intermediate-duration studies in animals report various signs of lung inflammation following periods ranging from 1 day to 10 months at manganese concentrations ranging from 0.7 to 69 mg/m^3 (Bergstrom 1977; Camner et al. 1985; Shiotsuka 1984; Suzuki et al. 1978; Ulrich et al. 1979a, 1979b). Bergstrom (1977) and Ulrich et al. (1979a, 1979b) determined NOAELs, which are reported in the levels of significant exposure (LSE) table and figure. Increased susceptibility to lung infection by bacterial pathogens following inhalation of manganese dusts has been noted in acute animal studies (Maigetter et al. 1976). Conversely, Lloyd Davies (1946) reported no increase in the susceptibility of manganese-treated mice to pneumococci or streptococci.

More recently, reversible inflammation (pleocellular inflammatory infiltrates and fibrinonecrotic debris) in the nasal respiratory epithelium (but not the olfactory epithelium) was observed in young adult male Crl:CD(SD)BR rats following 13 weeks of inhalation exposure to 0.5 mg manganese/m^3 as manganese sulfate, but not in rats exposed to 0.1 mg manganese/m^3 as manganese sulfate or manganese phosphate (hureaulite) (Dorman et al. 2004b). The lesions were not apparent in groups of rats assessed 45 days after the end of exposure, indicating their transient nature. In studies with young male rhesus monkeys exposed to 0, 0.06, 0.3, or 1.5 mg manganese/m^3 as manganese sulfate 6 hours/day, 5 days/week for 65 days, no nasal histological effects were found in exposed monkeys, but the high exposure level induced lesions in the lower respiratory tract (mild subacute bronchiolitus, alveolar duct inflammation, and proliferation of bronchus-associated lymphoid tissue) (Dorman et al. 2005b). The lower airway lesions from intermediate-duration exposure appear to have been transient, because they were not found in monkeys assessed 45 days after the end of exposure (Dorman et al. 2005b). These findings in rats and

monkeys are consistent with the understanding that inflammation of respiratory tissues from high-level exposure to inhaled manganese particulates is likely a consequence of the inhaled particulate matter.

Bredow et al. (2007) reported that nose-only inhalation exposure to 2 mg manganese/m^3 as manganese chloride aerosols 6 hours/day for 5 consecutive days did not cause lung lesions in female GVB/N mice, but induced a 2-fold increase in pulmonary levels of mRNA for vascular endothelial growth factor (VGEF), a regulator of proliferation, migration, and formation of new capillaries. Elevated levels of VGEF have been associated with respiratory diseases, but current understanding is inadequate to understand if this pulmonary gene expression response to manganese is adverse or benign.

There are limited evaluations of neurological end points in animals following intermediate-duration inhalation exposure to manganese. Neurological effects comparable to those observed in humans have been reported in monkeys exposed to manganese by parenteral routes (intravenous) for intermediate duration (Newland and Weiss 1992), but no reports of the application of sensitive neurobehavioral test batteries to animals following acute or intermediate-duration inhalation exposure to inorganic manganese were located.

In monkeys exposed to manganese oxide aerosol concentrations as high as 1.1 mg manganese/m^3 24 hours/day for 9 months, no exposure-related effects on limb tremor or electromyograms were observed, even though blood manganese levels were 5-fold higher in exposed compared with control monkeys (Ulrich et al. 1979a, 1979b, 1979c). No gross signs of neurological impairment were observed in rats exposed by the same protocol to manganese oxide aerosol concentrations as high as 1.1 mg manganese/m^3 (Ulrich et al. 1979a, 1979b, 1979c).

More recent studies of monkeys exposed to concentrations up to 0, 0.06, 0.3, or 1.5 mg manganese/m^3 as manganese sulfate 6 hours/day for 65 days reported: (1) no obvious signs of gross toxicity in the exposed monkeys; (2) about 2-fold higher manganese concentrations in most brain regions at 1.5 mg manganese/m^3, except for the globus pallidus which showed manganese concentrations 6-fold greater than control concentrations; and (3) a spectrum of exposure-related changes in biochemical markers of neurotoxicity in various regions of the exposed monkeys, compared with control monkeys (Dorman et al. 2006a, 2006b; Erikson et al. 2007, 2008). No published accounts of the application of sensitive neurobehavioral test batteries to these animals are available and there are no studies in monkeys reporting NOAELs and lowest-observed-adverse-effect level (LOAELs) for neurological effects following chronic-duration exposure.

Increased locomotor activity has been observed in Sprague-Dawley rats exposed for 90 days (6 hours/day, 5 days/week) to a manganese phosphate/manganese sulfate mixture at concentrations ≥0.03 mg manganese/m^3 (Salehi et al. 2003) and to manganese sulfate at concentrations ≥0.009 mg manganese/m^3 (Tapin et al. 2006), but this effect was not observed with exposure to hureaulite (manganese phosphate) at aerosol concentrations as high as 1 mg manganese/m^3 (Normandin et al. 2002). Significant neuronal cell loss in the globus pallidus and caudate putamen was also observed in Sprague-Dawley rats exposed for 90 day (6 hours/day, 5 days/week) to the manganese phosphate/manganese sulfate mixture at an aerosol concentration of 3 mg manganese/m^3; these changes, however, were not accompanied with signs of tremor as assessed with electromyographic techniques (Salehi et al. 2006).

MRL values for acute or intermediate durations based on animal studies were not derived, because an MRL based on animal data would be lower than the proposed chronic-duration inhalation MRL that is based on effects observed in humans. It is uncertain if this is due to species differences in susceptibility to the neurotoxic properties of inhaled manganese or to the testing of humans with sensitive neurobehavioral tests that have not been applied to animals following inhalation exposures to manganese. It is expected that the chronic MRL for inhaled inorganic manganese would provide protection for intermediate-duration exposure scenarios. The MRL is based on an analysis of dose-response data for subtle neurological deficits in occupationally exposed workers with durations of employment from about 5 to 24 years (see Appendix A); the average duration of employment in workers in the principal study was 5.3 years.

- An MRL of 0.0003 mg manganese/m^3 (manganese in respirable dust; 0.3 µg manganese/m^3) has been derived for chronic inhalation exposure (365 days or more) to manganese.

