



United States
Environmental Protection
Agency

Drinking Water Health Advisory for Manganese

**Drinking Water Health Advisory
for Manganese**

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<http://www.epa.gov/safewater/>

EPA-822-R-04-003
January, 2004

Acknowledgments

This document was prepared under the U.S. EPA Contract Number 68-C-02-009. Lead Scientist, Julie Du, Ph.D., Health and Ecological Criteria Division, Office of Science and Technology, Office of Water, U. S. Environmental Protection Agency.

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ABBREVIATIONS

ALS	Amyotrophic lateral sclerosis
CDC	Centers for Disease Control and Prevention
CJD	Creutzfeldt-Jakob Disease
g	gram
kg	kilogram
IOM	Institute of Medicine
L	liter
m ³	cubic meters
mg	milligram
mL	milliliter
mM	millimolar
Mn	manganese
min	minute
mmol	millimole
MMT	methylcyclopentadienyl manganese tricarbonyl
MND	motor neuron disease
NTP	National Toxicology Program
OST	Office of Science and Technology
OW	Office of Water
ppm	parts per million
PWS	public water system
RfD	Reference Dose
SDWA	Safe Drinking Water Act
SMCL	secondary maximum contaminant level
UCM	unregulated contaminant monitoring
µg	microgram
µmol	micromole

FOREWORD

The 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 (color, taste, odor, etc.) effects of contaminants in drinking water. This Drinking Water Health Advisory contains Health Advisories as well as aesthetic properties (e.g., taste, odor, color) of manganese in drinking water.

A Drinking Water Health Advisory is not an enforceable standard for action. This Health Advisory describes nonregulatory concentrations of the contaminant in water that are expected to be without adverse effects on both health and aesthetics. Health Advisories serve as technical guidance to assist Federal, State, and local officials responsible for protecting public health when emergency spills or contamination situations occur. They are not to be construed as legally enforceable Federal standards. They are subject to change as new information becomes available. This draft supersedes any previous draft advisories for this chemical.

This Document is based, in part, on the Health Effects Support Document for Manganese (U.S. EPA, 2003a), the ATSDR's final Toxicological Profile for Manganese (ATSDR, 2000), and the Institute of Medicine's Dietary Reference Intakes for Manganese (IOM, 2002). The sections on analytical method and treatment technology are based on the Contaminant Candidate List Preliminary Regulatory Determination Support Document for Manganese (U.S. EPA, 2001).

EXECUTIVE SUMMARY

The EPA Office of Water is issuing this health advisory to provide guidance to communities that may be exposed to drinking water contaminated with high manganese (Mn) concentrations. The advisory provides guidance on the concentrations below which potential health and organoleptic problems would unlikely occur. This Drinking Water Health Advisory does not mandate a standard for action; rather it provides practical guidelines for addressing Mn contamination problems. The advisory provides an analysis of the current health hazard information and information on the organoleptic (i.e., taste and odor) associated with Mn-contaminated water, because organoleptic problems will affect consumer acceptance of water resources.

Manganese is a naturally-occurring element that can be found ubiquitously in the air, soil, and water. Manganese is an essential nutrient for humans and animals. Adverse health effects can be caused by inadequate intake or over exposure. Manganese deficiency in humans is thought to be rare because manganese is present in many common foods.

The greatest exposure to manganese is usually from food. Adults consume between 0.7 and 10.9 mg/day in the diet, with even higher intakes being associated with vegetarian diets (Freeland-Graves et al., 1987; Greger, 1999; Schroeder et al., 1966).

Manganese intake from drinking water is normally substantially lower than intake from food. At the median drinking-water level of 10 : g/L determined in the National Inorganic and Radionuclide Survey (NIRS), the intake of manganese from drinking water would be 20 : g/day for an adult, assuming a daily water intake of 2 L. Exposure to manganese from air is generally several orders of magnitude less than that from the diet, typically around 0.04 ng/day on average (U.S. EPA, 1990), although this can vary substantially depending on proximity to a manganese source.

Although manganese is an essential nutrient at low doses, chronic exposure to high doses may be harmful. The health effects from over-exposure of manganese are dependent on the route of exposure, the chemical form, the age at exposure, and an individual's nutritional status. Regardless, the nervous system has been determined to be the primary target organ with neurological effects generally observed. Many of the reports of adverse effects from manganese exposures in humans are from inhalation exposures in occupational settings.

Although there are substantial data supporting the neurological effects of *inhaled* manganese in both humans and animals, there are few data for the association between *oral* exposure to manganese and toxic effects. For example, several epidemiological studies (Kondakis et al., 1989; He et al., 1994) associate adverse neurological effects with exposure to manganese from drinking water; however, due to a lack of qualitative and quantitative details of the exposure scenario, these studies cannot be used for quantitative assessment. On the other hand, rodents do not provide a good experimental model for manganese neurotoxicity. Therefore, the assessment in this document focuses more on what is believed to be a safe oral intake of manganese for the general human population. Finally, it is important to emphasize that

individual requirements for, as well as adverse reactions to, manganese may be highly variable. The lifetime health advisory derived from the reference dose is estimated to be an intake for the general population that is not associated with adverse health effects; this is not meant to imply that intakes above the reference dose are necessarily associated with toxicity. Some individuals may, in fact, consume a diet that contributes more than 10 mg Mn/day without any cause for concern.

There were no studies found that reported exposure to elevated inorganic manganese with cancer in humans. Cancer studies in animals have provided equivocal results. Therefore, there are little data to suggest that inorganic manganese is carcinogenic.

As an element, manganese cannot go through metabolic transformation, but it can exist in many oxidative states and can be converted from one oxidative state to another within the body. Manganese is almost entirely excreted in the feces, only a small proportion being eliminated in the urine (Davis and Greger, 1992). Fecal manganese is comprised of unabsorbed dietary manganese and manganese excreted in bile.

Groups possibly sensitive to manganese would be those who absorb greater amounts of manganese or those who excrete less. These would include the very young (who may absorb more and excrete less), the elderly, and those with liver disease (with impaired biliary excretion).

In order to enhance consumer acceptance of water resources, this advisory recommends reducing manganese concentrations to or below 0.050 mg/L, the EPA's Secondary Maximum Contaminant Level (SMCL) for Mn. The SMCL is based on staining and taste considerations. It is not a federally enforceable regulation, but is intended as a guideline for States. States may establish higher or lower levels depending on the local conditions, such as unavailability of alternate water sources or other compelling factors, provided that public health and welfare are not adversely affected. The lifetime health advisory value of 0.3 mg/L will protect against concerns of potential neurological effects. In addition, this document provides a One-day and 10-day HA of 1 mg/L for acute exposure. However, it is advised that for infants younger than 6 months, the lifetime HA of 0.3 mg/L be used even for an acute exposure of 10 days, because of the concerns for differences in manganese content in human milk and formula and the possibility of a higher absorption and lower excretion in young infants.

1.0 INTRODUCTION

Manganese is a naturally-occurring element that can be found ubiquitously in the air, soil, and water. Manganese is also an essential nutrient for humans and animals (Leach and Harris, 1997; U.S. EPA, 2003a). Adverse health effects can be caused by inadequate intake or over exposure (See a review by Keen et al., 1999 and Keen et al., 2000). The main exposure of humans to manganese is from ingestion of food. Manganese deficiency in humans appears to be rare because manganese is present in many common foods. Manganese is essential to the proper functioning of both humans and other animals as it is required by many cellular enzymes (e.g., manganese superoxide dismutase, pyruvate carboxylase) and can serve to activate many others (e.g., kinases, decarboxylases, transferases, hydrolases, etc.; Hurley et al., 1984; Wedler, 1994; WHO, 2002).

Although manganese is an essential nutrient at low doses, chronic exposure to high doses may be harmful. There are substantial data supporting the neurological effects of *inhaled* manganese in both humans and animals, however, there are little data for the association between *oral* exposure to manganese and toxic effects.

There is a need for EPA to issue a health advisory to provide guidance to communities on the concentrations for avoiding health and organoleptic problems. This Drinking Water Health Advisory does not mandate a standard for action; rather it provides practical guidelines for addressing Mn contamination problems. The advisory provides an analysis of the current health hazard and organoleptic (i.e., taste and odor) information associated with Mn-contaminated water, because organoleptic problems will affect consumer acceptance of water resources.

Uses

Manganese is used principally in the manufacture of iron and steel alloys, manganese compounds, and as an ingredient in various products (ATSDR, 2000; IPCS, 1999). Manganese dioxide and other manganese compounds are used in products such as batteries, glass, and fireworks. Potassium permanganate is used as an oxidant for cleaning, bleaching, and disinfection purposes (ATSDR, 2000; HSDB, 2001). Potassium and manganese greensands are used in some locations for potable water treatment (ATSDR, 2000). Methylcyclopentadienyl manganese tricarbonyl (MMT), an organic manganese compound, is used as an octane-enhancing agent in unleaded gasoline in Canada, the United States, Europe, Asia, and South America (Lynam et al., 1999). Other manganese compounds are used in fertilizers, varnish, fungicides, and as livestock feeding supplements (HSDB, 2001).

2.0 MANGANESE IN THE ENVIRONMENT

Manganese is one of the most abundant metals on the earth's surface, making up approximately 0.1% of the earth's crust. Manganese is not found naturally in its pure (elemental) form, but is a component of over 100 minerals (ATSDR, 2000).

2.1 Water

Manganese is naturally occurring in many surface and ground water sources and in soils that may erode into these waters. However, human activities are also responsible for much of the manganese contamination in water in some areas.

Ambient manganese concentrations in sea water have been reported to range from 0.4 to 10 : g/L (ATSDR, 2000), with an average of about 2 : g/L (Barceloux, 1999). Levels in freshwater typically range from 1 to 200 : g/L (Barceloux, 1999). ATSDR reported that a U.S. river water survey found dissolved manganese levels of less than 11 to more than 51 : g/L (ATSDR, 2000). The United States Geological Survey's National Ambient Water Quality Assessment (NAWQA) has gathered limited data since 1991 on representative study basins around the U.S. This report indicates a median manganese level of 16 : g/L in surface waters, with 99th percentile concentrations of 400 to 800 : g/L (Leahy and Thompson, 1994; USGS, 2001). Higher levels in aerobic waters are usually associated with industrial pollution.

Overall, the detection frequency of manganese in U.S. ground water is high (approximately 70% of sites assayed have measurable manganese levels) due to the ubiquity of manganese in soil and rock, but the levels detected in ground water are generally below levels of public health concern (U.S. EPA 2003a). Similarly, manganese is detected in about 97% of surface water sites (at levels far below those likely to cause health effects) and universally in sediments and aquatic biota tissues (at levels which suggest that it does not bioaccumulate; U.S. EPA 2003a).

Between 1984 and 1986, the National Inorganic and Radionuclide Survey (NIRS) collected data from 989 U.S. community public water systems (PWSs) served by ground water in 49 states and found that 68% of the ground water PWSs reported detectable levels of manganese, with a median concentration of 10 : g/L. Supplemental survey data from PWSs supplied by surface waters in five states reported occurrence ranges similar to those of ground water PWSs.

2.2 Soil

Manganese constitutes approximately 0.1% of the earth's crust, and is a naturally occurring component of nearly all soils (ATSDR, 2000). Natural levels of manganese range from less than 2 to 7,000 ppm, with a geometric mean concentration of 330 ppm (Shacklette and Boerngen, 1984). The estimated arithmetic mean concentration is 550 ppm. Accumulation of manganese occurs in the subsoil rather than on the soil surface (ATSDR, 2000). An estimated 60–90% of soil manganese is associated with the sand fraction (WHO, 1981, as cited in ATSDR, 2000).

