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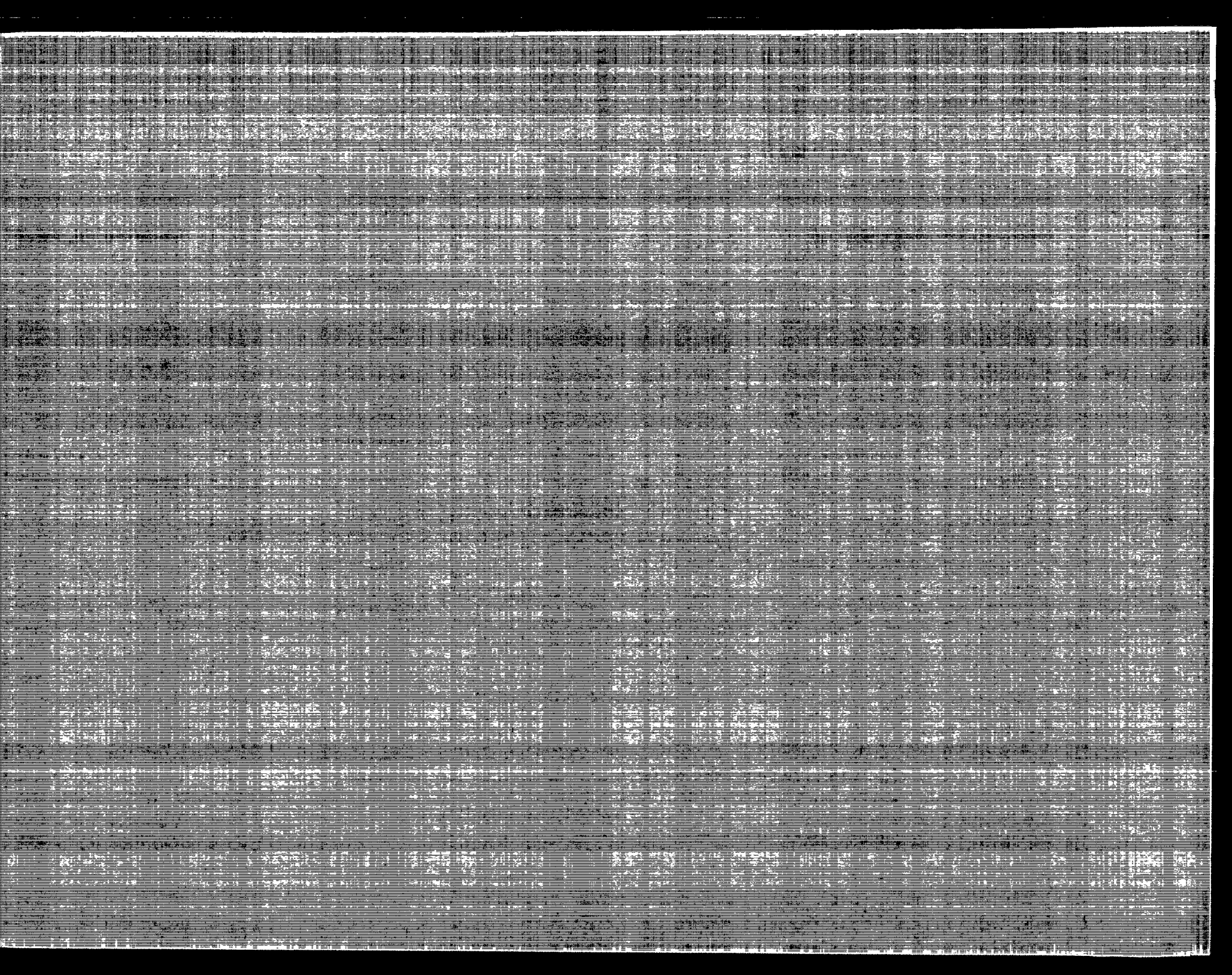
Health Effects Support Document for Manganese

External Review Draft



Health Effects Support Document for Manganese

EXTERNAL REVIEW DRAFT

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FOREWORD

The Safe Drinking Water Act (SDWA), as amended in 1996, requires the Administrator of the Environmental Protection Agency to establish a list of contaminants to aid the agency in regulatory priority setting for the drinking water program. In addition, SDWA requires EPA to make regulatory determinations for no fewer than five contaminants by August 2001. The criteria used to determine whether or not to regulate a chemical on the CCL are the following:

The contaminant may have an adverse effect on the health of persons.

The contaminant is known to occur, or there is a substantial likelihood that the contaminant will occur, in public water systems with a frequency and at levels of public health concern.

In the sole judgment of the administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems.

The Agency's findings for all three criteria are used in making a determination to regulate a contaminant. The Agency may determine that there is no need for regulation when a contaminant fails to meet one of the criteria. The decision not to regulate is considered a final agency action and is subject to judicial review.