The study chosen to derive the MRL is from an investigation of an occupational cohort involving 92 male workers in a dry alkaline battery plant (Roels et al. 1992). They and the 101 age- and area-matched controls (with no industrial exposure to manganese) were observed for performance on a battery of neurobehavioral tests. Manganese workers were exposed for an average (geometric mean) of 5.3 years (range: 0.2–17.7 years) to a respirable dust concentration of 215 µg manganese/m^3 and a total dust concentration of 948 µg manganese/m^3. Manganese concentrations were measured with personal samplers, with respirable dust being <5 microns in diameter. The authors noted that plant exposure conditions had not changed considerably in the last 15 years, suggesting that past exposures were consistent with those measured at the time of the study. Performance in measured neurobehavioral tests,

especially on measures of simple reaction time, eye-hand coordination, and hand steadiness, was significantly worse in manganese-exposed workers than in the comparison group.

Manganese-exposed workers performed significantly worse than the controls on the neurobehavioral tests, with particular differences in simple reaction time, eye-hand coordination, and hand steadiness. Dr. Harry Roels provided the data on the manganese-exposed group evaluated in this study. These data included individual exposure levels and whether the individual had an abnormal performance in the neurobehavioral tests (scores below the 5^{th} percentile score of the control group). Percent precision score in the eye-hand coordination test was the most sensitive end point among the end points showing statistically significantly elevated incidences of abnormal scores and was selected as the basis of the MRL. Average exposure concentration for each worker was calculated by dividing the individual lifetime integrated respirable concentration (LIRD; calculated by Dr. Roels from occupational histories and measurements of workplace air manganese concentrations) by the individual's total number of years working in the factory. Individuals were grouped into six exposed groups and the control group, and the average of the range in each group was used in benchmark dose (BMD) modeling of the incidence data for number of workers with abnormal percent precision eye-hand coordination scores (see Table A-1 in Appendix A).

Available dichotomous models in the EPA Benchmark Dose Software (BMDS version 1.4.1c) were fit to the incidence data for abnormal eye-hand coordination scores in workers exposed to respirable manganese (Roels et al. 1992, Table A-1). Results from the modeling using a benchmark dose response of 10% are shown in Table A-2 in Appendix A. Based on the chi-square and Akaike's Information Criterion (AIC) measures of fit, all of the models provided adequate and comparable fits to the data (the quantal linear and Weibull models had the same parameter values). $BMCL_{10}$ estimates from the different models showed an approximate 2-fold range from 73 $\mu g/m^3$ from a one-stage multistage model to 142 $\mu g/m^3$ from the logistic model. The logistic model was indicated as the best fitting model by the AIC measure (Table A-2) and was used to provide the point of departure (POD) for the MRL. Previous BMD analyses of exposure data and incidence data for abnormal eye-hand coordination test scores from the Roels et al. (1992) study used a quantal linear model to arrive at a $BMCL_{10}$ value of about 74 μg respirable manganese/m^3 (Agency for Toxic Substances and Disease Registry 2000; EPA 1994c; WHO 2001). This value is virtually the same as the $BMCL_{10}$ of 73.2 μg manganese/m^3 obtained from the equivalent multistage model in the current analysis (Table A-2).

The MRL of 0.3 µg manganese/m^3 was derived by adjusting the POD to a continuous exposure basis (142 µg manganese/m^3 x 5/7 x 8/24) and dividing by an uncertainty factor of 100.

An uncertainty factor of 10 was used for uncertainty about human variability including possibly enhanced susceptibility of the elderly, infants, and children; individuals with chronic liver disease or diminished hepatobiliary function; and females and individuals with iron deficiency. The current assessment does not use an additional modifying factor of 5 for potentially increased susceptibility in children based on differential kinetics in the young (which was used in the Agency for Toxic Substances and Disease Registry [2000] assessment), because recent toxicokinetic studies in lactating rats and their offspring exposed to manganese by the oral or inhalation routes suggest that the human variability factor of 10 provides sufficient protection for differential kinetics in children and adults. For example, in neonatal rats orally exposed to 25 or 50 mg manganese/kg/day (as manganese chloride) from postnatal day (PND) 1 through 21, manganese concentrations in various brain regions were about 2-fold higher than brain manganese concentrations in adult rats exposed to the same oral dose levels for 21 days (Dorman et al. 2000). Similarly, 18-day-old neonatal rats exposed from birth to aerosols of manganese sulfate at 1 mg manganese/m^3, 6 hours/day showed a 2.6-fold increase in striatum manganese concentrations, compared with controls, while lactating adults exposed to the same aerosol concentration showed a 1.7-fold increase compared with controls (Dorman et al. 2005a). Likewise, simulations with physiologically based pharmacokinetic (PBPK) models for inhaled manganese in lactating rat dams and offspring indicate that manganese concentrations in the striatum and olfactory bulb of the brains of PND 19 offspring begin to increase when air concentrations exceed 50–100 µg manganese/m^3, whereas maternal concentrations begin to increase at air concentrations between 100 and 300 µg manganese/m^3 (Yoon et al. 2009b). These results indicate that at air concentrations above about 0.05–0.1 mg/m^3, brain concentrations in neonates may be elevated, compared with controls, to a greater degree than in lactating dams, but the age-specific difference in the tested air concentration range does not appear to be large. Simulations from a human PBPK model for inhaled manganese in lactating mothers and their offspring indicate that average daily areas under the curve (AUCs) for manganese concentrations in the globus pallidus of the fetus, suckling neonate, and 3-year-old child from manganese air concentrations increased beyond 10% of background concentrations in fetuses and 3-year-old children when air concentrations exceeded 0.01 mg/m^3 (10 µg/m^3) and in suckling neonates when air concentrations exceeded 0.001 mg/m^3 (1 µg/m^3) (Yoon et al. 2011). Thus, the inhalation MRL derived herein, 0.3 µg/m^3, is below the air concentrations at which brain concentrations in human fetuses (10 µg/m^3) and nursing infants (1 µg/m^3) are predicted to begin to rise under normal dietary manganese exposure conditions.