No published reports quantify exposure to manganese associated with soil ingestion. Assuming a concentration range of < 2 to 7,000 mg/kg soil and average ingestion of 50 mg soil/day, the average manganese intake of a 70-kg adult would be <0.0014 to 5 : g/kg-day. The corresponding intake for a 10-kg child consuming 100 mg of soil/day would be <0.02 to 70 : g/kg-day (U.S. EPA, 2003a).

2.3 Air

Air levels of manganese compounds vary widely depending on the proximity of point sources such as ferroalloy production facilities, coke ovens, or power plants. Average ambient levels near industrial sources have been reported to range from 220 to 300 nanograms of manganese per cubic meter (ng Mn/m^3), while levels in urban and rural areas without point sources have been reported to range from 10 to 70 ng Mn/m^3 (Barceloux, 1999). Existing data indicate that little difference is found between ambient manganese levels in areas where MMT is used in the gasoline and areas where MMT is not used (Lynam et al., 1999). The U.S. EPA estimated 40 ng Mn/m^3 as an average annual background concentration in urban areas based on measurements in 102 U.S. cities (U.S. EPA, 1990).

2.4 Food

Manganese is found in a variety of foods including many nuts, grains, fruits, legumes, tea, leafy vegetables, infant formulas, and some meat and fish. Food is the most important source of manganese exposure in the general population (ATSDR, 2000; IOM, 2002; U.S. EPA, 2003a).

Heavy tea drinkers may have a higher manganese intake than the general population. An average cup of tea may contain 0.4 to 1.3 mg manganese (ATSDR, 2000). In addition to dietary sources, approximately 12% of the adult population of the U.S. consumed manganese supplements in 1986 (Moss et al., 1989). The median amount of manganese in these dietary supplements was determined to be 2.4 mg/day, similar to the amount of the element consumed in the diet (based on survey information from the Third National Health and Nutrition Estimation Survey; IOM, 2002).

Freeland-Graves et al. (1987) have suggested a daily intake range of 3.5 to 7 mg Mn/day for adults based on a review of human studies. After reviewing dietary surveys, Greger (1999) presented a range for average intakes from adult Western and vegetarian diets of 0.7 to 10.9 mg Mn/day.

Infant formulas contain 50 to 300 : g/L manganese (Collipp et al., 1983), compared to human milk which contains approximately 3.5 to 15 : g/L manganese (ATSDR, 2000; U.S. EPA, 1997). Assuming an intake of 742 millilitres (mL) of breast milk/day (U.S. EPA, 1996a), a breast-fed infant would have an estimated daily manganese intake of 2.6 to 11.1 : g/day. An infant consuming the same volume of infant formula would have an estimated daily manganese intake of 37.1 to 223 : g/day. Assuming an average weight of 6 kg for an infant of age 6 months, the weight-adjusted average daily intake would range from 0.4 to 1.85 : g/kg-day for breast-fed infants. The corresponding weight-adjusted intake for a formula-fed infant would be 6.2 to 37.2 : g/kg-day. Given the high manganese content of milk-based formula, the underexposure of infants to manganese appears less probable than their overexposure (Davidsson et al., 1989a; Dörner et al., 1987; Keen et al., 1986). Once solid foods are introduced, however, the contribution of manganese intake from milk becomes less significant.

In addition to concentration, an important consideration for determining human exposure to manganese from food is bioavailability (Kies, 1994). Several factors can influence the degree to which manganese in foods is absorbed following ingestion. These include intake of dietary fiber, oxalic acids, tannins, and phytic acids, which tend to decrease manganese absorption (Gibson, 1994; U.S. EPA, 2003a), as well as possibly sex-specific iron status (low iron can result in increased manganese absorption; Finley, 1999 while high levels of iron can inhibit manganese uptake). In addition, the status of the GI tract (e.g., the presence of material in the GI tract - fed vs fasted) also affects bioavailability.

Manganese Intake

Adequate Intake (AI) values have been determined for manganese by the Food and Nutrition Board of the Institute of Medicine as follows: 3 : g/day for infants 0-6 months, 0.6 mg/day for infants 7-12 months, 1.2 mg/day for children 1-3 years, 1.5 mg/day for children 4-8 years, 1.9 mg/day for boys 9-13 years, 2.2 mg/day for boys 14-18 years, 1.6 mg/day for girls 9-18 years, 2.3 mg/day for men 19 years or older, 1.8 mg/day for women 19 years or older, 2 mg/day during pregnancy, and 2.6 mg/day during lactation (IOM, 2002).

Adequate Manganese Intakes for Men, Women and Children

Age Group	Males	Females
Infants, 0-6 months	3 : g/day	3 : g/day
Infants, 7-12 months	0.6 mg/day	0.6 mg/day
Children, 1-3 years	1.2 mg/day	1.2 mg/day
Children, 4-8 years	1.5 mg/day	1.5 mg/day
Boys, 9-13 years	1.9 mg/day	--
Boys, 14-18 years	2.2 mg/day	--
Girls, 9-18 years	--	1.6 mg/day
Adults, \$19 years	2.3 mg/day	1.8 mg/day
Women, pregnant (lactating)	--	2 mg/day (2.6 mg/day)

According to IOM, the AI for infants (newborn to 6 months) was set based on “an average manganese concentration of 0.0035 mg/L in human milk” and an average milk consumption of 0.78 L/day. As indicated previously, the manganese concentration in human milk varies. For example, ATSDR (2000) listed a manganese concentration in human milk ranging from 0.003 to 0.01 mg/L, and U.S. EPA (1997), from 0.007 to 0.015 mg/L. Assuming an intake of 0.78 liters milk per day, an infant (0 to 6 months) would ingest 0.003 to 0.012 mg Mn/day from human milk (using the minimum and maximum values in the two concentration ranges); the AI set by the IOM (i.e., 0.003 mg/day) is at the lower end of this range.

Tolerable Upper Intake

The IOM (2002) also set a tolerable upper intake level of 11 mg/day for adults, based on a recent review (Greger, 1999) which stated that the average manganese intake for adults eating typical Western and vegetarian diets in various surveys ranged from 0.7 to 10.9 mg Mn/day. Davis and Greger (1992) reported that women given daily supplements of 15 mg manganese (as an amino acid-chelated manganese supplement) for 90 days experienced no effects other than a significant increase in lymphocyte manganese-dependent superoxide dismutase, a “biomarker” that increases in direct relation to manganese exposure (Greger 1998, 1999). There are insufficient data to set tolerable upper intakes for infants or children.

2.5 Environmental Fate

Manganese compounds may be present in the atmosphere as suspended particulates resulting from industrial emissions, soil erosion, volcanic emissions, application of manganese-containing pesticides, and the burning of MMT-containing gasoline (IPCS, 1999). Early analysis of emissions suggested that manganese from combustion of MMT is emitted primarily as manganese tetroxide (Mn_3O_4 ; Ter Haar et al., 1975, as cited in ATSDR, 2000). However, more recent testing suggests that when very low levels of MMT are combusted (i.e., concentrations comparable to the currently allowed levels), manganese is emitted primarily as manganese phosphate and sulfate. The reported formal charge of the emitted manganese is +2.2, with a mass median aerodynamic diameter of 1 to 2 microns (Ethyl Corporation, 1997, as cited in Lynam et al., 1999). Uncombusted MMT rapidly decomposes to manganese oxide, carbon dioxide, and organic compounds in the atmosphere and has a half-life of only a few seconds in the presence of sunlight (Lynam et al., 1999; Zayed et al., 1999). Because particle size is small, atmospheric manganese distribution can be widespread. These particles will eventually settle out into surface waters or onto soils via the process of dry deposition. Little information is available on the chemical reactions of atmospheric manganese, but it is expected to react with sulfur and nitrogen dioxide. The half-life of manganese in air is only a few days (ATSDR, 2000).

The primary sources for surface and ground water releases are industrial facility effluent discharge, landfill and soil leaching, and underground injection. Manganese, in the form of potassium permanganate, may be used in drinking water treatment to oxidize and remove iron, manganese, and other contaminants (ANSI/NSF, 2000). Transport and partitioning of manganese in water is dependent on the solubility of the manganese form. In surface waters, manganese occurs in both dissolved and suspended forms, depending on such factors as pH, anions present, and oxidation-reduction potential (ATSDR, 2000). Often, manganese in water will settle into suspended sediments. Anaerobic groundwater often contains elevated levels of dissolved manganese. The divalent form (Mn^{2+}) predominates in most water at pH 4–7, but more highly oxidized forms may occur at higher pH values or result from microbial oxidation (ATSDR, 2000). It can bioaccumulate in lower organisms (e.g., phytoplankton, algae, mollusks, and some fish), but not in higher organisms, and biomagnification in food-chains is not expected to be significant (ATSDR, 2000). Little information is available on the biodegradation of manganese-containing compounds in water, but factors such as pH and temperature are important for microbial activities.

Approximately 91% of environmental manganese is released to soil. The main source of this release is land disposal of manganese-containing wastes. The ability of manganese compounds to adsorb to soils and sediments is contingent upon the cation exchange capacity and organic content of the soil or sediment. Adsorption can vary widely based on differences in these two factors. Oxidative microbial activity may increase the precipitation of manganese minerals and increase the dissolution of manganese in subsurface environments.

2.6 Summary

The greatest exposure to manganese is usually from food. Adults consume between 0.7 and 10.9 mg/day in the diet, with even higher intakes being associated with vegetarian diets (Freeland-Graves et al., 1987; Greger, 1999.; Schroeder et al., 1966) or the consumption of large amounts of tea.

Manganese intake from drinking water is normally substantially lower than intake from food. At the median drinking-water level of 10 : g/L determined in the National Inorganic and Radionuclide Survey (NIRS), the intake of manganese would be 20 : g/day for an adult, assuming a daily water intake of 2 L. Exposure to manganese from air is generally several orders of magnitude less than that from the diet, typically around 0.04 ng/day on average (U.S. EPA, 1990), although this can vary substantially depending on proximity to a manganese source.

3.0 CHEMICAL AND PHYSICAL PROPERTIES

Manganese can exist in multiple oxidative states; the most environmentally and biologically important manganese compounds are those that contain Mn^{2+} , Mn^{4+} , and Mn^{7+} (U.S. EPA, 1994). The physical and chemical properties of different manganese compounds vary substantially, as demonstrated in Table 1 on the next page.

ORGANOLEPTIC PROPERTIES

At concentrations exceeding 0.1 milligrams per litre (mg/L), the manganese ion imparts an undesirable taste to beverages and stains plumbing fixtures and laundry (Griffin, 1960). When manganese (II) compounds in solution undergo oxidation, manganese precipitates, resulting in encrustation problems. At concentrations as low as 0.02 mg/L, manganese can form coatings on water pipes that may later slough off as a black precipitate (Bean, 1974). The U. S. and a number of other countries have set secondary standards of 0.05 mg/L for manganese. This is an aesthetic level above which problems with discoloration may occur.