This document provides the health effects basis for the regulatory determination for manganese. In arriving at the regulatory determination, data on toxicokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology, and mechanisms of toxicity were evaluated. In order to avoid wasteful duplication of effort, information from the following risk assessments by the EPA and other government agencies were used in development of this document:

U.S. EPA 1994a. U.S. Environmental Protection Agency. Drinking Water Criteria Document for Manganese. Office of Health and Environmental Assessment, Cincinnati, OH CEAO-CIN-D008, prepared September, 1993, revised March 31, 1994.

ATSDR. 2000. Agency for Toxic Substances and Disease Registry. Toxicological Profile for Manganese (Update). Department of Health and Human Services. Atlanta, GA. Available at http://www.atsdr.cdc.gov.

U.S. EPA 1996a. U.S. Environmental Protection Agency. Integrated Risk Information System (IRIS): Manganese. Available at http://www.epa.gov/iris. Last revised December 1, 1996.

In addition, primary references of studies published in peer-reviewed scientific journals relevant to human risk assessment of manganese were also used in preparing this Drinking Water

Support Document for Manganese. Recent studies of manganese were identified by literature searches conducted in 1999 and 2000.

Generally a Reference Dose (RfD) is provided as the assessment of long-term toxic effects other than carcinogenicity. RfD determination assumes that thresholds exist for certain toxic effects such as cellular necrosis. It is expressed in terms of milligrams per kilogram per day (mg/kg-day). In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime.

The carcinogenicity assessment for manganese includes a formal hazard identification. Hazard identification is a weight-of-evidence judgment of the likelihood that the agent is a human carcinogen via the oral route.

Guidelines that were used in the development of this assessment may include the following: the *Guidelines for Carcinogen Risk Assessment* (U.S. EPA, 1986a), *Guidelines for the Health Risk Assessment of Chemical Mixtures* (U.S. EPA, 1986b), *Guidelines for Mutagenicity Risk Assessment* (U.S. EPA, 1986c), *Guidelines for Developmental Toxicity Risk Assessment* (U.S. EPA, 1991a), *Proposed Guidelines for Carcinogen Risk Assessment* (1996b), *Guidelines for Reproductive Toxicity Risk Assessment* (U.S. EPA, 1996c), and *Guidelines for Neurotoxicity Risk Assessment* (U.S. EPA, 1998a); *Recommendations for and Documentation of Biological Values for Use in Risk Assessment* (U.S. EPA, 1988); and Health Effects Testing Guidelines (OPTS series 870, 1996 drafts; U.S. EPA 40 CAR Part 798, 1997); *Peer Review and Peer Involvement at the U.S. Environmental Protection Agency* (U.S. EPA, 1994b); *Use of the Benchmark Dose Approach in Health Risk Assessment* (U.S. EPA, 1995).

The chapter on occurrence and exposure to manganese through potable water was developed by the Office of Ground Water and Drinking Water. It is based primarily on unregulated contaminant monitoring (UCM) data collected under SDWA. The UCM data are supplemented with ambient water data as well as information on production, use, and discharge.

ACKNOWLEDGMENTS

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1.0 EXECUTIVE SUMMARY

The U.S. Environmental Protection Agency (EPA) has prepared this Health Effects Support Document to assist in determining whether to establish a National Primary Drinking Water Regulation (NPDWR) for manganese. At high doses by inhalation, manganese is very toxic, as seen by occupational exposure in miners. On the other hand, manganese is essential for normal physiological function of animals and humans. The Food and Nutrition Board of the National Academy of Science (NAS) sets an adequate intake for manganese at 2.3 mg/day for men and 1.8 mg/day for women, and an upper limit for daily intake at 11 mg for adults (Food and Nutrition Board, 2002). Manganese has a low aesthetic threshold in water. Based on staining and taste, EPA has set a secondary level for manganese at 0.05 mg/L which is below the level that may present a health concern. Available data suggest that regulation of manganese in public water does not present a meaningful basis for health risk reduction. EPA will present a determination and further analysis in the Federal Register Notice covering the Contaminant Candidate List proposals.

Manganese (Chemical Abstracts Services Registry Number 7439-96-5) is an abundant elemental metal that does not exist naturally in its pure form, but rather is found as a component of over 100 minerals. It is also an essential nutrient, and a certain level of intake is necessary for good health. The Food and Nutrition Board of the NAS has determined that the Adequate Intake for manganese (AI) is 1.8 to 2.3 milligrams per day for an adult woman and man, respectively, although others have argued that it may be higher. Manganese occurs naturally in soil, air, water, and food at low levels.

Manganese and manganese compounds are used mostly in the production of manganese-iron alloy through a smelting process. They are also used in fertilizer, fungicide, livestock feed, and in unleaded gasoline as an anti-knock additive in the form of methylcyclopentadienyl manganese tricarbonyl (MMT). Any of these uses may result in substantial releases of manganese to the environment. Manganese is listed as a Toxic Release Inventory (TRI) chemical, with releases to soil constituting most of the on-site releases, although air, surface water and ground water are also important sinks for manganese release.