An uncertainty factor of 10 was applied for limitations/uncertainties in the database including the lack of epidemiological data for humans chronically exposed to soluble forms of manganese and the concern that the general population may be exposed to more soluble forms of manganese than most of the manganese-exposed workers in the principal and supporting studies. Evidence from several rat studies indicate that inhalation of more soluble forms of manganese (e.g., manganese sulfate and manganese chloride) results in higher brain manganese concentrations in brains than inhalation of less soluble forms, such as manganese phosphate, manganese tetroxide or manganese dioxide (Dorman et al. 2004a, 2001a; Roels et al. 1997). In addition, data on developmental toxicity for this route and duration of exposure are lacking. There is limited information on reproductive effects in females (one study in rat dams) and reported effects on male reproductive organs have not been clearly associated with decreased reproductive function. Though it is clear that the neurological system is the most sensitive identified target organ for effects from sub-chronic to chronic-duration inhalation exposure to manganese, data are lacking to fully characterize the potential risk for all organ systems from chronic inhalation exposure.

Several BMD analyses of results from other epidemiological studies of neurobehavioral end points in manganese-exposed workers provide support for the MRL (Clewell and Crump 1999; Clewell et al. 2003; Health Canada 2010). Estimated $BMCL_{10}$ values in these analyses were within an approximate 2–4-fold range of the POD (142 µg manganese/m^3) selected for the chronic inhalation MRL herein.

Dr. Anders Iregren provided ATSDR with individual worker data on total dust manganese exposure and performance on neurobehavioral tests for the occupational cohort that participated in his study (Iregren 1990; Wennberg et al. 1991). A BMD analysis was performed with these data under contract with ATSDR (Clewell and Crump 1999) and the lowest $BMCL_{10}$ value among the end points analyzed was 0.07 mg respirable manganese/m^3 for a 10% change in simple reaction time. The BMD analysis applied K-power and Weibull models to continuous variable data (from 11 different test scores collected by Dr. Iregren) using current respirable manganese exposure estimates, age, and vocabulary test results as explanatory variables, an assumption that 5% of unexposed subjects had adverse responses, and a benchmark response of 10% change from unexposed mean scores. For each dataset, $BMCL_{10}$ values from the Weibull model were lower (by 2–3-fold at the most) than $BMCL_{10}$ values from the K-Power model. Weibull $BMCL_{10}$ values for the different test score datasets ranged from 0.07 to 0.67 mg respirable manganese/m^3. Thus, the lowest $BMCL_{10}$ value from this analysis of test score data from manganese-exposed workers collected by Iregren (1990; Wennberg et al. 1991) is within a 2-fold range of the selected POD of 142 µg manganese/m^3 for the MRL.

Clewell et al. (2003) conducted BMD analyses on data from three neuromotor tests in the Roels et al. (1992) study (visual reaction time, eye-hand coordination, and hand steadiness) and from five neuromotor tests in the Gibbs et al. (1999) study (hole 6 of the hand steadiness test, percent precision of the eye-hand coordination test, reaction time in the complex reaction test, root mean square amplitude in the steady test, and tap time). Exposure measures in these analyses were recent measures of manganese concentrations in respirable dust. $BMCL_{10}$ values were 0.257, 0.099, and 0.202 mg manganese/m^3 for the visual reaction time, eye-hand coordination, and hand steadiness data from the Roels et al. (1992) study; these results were obtained after fitting incidence data for abnormal scores in these tests to a Weibull model for dichotomous data. The reported $BMCL_{10}$ value of 0.099 mg manganese/m^3 for the eye-hand coordination test is similar to the $BMCL_{10}$ value of 0.091 mg manganese/m^3 obtained with the Weibull model in the current ATSDR analysis (Table A-2). $BMCL_{10}$ values from the analyses of outcomes from the Gibbs et al. (1999) study ranged from 0.09 to 0.27 mg manganese/m^3 (averaging the BMCLs within end points across different BMD models applied to the data). Clewell et al. (2003) did not have individual worker data from the Iregren (1990) or Mergler et al. (1994) studies, but based on some assumptions about exposures (e.g., all workers were exposed to average concentrations for the facilities and respirable manganese concentrations were calculated for the workers in the Iregren [1990] study based on an assumption that 50% of total dust manganese was respirable), they calculated $BMCL_{10}$ values for six end points from the Mergler et al. (1994) study and the simple reaction time end point in the Iregren (1990) study. $BMCL_{10}$ values ranged from about 0.1 to 0.3 mg manganese/m^3 from the Mergler et al. (1994) study end points to 0.1 mg manganese/m^3 for the reaction time end point in the Iregren (1990) study.

Health Canada (2010) published a human health risk assessment for inhaled manganese in which BMD analyses were conducted on data for neurobehavioral end points from the study of manganese alloy workers by Lucchini et al. (1999). Dose-response data for six tests of fine motor control, two aspects of memory tests, one test of mental arithmetic, and measured serum prolactin levels were fit to linear models using exposure metrics based on an average overall occupational history (ARE) or an average over the latest 5 years of occupation (ARE5). Using a linear model, $BMCL_{10}$ values for the various end points were 32–59 and 85–98 µg manganese/m^3 for the ARE5 and ARE exposure metrics, respectively. Regardless of exposure metric, the values are within an approximate 2–4-fold range of the selected POD of 142 µg manganese/m^3, based on eye-hand coordination test scores in workers in the Roels et al. (1992) study.