Table 1.
Chemical and Physical Properties of Manganese and Common Manganese Compounds

	Mn	MnCl ₂	Mn ₃ O ₄	MnO ₂	KMnO ₄
CAS No.	7439-96-5	2145-07-3	1317-35-7	479-93-7	7722-64-7
Valance	0	+2	+2 and +3	+4	+7
Molecular Weight	54.9	125.8	228.8	86.9	158
Synonyms	Elemental manganese	Manganese dichloride; Manganese chloride; Manganese (II) chloride	Manganese oxide; Manganese (II,III) oxide; Manganese tertoxide	Manganese dioxide; Black dioxide; Cement black; Manganese peroxide; Manganese (IV) oxide	Potassium permanganate; permanganic acid, potassium salt
Physical State (25°C)	Solid	Solid	Solid	Solid	Solid
Boiling Point (°C)	1962	1190	—	—	—
Melting Point (°C)	1244	650	1564	535 (loses oxygen)	240
Density (g/cm ³)	7.4	2.98	4.86	5.026	2.703
Vapor Pressure (20°C)	1.9	—	—	—	—
Water Solubility (g/100 mL)	Decomposes	723 (25°C)	insoluble	insoluble	63.8 (20°C)
Log Octanol/Water Partition – Coefficient (Log K _{ow})	—	—	—	—	—
Taste Threshold	—	—	—	—	—
Odor Threshold (air)	—	—	—	—	—
Conversion Factor	—	—	—	—	—

— No date available.

4.0 TOXICOKINETICS

The absorption, distribution, metabolism and excretion of manganese in the body are reviewed, discussed, and summarized in Greger (1999), Kies (1994), U.S. EPA (1984; 1993; 2003a), and ATSDR (2000). Age, chemical species, dose, route of exposure, and dietary conditions all affect manganese absorption and retention (Lönnerdal et al., 1987). Uptake of dietary manganese appears to be influenced by several dose-dependent processes: biliary excretion, intestinal absorption, and intestinal elimination.

4.1 Absorption

Manganese speciation and the route of exposure affects its absorption (Andersen et al., 1999; Tjälve et al., 1996). Thomson et al. (1971) and Gibbons et al. (1976) reported that the divalent form of manganese is absorbed most efficiently. However, as Bales et al. (1987) reported, the efficiency of absorption also varies for different manganese salts with manganese

chloride more efficiently absorbed than the sulfate or acetate salts. Recent studies show that significant differences exist in the amounts of manganese that are absorbed across different exposure routes, with inhaled manganese being absorbed more rapidly and to a greater extent than ingested manganese (Roels et al., 1997; Tjälve et al., 1996). Very little manganese is absorbed through the skin. Absorption of manganese via inhalation, intratracheal instillation, or intravenous infusion bypasses the control processes of the gastrointestinal tract. Absorption from inhalation exposure is mainly a function of particle size with smaller particles reaching the lower airways where they can be absorbed and larger particles deposited in the upper airways where they are subject to possible mucociliary transport to the throat followed by entrance into the gastrointestinal tract.

From animal experiments, it is known that inhaled manganese (even the insoluble MnO_2) is transported in a retrograde direction from the olfactory epithelium to the striatum of the brain (Gianutsos et al., 1997; Roels et al., 1997). During its uptake through the olfactory nerve endings (Bench et al., 2001; Brenneman et al., 2000; Tjälve et al., 1996; Vitarella et al., 2000) it may damage the astrocytes (Henriksson and Tjälve, 2000). After peroral uptake, manganese, like all other metals, is filtered from the blood by the choroid plexus (Ingersoll et al., 1995; Zheng et al., 1991). The retrograde transport of manganese through the olfactory epithelium directly into certain regions of the central nervous system or the brain could explain why the safe dose following inhalation exposure is much lower than after oral ingestion (Wang et al., 1989). The following sections discuss absorption of manganese following oral exposure only.

Absorption of manganese across the gastrointestinal tract is regulated by normal physiological processes to help maintain manganese homeostasis. Manganese absorbed in the divalent form from the gut via the portal blood is complexed with plasma proteins that are efficiently removed by the liver. A 7-week study in which 7 adult males ingested high-fiber diets containing 12.0 to 17.7 mg Mn/day (0.17 to 0.25 mg/kg-day) found that an average of $7.7\% \pm 6.3\%$ of the manganese was absorbed during weeks 5 to 7, with no measurable net retention of manganese (Schwartz et al., 1986). Similarly, an average absorption of $8.4\% \pm 4.7\%$ was observed in 7 adults ingesting infant formula containing manganese (Sandström et al., 1986).

Manganese retention may be greater for young animals and infants (Keen et al., 1986) due to the fact that the biliary system, the primary route of excretion, is not completely developed in human infants (Lönnerdal, 1994). Keen et al. (1986) demonstrated a strong effect of age on intestinal manganese uptake and retention. Sprague-Dawley rat pups were fasted overnight and then intubated with 0.5 mL of human milk containing 0.005 mg $^{54}Mn/mL$. Manganese retention was highest ($\geq 80\%$) in pups less than 15 days old. In older pups (16-19 days old), the average retention was 40%. Lönnerdal et al. (1987) showed that manganese uptake from brush border membranes was higher in 14-day-old rats than in 18-day-old rats. Although Rehnberg et al. (1985) found that younger animals had a slower distal intestinal transit time than older animals (potentially contributing to a higher proportional uptake), Bell et al. (1989) showed that the uptake rate was similar in pre- and post-weanling animals, suggesting that age-dependent differences in manganese retention were not due to immature intestinal transport mechanisms. Fechter (1999) determined that neonatal mice are unable to maintain manganese homeostasis until 17-18 days of age. When considered together, these data indicate that human infants, at

certain ages, may not have developed the capacity to completely excrete manganese following ingestion.

Davidsson et al. (1989b) studied whole-body retention of ^{54}Mn in adult humans after intake of radiolabeled infant formula. These authors observed reproducible retention figures at day 10, after repeated administrations of the labeled formula to six subjects. Absorption ranged from 0.8-16%, with a mean value of $5.9 \pm 4.8\%$. This range corresponds to a 20-fold difference between the highest and lowest values. Retention at day 10 ranged from 0.6-9.2%, with a mean value of $2.9 \pm 1.8\%$, when measured in 14 healthy individuals. These results suggest substantial variation in absorption between individuals.

The absorption of manganese is closely linked to iron absorption; iron-deficient diets lead to an increased absorption of both iron and manganese (Finley, 1999; Sandström et al., 1986; Thomson et al., 1971). Rehnberg et al. (1982) administered dietary Mn_3O_4 (450, 1,150, or 4,000 ppm Mn) to young rats. These authors amended the basal diets with varying levels of iron, and demonstrated that iron deficiency promoted the intestinal absorption of manganese. Conversely, manganese absorption was inhibited by large amounts of dietary iron. Absorption is also related inversely to the level of calcium in the diet (Lutz et al., 1993; McDermott and Kies, 1987; Kies, 1994; Schroeder et al., 1966). Johnson et al. (1991) studied the absorption of radiolabeled manganese from various plant foods in adult men and women and reported that the absorption values ranged from 1.4 to 5.5% and were significantly lower than the mean values of 7.8 to 10.2% from controls (MnCl_2 dissolved in water). Certain constituents of tea, such as tannins, can result in reduced manganese absorption (Freeland-Graves and Llanes, 1994). Other factors can also influence the degree to which manganese in foods is absorbed upon ingestion. These include intake of dietary fiber, oxalic acids, and phytic acids as well as contents in the gut, which tend to decrease manganese absorption (Gibson, 1994; U.S. EPA, 2003a).

4.2 Distribution

Manganese is present in all tissues of the body, the highest levels usually being found in the liver, kidney, pancreas, and adrenals (Sumino et al., 1975; Tipton and Cook, 1963). Intermediate concentrations occur in the brain, heart and lungs (ATSDR, 2000), with accumulations preferential in certain regions of the brain in infants and young animals (Kontur and Fechter, 1988; Zlotkin and Buchanan, 1986). The lowest concentrations of manganese are observed in bone and fat. Some data suggest that tissues rich in mitochondria (for example, liver, kidney, and pancreas) contain higher levels of manganese (Kato, 1963; Maynard and Cotzias, 1955).

After absorption into the blood system by routes other than the gastrointestinal tract, manganese is apparently oxidized, and the trivalent manganese binds to transferrin. Transferrin-bound trivalent manganese is not as readily removed by the liver, as are protein complexes with divalent manganese. Thus, manganese delivered by routes other than the gastrointestinal tract would be available for uptake into tissues for a longer period of time than the orally administered manganese, leading to quantitative differences in tissue uptake (Andersen et al., 1999).

Factors that may alter tissue distribution include co-exposure to other metals (Shukla and Chandra, 1987) and the chemical form (Gianutsos et al., 1985). Age may also be a factor. Animal studies have shown that manganese crosses the blood-brain barrier in neonates at a rate four times higher than that in adults (Mena, 1974).

4.3 Metabolism

As a metallic element, manganese does not undergo metabolic conversion to other products. However, manganese has the potential to exist in several oxidation states in biological systems. Circumstantial evidence from the study of manganese-containing enzymes and from electron spin trapping experiments suggests that manganese undergoes conversion from Mn(II) to Mn(III) within the body (ATSDR, 2000). The conversion from Mn(II) to Mn(III) appears to be catalyzed by the α -globulin protein ceruloplasmin (Andersen et al., 1999).

A small fraction of absorbed manganese is present as the free ion. However, manganese readily forms complexes with a variety of organic and inorganic ligands. The complexes formed include 1) low molecular weight complexes with bicarbonate, citrate or other ligands; 2) an exchangeable complex with albumin; and 3) tightly bound complexes with proteins such as transferrin and α_2 -macroglobulin. In addition, manganese can assume a structural role in metalloproteins such as mitochondrial superoxide dismutase, pyruvate decarboxylase, and liver arginase. Manganese also plays a catalytic or regulatory role in enzymatic reactions involving select hydrolases, dehydrogenases, kinases, decarboxylases and transferases.

4.4 Excretion

Manganese is almost entirely eliminated in the feces, with only a small proportion (0.1-2%) being excreted in the urine (Davis and Greger, 1992). Fecal manganese is comprised of unabsorbed dietary manganese plus manganese excreted in bile. In humans, elimination is biphasic, with half-lives of 13 and 37 days (Davidsson et al., 1989b; Sandström et al., 1986). Sweat, hair and the milk of lactating mothers also contribute to excretion (Roels et al., 1992).

5.0 HEALTH EFFECTS DATA

Manganese is an essential element for many living organisms, including humans. It is necessary for proper functioning of some enzymes (manganese superoxide dismutase) and for the activation of others (kinases, decarboxylases, etc). Adverse health effects can be caused by inadequate intake or over exposure. Manganese deficiency in humans appears to be rare because manganese is present in many common foods. Animals experimentally maintained on manganese-deficient diets exhibit impaired growth, skeletal abnormalities, reproductive deficits, ataxia of the newborn, and defects in lipid and carbohydrate metabolism (Keen et al., 1999; Hurley and Keen, 1987; U.S. EPA, 1984).

The health effects from over-exposure of manganese are dependent on the route of exposure, the chemical form, the age at exposure, and an individual's nutritional status.

Irrespective of the exposure route, the nervous system has been determined to be the primary target with neurological effects generally observed.

5.1 Human Studies

Humans are exposed to inorganic manganese compounds in food and water, but there are few reports of adverse effects in humans from ingesting excess manganese. Most human studies reporting adverse effects are of inhalation exposure. There is conclusive evidence from occupational studies in humans that inhalation exposure to high levels of manganese compounds can lead to a disabling syndrome of neurological effects referred to as “manganism.” Although it is typical for symptoms to occur after several years of exposure, some individuals may begin to show signs after 1-3 months of exposure (Rodier, 1955).