Human exposure to manganese occurs primarily through ingestion of foods containing manganese. These include many nuts, grains, fruits, legumes, tea, leafy vegetables, infant formulas, and some meat and fish. The relatively high levels of manganese in nuts, grains, and many plant products and infant formulas are not well absorbed upon ingestion because these foods also contain inhibitors of manganese absorption such as phytates, fiber, plant protein and polyphenolic compounds (tannins). Manganese absorption is affected by other factors including age (neonate compared to the adult), chemical species of manganese, dose, and route of exposure in addition to the dietary factors mentioned above. Human exposure to manganese may also occur through inhalation of manganese dust, intake of soil containing manganese compounds, or drinking water contaminated with manganese.

The primary target of manganese toxicity is the nervous system, and common symptoms of toxic exposure include ataxia, dementia, anxiety, a "mask-like" face, and manganism, a syndrome similar to Parkinson's disease. These effects, when observed, are generally the result of very high exposures via inhalation, as might occur in an industrial setting, and are not seen among the general population exposed to low or moderate manganese levels. Manganese has very low toxicity by oral ingestion and reports of adverse effects by this route are rare. Because manganese is an essential nutrient, concern for toxic over-exposure must be balanced against the potentially negative effects of nutritional deficiency resulting from under-exposure.

An epidemiological study performed in Peloponnesus, Greece (Kondakis et al., 1989) showed that lifetime consumption of drinking water containing naturally high concentrations of manganese oxides may lead to neurological symptoms and increased manganese retention (through the concentration of manganese in hair) for people over 50 years old. For the group consuming the highest concentration (around 2 mg/L) for more than ten years, the authors suggested that some neurologic impairment may be apparent. The study raises concerns about possible adverse neurological effects following chronic ingestion from drinking water at doses within ranges deemed essential. However, the study did not examine manganese intake data from other routes/sources (i.e., dietary intake, inhalation from air, etc.), precluding its use as a basis for the RfD.

Another long-term drinking water study in Germany (Vieregge et al., 1995) found no neurological effects in people older than 50 years of age who drink water containing 0.3 to 2.16 mg/L of manganese for more than ten years. However, this study also lacks exposure data from other routes and sources, and the manganese concentration range in water is very wide. Thus, the study cannot be used for quantitative assessment.

A small Japanese community (total 25 individuals) ingested high levels of manganese in contaminated well water (that leaked from dry cell batteries buried near the wells) over a three-month period (Kawamura et al., 1941). Manganese intake was not determined at the time of intoxication, but when assayed months later, it was estimated to be close to 29 mg/L (i.e., 58 mg/day or approximately 1 mg/kg-day assuming a body weight of 60 kg). Symptoms included lethargy, increased muscle tonus, tremor, mental disturbances, and even death. Autopsies revealed macroscopic and microscopic changes in the brain tissue. In contrast, six children (1- to 10-yr-old) were not intoxicated as were the adults by this exposure. The elderly were more severely affected. Some effects may have resulted from factors other than manganese exposure.

There is no information available on the carcinogenic effects of manganese in humans, and animal studies have reported mixed results. EPA considers manganese to be not classifiable with respect to carcinogenicity, Group D, according to the1986 Guidelines for Carcinogen Risk Assessment. Data from oral exposure suggest that manganese has a low developmental toxicity.

In various surveys, manganese intakes of adults eating western-type and vegetarian diets ranged from 0.7 to 10.9 mg per day (Freeland-Graves, 1994; Gibson, 1994 as cited by Food and Nutrition Board, 2002). Depending on individual diets, a normal intake may be well over 10 mg

per day, especially from a vegetarian diet (Schroder et al. 1966). Thus, from the dietary surveys taken together, EPA concludes that an appropriate reference dose (RfD) for manganese is 10 mg/day (0.14 mg/kg-day; IRIS, 1996). This RfD is unique, with an uncertainty factor (UF) of 1 applied to a human chronic NOAEL of 0.14 mg/kg-day. The UF of 1 is used because the NOAEL (with no apparent LOAEL) is based on chronic human dietary intake surveys, not the typical toxicity studies, and because of the essentiality of the trace element.

EPA derived a health-related benchmark for evaluating the occurrence data, called the health reference level (HRL), of 0.30 mg/L. The HRL is six times the s-MCL of 0.05 mg/L. The HRL is based on the dietary RfD and application of a modifying factor (MF) of three for drinking water as recommended by IRIS (IRIS, 1996), and on an allocation of an assumed 20% relative source contribution from water ingestion as opposed to total manganese exposure. The modifying factor accounts for concerns raised by the Kondakis study (1989), the potential for higher absorption of manganese in water compared to food, consideration of fasting individuals, the concern for infants with potentially higher absorption and lower excretion rates of manganese, and the potential for increased susceptibility to neurotoxic effects of ingested manganese as compared to adults. For example, Dorman et al. (2000) reported that rat pups dosed for 21 days postnatally with 11 or 22 mg Mn/kg-day (by mouth in drinking water) exhibited significant increases in the startle response compared to controls. Significant increases in striatal DA (dopamine) and DOPAC (dihydroxyphenylacetic acid) concentrations, in the absence of pathological lesions, were also observed in the high-dose treated neonates. 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. Potentially sensitive sub-populations include children, the elderly, pregnant women, iron-deficient individuals, and individuals with impaired liver function.