Neurological effects from repeated inhalation exposure to manganese are well recognized as effects of high concern based on case reports and epidemiological studies of groups of occupationally exposed workers. A number of epidemiological studies have used batteries of neurobehavioral tests of neuromotor, cognition, and mood states to study the psychological or neurological effects of exposure to low levels of manganese in the workplace (Bast-Pettersen et al. 2004; Beuter et al. 1999; Blond and Netterstrom 2007; Blond et al. 2007; Bouchard et al. 2003, 2005, 2007a, 2007b; Chia et al. 1993a, 1995; Crump and Rousseau 1999; Deschamps et al. 2001; Gibbs et al. 1999, Iregren 1990; Lucchini et al. 1995, 1999; Mergler et al. 1994; Myers et al. 2003a, 2003b; Roels et al. 1987a, 1992, 1999; Summers et al. 2011; Wennberg et al. 1991). Some of these studies found statistically significant differences between exposed and non-exposed groups or significant associations between exposure indices and neurological effects (Bast-Pettersen et al. 2004; Chia et al. 1993a; Iregren 1990; Lucchini et al. 1995, 1999; Mergler et al. 1994; Roels et al. 1987a, 1992; Wennberg et al. 1991), whereas others have not found significant associations (Deschamps et al. 2001; Gibbs et al. 1999; Myers et al. 2003a, 2003b; Summers et al. 2011; Young et al. 2005). Table A-3 in Appendix A summarizes results from these studies. The neurological effects associated with prolonged low-level manganese exposure generally have been subtle changes including deficits in tests of neuromotor or cognitive functions and altered mood states; they have been referred to by various authors as preclinical or subclinical neurological effects. Manganese air concentrations associated with these effects in chronically exposed workers range from about 0.07 to 1.59 mg manganese/m^3 (manganese in total or inhalable dust measurements; values for manganese in respirable dust are noted in parentheses in Table A-3). Comparison of the effect levels in these studies provides support for selection of the Roels et al. (1992) as the basis of the MRL. The advantage of the Roels et al. (1992) study is that individual worker data were available to support a BMD analysis, but BMD analyses of other epidemiological data for performance on tests of neurobehavior provided potential PODs within about 2–4-fold of the POD selected as the basis of the MRL.

Studies in communities surrounding manganese industries have also reported evidence for associations between deficits in neurological end points (such as attention impairments, postural stability, and motor impairments) and increasing biomarkers of manganese exposure in adults and children, but all potential sources of exposure (e.g., air, diet, drinking water) could not be accounted for in these studies and they do not provide useful dose-response data for deriving an MRL for inhaled manganese (Baldwin et al. 1999; Beuter et al. 1999; Bowler et al. 1999; Hernández-Bonilla et al. 2011; Kim et al. 2011; Menezes-Filho et al. 2011; Mergler et al. 1999; Solís-Vivano et al. 2009; Standridge et al. 2008; Riojas-Rodríguez et al. 2010; Rodríguez-Agudelo et al. 2006).

Oral MRLs for Inorganic Manganese

Overview. No oral MRLs were derived for acute-, intermediate-, or chronic-duration oral exposure to manganese, even though the limited human data and extensive animal data clearly identify neurobehavioral changes as the most sensitive effect from intermediate- and chronic-duration oral exposure to excess inorganic manganese. However, inconsistencies in the dose-response relationship information across studies evaluating different neurological end points under different experimental conditions in different species, as well as a lack of information concerning all intakes of manganese (e.g., dietary intakes plus administered doses), make it difficult to derive intermediate- or chronic-duration MRLs using standard MRL derivation methodology from the human or animal studies. New reports of neurobehavioral effects in children associated with elevated concentrations of manganese in drinking water were evaluated as the possible basis of an oral MRL for intermediate and/or chronic durations of exposure. However, the data were assessed to be unsuitable for MRL derivation due to uncertainties about other possible confounding exposures to neurotoxic agents in the drinking water or via food and/or the lack of information about dietary intakes of manganese by the children. An interim guidance value of 0.16 mg manganese/kg/day, based on the Tolerable Upper Intake Level for 70 kg adults of 11 mg manganese/day (established by the U.S. FNB/IOM [2001]) is recommended to be used for ATSDR public health assessments of oral exposure to inorganic forms of manganese.

Acute Oral Exposure. Although neurological effects are expected to be the most sensitive end points based on epidemiological studies in humans (see Section 3.1), only two acute studies reported neurological end points in rodents. Moreno et al. (2009) administered 0, 4.4, or 13.1 mg manganese/kg/day (as manganese chloride) via gavage for 2 weeks to juvenile C57Bl/6 mice. Increased novelty seeking behavior in an open arena was reported in males exposed to 4.4 or 13.1 mg/kg/day (time in center increased 10 and 8%, respectively; 8–10 animals/group). These data identify a free-standing LOAEL of 4.4 mg/kg/day for behavioral alterations; however, the response did not increase with increasing dose. Additionally, mice receiving 13.1 mg/kg/day had significantly increased concentrations of dopamine, decreased concentration of its metabolite dihydroxyphenylacetic acid (DOPAC), and increased concentration of the serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA) in the stratum compared with control mice (altered 60, 20, and 68%, respectively; 3–4 mice/group). Additionally, Shukakidze et al. (2003) reported that a single dose of 50 mg manganese chloride/kg (13.9 mg manganese/kg) to a group of 10 white rats caused worsened acquisition of an avoidance reaction in response to unconditioned and condition stimuli, increased latent period of a conditioned reflex activity, and increased numbers of errors

and time taken to navigate a maze (compared with controls), beginning on day 5 after dose administration and lasting until days 10–15.

Other acute-duration oral studies found only decreased liver and body weight and decreased leukocyte and neutrophil counts in rats at dietary doses of 1,300 mg manganese/kg/day and no effects in mice at dietary doses up to 2,600 (males) or 3,900 (females) mg manganese/kg/day after 14 days of exposure to manganese sulfate in the diet (NTP 1993). No signs of developmental or maternal toxicity were observed in a standard developmental toxicity study of pregnant rats given daily gavage doses of 2,200 mg manganese/kg/day as manganese chloride on gestation days (GDs) 6–17 (Grant et al. 1997a). With intermediate-duration, no exposure-related effects on fetal body weight or skeletal development or anomalies were found in pregnant rabbits exposed to 33 mg manganese/kg/day on GDs 6–20, but some evidence for delayed fetal skeletal development was found in pregnant Sprague-Dawley rats exposed to the same dose of manganese chloride on GDs 0–21 (Szakmáry et al. 1995).

Of the acute studies, the lowest LOAEL identified was 4.4 mg manganese/kg/day for decreased increased novelty-seeking behavior in an open field in male juvenile C57Bl/6 mice exposed for 2 weeks by gavage (Moreno et al. 2009). If this was used as the POD for the intermediate-duration oral MRL, a value of 0.004 mg manganese/kg/day would be derived if an uncertainty factor of 1,000 was used (10 for use of a LOAEL, 10 for extrapolating across species, and 10 for human variability). However, this rodent-based value of 0.004 mg manganese/kg/day would be 7.5-fold below the FNB/IOM (2001) recommended AI of 1.8 and 2.3 mg manganese/day for women and men, respectively (approximately 0.03 mg manganese/kg/day) and 40-fold below the FNB/IOM (2001) recommended Tolerable Upper Intake Level (UL) of 11 mg/day for adults ≥19 years of age (approximately 0.16 mg manganese/kg/day). Part of the apparent discrepancy between this prospective MRL and the recommended dietary intakes is that the MRL is based only on manganese intakes above the normal dietary intakes. Unfortunately, the dietary intakes of manganese by the rats in the Moreno et al. study (2009) cannot be estimated from the information provided in the published report.