5.1.1 Short-term Exposure Studies

Neurological

Kawamura et al. (1941) reported health effects resulting from the ingestion of manganese-contaminated well water for an estimated 2-3 months by 25 individuals. The source of contamination was identified as leachate from approximately 400 dry cell batteries buried near the drinking water well. The concentration of manganese in the well water was analyzed 7 weeks after the first case appeared and was determined at that time to be ~14 mg Mn/L (as Mn_3O_4). However, when re-analyzed 1 month later, the levels were decreased about half. Therefore, the actual exposure was probably to drinking water containing ~28 mg Mn/L or higher. Assuming a daily water intake of 2 L, with a minimum of 2 mg Mn from food, a dose of at least 58 mg Mn/day is estimated. This exposure level is quite uncertain and it is estimated that it is around 25-30 times the level considered to be safe and adequate by the Food and Nutrition Board of the Institute of Medicine (IOM, 2002).

Health effects reported by Kawamura et al. (1941) included lethargy, increased muscle tonus, tremor and mental disturbances. Out of 25 people examined, 15 had symptoms. Five cases were considered severe, 2 cases were categorized as moderate, and 8 cases were described as mild. The most severe symptoms were observed in the elderly. Younger people were less affected, and symptoms of intoxication were completely absent in young children (age 1 to 6 years). Three deaths occurred, including one from suicide. Upon autopsy, the concentration of manganese in the brain of one person was found to be 2 to 3 times higher than concentrations measured in two unexposed individuals (controls). Extreme macroscopic and microscopic changes were seen in the brain tissue, especially in the globus pallidus. Although there were also elevated levels of zinc in the well water, the authors concluded that the zinc appeared to have no relation to the observed symptoms or tissue pathology. This conclusion was largely based on the observation of morphological changes in the corpus striatum, which are characteristic of manganese poisoning, but are not a feature of zinc poisoning.

While toxicity in the Kawamura et al. (1941) study is attributed to manganese, several

aspects of the observed health effects are inconsistent with traits of manganism observed in humans following chronic inhalation exposure. Inconsistencies include the rapid onset of symptoms and rapid progression of the disease. Two adults who came to tend the members of one family developed symptoms within 2 to 3 weeks. The course of the disease was very rapid, progressing in one case from initial symptoms to death in 3 days. Some survivors recovered prior to significant decreases in the manganese concentration of the well water which resulted when the dry-cell batteries were removed from the site. This pattern contrasts with the longer latency period and irreversible damage caused by inhalation exposure to manganese (as observed in several occupational exposure studies; ATSDR, 2000). These observations may represent differences in the pharmacokinetics of ingested versus inhaled manganese, but there is little information to support this conclusion. Although the individuals in the Kawamura et al. (1941) study were clearly exposed to high levels of manganese, it is possible that additional factors contributed to the observed effects (ATSDR, 2000; U.S. EPA, 1993).

Symptoms resembling Parkinson's disease have also been noted in an individual who ingested 1.8 mg/kg-day potassium permanganate for 4 weeks (Bleich et al., 1999; Holzgraefe et al., 1986). The symptoms occurred 9 months after the exposure.

5.1.2 Long-term Exposure Studies

Neurological

The neurological effects of inhaled manganese have been well documented in humans chronically exposed to elevated levels in the workplace (ATSDR, 2000; Canavan et al., 1934; Cook et al., 1974; Roels et al., 1999). The syndrome known as “manganism” is caused by exposure to very high levels of manganese dusts or fumes and is characterized by a “Parkinson-like syndrome” including weakness, anorexia, muscle pain, apathy, slow speech, monotonous tone of voice, emotionless “mask-like” facial expression, and slow clumsy movement of the limbs. In general, these effects are irreversible. Some motor functions may already be affected following chronic exposure to levels of manganese $\leq 1 \text{ mg/m}^3$ (if the inhaled manganese is respirable), but individuals in these situations have not shown the overt, clinical symptoms of those exposed to much higher levels (Mergler et al., 1994; Roels et al., 1992).

By the oral route, manganese is often regarded as one of the least toxic elements, although there is some controversy as to whether the neurological effects observed with inhalation exposure also occur with oral exposure. Several case reports of oral exposure to high doses of manganese have described neurological impairment as an effect, but the quantitative and qualitative details of exposure necessary to establish direct causation are lacking. An individual who took large mineral supplements over several years displayed symptoms of manganism (Banta and Markesbery, 1977).

An epidemiological study was conducted in Greece to investigate the possible correlation between long-term (i.e., more than 10 years) manganese exposure from drinking water and neurological effects in elderly people (Kondakis et al., 1989). The levels of manganese in the drinking-water of 3 different geographical areas were 3.6-14.6 : g/L in the control area and 81-253 : g/L and 1800-2300 : g/L in the manganese-containing areas. The total population in

the three areas being studied range from 3200 to 4350 people. The study included only individuals over the age of fifty drawn from a random sample of 10% of all households. The number of subjects sampled were 62, 49, and 77 for control, low-, and high-exposed groups. The authors performed a neurological examination of the subjects (weakness/fatigue, gait disturbances, tremors, dystonia, etc.) and expressed the results as composite scores. They found no differences in the manganese content in the blood, but a statistically-significant difference in both the manganese content in the hair and composite neurological scores between the high-exposed area (concentrations 1800-2300 : g/L) and the control area, suggesting neurological impairment in the high exposed area. The investigators estimated a dietary intake of 5-6 mg/day (personal communication), but data were not provided. Because of the uncertainty in the amount of manganese in the diet, and possible exposure from other sources such as dust, and little information on nutritional status and other possible confounding variables, it is difficult to estimate the total exposure to manganese.

The incidence of motor neuron disease (MND) in a small Japanese town was positively correlated with a significantly increased manganese concentration in local rice and a low magnesium concentration in the drinking-water (Iwami et al., 1994). The study did not provide good estimates of overall exposure to manganese in either the control population or the population with MND; therefore, development of the disease could not be conclusively attributed to manganese exposure. The simultaneous exposure to manganese and the deficiency of other essential minerals was possibly the reason for the enhanced incidence of neurotoxicological symptoms found in this study in Japan and in another population in Guam (Florence and Stauber, 1989; Yoshida et al., 1988).

There was also some speculation on a link between mineral deficiency, enhanced oral manganese uptake and Mn-catalyzed denaturation of copper-free prion protein to the pathogenic prion protein (Brown et al., 2000), which might explain the enhanced occurrence of some prion diseases in certain world regions (Purdey, 2000).

Goldsmith et al. (1990) investigated a Parkinson's disease cluster within southern Israel in which the prevalence of the disease was increased among persons 50 to 59 years old, suggesting an early onset. Well water and soils in the region reportedly contained high levels of manganese, although no quantitative data were provided. In addition, the manganese-containing fungicide Maneb was commonly used in the area. Several factors limit the use of this study for evaluation of the human health effects of excess manganese exposure. Lack of environmental concentration data prevent reliable estimation of exposure rates. Potentially confounding factors include the high levels of aluminum, iron, and other metals in the soil and water, and the use of the herbicide paraquat in the area (ATSDR, 2000). Paraquat is structurally related to N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), which causes irreversible symptoms of parkinsonism in humans.

Contrary to the above studies, another long-term drinking-water study in a rural northern area of Germany (Vieregge et al., 1995) found no neurological effects following ingestion of increased manganese. No significant differences in neurological tests were found in older people (41 subjects older than 40 years with a mean age of 57.5) consuming well water containing at least 0.3 mg/L of manganese (0.3 to 2.16 mg/L of manganese) for 10 to 40 years. The control

group (74 subjects, mean age 56.9 years) was exposed to water containing less than 0.05 mg/L of manganese. Subjects of both groups were randomly selected and matched with respect to age, sex, nutritional habits, and drug intake. However, like the Kondakis et al. (1989) study, this study lacks exposure data from other routes and sources, and the manganese concentration range in the water was very broad.

Two other studies involving ingestion exposure to manganese reported no increases in adverse health effects. In one area of Japan, a manganese concentration of 0.75 mg/L in the drinking-water supply had no apparent adverse effects on the health of consumers (Suzuki, 1970). No signs of toxicity were observed in patients given 30 mg of manganese citrate (9 mg of manganese) per day for many months (Schroeder et al., 1966).

One epidemiological study has been identified which attempts to link potential overexposure to ingested manganese with neurotoxicity in children. Adverse neurological effects (decreased performance in school and in neurobehavioral examinations of the WHO core test battery) were reported in 11- to 13-year-old children who were exposed to excess manganese through ingestion of contaminated water and consumption of food made of wheat fertilized with sewage water (He et al., 1994; Zhang et al., 1995). The exposed and control groups were both from farming communities and were matched for age, sex, grade, family income level, and parental education level. The average manganese concentration of the drinking-water was 0.241 mg/L for the exposed area compared to the control level of 0.04 mg/L. However, the total exposure data, including manganese exposure from food, water and air, exposure duration, as well as other confounding factors and the nutritional status of the children were not well-characterized.

A recently published case study (Woolf et al., 2002) reported increased manganese levels in the hair and blood of a 10-year-old child exposed to increased manganese in drinking water. The child had been ingesting drinking water supplied by a well for 5 years prior to a clinic visit for evaluation of over-exposure to manganese. In addition, the family lived in a house near a toxic waste dump. An evaluation of the well water performed four months prior to the child's health assessment indicated that manganese and iron levels in the water were both elevated, with concentrations of 1.21 (reference level, 0.05 mg/L) and 15.7 mg/L, respectively. The child's whole blood and serum manganese levels were 3.82 : g/100 mL (reference normal, <1.4 : g/100 mL) and 0.90 : g/100 mL (reference normal, <0.265 : g/100 mL), respectively. The child's hair manganese level was 3,091 ppb of washed, acid-digested hair (reference normal, <260 ppb hair). Although the child's 16-year-old brother did not exhibit elevated blood manganese, he did have increased manganese in his hair. The 10-year-old did not exhibit any clinical effects of manganese over-exposure (cogwheeling, abnormally high muscle tone, fixed facies, etc.) and had good balance with closed eyes, although he did have trouble coordinating rapid alternating motor movements (this deficiency is consistent with the test performance of occupational workers chronically exposed to airborne manganese). Magnetic resonance imaging (MRI) of the child's brain did not indicate any hyperintense signaling of the globus pallidus, basal ganglia, mid-brain or pons, which would indicate manganese deposition in these areas of the brain. Selective deposition of manganese in the globus pallidus and basal ganglia has been shown to occur in children and adults with chronic manganese overexposure (Devenyi et al., 1994; Hauser et al., 1996). The absence of the signaling argues against manganese toxicity. Results from a battery of neuropsychologic tests on the child indicated that global cognition was unimpaired.

However, the child had difficulties in both visual and verbal memory, which the study authors considered consistent with a deficit in free retrieval skills. The family was counseled to use bottled water for drinking and cooking; one month after the initial test, the child's whole blood manganese level was reduced to 1.71 : g/100 mL (Woolf et al., 2002). It is difficult to determine the total exposure from this study.