Exposure to manganese in drinking water is ubiquitous in the United States. Data from the National Inorganics and Radionuclide Survey (NIRS), conducted between 1984 and 1986 by EPA, were used to characterize manganese occurrence in public water systems (PWSs). Although somewhat out of date, these data indicate that occurrence estimates are relatively high, with approximately 68% of ground water PWSs (an estimate of approximately 40,000 systems nationally) showing detections of manganese, affecting about 55% of the ground water PWS population served (approximately 47.5 million people nationally). The median levels for detects and the 99th percentile concentration for all samples were 0.01 milligram per liter (mg/L) and 0.63 mg/L, respectively. Based on this survey information (which consisted only of ground water and not surface water sampling), and using supplemental surface water levels from Safe Drinking Water Act (SDWA) compliance monitoring data from five States, EPA concluded that population exposure to manganese in PWSs is potentially high.

When the detected concentrations are evaluated at a draft health reference level (HRL) of 0.3 mg/L, approximately 6.2% of the NIRS PWSs have detections > ½ HRL (> 0.15 mg/L), consisting of about 3,700 ground water PWSs nationally, and affecting approximately 4.6% of the population served (estimated at four million people nationally). The percentage of NIRS PWSs with detections > HRL of 0.3 mg/L is approximately 3.6% (about 2,200 ground water PWSs

nationally), affecting 2.7% of the population served (estimated at approximately 2.3 million people nationally). It is important to note, however, that when average daily drinking water intakes for manganese are compared with intakes from a normal diet, drinking water accounts for a relatively small proportion of total manganese intake.

2.0 IDENTITY: CHEMICAL AND PHYSICAL PROPERTIES

Manganese is an abundant element which makes up about 0.1% of the earth's crust (ATSDR, 2000). Although the elemental (metal) form of manganese does not occur naturally in the environment, manganese is a component of over 100 minerals (ATSDR, 2000).. The most common mineral forms include manganese dioxide, manganese carbonate, and manganese silicate (ATSDR, 2000). Manganese exists in 11 oxidative states, with the most common valences being 2+, 4+, and 7+ (U.S. EPA, 1994a). Although there is no recommended daily allowance (RDA) for manganese, it is essential for the proper function of several enzymes and is necessary for normal bone structure and brain function (U.S. EPA, 1994a). The chemical and physical properties of elemental manganese are presented in Table 2-1. Chemical and physical properties for manganese compounds are summarized in Table 2-2.

Table 2-1. Chemical and Physical Properties of Manganese.

Property	Information
Chemical Abstracts Services (CAS) Registry No.	7439-96-5
Chemical Formula	Mn
Atomic Number	25
Molecular Weight	54.94
Synonyms	Elemental manganese; Collodial manganese; Cutaval; Magnacat; Tronamang
NIOSH Registry of Toxic Effects of Chemical Substances (RTECS) No.	009275000
Hazardous Substances Data Bank (HSDB) No.	00550
Boiling Point	1,962°C
Melting Point	1,244°C
Vapor Pressure (at 1,292°C)	1 mm Hg
Density (at 20°C)	7.21–7.44 g/cm^3
Water Solubility	Decomposes
Acid Solubility	Dissolves in dilute mineral acids

Sources: ATSDR (2000); U.S. EPA (1994a); ChemID*plus* (2000)

Table 2-2. Chemical and Physical Properties of Manganese Compounds.

Name	CAS Registry No.	Synonyms	Valence	Chemical Formula	Molec. Wt.	Specific Gravity or Density	Melting Point (°C)	Boiling Point (°C)	Soluble in Water?
Methylcyclopentadienyl manganese tricarbonyl (MMT)	12108-13-3	Pi-methylcyclopentadienylmanganese tricarbonyl; Tricarbonyl(methylcyclopentadienyl) manganese; Tricarbonyl(2-methylcyclopentadienyl)manganese; Tricarbonyl(eta(5)-methylcyclopentadienyl)manganese; Manganese, tricarbonyl (methyl-pi-cyclopentadienyl); Manganese, tricarbonyl((1,2,3,4,5-eta)-1-methyl-2,4-cyclopentadien-1-yl); Manganese, tricarbonyl(2-methylcyclopentadienyl); pi-(Methylcyclopentadienyl)manganese tricarbonyl; 2-Methylcyclopentadienylmanganese tricarbonyl; (Methylcyclopentadienyl)tricarbonylmanganese; Methylcymantrene	+1	$CH_3C_5H_4Mn(CO)_3$	218.09	1.39	1.5	233	No
Manganous carbonate	598-62-9	Carbonic acid, manganese(2+) salt; Manganese(2+) carbonate; Manganese carbonate; Manganese(II) carbonate; Natural rhodochrosite	+2	$MnCO_3$	114.95	3.125	Decom-poses	NS	Yes
Manganous chloride	7773-27-01-5	Manganese chloride; manganese dichloride; manganese bichloride; manganese(II) chloride	+2	$MnCl_2$	125.84	NS	650	1190	Yes
Manganous acetate	15243-27-3	--	+2	$Mn(C_2H_3O_2)_2 \cdot 4H_2O$	245.08	1.589	NS	NS	Yes; Cold H_2O