Intermediate Oral Exposure. With intermediate-duration oral exposure, effects on neurobehavior are expected to be the most sensitive effects from excessive manganese, particularly during early developmental periods, based on findings for subtle neurobehavioral effects in epidemiological studies on manganese-exposed workers (see Section 3.1), higher brain manganese levels and altered brain dopamine levels in neonatal rats, compared with adult rats, due to immaturity of the blood-brain barrier and the lack of biliary excretion in preweanling rats (Aschner et al. 2005; Dorman et al. 2000, 2005a; Kontur and

Fechter 1985, 1988), and results from studies of the effects of intermediate-duration oral exposure on systemic toxicity end points and neurobehavioral, neurochemical, and neurodevelopmental end points in adult and young laboratory animals (Anderson et al. 2007a, 2009; Avila et al. 2008; Calibresi et al. 2001; Kern and Smith 2011; Kern et al. 2010; Moreno et al. 2009; Reichel et al. 2006; Tran et al. 2002a, 2002b).

The discussion that follows provides evidence that, while systemic effects of manganese are not typically the most sensitive end point of action, some evidence exists to support adverse cardiovascular effects of manganese at relatively low dose levels, followed by a review of the large number of studies that most consistently support neurobehavior effects as the most sensitive effects from excessive oral manganese exposure.

In standard toxicity studies of intermediate-duration oral exposure to inorganic manganese, marginal evidence for systemic toxicity was found in rats at doses ≥33 mg manganese/kg/day (increased neutrophil count and decreased liver weight in males; decreased body weights at higher doses) and in mice at the highest administered dose of 1,950 mg manganese/kg/day (decreased hemoglobin, mild hyperplasia of forestomach, decreased liver and body weight) (NTP 1993). Corroborative evidence comes from reports of decreased red blood cell counts and body weight in mice following 100 days of dietary exposure to one of several forms of inorganic manganese (manganese acetate, carbonate, oxide, or chloride) at a dose level of 284 mg manganese/kg/day (Komura and Sakamoto 1991).

However, other animal studies indicate that excessive oral intake of manganese may present a cardiovascular hazard. Under magnesium deficiency conditions (4.1 mmol Mg/kg diet), swine fed moderately elevated levels of manganese (about 500 mg manganese/kg diet) died suddenly within 5 weeks and showed necrosis and mineralization of the heart (Miller et al. 2000). This finding was supported with subsequent findings of myocardial necrosis and mitochondrial swelling in magnesium-deficient pigs fed a diet high in manganese (500 mg manganese/kg diet) for 8 weeks (Miller et al. 2004) and of depressed heart muscle mitochondrial O_2 consumption and decreased red blood cells in rats consuming a high manganese diet (250 mg manganese/kg diet) under marginal magnesium dietary conditions; the manganese-induced effects on hematological end points in rats were absent when adequate dietary magnesium was provided (Miller et al. 2006). In another study involving rats supplied with adequate and excessive Mn in the diet (10–15 and 45–50 mg manganese/kg diet), aortas from rats with excessive dietary manganese showed less expression and sulfation of heparin sulfate glycosaminoglycans, compared with the adequate condition (Kalea et al. 2006). The results from these studies suggest that

excessive intermediate-duration oral intake of manganese may present a cardiovascular hazard, especially under magnesium-deficient dietary conditions, but their use as the basis of an intermediate-duration oral MRL for inorganic manganese is limited due to the lack of reported information to accurately calculate daily intakes. Myocardial lesions were not found in rats or mice provided manganese sulfate in the diet for 2 years at dose levels up to 232 or 731 mg manganese/kg/day, respectively (NTP 1993).

Numerous studies support the sensitivity of neurobehavioral end points to intermediate-duration oral doses of manganese. In humans and nonhuman primates exposed orally for intermediate durations, neurobehavioral end points have been examined in healthy adult female subjects given low (0.01 mg manganese/kg/day) or high (0.3 mg manganese/kg/day) manganese diets for 8 weeks (Finley et al. 2003) and in infant monkeys fed either a commercial cow's milk formula (17.5 mg manganese/kg/day), a commercial soy formula (107.5 mg manganese/kg/day), or a soy formula with added magnesium chloride (328 mg manganese/kg/day) for 4 months with monkeys tested through 18 months of age (Golub et al. 2005). No differences between the low and high dietary-intake states were found in the adult females on scores for hand-steadiness and self-reported traits such as assertiveness and anger (Finley et al. 2003). Monkeys provided the highest manganese dose level showed no marked differences from the cow's milk controls in gross motor maturation, growth, cerebrospinal fluid levels of dopamine or serotonin metabolites, or performance on tests of cognitive end points, but showed decreased activity during sleep at 4 months and decreased play activity between 1 and 1.5 months. These results suggest that daily intakes of 328 mg manganese/kg/day (but not 107.5 mg manganese/kg/day) during neonatal periods may cause subtle neurobehavioral changes in primates.

In neurobehavioral assessments of rodents orally exposed to inorganic manganese for intermediate durations during neonatal periods, subtle neurobehavioral effects have been observed at supplemental dose levels as low as about 10–20 mg manganese/kg/day (Brenneman et al. 1999; Dorman et al. 2000; Kern et al. 2010; Kristensson et al. 1986; Moreno et al. 2009; Pappas et al. 1997; Reichel et al. 2006; Tran et al. 2002a, 2002b). Although there are some inconsistencies in the results obtained in these studies (e.g., Brenneman et al. [1999] found increased motor activity with exposure to 22 mg manganese/kg/day after exposure on PNDs 1–49, but Dorman et al. [2000] found no effects of the same dose level on motor activity after exposure on PNDs 1–21), the weight of evidence suggests that subtle neurobehavioral effects can occur in rats with intermediate-duration neonatal exposures at doses ≥10–20 mg manganese/kg/day.