Results from studies of an Aboriginal population in Groote Eylandt have been cited as additional evidence for a relationship between elevated manganese exposure, violent behavior, and adverse health effects. The soil on this Australian island is exceptionally high in manganese (40,000 to 50,000 ppm), and the fruits and vegetables grown in the region are reported to contain elevated concentrations of the element. High alcohol intake, anemia, and a diet deficient in zinc and several vitamins (Florence and Stauber, 1989) may contribute to increased uptake and retention of manganese. The proportion of arrests in this native population is the highest in Australia, and high incidences of stillbirths and congenital malformations, as well as a high occurrence of Parkinson-like neurobehavioral syndrome, have been observed (Cawte and Florence, 1989; Kilburn, 1987). Clinical symptoms consistent with manganese intoxication are present in about 1% of the inhabitants. Quantitative data on oral intake have not been reported, but elevated concentrations of manganese have been determined in the blood and hair of the Aborigines (Stauber et al., 1987). However, Stauber et al. (1987) did not find a correlation between hair levels of manganese and the severity of neurological symptoms in individuals. A study of the neurologic status of the Aborigines in Groote Eylandt identified two general syndromes. One syndrome is characterized by muscle atrophy and weakness, while the other is characterized by ataxia and oculomotor disturbances (Kilburn, 1987). Although an association of adverse health effects with elevated manganese exposure is suggested by these observations, the small population of Groote Eylandt and the difficulty in defining an appropriate control population have prevented the identification of statistically-significant trends (U.S. EPA, 1993).

Several of the studies above utilized hair analysis as a method for estimating exposure to manganese. ATSDR (2000) has outlined several potential limitations to the use of hair analysis. The normal cycle of hair growth and loss restricts its usefulness to a period of a few months following exposure. External contamination of hair by dye, bleaching agents, or other materials may result in values which are not representative of absorbed doses. Further, the affinity of manganese for pigmented tissue may result in variations of manganese concentration with hair color.

Kihira et al. (1990) have associated manganese with amyotrophic lateral sclerosis (ALS). Spinal cord samples from ALS patients were found to have higher manganese concentrations in the lateral fasciculus and anterior horn than in the posterior horn. ALS patients also exhibited a positive correlation between manganese and calcium spinal cord content, while controls exhibited a negative correlation. It was suggested that an imbalance between manganese and calcium in ALS patients plays a role in functional disability and neuronal death. This study needs to be interpreted with caution, however, because it is not conclusive that the high manganese concentrations in these patients preceded the onset of the disease.

Creutzfeldt-Jakob Disease (CJD) clusters in central Slovakia have occurred near areas of

high manganese in conjunction with low copper (Purdey, 2000). The level of manganese in natural uncultivated pasture in CJD-endemic areas was 210 ppm dry weight in comparison to CJD-free areas where the level was 85 ppm dry weight. The levels of manganese in pine needles and some specific crops were also measured and were approximately 1.5-16 times greater in the CJD-endemic regions (Purdey, 2000). It was suggested that manganese replaces copper in CNS prion proteins (PrP) causing a protease-resistant, misfolded PrP. Brown et al. (2000) determined that manganese can replace copper in recombinant PrP and reported that the PrP appears less stable and quickly converts to a misfolded form. Although the manganese-loaded PrP initially had a similar structure and activity as copper-loaded PrP, aging of the manganese-loaded PrP caused it to become proteinase-resistant and lose function.

Reproductive and Developmental Studies

Male workers afflicted with clinically identifiable symptoms of manganism also have loss of libido and impotence from occupational exposure to manganese for 1-21 years (Emara et al., 1971; Mena et al., 1967; Rodier, 1955; Schuler et al., 1957). Impaired fertility, as measured by fewer children/married couple, has been observed in male workers exposed for 1-19 years to manganese dust at levels that did not produce obvious manganism (0.97 mg/m^3 ; Lauwerys et al., 1985).

Three groups of men occupationally exposed to manganese for 1 or more years (63 miners or ore processors, 38 electric welders in mechanical fields, and 110 electric welders in shipbuilding) were reported to have increased semen liquification time and decreased sperm count and viability (Wu et al., 1996). Matched controls consisted of 99 men who were employed in the same occupation, but were not exposed to manganese or other reproductive toxins. Manganese levels, as well as those of a few other metals, were increased in the semen of the exposed group. Although this study suggests that manganese exposure may cause sperm toxicity, a stepwise regression analysis of the other metals present indicated that the higher nickel concentrations were also associated with lesser semen volume and a greater percentage of deformed sperm. This prevents any conclusive link between manganese and reproductive function.

By contrast, no significant differences in reproductive outcome were found between exposed men and matched controls in a reproductive epidemiology study involving 314 men in a manganese plant (Jiang et al., 1996). The geometric mean airborne manganese concentration was 0.145 mg/m^3 as MnO_2 . The incidences of sexual dysfunction were evaluated through researchers' questions and judged by the occurrence of two positive responses to three potential conditions: impotence, abnormal ejaculation (early ejaculation or non-ejaculation), and lack of sexual desire.

No information was found regarding reproductive effects in women following manganese exposure.

Studies are limited regarding developmental toxicity in humans following oral exposures to manganese. Kilburn (1987) reported an increased incidence in birth defects and stillbirths in a

small population of indigenous peoples in Groote Eylandt, Australia. Although the area was rich in manganese deposits and ingestion of excess amounts of the metal was suspected, the study

suffered from a lack of exposure data, small sample sizes, and no suitable control group. Further, inhalation exposure to manganese could not be ruled out.

Cancer and Mutagenicity Studies

Mutagenicity

The genotoxic potential of high manganese exposure in humans is not known (IPCS, 1999). Elias et al. (1989) found an increase in the incidence of chromosomal aberration in metal active gas welding workers who had been welding for 10-24 years. Occupational exposure to nickel, as well as manganese, was reported. Since nickel is known to cause chromosomal aberration via inhalation, the results could not be attributed solely to the influence of manganese.

Carcinogenicity

No studies are available on the potential carcinogenicity of high exposure to manganese in humans (ATSDR, 2000).

Variation In Human Sensitivity

Individuals that have an impaired excretion and increased retention would be sensitive to manganese toxicity. Reasons for such susceptibility are genetic make-up, developmental stage, age, health and nutritional status. First, individuals with decreased excretion or impaired liver function can be at risk from exposure to excess manganese because the liver is the main organ for excreting manganese. This group may include the elderly who may have declining organ function, the very young who may have immature and developing organs, and those with liver disease. For example, Devenyi et al. (1994) reported observable neurological signs associated with manganese toxicity in individuals with chronic liver disease. Hauser et al. (1996) reported changes in brain MRI scans in liver failure patients which were identical to those observed in cases of manganese intoxication. Second, individuals with increased retention of manganese may be more sensitive to manganese toxicity including those whose nutritional status causes increased uptake of manganese. For example, the very young are considered a potential sensitive population due to the increased retention of manganese in animals (Keen et al., 1986; Kostial et al., 1978; Rehnberg et al., 1980) and humans (Zlotkin and Buchanan, 1986). This increased retention leads to increased manganese in the tissue, especially in the brain (Kontur and Fechter, 1985, 1988; Kostial et al., 1978; Kristensson et al., 1986; Miller et al., 1975; Rehnberg et al., 1981). This is a concern because the nervous system is the primary target organ. Although some data suggest that infants are potentially more susceptible to the toxic effects of manganese, evidence indicates that individual susceptibility varies greatly. The Kawamura et al. (1941) study suggested that young children (age 1 to 6 years) may be less sensitive to manganese toxicity than adults or older people. Current information is not sufficient to quantitatively assess the susceptibility of the young compared to adults.

Although studies are mixed, the majority have also suggested that the elderly (50 years of age or over) are more susceptible to manganese neurotoxicity than the general population (Kawamura et al., 1941; Rodier, 1955; Tanaka and Lieben, 1969). Loss of neuronal cells due to

aging and/or accumulated damage from other environmental neurotoxicants, as well as less effective homeostatic control, may contribute to this increased susceptibility (Silbergeld, 1982).

5.2 Animal Studies

5.2.1 Short-term Exposure Studies

Lethality

Acute lethality of manganese in animals appears to vary depending on the chemical species and whether exposure is via gavage or dietary ingestion (ATSDR, 2000). Single-dose oral LD₅₀ values in adult rats exposed by gavage ranged from 331 mg Mn/kg-day (as manganese chloride; Kostial et al., 1989) to 1,082 mg Mn/kg-day (as manganese acetate; Smyth et al., 1969), while a 14-day exposure of rats to 1,300 mg Mn/kg-day (as manganese sulfate) in feed resulted in no deaths (NTP, 1993).

Manganese compounds administered by parenteral routes generally result in mortality at lower doses. For example, Larsen and Grant (1997) administered a single intravenous dose of 150, 200, 300, or 400 : mol/kg in saline to male mice (5/group). These doses correspond to 8.2, 11, 16, and 22 mg Mn/kg, respectively. These study authors reported an LD₅₀ value of 300 : mol/kg (16 mg Mn/kg). LD₅₀ values for the intraperitoneal route ranged from 14 to 64 mg Mn/kg.

Age may be a factor in susceptibility to acute manganese toxicity. Kostial et al. (1978) found that MnCl₂ produced the greatest oral toxicity in the youngest and oldest groups of exposed rats. Roth and Adleman (1975) proposed that the increased susceptibility of older rats may result from a decrease in adaptive responsiveness, which is characteristic of the aging process. Increased susceptibility of younger rats may reflect high intestinal absorption and body retention of manganese.

General Toxicity

In a 14-day oral exposure study, NTP (1993) administered diets containing 0, 3, 130, 6,250, 12,500, 25,000, or 50,000 ppm manganese sulfate monohydrate to F344 rats (5/sex/dose). All rats survived the exposure period. Statistically-significant differences in manganese-treated rats included reduced body weight gain (57% decrease) and final body weight (13% decrease) in the high-dose males when compared to the control group. Decreased leukocyte and neutrophil counts and reduced liver weight were observed in high-dose males and females. The high-dose groups also exhibited diarrhea during the second week of the study. Manganese concentrations in the livers of animals receiving the 50,000 ppm diet were more than twice those of the controls. The NOAEL and LOAEL values based on decreased weight gain (males) and hematological changes were approximately 650 and 1,300 mg Mn/kg-day, respectively.

NTP (1993) also administered diets containing 0, 3, 130, 6,250, 12,500, 25,000, or 50,000 ppm manganese sulfate monohydrate to B6C3F₁ mice (5/sex/dose) for 14 days. However, study animals were poorly randomized at the beginning of the study, and no effects clearly attributable to manganese exposure were identified.

Exon and Koller (1975) reported that rats administered as little as 6 mg Mn/kg-day as Mn_3O_4 in feed for 28 days gained only 44% as much weight as control rats over the duration of the study. Since no histopathological changes were observed in the exposed animals, the authors suggested that the decrease in body weight gain might have been due to manganese interference in metabolism of calcium, phosphorous, and iron.

Hepatic

Shukla et al. (1978) administered a dose of 16 mg $MnCl_2 \cdot 4H_2O$ /kg (4.4 mg Mn/kg) in drinking water (dose calculated by investigators) to rats for 30 days and evaluated the effect on hepatic enzyme activity. Treated rats revealed significantly decreased succinic dehydrogenase, alcohol dehydrogenase, and α -amylase activity when compared with controls. In contrast, manganese exposure resulted in significantly increased activities of monoamine oxidase (MAO), adenosine triphosphatase, arginase, glutamate pyruvate transaminase (alanine aminotransferase or ALT), ribonuclease, glucose-6-phosphatase, and β -amylase activity in the livers of treated rats.