Table 2-2. Chemical and Physical Properties of Manganese Compounds (continued).

Name	CAS Registry No.	Synonyms	Valence	Chemical Formula	Molec. Wt.	Specific Gravity or Density	Melting Point (°C)	Boiling Point (°C)	Soluble in Water?
Manganous acetate	638-38-0	Acetic acid, manganese(II) salt; Diacetyl manganese; Manganese(2+) acetate; Manganese acetate; Manganese diacetate; Manganese(II) acetate; Manganous acetate; Octan manganaty	+2	$Mn(C_2H_3O_2)_2$	173.02	1.74	NS	NS	Decom-poses
Manganese ethylenebisdithio-carbamate	12427-38-2	Carbamic acid, ethylenebis(dithio-, manganese salt; Carbamodithioic acid, 1,2-ethanediylbis-, manganese(2+)salt; 1,2-Ethanediylbis(carbamodithioato) (2-)-manganese; Manganous ethylenebis(dithiocarbamate); Maneb	+2	$(CH_2NHCS_2)_2Mn$	265.24	NS	NS	NS	Moder-ately
Manganous oxide	1344-43-0	Manganese monoxide; Manganese oxide; Manganese protoxide	+2	MnO	70.94	5.43–5.46	1,945	NS	No
Manganous phosphate	10124-54-6	Manganese orthophosphate; Phosphoric acid, manganese salt	+2	$Mn_3(PO_4)_2$	259.78	NS	NS	NS	NS
Manganous sulfate	7785-87-7	Manganese sulfate; Sulfuric acid, manganese (II) salt	+2	$MnSO_4 \cdot H_2O$	169.01	2.95	Stable; 57–117	NS	NS
Manganous difluoride	7782-64-1	Manganese difluoride Manganese fluoride; Manganese fluorure	+2	MnF_2	92.93	3.98	856	NS	Yes
Manganous trifluoride	7782-53-1	--	+2	MnF_3	111.93	3.54	Decom-poses; 600	NS	Decom-poses
Manganese borate	12228-91-0	Boron manganese oxide; Tetraboron manganese heptaoxide	+2	$MnB_4O_7 \cdot 8H_2O$	354.17	NS	NS	NS	No

Table 2-2. Chemical and Physical Properties of Manganese Compounds (continued).

Name	CAS Registry No.	Synonyms	Valence	Chemical Formula	Molec. Wt.	Specific Gravity or Density	Melting Point (°C)	Boiling Point (°C)	Soluble in Water?
Manganese formate	--	--	NS	$Mn(CHO_2)_2 \cdot 2H_2O$	181.00	1.953	Decom-poses	NS	Yes
Manganese glycerophosphate	1320-46-3	1,2,3-Propanetriol, mono(dihydrogen phosphate), manganese(2++) salt Glycerol, dihydrogen phosphate, manganese(2+) salt; Manganese(2+) 1,2,3-propanetriol mono(dihydrogen phosphate); Manganese(2+) glycerol dihydrogen phosphate	+2	$MnC_3H_7O_6P$	225.00	NS	NS	NS	Slightly in Cold H_2O
Manganous hydroxide	--	Pyrochaotite	+2	$Mn(OH)_2$	88.95	3.258 (13°C)	Decom-poses	NS	Slightly in Cold H_2O
Manganous nitrate	10377-66-9	Manganese dinitrate; Nitric acid, manganese(2+) salt	+2	$Mn(NO_3)_2 \cdot 4H_2O$	215.01	1.82	25.8	129.4	Yes
Manganous sulfide	--	--	+2	MnS	87.00	3.99	Decom-poses	NS	Slightly in Cold H_2O
Manganese dioxide	1313-13-9	Manganese peroxide; manganese binoxide; manganese black; battery manganese; pyrolusite	+4	MnO_2	86.94	5.026	535	NS	No
Potassium permanganate	7722-64-7	Permangnaic acid; potassium salt; chameleon mineral	+7	$KMnO_4$	158.03	2.7	Decom-poses 240	NS	Yes

Sources: ATSDR (2000); U.S. EPA (1994a); ChemID*plus* (2000).
Log K_{ow} and threshold information was not available for manganese compounds.
NS = Not Specified

3.0 USES AND ENVIRONMENTAL FATE

The uses and environmental fate of manganese in air, water, and soil have been extensively reviewed by ATSDR (2000) and U.S. EPA (1994a). Information from these documents and other sources is summarized below.