Findings for histopathological changes in the rat brain following intermediate-duration oral exposure to inorganic manganese during neonatal periods are less consistent than the findings for subtle neurobehavioral effects. Chandra and Shukla (1978) reported neuronal degeneration in cortical and cerebellar sections from the brains of young rats orally exposed to 0.3 mg manganese/kg/day as manganese chloride between PND 21 and 51. In contrast, Kristensson et al. (1986) reported no adverse histological changes in cerebellum or hippocampus in rats exposed to a much higher dose level of manganese chloride (150 mg manganese/kg/day) between PND 3 and 44. Pappas et al. (1997) reported a decreased cortical thickness in the offspring of rat dams exposed to 120 or 650 mg manganese/kg/day from GD 1 through PND 30, but found no immunohistological evidence for increased glial fibrillary acidic protein in the cortex, caudate, or hippocampus. Dorman et al. (2000) reported that no adverse histological changes were found in sections of the following brain regions in Sprague-Dawley rats exposed to 11 or 22 mg manganese/kg/day on PNDs 1–21: olfactory bulbs, cerebral cortex, hippocampus, basal ganglia, thalamus, hypothalamus, midbrain, and cerebellum. However, Lazrishvilli et al. (2009) reported neuronal damage in small proportion of cells (7–10%) and marked gliosis throughout the brain in the offspring of rat dams exposed to 10 mg manganese/kg/day in feed for 15–20 days before pregnancy, during pregnancy, and for 1 month after parturition. The weight of evidence from these studies indicates that subtle neurobehavioral effects in neonatally exposed rats are not consistently associated with histological changes in the brain.

Neurobehavioral effects have also been observed in adult rats orally exposed to inorganic manganese for intermediate durations. In several studies, doses inducing these effects were higher than those inducing subtle neurobehavioral effects after neonatal exposure (Calabresi et al. 2001; Centonze et al. 2001; Torrente et al. 2005), but in two other studies, neurobehavioral effects were observed at doses as low as 5.6 mg manganese/kg/day (Shukakidze et al. 2003) and 6.5 mg manganese/kg/day (Vezér et al. 2005, 2007). Increased open field activity, increased interest in a novel object, and increased signs of fear were observed in adult male Wistar rats exposed to drinking water containing 20 mg manganese chloride/L for 10 weeks (estimated doses of 1,310 mg manganese/kg/day), but no effects on radial maze performance, numbers of neuronal cells or levels of glial fibrillary acidic protein in striatum, or intrinsic electrophysiological membrane properties of striatal neurons with the exception of a manganese-induced increase in the frequency and amplitude of spontaneous excitatory postsynaptic potentials (Calabresi et al. 2001; Centonze et al. 2001). In an earlier study of adult male Wistar rats exposed to 20 mg manganese chloride/L for 13 weeks, no neuronal loss or gliosis was evident in the globus pallidus by either histological or immunohistochemical examination (Spadoni et al. 2000). Decreased open field activity and impaired spatial learning were observed in restraint stressed adult male Sprague-Dawley rats exposed

to 153 mg manganese/kg/day (but not 76 mg manganese/kg/day) as manganese chloride in drinking water for 19 weeks (Torrente et al. 2005). Similarly, decreased locomotor activity, as well as decreased tongue protrusion frequency (orofacial dyskinesia measure), were reported in adult Wister rats exposed to 1,280 mg manganese/kg/day (as manganese chloride in drinking water) for 30 days (Avila et al. 2008). No changes in motor activity or performance in a passive avoidance test were observed in adult male Sprague-Dawley rats exposed to 11 or 22 mg manganese/kg/day for 21 days; these doses induced increased pulse-elicited acoustic startle response with neonatal exposure, but exposure during adulthood did not (Dorman et al. 2000). The lowest intermediate-duration daily dose associated with neurobehavioral effects in adult rats is 5.6 mg manganese/kg/day for severely impaired cognitive performance in a maze test following a 30-day exposure of white rats to manganese chloride in the diet (strain not otherwise indicated) (Shukakidze et al. 2003). In another study, decreased open-field locomotor activity and acoustic startle response and impaired performance in maze learning (a test of spatial memory) were observed in male adult Wistar rats exposed to gavage doses of 6.5 or 25.9 mg manganese/kg/day for 10 weeks, compared with controls (Vezér et al. 2005, 2007). Decreased acoustic startle response and impaired spatial memory were still evident in exposed rats, compared with controls, after 5–7 weeks without exposure (Vezér et al. 2005, 2007). The only intermediate-duration study in mice reported no changes in open field activity following adult exposure up to 13.1 mg/kg/day (as manganese chloride) via gavage for 8 weeks (Moreno et al. 2009). However, if adults were previously exposed as juveniles (PNDs 20–34), subsequent exposure in males (but not females) at 4.4 mg/kg/day for 8 weeks resulted in decreased novelty seeking behavior in the open field. Additionally, at 13.1 mg/kg/day, total overall movement in the open field was decreased in males.

Several types of reproductive effects have been reported for manganese. A study by Hafeman et al. (2007) reported a high mortality rate among infants <1 year of age in a Bangladesh community where manganese levels in drinking water were high, but the actual association between the manganese levels in drinking water and infant mortality is difficult to make with certainty. The average level of manganese intake was calculated to be 0.26 mg manganese/kg/day. Similarly, Spangler and Spangler (2009) reported that with every log increase in groundwater manganese concentration in North Carolina counties, there was a 2.074 increase in county level infant deaths per 1,000 live births. Other reproductive effects reported for manganese in intermediate-duration animal studies include 25% decreased pregnancy rate in Long-Evans rats (males and females) exposed to manganese oxide in the diet at 180 mg manganese/kg/day (but not 55 mg manganese/kg/day) for 100–224 days (Laskey et al. 1982), increased incidence of testicular degeneration in male Sprague-Dawley rats exposed to manganese acetate at gavage doses of 137 (but not 69) mg manganese/kg/day for 63 days (Ponnapakkam et al. 2003c), and delayed growth of

testes and sex accessory glands in CD-1 mice exposed to manganese oxide in the diet at 205 mg manganese/kg/day (Gray and Laskey 1980). In Swiss mice exposed for 12 weeks to manganese chloride in drinking water, impaired fertility was observed in males at 309 mg manganese/kg/day (but not a 154 mg manganese/kg/day) and in females at 277 mg manganese/kg/day (Elbetieha et al. 2001). Decreased sperm motility and sperm counts were observed in CD-1 mice exposed to 4.8 or 9.6 mg manganese/kg/day as manganese acetate, but no effects on the ability of exposed males to impregnate unexposed female mice were found at these doses (Ponnapakkam et al. 2003a). The results from the intermediate-duration animal studies suggest that oral exposure to manganese may produce adverse effects on reproduction, but at much higher doses than those inducing subtle neurobehavioral effects in adult or neonatal rats.