Hietanen et al. (1981) studied the effect of manganese on hepatic and extrahepatic enzyme activities. Male Wistar rats were exposed to 0.5% Mn (as $MnCl_2$) in the drinking water for 1, 4, or 6 weeks. Assuming an average body weight of 0.35 kg and average water consumption of 0.045 L/day (U.S. EPA, 1986a), this corresponds to an exposure of 0.7 mg Mn/kg-day. Changes in the activity of several enzymes, including aryl hydrocarbon hydroxylase, ethoxycoumarin γ -deethylase, and epoxide hydrolase, were observed at 1 week but not at 6 weeks. Enzyme activities were increased in the liver, and decreased in the intestines and kidney.

Neurological

The central nervous system is the chief target of manganese toxicity. Oral doses ranging from 1 to 150 mg per kg of body weight per day produced a number of neurological effects in rats and mice, mainly involving alterations in neurotransmitter and enzyme levels in the brain. These changes were sometimes accompanied by clinical signs, such as changes in coordination and activity level (ATSDR, 2000).

Deskin et al. (1980) studied neurological alteration induced by manganese chloride in neonatal CD rats. Rats were intubated with 1, 10 or 20 mg Mn/kg-day from birth to 24 days old. Manganese administration (10 and 20 mg/kg-day) resulted in a significant elevation of manganese in the hypothalamic area and corpus striatum, but neurochemical alterations (a decrease in dopamine concentration and turnover) were observed only in the hypothalamic area. The highest dose also resulted in an increase in monoamine oxidase activity in the hypothalamus of treated rats. A subsequent study by Deskin et al. (1981) using the same protocol (but doses of 10, 15 or 20 mg/kg-day) reported a significant elevation in serotonin levels in the hypothalamus, but not the striatum, following exposure to 20 mg/kg-day.

Kontur and Fechter (1988) intubated neonatal Long-Evans rats daily with 0, 25 or 50 mg/kg-day manganese chloride ($\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$) for 14 or 21 days. The level of manganese in the brain was increased at both 14 and 21 days, but was greater at 14 days. However, monoamine and metabolite levels were not altered by manganese treatment in any brain region. The authors suggest that the different results from short-term studies reported by different laboratories may be because of species or strain differences, the dosing regimen or vehicle, the route of administration, or the time points chosen for testing.

Kimura et al. (1978) provided rats with diets supplemented with 564 ppm of manganese as MnCl_2 for 3 weeks. Assuming a food consumption factor of 5% (i.e., 5g diet per 100 g body weight per day), this corresponds to a daily dose of 28 mg Mn/kg-day. The study authors reported that brain serotonin levels were decreased in manganese-treated rats. Monoamine oxidase activity was unchanged, but l-amino-acid decarboxylase activity in the brain was decreased by manganese treatment. Histopathological analysis of the brain was not conducted. Blood serotonin levels were increased in treated rats, and this change was accompanied by decreased blood pressure.

5.2.2 Long-term Exposure Studies

General Toxicology

Chronic ingestion of 1-2 mg Mn/kg-day produced changes in appetite and reduction in hemoglobin synthesis in rabbits, pigs, and cattle (Hurley and Keen, 1987). Transient effects on biogenic amine levels and activities of dopamine β -hydroxylase and monoamine oxidase in rat brain have been noted with long-term exposures to manganese (Eriksson et al., 1987; Lai et al., 1984; Subhash and Padmashree, 1990). An increase in physical activity level and a transient increase in dopaminergic function were observed in rats given 40 mg Mn/kg-day for 65 weeks (Nachtman et al., 1986). Two-year oral exposures to extremely high doses (1800-2250 mg/kg-day as MnSO_4) in male and female mice resulted in hyperplasia, erosion, and inflammation of the forestomach; no effects were seen in rats (NTP, 1993).

Mitochondria-rich organs, such as the liver and pancreas, are hypothesized to be most affected by oral exposure to manganese because of the interaction of manganese with mitochondrial enzymes. Wassermann and Wassermann (1977) reported ultrastructural changes of the liver cells in rats exposed to 200 mg/L of manganese chloride in their drinking water for 10 weeks. Assuming water consumption of 0.05 L/day and an average body weight of 0.35 kg (U.S. EPA, 1986a), this level of exposure corresponds to an average daily dose of approximately 12 mg Mn/kg-day. Increased metabolic activity was inferred from an increased amount of rough endoplasmic reticulum, the occurrence of multiple rough endoplasmic cisternae and prominent Golgi apparatus, and large Golgi vesicles filled with osmiophilic particles in the biliary area of the liver cell. The authors attributed this apparent increase in metabolic activity to biochemical processes related to the nutritional requirement for manganese, and homeostatic processes triggered by increased exposure. They noted that other observed liver effects, including the presence of glycogenosomes in the biliary area, groups of collagen fibers in the Disse's spaces, and degenerative changes in some centrilobular liver cells, may either be direct toxic phenomena or secondary responses to the effect exerted by manganese on other target tissues. ATSDR (2000) evaluated these data and designated 12 mg Mn/kg-day as the NOAEL in

this study.

In a 13-week study, NTP (1993) administered diets containing manganese sulfate at 0, 1,600, 3,130, 6,250, 12,500, or 25,000 ppm ($\text{mg MnSO}_4 \cdot \text{H}_2\text{O}$ per kg diet) to F344 rats (10/sex/dose). The baseline concentration of manganese in the control diets was approximately 92 ppm. Mean daily intake of manganese sulfate monohydrate ranged from 98 mg/kg-day (32 mg Mn/kg-day) for the low-dose to 1,669 mg/kg-day (542 mg Mn/kg-day) for the high-dose males. For females, the range was 114 mg/kg-day (37 mg Mn/kg-day) for the low-dose group and 1,911 mg/kg-day (621 mg Mn/kg-day) for the high-dose group. No rats died during the study, and no clinical or histopathology findings were attributed to manganese exposure. Females receiving diets with $\geq 6,250$ ppm manganese sulfate experienced decreased body weight gain. Absolute and relative liver weights were decreased in males receiving diets with $\geq 1,600$ ppm, and in females in the highest dose group only. Hematological effects were also reported. All groups of exposed males exhibited a significantly increased neutrophil count. Lymphocyte counts were decreased in males receiving $\geq 6,250$ ppm in the diet and females in the three highest dose groups. The low dose of 1,600 ppm (about 32 mg Mn/kg-day) was identified as the LOAEL for this study, based on effects on liver weight and neutrophil counts in male rats.

In a concurrent 13-week study, NTP (1993) administered diets containing manganese sulfate (monohydrate) at 0, 3,130, 6,250, 12,500, 25,000, or 50,000 ppm to B6C3F₁ mice (10/sex/dose). The baseline concentration of manganese in the control diets was approximately 92 ppm. Mean daily intake of manganese sulfate monohydrate ranged from 328 mg/kg-day (107 mg Mn/kg-day) for the low-dose to 8,450 mg/kg-day (2,746 mg Mn/kg-day) for the high-dose group. No deaths were attributed to manganese exposure. Both male and female mice in the highest dose group exhibited significantly decreased body weight gain. The male mice in the highest dose group also had decreased relative and absolute liver weights. Both sexes at the highest dose exhibited decreased hematocrit and hemoglobin concentrations. The NTP report suggests that these findings may indicate microcytic anemia, which may have resulted from a sequestration or deficiency of iron. Males receiving $\geq 25,000$ ppm also exhibited significantly lower leukocyte counts, although this finding was of questionable relevance to manganese exposure. No clinical findings were attributed to manganese exposure. The LOAEL for this study was 3,130 ppm (107 mg Mn/kg-day), based on significantly decreased body weight gain in male mice.

Komura and Sakamoto (1991) investigated the effect of different forms of manganese on potential adverse effects following ingestion exposure to the element. Male mice (8/group) were exposed either to a control diet containing 130 mg Mn/kg, or a diet supplemented with an additional 2,000 mg Mn/kg as $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{Mn}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$, MnCO_3 , or MnO_2 . Assuming an average food consumption of 13% of body weight, the average daily dose from the control diet was approximately 17 mg Mn/kg-day, while the average daily dose from the manganese-enriched diet was 276 mg Mn/kg-day. The duration of treatment was 100 days. The mice were tested for spontaneous motor activity after 30 days. Blood and tissues were analyzed at the termination of the experiment. No significant difference in food intake among groups was seen. Body weight gain and red and white blood cell count was decreased in groups that received $\text{Mn}(\text{CH}_3\text{COO})_2 \cdot 4\text{H}_2\text{O}$ or $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$. Motor activity was reduced in the MnCO_3 group. Tissue manganese concentrations in groups receiving supplemental manganese were 2 to 3 times that of controls. A LOAEL of 276 mg Mn/kg-day was identified in this study based on

decreased weight gain and hematological effects.

Hepatic

Leung et al. (1982) administered 1,000, 10,000, or 20,000 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}/\text{L}$ in drinking water to female Wistar rats. Exposure was initiated at conception by administration of manganese-containing drinking water to the dams, and continued through age 60 days. The estimated doses were 38.9, 389, and 778 mg Mn/kg-day (U.S. EPA, 1993). Treated rats exhibited liver necrosis and ultrastructural alterations that resembled human cholestasis. A LOAEL of 38.9 mg Mn/kg-day was identified in this study based on hepatic necrosis.

Suzuki et al. (1975) administered 250, 500, or 1,000 mg of MnO_2 in saline to 4 kg monkeys (*Macaca mullata*, age not specified) by subcutaneous injection. Injections were given once a week for 9 weeks. Estimated time-averaged doses correspond to 5.6, 11, and 23 mg Mn/kg-day. At autopsy, manganese-treated monkeys had irregular arrangement of hepatic cords and lymphocytic infiltration.

Neurological

Neurotoxicity is a known effect of long-term exposure to inhaled manganese in humans and animals, but the potential for neurotoxicity resulting from oral exposure is less well characterized. The only report of neurobehavioral toxicity in primates from orally administered manganese is by Gupta et al. (1980). Muscular weakness and lower limb rigidity were observed in 4 male rhesus monkeys given oral doses of manganese chloride (25 mg $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}/\text{kg}$, 6.9 mg Mn/kg-day) for 18 months. Histologic analysis showed degenerated neurons in the substantia nigra of the exposed animals at autopsy. There were no biochemical data. This study is of limited use for risk assessment because only one dose level was evaluated.

Studies involving oral exposures of manganese in drinking water or by gavage in neonatal rodent pups have reported changes in brain neurochemistry but generally do not show significant adverse effects on neurological development (ATSDR, 2000). Dorman et al. (2000) reported on neurological changes in rat pups dosed for 21 days postnatally with 11 or 22 mg Mn/kg-day by mouth in drinking water. The high dose group had significant increases in brain striatal DA (dopamine) and DOPAC (dihydroxyphenylacetic acid) concentrations and exhibited significant increases in the startle response, in the absence of pathological lesions. Because manganese is an essential nutrient in developing infants, the potential adverse effects from manganese deficiency may be of greater concern than potential toxicity from over-exposure.