3.1 Production and Uses

Manganese is a naturally occurring element that constitutes approximately 0.1% of the earth's crust. It does not occur in the environment in its pure metal form, but is ubiquitous as a component of over 100 minerals, including many silicates, carbonates, sulfides, oxides, phosphates, and borates (ATSDR, 2000). Manganese occurs naturally at low levels in soil, water, air, and food. Of the heavy metals, manganese is surpassed in abundance only by iron (ATSDR, 2000).

In the United States, most manganese ore is smelted to produce ferromanganese, which is a manganese-iron alloy (ATSDR, 2000). The latter is used primarily in the production of steel to improve stiffness, hardness, and strength. The ore is mined in open pit or shallow underground mines, though little has been mined in the U.S. since 1978 (ATSDR, 2000; USGS, 2000). Almost all of the manganese ore used in steel production in the United States is imported (see Table 3-1; ATSDR, 2000). Large quantities of ferromanganese are imported as well (USGS, 2000). Table 3-2 provides further information by State of the widespread manufacture and processing of manganese.

Table 3-1. Imports of Manganese and Ferromanganese to the United States (thousand metric tons, gross weight).

Compound	1984	1988	1995	1996	1997	1998	1999 †
manganese ore	308	499	394	478	355	332	535
ferromanganese	330	449	310	374	304	339	325

years 1984 and 1988: ATSDR, (1997)
years 1995 to 1999: USGS, (2000)
† *estimated*

Manganese compounds are produced through reactions of various elements and compounds with either manganese ores or ferromanganese (ATSDR, 2000). Some common manganese compounds include manganese chloride, manganese sulfate, manganese (II, III) oxide, manganese dioxide, and potassium permanganate (ATSDR, 2000). Uses of these compounds are varied, implying widespread environmental release. Significantly, approximately 80% of the potassium permanganate used in the United States is expended in wastewater and drinking water treatment (U.S. EPA, 1984). Manganese dioxide is used in the production of matches, dry-cell batteries, fireworks, and as a precursor for other manganese compounds. Manganese chloride is also used as a precursor for manganese compounds. A large

Table 3-2. Manganese Manufacturers and Processors by State.

State[a]	Number of facilities	Range of maximum amounts on-site in thousands of	Activities and uses[c]
AL	60	0-50,000	1,2,3,6,7,8,9,12
AR	29	0-50,000	1,2,3,5,7,8,9,12,13
AZ	8	1-1,000	1,4,5,7,8,9,10,12
CA	55	0-500,000	1,2,3,4,5,6,7,8,9,10,11,12,13
CO	14	1-10,000	2,3,4,9,12
CT	16	1-1,000	2,3,9,10
DE	1	10-100	1,5,8
FL	26	0-10,,000	8,9,10,13
GA	42	0-10,000	1,2,3,5,7,8,9,10,12,13
HI	1	10-100	9
IA	51	0-10,000	1,2,3,5,7,8,9,10,12
ID	3	1-1,000	9
IL	121	0-50,000	1,2,3,4,5,8,9,10,11,12,13
IN	161	0-50,000	1,2,3,4,5,6,7,8,9,10,11,12,13
KS	30	1-500,000	1,3,4,5,8,9,12,13
KY	63	0-500,000	1,2,3,4,5,6,7,8,9,10,12,13
LA	17	0-10,000	1,2,3,5,7,8,9,10,12,13
MA	26	0-1,000	1,2,3,4,5,9,10
MD	17	1-50,000	2,4,9,10,13
ME	8	1-100	1,3,9
MI	128	0-10,000	1,2,3,4,5,6,7,8,9,10,12,13
MN	28	0-10,000	8,9,10,12
MO	49	0-10,000	1,5,8,9,12
MS	23	0-50,000	8,9,13
MT	1	100,000-500,000	1,2,3,4,5,6,7
NC	57	0-10,000	1,2,3,5,8,9,10,11,12,13
ND	5	1-100	2,3,9
NE	18	0-10,000	1,2,3,8,9,12,13
NH	4	1-1,000	8,9
NJ	27	1-10,000	1,2,3,4,7,8,9,10
NM	1	10-100	9
NV	2	100-50,000	2,3,7
NY	63	0-10,000	1,2,3,4,5,7,8,9,10,12,13
OH	231	0-500,000	1,2,3,4,5,6,7,8,9,10,12,13
OK	48	0-10,000	1,2,3,4,5,6,8,9
OR	17	1-10,000	2,3,9,12,13
PA	179	0-100,000	1,2,3,4,5,7,8,9,10,11,12,13
PR	5	0-1,000	9
RI	5	1-1,000	2,3,9,10
SC	57	0-10,000	1,2,3,5,7,8,9,10,13
SD	7	1-100	9,13
TN	54	0-50,000	1,2,3,4,5,6,7,8,9,10,12,13
TX	85	0-10,000	1,2,3,4,5,6,8,9,10,12,13
UT	23	1-100,000	2,3,7,9,12,13
VA	23	0-1,000	1,3,5,7,8,9
VT	1	10-100	9
WA	27	0-1,000	1,2,3,6,8,9
WI	126	0-50,000	1,2,3,5,6,7,8,9,10,12,13
WV	15	1-500,000	8,9,10,13
WY	2	0-1,000	1,5