In summary, results from animal studies identify subtle neurobehavioral effects as the critical effect in rodents from intermediate-duration oral exposure to inorganic manganese. Potential points of departure for an intermediate-duration oral MRL include LOAEL values of 5.6 mg manganese/kg/day for severely impaired cognitive performance in a maze test following 30-day dietary exposure of adult white rats (Shukakidze et al. 2003); 6.5 mg manganese/kg/day for decreased open-field locomotor activity and acoustic startle response and impaired performance in maze learning (a test of spatial memory) in male adult Wistar rats exposed for 10 weeks by gavage (Vezér et al. 2005, 2007); and 11 mg manganese/kg/day for increased pulse-initiated acoustic startle response in Sprague-Dawley rats exposed (orally by pipette) on PNDs 1–21 (Dorman et al. 2000). In contrast, hand steadiness or self-reported scales for assertiveness or anger were not different in adult female subjects following 8 weeks of exposure to dietary doses of 0.01 or 0.3 mg manganese/kg/day (Finley et al. 2003). In young monkeys, decreased activity during sleep at 4 months and decreased play activity between 1 and 1.5 months were observed following daily intakes of 328 mg manganese/kg/day (but not 107.5 mg manganese/kg/day), but no effects on gross motor maturation or performance in cognitive tests were observed at either dose level compared with controls (Golub et al. 2005).

The effects noted in the rat study by Shukakidze et al. (2003) are much more severe than effects noted in adult rats at reportedly higher dose levels of 1,310 mg manganese/kg/day (Calabresi et al. 2001; Centonze et al. 2001) or 153 mg manganese/kg/day (Torrente et al. 2005) or in adult rats at comparable reported doses of 6.5 mg manganese/kg/day (Vezér et al. 2005, 2007). Shukakidze et al. (2003) reported that the exposed rats “showed increased aggresivity, frequently fell from the platform in the maze, and were unable to perform the maze test.” Because the reporting of the experimental conditions in the Shukakidze et al. (2003) study is sparse and the severity of effects is so unusual, the results are considered to be

outlying results that are not consistent with the rest of the database and not appropriate as the basis of an MRL.

If the LOAEL of 6.5 mg manganese/kg/day for decreased open-field locomotor activity and acoustic startle response and impaired performance in maze learning in male adult Wistar rats exposed for 10 weeks by gavage (Vezér et al. 2005, 2007) was used as the POD for the intermediate-duration oral MRL, a value of 0.007 mg manganese/kg/day would be derived if an uncertainty factor of 1,000 were used (10 for use of a LOAEL, 10 for extrapolating across species, and 10 for human variability). However, this rodent-based value of 0.007 mg manganese/kg/day would be about 4-fold below the FNB/IOM (2001) recommended AI of 1.8 and 2.3 mg manganese/day for women and men, respectively (approximately 0.03 mg manganese/kg/day) and about 23-fold below the FNB/IOM (2001) recommended UL of 11 mg/day for adults ≥19 years of age (approximately 0.16 mg manganese/kg/day). Part of the apparent discrepancy between this prospective MRL and the recommended dietary intakes is that the MRL is based only on manganese intakes above the normal dietary intakes. Unfortunately, the dietary intakes of manganese by the rats in the Vezér et al. study (2005, 2007) cannot be estimated from the information provided in the published report.

Alternatively, using the monkey NOAEL of 107 mg manganese/kg/day for decreased activity during sleep at 4 months and decreased play activity between 1 and 1.5 months in formula-fed infant monkeys provided soy-based formula from birth to 4 months of age (Golub et al. 2005), a value of 1 mg manganese/kg/day would be derived if an uncertainty factor of 100 were used (10 for extrapolating across species and 10 for human variability). The monkey-based value would be about 6-fold higher than the FNB/IOM (2001) UL of 11 mg manganese/day for adults (0.16 mg manganese/kg/day assuming a 70-kg body weight). The formulas fed to the infant monkeys in this study are expected to have been the principal source of manganese.

For children and adolescents, FNB/IOM (2001) scaled the adult UL values according to reference body weights for children and adolescents, noting that there were no reports of manganese toxicity in children and adolescents and that it was not possible to establish UL values for infants (0–12 months).

Based on several surveys, FNB/IOM (2001) reported that average intakes of adults with typical "Western-type" and vegetarian diets ranged from 0.7 to 10.9 mg/day (0.01–0.156 mg manganese/kg/day, assuming a 70-kg body weight). WHO (2004b) recently calculated an estimated daily intake of about 0.0003 mg

manganese/kg/day for 70-kg subjects drinking 2 L of water per day at a concentration of 0.010 mg manganese/L, the median of a survey of manganese in drinking water.

Chronic Oral Exposure. Data on the effects of manganese following chronic oral exposure are less extensive than intermediate-duration data, but these reports do suggest that neurological effects similar to those seen after intermediate-duration exposure may be anticipated following chronic oral exposure to excess manganese. In the reports of neurological effects in humans following chronic oral exposure, there is either uncertainty regarding the exposure level (He et al. 1994; Zhang et al. 1995) or uncertainty that the effects observed were solely attributable to manganese (Bouchard et al. 2007c, 2011; Holzgraefe et al. 1986; Kawamura et al. 1941; Kilburn 1987; Kondakis et al. 1989; Wasserman et al. 2006, 2011; Wright et al. 2006). There is also no clear understanding of the threshold for manganese deficiency/sufficiency or toxicity. Males consuming 0.35 and 0.11 mg manganese/day exhibited symptoms of manganese deficiency (Doisy 1973; Friedman et al. 1987, respectively). But Davis and Greger (1992) did not report any deficiency symptoms among female subjects, 20% of whom consumed <1 mg manganese/day, and Finley et al. (2003) did not observe signs of manganese deficiency or toxicity in adult females with dietary intakes of 0.8 or 20 mg manganese/day for 8 weeks. Authors of a case study suspected abuse of vitamin and mineral preparations to be the source for excess manganese and neurological symptoms observed in their patient (Banta and Markesbery 1977).