Chandra et al. (1979) evaluated the neurological effects of manganese in mice exposed from birth. Neonatal mice were initially exposed by nursing from dams given 5 mg/mL MnCl_2 in their drinking water. After weaning at 25 days, the mice received manganese in their drinking water. Average exposures to manganese were determined to be 0.030 mg Mn/day for 60 days, 0.036 mg Mn/day through the 90th day, 0.075 mg Mn/day through the 120th day and 0.090 mg Mn/day for the interval between 150 and 180 days. Assuming a body weight of 0.03 kg at adulthood, the average daily dose at the termination of the experiment was approximately 3 mg Mn/kg-day. Elevated levels of striatal dopamine, norepinephrine, and homovanillic acid were

observed at 60 and 90 days of age, with a concomitant increase in spontaneous locomotor activity. Exposure past 90 days did not influence motor activity. Chandra et al. (1979) proposed that the hyperactivity observed in these mice was an early behavioral effect of excess manganese exposure that resulted from elevated dopamine and norepinephrine levels. The study authors further suggested that the observed hyperactivity may be comparable to the psychomotor excitement observed in the early stages of human manganese.

Chandra and Shukla (1981) exposed male albino rats to 1,000 mg/L $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (436 mg Mn/L) in drinking water. Assuming water consumption of 0.049 L/day and an average adult body weight of 0.35 kg, this level of exposure corresponds to an average daily dose of 61 mg Mn/kg-day. Levels of catecholamines, homovanillic acid, manganese, and the activity of monoamine oxidase were determined in the corpus striatum at time intervals up to 360 days. The investigators found initial increases in dopamine, norepinephrine, and homovanillic acid levels. This initial increase was followed by a period of normal levels. After 300 days, a decrease in all levels was observed. These changes were not correlated with the tissue concentration of manganese. The authors suggested that the decreased locomotor activity observed during later periods of manganese exposure may be related to lowered dopamine and norepinephrine levels in the brain, and that this stage of chronic toxicity may correspond to the later neurologic phase of motor dyskinesia in humans. Ali et al. (1981) conducted concurrent behavioral studies, and found an initial increase in spontaneous locomotor activity followed by a decrease during later periods of manganese exposure.

Purdey (2000) examined an endemic of sheep scrapie (a form of transmissible spongiform encephalopathy) in North Central/Eastern Iceland. Purdey reported high (200 ppm dry weight) levels of manganese in the herbage of areas where the sheep had suffered from a high incidence of scrapie for decades. Areas that were scrapie free had a mean level of 80 ppm dry weight of manganese in the herbage. These data, along with the data on CJD in humans (Purdey, 2000; Brown et al., 2000), suggest a link between high manganese and low copper in the etiology of these degenerative neurologic diseases, but further data are needed to support the hypothesis.

Reproductive and Developmental Studies

Reproductive Effects

The results of several studies in rats and mice indicate that the ingestion of high dose of manganese can delay reproductive maturation in male animals (ATSDR, 2000). Testosterone levels were reduced in male rats given an oral dose of 13 mg Mn/kg-day for 100-224 days (Laskey et al., 1982), while delayed growth of the testes was observed in young rats ingesting 140 mg Mn/kg-day for 90 days (Gray and Laskey, 1980). These effects do not appear to be severe enough to affect male reproductive function (ATSDR, 2000). Several studies which found effects on male reproductive organs, however, did not assess reproductive performance (IPCS, 1999).

Laskey et al. (1982) found a slight decrease in pregnancy rate but no significant effect on litter size, ovulations, resorption, or fetal weight when male and female rats were exposed to 130 mg Mn/kg-day (as Mn_3O_4) in the diet for 90-100 days prior to breeding.

The results of most studies indicate that oral exposure to manganese does not result in reproductive toxicity in the female rodent (e.g., rats and mice) and rabbit (See also ATSDR, 2000), although increased postimplantation loss was observed in female rats in at least one study (Szakmáry et al., 1995).

Developmental Effects

Results from several developmental studies in rodents and rabbits are equivocal. Data from the majority of these studies indicate that manganese exposure during part or all of gestation results in increased manganese levels in the pups (Järvinen and Ahlström, 1975; Kontur and Fechter, 1988), but generally causes either no measurable effect (Grant et al., 1997), transient effects such as weight decreases and hyperactivity (Pappas et al., 1997), or reversible effects on skeletal and organ development (Szakmáry et al., 1995). Joardar and Sharma (1990) administered varying levels of MnSO₄ (10.25, 20.25, and 61.00 mg/100 g bw) and KMnO₄ (6.5, 13, and 36 mg/100 g bw) to mice by gavage over a 3-week period. Sperm head abnormalities and the percentage of abnormal sperm were significantly increased in all treated groups.

Cancer and Mutagenicity Studies

Mutagenicity

Laboratory evidence for the mutagenicity and genotoxicity of high dose manganese exposure is equivocal. Joardar and Sharma (1990) administered varying levels of MnSO₄ (10.25, 20.25, and 61.00 mg/100 g bw) and KMnO₄ (6.5, 13, and 36 mg/100 g bw) to mice over a 3-week period. The frequencies of chromosomal aberrations and micronuclei in bone marrow cells were significantly increased. Dikshith and Chandra (1978) administered repeat oral doses of 0.014 mg Mn/kg-day (as MnCl₂) to albino rats for 180 days with no significant chromosomal damage noted in either bone marrow or spermatogonial cells.

In vitro bacterial gene mutation tests have yielded both positive and negative results, while *in vitro* tests with fungi and mammalian cells have been predominantly positive. Manganese chloride produced an increased frequency of mutations in *Salmonella typhimurium* strain TA1537, but induced negative results in other strains; manganese sulfate was reported to be both positive and negative in separate studies in *Salmonella* strain TA97, but negative in other strains (IPCS, 1999). Positive results were obtained with various manganese compounds in *Phytobacterium fischeri* and *Escherichia coli*, as well as in *Saccharomyces cerevisiae* and hamster embryo cells (ATSDR, 2000). In spite of these results, the genotoxic potential of manganese in humans is not known (IPCS, 1999).

Carcinogenicity

No animal studies are available that have investigated the potential carcinogenicity of manganese following inhalation or dermal exposure (ATSDR, 2000). A 2-year oral study of manganese sulfate in rats and mice produced equivocal evidence of carcinogenicity (NTP, 1993). In rats fed manganese sulfate (30-331 mg Mn/kg-day in males, 26-270 mg Mn/kg day in females), no treatment-related increases in tumor incidence were reported. In mice fed manganese sulfate (63-722 mg Mn/kg-day in males, 77-905 mg Mn/kg-day in females), the incidence of follicular cell adenoma of the thyroid was increased slightly in high-dose animals compared to controls. These increases were not statistically significant, and the tumors were observed at the end of the study only. However, follicular cell adenoma of the thyroid appears with low frequency in historical control male mice of this strain. Thus, the significance of these results and their relevance to typical human oral exposure to manganese is questionable.

Stoner et al. (1976) tested manganese sulfate in a mouse lung adenoma screening bioassay. These investigators exposed 6- to 8-week-old Strain A/Strong mice of both sexes (10/sex) to 6, 15 or 30 mg MnSO₄/kg via intraperitoneal injection. Doses were administered three times a week for a total of 21 injections. The cumulative doses were 132, 330 and 660 mg MnSO₄/kg, corresponding to 42.9, 107.2 and 214.4 mg Mn/kg. Observation continued for 22 weeks after the dosing period, and the mice were sacrificed at 30 weeks. The percentage of mice with tumors was elevated at the highest dose level, but the difference was not significant when compared with the vehicle controls. An apparent increase in the average number of pulmonary adenomas per mouse was noted both at the middle and high doses, but the increase was significant only at the high dose (660 mg MnSO₄/kg; $p < 0.05$). Although the study results are suggestive of carcinogenic activity, they do not conclusively meet the positive-response criteria (increased tumor incidence and an observable dose-response relationship) for the interpretation of lung tumor data in this mouse strain (Shimkin and Stoner, 1975).

6.0 QUANTIFICATION OF TOXICOLOGICAL EFFECTS

Limitations of Using Rodent Data In Assessing Neurotoxicity of Manganese

There are considerable species differences between rodents and primates in nutritional requirements as well as neurotoxicity of manganese. Therefore, rodents are of limited value in assessing the neurobehavioral effects associated with extrapyramidal deficits (Chandra, 1983).

Manganese has a propensity for accumulation in the melanin pigment (Lyden et al., 1985) and there is a relative lack of neuromelanin in rodents. This may explain the fact that neurologic effects (e.g., tremor, gait disorders) seen in primates are often preceded or accompanied by psychologic symptoms (e.g., irritability, emotional lability) but are not apparent in rodents.

Contributing to the difficulties in interpreting the toxicologic data from exposure of rodents to manganese is the substantial difference in species' requirements for this dietary element. The estimated requirement for rats is 50 mg Mn/kg diet (Rogers, 1979). Assuming a

food consumption equivalent to 5% of body weight (U.S. EPA, 1986a), this corresponds to a requirement for about 2.5 mg Mn/kg body weight (bw)/day. In contrast, the adequate intake for men and women (including lactating women) is about 2.3 -2.6 mg Mn/day, or about 0.03 - 0.07 mg Mn/kg bw/day, assuming a reference body weight of 70 kg. The dietary requirement for manganese in humans, then, is about two orders of magnitude lower than for rodents, suggesting that data derived from rodent studies may not be appropriate for use in deriving quantitative estimates of manganese levels that might be expected to result in adverse effects in humans.

As discussed above, rodent studies are limited in their use as a database from which to extrapolate effects in humans from over-exposure to manganese, because rodents do not exhibit the same neurologic deficits that humans do following exposure to manganese. On the other hand, the optimal levels of oral exposure to manganese for humans have not been well defined. For example, the available epidemiological studies in drinking water are of limited use in quantitative assessment of manganese toxicity, because of a lack of total exposure data. Balance studies are also not useful because short and moderate-term manganese balance studies are found not to be proportional to manganese intakes (Greger, 1999). Therefore, the health advisories (acute and chronic) are based on human dietary studies (See Sections below).

Dose Response and Risk Characterization

Manganese is a ubiquitous element that is essential for normal physiologic functioning in all animal species. Several disease states in humans have been associated with both deficiencies and excess intakes of manganese. Thus any quantitative risk assessment for manganese must take into account aspects of both the essentiality and the toxicity of manganese. In humans, many data are available providing information about the range of essentiality for manganese. In addition, there are many reports of toxicity to humans exposed to manganese by inhalation; much less is known, however, about oral intakes resulting in toxicity. As discussed above, rodents do not provide a good experimental model for manganese toxicity, and only one limited study in primates by the oral route of exposure is available (Gupta et al., 1980). The following assessment, therefore, focuses more on what is known to be a safe oral intake of manganese for the general human population. Finally, it is important to emphasize that individual requirements for, as well as adverse reactions to, manganese may be highly variable. The reference dose is estimated to be an intake for the general population that is not associated with adverse health effects; this is not meant to imply that intakes above the reference dose are necessarily associated with toxicity. Some individuals may, in fact, consume a diet that contributes more than 10 mg Mn/day without any cause for concern.

Determination of Health Advisories

Health Advisories (HAs) are generally determined for one-day, ten-day and life time exposure if adequate data are available that identify a sensitive noncarcinogenic end point of toxicity. The HAs for noncarcinogenic toxicants are derived using the following formula:

$$HA = \frac{(\text{NOAEL or LOAEL}) \times (\text{BW})}{(\text{UF}) (\text{L/day})} = \text{mg/L} (\text{: g/L})$$

where:

NOAEL or LOAEL = No- or Lowest-Observed-Adverse-Effect Level (in mg/kg bw/day).

BW = assumed body weight of a child (10 kg) or an adult (70 kg).