[a]Post office State abbreviations used
[b]Data in TRI are maximum amounts on-site at each facility
[c]Activities/Uses

1. Produce
2. Import
3. On-site use/processing
4. Sale/distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation component
9. Article component
10. Repackaging
11. Chemical processing aid
12. Manufacturing aid
13. Ancillary/other uses

source: ATSDR (2000) compilation of 1996 TRI data

proportion (60%) of U.S. manganese sulfate is used as a fertilizer, while the remainder is used in varnish, fungicides, and as a livestock feed supplement. An organic manganese compound, methylcyclopentadienyl manganese tricarbonyl (MMT), was used as an anti-knock additive in unleaded gasoline before it was banned in 1977. However, a 1995 court decision required EPA to reregister MMT and its use is ongoing (ATSDR, 2000).

The uses of manganese compounds vary widely depending on the chemical form. Table 3-3 summarizes key uses of selected manganese compounds.

3.2 Sources and Environmental Fate

Manganese compounds are widely distributed in air, soil, and water. Sources of atmospheric manganese include industrial emissions, fossil fuel combustion, and erosion of manganese-containing soils. Volcanic eruptions can also contribute to levels of manganese in air. Almost 80% of industrial emissions of manganese are attributable to iron and steel production facilities. Power plant and coke oven emissions contribute about 20%. Although soil erosion is considered an important source of atmospheric manganese, quantitative data for contributions from this source are not available. Due to generally low vapor pressure, manganese compounds in air exist primarily as suspended particulate matter. Because particle size is small, atmospheric manganese distribution can be widespread. These particles will eventually settle out via the process of dry deposition into surface waters or onto soils. 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 fuel additive methylcyclopentadienyl manganese tricarbonyl (MMT) is expected to contribute to urban air concentrations of manganese compounds. The fuel-enhancing properties of MMT were first discovered in the 1950s, and the compound has been used as an additive in leaded and unleaded gasoline since the 1970s in the United States and Canada (Lynam et al., 1999). MMT was banned for use in unleaded gasoline in the United States in 1977 in accordance with provisions in the Clean Air Act, which stated that all gasoline additives that were not "substantially similar" to gasoline were required to obtain a waiver proving that the additive did not "cause or contribute to the failure of emission control systems" (Lynam et al., 1999). The U.S. EPA lifted this ban under court order in 1995, and MMT has been used freely since that time.

Gasoline without MMT contains virtually no manganese (Lynam et al., 1999). The currently allowed maximum level of MMT in unleaded fuel is 0.03125 gram of manganese per U.S. gallon of gasoline (0.0083 g/L or 10.4 ppm). The amount of manganese emitted from the tailpipe of an automobile using MMT-containing fuel depends upon the type of engine, driving cycle, and age of the vehicle. Estimates for manganese in vehicular exhaust vary between 4% and 41% of the manganese consumed (Ardeleanu et al., 1999). The remaining fraction apparently remains in the vehicle (Ardeleanu et al., 1999). Early analysis of emissions suggested

Table 3-3. Summary of Uses for Selected Manganese Compounds.

Compound	Use
Methylcyclopentadienyl manganese tricarbonyl (MMT)	Fuel additive
Manganous carbonate	Ferrites; animal feeds; ceramics; acid soluble manganese source
Manganese chloride	Catalyst in organic compound chlorination; trace mineral supply for animal feed; brick colorant; dye; dry-cell batteries; linseed oil drier; disinfecting; purifying natural gas
Manganous acetate	Mordant in dyeing; drying agent for paint and varnish; bister
Manganese ethylenebisdithiocarbamate	Agricultural fungicide
Manganese oxide	Ferrites; ceramics; fertilizer; livestock feed additive
Manganese phosphate	Ingredient of proprietary solutions for phosphating iron and steel
Manganese sulfate	Livestock feed additive; fertilizer; glazes; varnishes; ceramics; fungicides
Manganous trifluoride	Fluorinating agent in organic chemistry
Manganese borate	Drying agent for varnish and oil; linseed oil drier; leather industry
Manganous nitrate	Porcelain colorants; manufacture of reagent grade manganese dioxide
Manganese dioxide (electrolytic manganese, pyrolusite)	Dry-cell batteries; matches; fireworks; porcelain; glass bonding materials; amethyst glass; manufacturing manganese steel; oxidizer
Potassium permanganate	Oxidizing agent; water and air disinfectant; antialgal agent; metal cleaning, tanning, and bleaching agent; fresh flower and fruit preservative