Four epidemiological reports of manganese neurotoxicity in children resulting from manganese exposure in drinking water have been recently published. In two separate cross-sectional studies, Wasserman et al. (2006, 2011) reported statistically significant relationships for decreasing intelligence scores with increasing manganese levels in drinking water in 142–151 children (ages 8–11 years) in Bangladesh. Similarly, in a cross-sectional study conducted by Bouchard et al. (2011), a significant negative association was found between manganese levels in the home tap water and intelligence scores in 362 children from Quebec, Canada. In previous study by Bouchard et al. (2007c), a statistically significant relationship between increased levels of oppositional behaviors and hyperactivity and increased levels of manganese in drinking water in an epidemiological study of 46 children (ages 6–15 years), also from Quebec, Canada.

Additionally, three recent case studies suggest that certain children are particularly susceptible to manganese neurotoxicity from high levels in drinking water, including: (1) severe neurotoxic symptoms (inability to walk independently, tendency to fall backward, and development of a “cock-like” walk) and MRI scan findings consistent with a diagnosis of hypermanganism in a previously healthy 5-year-old

female that were associated with elevated drinking water concentrations of manganese (1.7–2.4 mg manganese/L), pica, emotional lability, polycythemia, iron deficiency, and elevated levels of plasma manganese (Brna et al. 2011); (2) a similar case of severe manganism-like neurotoxic symptoms in a previously healthy 6-year-old female that were associated with elevated drinking water concentrations of manganese (1.7–2.4 mg manganese/L), pica, a diet high in manganese-rich foods, and elevated levels of plasma manganese (Sahni et al. 2007); and (3) inattentiveness and lack of focus in the classroom and low-percentile performance in tests of memory in a 10-year-old male with no history of learning problems associated with elevated manganese in drinking water (1.21 mg manganese/L) (Woolf et al. 2002). Although these recent reports cannot causally link the observed neurotoxic effects to excessive manganese intakes, they provide added weight to the evidence for the neurotoxic potential of excessive manganese in children.

As shown in the chronic exposure section of the oral LSE table and figure in Chapter 3, estimated daily intakes from drinking water were calculated as 0.05 mg manganese/kg/day based on the mean manganese drinking water concentration for high exposure group of Bangladesh children ages 8–11 (1.111 mg manganese/L), reference daily water intakes (1.3 L/day), and reference body weights (31.19 kg); 0.07 mg manganese/kg/day based on the mean manganese drinking water concentration for the fourth quartile group of Bangladesh 10-year-old children (1.923 mg manganese/L), reference daily water intakes (1.3 L/day), and average body weights (22.4 kg) (Wasserman et al. 2006); 0.0003 mg manganese/kg/day based on the reported 50th percentile monthly exposure value (8.0 µg/kg/month), assuming 30 days in a month (Bouchard et al. 2011); 0.02 mg manganese/kg/day for the high-manganese intake children in Quebec (0.5 mg manganese/L), reference daily water intakes (1.3 L/day) and reference body weights (37.2 kg) (Bouchard et al. 2007c); 0.104 mg/ manganese/kg/day for the 5-year-old female (Brna et al. 2011); 0.103 mg manganese/kg/day for the 6-year-old female (Sahni et al. 2007), and 0.06 mg manganese/kg/day for the 10-year-old male (Woolf et al. 2002).

To derive an oral MRL for intermediate and chronic durations, an average of the drinking water LOAELs for neurobehavioral effects in the three case reports (Brna et al. 2011; Sahni et al. 2007; Woolf et al. 2002), the cross-sectional studies of children in Bangladesh (Wasserman et al. 2006, 2011), and the studies of children in Quebec (Bouchard et al. 2007c, 2011) could potentially serve as a POD for the MRL. However, one or more of the following uncertainties associated with these studies of children preclude their use as the basis for an intermediate- or chronic-duration MRL: (1) whether or not the observed effects were solely due to excess manganese alone or could have been influenced by other

drinking water or dietary components; (2) the lack of information about manganese levels in food and air; and (3) the small sample sizes.

Interim Guidance Value for Oral Exposure to Inorganic Manganese. As discussed in the preceding sections, no oral MRLs were derived for acute-, intermediate-, or chronic-duration exposure to inorganic manganese, but it is recommended that an interim guidance value of 0.16 mg manganese/kg/day be used for ATSDR public health assessments. The interim guidance value is based on the Tolerable Upper Intake Level for adults of 11 mg manganese/day established by the U.S. Food and Nutrition Board/ Institute of Medicine (FNB/IOM 2001) based on a NOAEL for Western diets (0.16 mg manganese/kg/day assuming an adult body weight of 70 kg). The interim guidance value is well above the FNB/IOM AI value for manganese for men and women of 2.3 and 1.8 mg manganese/day, respectively (for 70-kg individuals, this would result in exposures of 0.033 and 0.026 mg manganese/kg/day, respectively). The interim guidance value is necessary because of the prevalence of manganese at hazardous waste sites and the fact that manganese is an essential nutrient. It is recommended that this value be used until more information on actual intake levels across environmental media can be obtained.

MRLs for MMT

Inhalation and oral MRL values for acute, intermediate, or chronic exposures to MMT have not been derived. There are currently insufficient data regarding the systemic toxicity and carcinogenicity of this compound via inhalation or oral exposures and no reliable data concerning current environmental or occupational exposures with appropriate dose-response information.

MRLs for Mangafodipir

MRL values for mangafodipir are not believed to be warranted. This compound is used in a clinical environment, is administered intravenously only, and is restricted to a very limited population. Thus, it is believed unlikely that this compound would be found at hazardous waste sites or other environmental settings.