UF = uncertainty factor (10, 100, 1,000 or 10,000) in accordance with EPA or NAS/ODW guidelines.

L/day = assumed daily water consumption of a child (1 L/day) or an adult (2 L/day).

One-day HA

No suitable information was found in the available literature for determining the One-day HA for manganese. The Ten-day HA for a child of 1 mg/L, calculated below is recommended for use as a conservative estimate for a 1-day exposure for both children and adults.

Ten-day HA

The adequate intake for a child 7 to 12 months old is 0.6 mg/day, and that from a 1 to 3-year-old is 1.2 mg/day (IOM, 2002). Taking the upper end of the adequate intake for a 10 kg child (up to 1 mg/day), and assuming the manganese comes from a maximum of 1 liter of formula per day, this would correspond to a manganese concentration of 1 mg/L. This 10-day HA for a child should also be protective of adults.

The Ten-day HA for a 10-kg child is calculated as follows:

$$1\text{- and }10\text{-day HA} = \frac{1 \text{ mg/day}}{1 \text{ L/day}} = 1 \text{ mg/L}$$

However, it is advised that for infants younger than 6 months, the lifetime HA of 0.3 mg/L be used even for an acute exposure of 10 days, because of the concerns for differences in manganese content in human milk and formula and the possibility of a higher absorption and lower excretion in young infants.

Lifetime Health Advisory

Lifetime health advisories are only developed for chemicals that are not likely to be carcinogenic to humans. The Lifetime HA represents that portion of an individual's total exposure that is attributed to drinking water and is considered protective of noncarcinogenic adverse health effects over a lifetime exposure. The Lifetime HA is derived in a three-step process. Step 1 determines the Reference Dose (RfD), formerly called the Acceptable Daily Intake (ADI). 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 appreciable risk of deleterious health effects during a lifetime, and is derived from the NOAEL (or LOAEL), identified from a chronic (or subchronic) study, divided by an uncertainty factor(s). From the RfD, a Drinking Water Equivalent Level (DWEL) can be determined (Step 2). A DWEL is a medium-specific (i.e., drinking water) lifetime exposure level, assuming 100% exposure from that medium, at which adverse, noncarcinogenic health effects would not be expected to occur. The DWEL is derived from the multiplication of the RfD by the assumed body weight of an adult and divided by the assumed daily water consumption of an adult. The Lifetime HA in drinking water alone is determined in Step 3 by factoring in other sources of exposure, e.g., the relative source contribution (RSC). The RSC from drinking water is based on actual exposure data or, if data are not available, a value of 20% is assumed.

Step 1: Determination of Reference Dose (RfD)

Choice of Principal Study and Critical Effect

Manganese is an essential trace element that is required for normal physiologic function in humans and animals. Excess exposure to manganese, particularly via the inhalation route, is associated with neurotoxicological symptoms that resemble parkinsonism. Thus, derivation of the RfD must consider issues of both essentiality and toxicity.

The RfD is not based on rodent studies, because rodents do not exhibit the same neurologic deficits that humans do following exposure to manganese. For example, manganese at high doses induces Parkinson-like symptoms in humans and primates, but not in rodents. Because of the species difference in the response to manganese exposure, rodents are not good models for manganese toxicity studies. More details on these species differences can be seen in IRIS (U.S. EPA, 1997).

The oral toxicity data on which risk assessments may be based are quite limited in scope. It is recognized that the information available in humans is inherently more useful than data obtained from laboratory animals, especially non-primates. However, the toxicity data in humans following ingestion of large amount of manganese are not suitable for a quantitative assessment (For details, See Section 5.1.2 Long-term Exposure).

Dose-Response Assessment

Based on the dietary information described by WHO (1973), Schroeder et al. (1966), and NRC (1989), EPA estimated that an intake of 10 mg Mn/day (0.14 mg Mn/kg-day, assuming a body weight of 70 kg) in the diet is safe for a lifetime of exposure. This level of manganese represents

a NOAEL for chronic ingestion of manganese by humans. Application of a UF of 1 was used to derive the dietary RfD of 0.14 mg Mn/kg-day (U.S. EPA, 1997). The use of 1 as the UF is based on the following considerations. Manganese is an essential trace element for human health. The information used to derive the RfD was collected from many large human populations consuming normal diets over an extended period of time. The available data suggest that as long as physiological systems are not overwhelmed, humans exert effective homeostatic control over manganese so that body burden is kept relatively constant even when the concentration of manganese in the diet varies.

Application of Modifying Factor in Water

U.S. EPA (1997) has recommended the use of a modifying factor of 3 when assessing exposure to manganese from drinking water. Four reasons for this recommendation have been outlined:

- While toxicokinetic data suggest that there is no significant difference in absorption of manganese from food versus water, uptake of manganese from water appears to be greater in fasted individuals.
- The study by Kondakis et al. (1989) raises concern for possible adverse health effects associated with a lifetime consumption of drinking water containing 2 mg/L of manganese.
- Evidence exists that neonates absorb more manganese from the gastrointestinal tract, and excrete less of the absorbed manganese. Additional evidence suggests that absorbed manganese more easily crosses the blood-brain barrier in neonates. However, this evidence comes from animal studies; similar absorption studies in human neonates have not been performed, although Collipp et al. (1983) observed increased hair manganese levels in infants fed prepared formula compared with infants fed breast milk.
- Infant formula typically contains a much higher concentration of manganese than human or cows' milk. Powdered formula reconstituted with drinking water represents an additional source of manganese intake for a potentially sensitive population.

The potential impacts on children, when considered in conjunction with the likelihood that the most adverse effects of manganese (e.g., those seen in manganese miners or others with chronic overexposure to inhaled manganese) are likely to be irreversible and not manifested for many years after exposure, warrant caution until more definitive data are available (U.S. EPA, 1997). Recent data indicate, however, that in contrast to the symptoms of manganism, preclinical neurological effects of inhalation exposure of occupational workers to excess manganese are reversible (Roels et al., 1999). Similarly, symptoms of oral exposure to excess manganese in compromised individuals (e.g., individuals with liver disease who could not excrete manganese in the bile) were resolved when the exposure to excess manganese was decreased (Devenyi et al., 1994; Fell et al., 1996). These data indicate that the human body can recover from certain adverse effects of overexposure to manganese if the exposure is stopped

and the body can clear the excess. Significant uncertainty still exists, however, concerning at what level of manganese intake these preclinical neurological symptoms might occur.

The RfD for chronic exposure to manganese in drinking water is therefore calculated as follows:

$$\text{RfD} = \frac{10 \text{ mg/day}}{1 \times 70 \text{ kg}} = 0.14 \text{ mg/kg-day}$$

where:

10 mg/person-day = chronic no adverse effect level per person from dietary intake

1 = uncertainty factor

70 kg = assumed body weight of adult

Step 2: Determination of the Drinking Water Equivalent Level (DWEL)

$$\text{DWEL} = \frac{(0.14 \text{ mg/kg-day})(70 \text{ kg})}{3 (2 \text{ L/day})} = 1.6 \text{ mg/L (1600 : g/L)}$$

where:

0.14 mg/kg-day = RfD

70 kg = assumed body weight of adult

2 L/day = assumed water consumption by 70-kg adult

3 = modifying factor for assessing exposure to manganese from drinking water (mainly for bioavailability concerns)

Step 3: Determination of the Lifetime HA

The Lifetime HA = (1.6 mg/L)(20%) = 0.3 mg/L (rounded from 0.32 mg/L)

where

1.6 mg/L = DWEL

20% = relative source contribution for manganese in drinking water

Evaluation of Carcinogenic Potential

- Available data are equivocal regarding carcinogenic potential of manganese.
- Based on the Draft Guidelines for Carcinogen Risk Assessment (U.S. EPA, 2003b; 1999), there is inadequate information to assess the human carcinogenic potential for manganese.
- Based on 1986 Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986b) manganese has been classified in Group D: Not classified as to human carcinogenicity.

7.0 ANALYTIC METHODS AND TREATMENT TECHNOLOGY

Analytical Methods

Manganese can be measured by several well-documented analytical methods as shown in the Table 7-1.

Treatment Technology

The technologies include conventional treatment, ion exchange, reverse osmosis, lime softening, and chemical precipitation.

Conventional treatment usually includes pre-treatment steps of chemical coagulation, rapid mixing, and flocculation, followed by flocculation removal via sedimentation or flotation. After clarification, the water is then filtered. Common filter media include sand, and dual- and tri-media (e.g., silica sand, garnet sand, or anthracitic coal).

Ion exchange involves the selective removal of charged inorganic species from water using an ion-specific resin. The surface of the ion exchange resin contains charged functional groups that hold ionic species by electrostatic attraction. As water containing contaminant ions passes through a column of resin beds, charged ions on the resin surface are exchanged for the contaminant species in the water.

Reverse osmosis (RO) is similar to other membrane processes, such as ultrafiltration and nanofiltration, since water passes through a semi-permeable membrane. However, in the case of RO, the principle involved is not filtration. Instead, it involves the use of applied hydraulic pressure to oppose the osmotic pressure across a non-porous membrane, forcing the water from the concentrated solution side to the dilute solution side. The water does not travel through pores, but rather dissolves into the membrane, diffuses across, then dissolves out into the permeate. Most inorganic and many organic contaminants are rejected by the membrane and will be retained in the concentrate.

In the lime-softening process, the pH of the water being treated is raised sufficiently to precipitate calcium carbonate and, if necessary, magnesium hydroxide. Calcium and magnesium

ions in water cause hardness. After mixing, flocculation, sedimentation, and pH readjustment, the softened water is filtered.

Results of a preliminary technology assessment and review indicate that all of the above-mentioned techniques remove manganese from water. However, data indicate that chemical precipitation is the most effective option.

Table 7-1: Analytical Methods for Manganese

Method	Type	Method Detection Limit (µg/L)
EPA 200.7	Inductively Coupled Plasma Optical Emission Spectrometry (ICP)/Atomic Emission Spectrometry	1.0
SM 3120 B	ICP/Atomic Emission Spectrometry	Estimated Detection Limit (EDL) 2.0
EPA 200.8	ICP/Mass Spectrometry	0.02
SM 3111B	Atomic Absorption, direct aspiration	Instrument Detection Level (IDL) 10 Optimum conc. range 100-10,000
EPA 200.9	Stabilized Temperature Graphite Furnace AA Spectrometry	0.3
SM 3113 B	Atomic Absorption, Furnace	EDL 0.2 Optimum conc. range 1-30

8.0 OTHER CRITERIA, GUIDANCE AND STANDARDS

- There is no current Maximum Contaminant Level (MCL) for manganese.
- OSHA (1998) has established a maximum permissible air exposure limit for manganese fumes at no greater than 5 mg/m³ and elemental or inorganic manganese at no greater than 0.2 mg/m³, averaged over any 8-hour period in the workplace environment.

- The World Health Organization (WHO) has established a provisional guideline value for manganese of 0.5 mg/L. This guideline is provisional because there is some evidence of a potential hazard, but available information on health effects is limited. Concentrations of this substance at or below the health-based guideline value may affect appearance, taste, or odor of water.
- EPA recommends a concentration of manganese in drinking water not to exceed 0.05 mg/L (ppm). This recommendation is to avoid staining of clothing and fixtures and is believed to be more than adequate to protect human health.
- The Food and Drug Administration (FDA) also recommends 0.05 mg/L of manganese in bottled water.
- EPA has also established rules setting limits on the amount of manganese factories can discharge to the water.

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