Sources: U.S. EPA (1994a); ATSDR (2000); Merck (1983).

that manganese from combustion of MMT is emitted primarily as manganese tetroxide (Mn_3O_4) (der Haar et al., 1975d as cited in Lynam et al., 1995). 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 valence of the emitted manganese is +2.2, with a mass median aerodynamic diameter of 1 to 2 microns (Ethyl Corporation, 1997; Ressler et al., 1999; Wong et al., 1998; all 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 (Lyman et al., 1999; Zayed et al., 1999a). Data on the occurrence of manganese in air resulting from combustion of MMT and other sources are presented in Section 4.2.

Manganese is listed as a Toxic Release Inventory (TRI) chemical. In 1986, the Emergency Planning and Community Right-to-Know Act (EPCRA) established the TRI of hazardous chemicals. Created under the Superfund Amendments and Reauthorization Act (SARA) of 1986, EPCRA is also sometimes known as SARA Title III. The EPCRA mandates that larger facilities publicly report when TRI chemicals are released into the environment. This public reporting is required for facilities with at least 10 full-time employees that annually manufacture or process more than 25,000 pounds, or use more than 10,000 pounds, of TRI chemical (U.S. EPA, 1996e, 2000a).

Under these conditions, facilities are required to report the pounds per year of manganese released into the environment both on- and off-site. The on-site quantity is subdivided into air emissions, surface water discharges, underground injections, and releases to land (see Table 3-4). For manganese, releases to land constitute most of the on-site releases, with an abrupt decrease occurring in 1989. It is unclear whether this sharp decrease is real or a function of changes in TRI reporting requirements in the late 1980s and early 1990s (see discussion below). Land releases have fluctuated modestly since that year with no trend evident. Air emissions are also an important mode of on-site release. Though the first four years of record for air emissions are markedly higher, no trend is apparent for the remainder. Surface water discharges and underground injections are less significant on-site releases, with underground injections sharply decreasing in 1994. Low levels of underground injection have continued to the present. Off-site releases of manganese are considerable. Though in 1990 there was a large drop when compared to previous years, the late 1990s showed a steady increase in pounds released. These TRI data for manganese were reported from 49 States, excluding Alaska (U.S. EPA, 2000b).

Only 1% of environmental manganese is released to water (Table 3-4). 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), in addition to its use in industrial wastewater purification and odor abatement (ATSDR, 2000; U.S. EPA, 1984). Transport and partitioning of manganese in water is dependent on the solubility of the manganese form. The chemical form is controlled by factors such as pH, oxidation-reduction potential (Eh), and the available anions. Often, manganese

in water will settle into suspended sediments. 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. Data for occurrence of manganese in drinking water are presented in Section 4.3.

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. Occurrence data for manganese in soils are presented in Section 5.3.

TRI data are also available for the release of manganese compounds (Table 3-5). Releases to land again constitute the largest proportion of on-site releases. With the exception of 1997 and 1998, releases to land have generally decreased over the period of record. Air emissions are also an important mode of release and no trends are evident in the data. Significantly, surface water discharges and underground injections are much more substantial for the compounds than for elemental manganese, and have been generally increasing (dramatically in some years) since the early 1990s. These data must be interpreted with caution, however, as they reflect changes in the requirements for reporting releases. In 1998, only releases of 75,000 lbs/yr were required to be reported; this value is now 25,000 lbs/yr. Therefore, although the values may seem to be increasing, they are likely comparable to past releases that were previously unreported. Further, the TRI data are meant to reflect releases and should not be used to estimate general exposure to a chemical (U.S. EPA, 2000c, d).

Increases in surface water discharges and underground injections of manganese compounds have contributed to increases in total on- and off-site releases in recent years. The latter have returned to, or exceeded, the higher levels seen in the late 1980s and early 1990s. Off-site releases, a large component of total releases, are also at their highest levels since reporting began in 1988. These TRI data for manganese compounds were reported from all 50 States (U.S. EPA, 2000b).

Table 3-4. Environmental Releases (in pounds) for Manganese in the United States, 1988–1998.

Year	On-Site Releases				Off-Site Releases	Total On- & Off-Site Releases
	Air Emissions	Surface Water Discharges	Underground Injection	Releases to Land		
1998	970,658	260,403	3	9,995,895	15,967,545	27,194,504
1997	751,743	146,364	7	9,920,481	16,209,483	27,028,078
1996	816,733	117,571	8	10,111,563	15,191,636	26,237,511
1995	699,897	117,277	17	8,279,054	12,753,204	21,849,449
1994	818,600	89,332	10	8,452,582	14,076,682	23,437,206
1993	901,827	243,999	504	7,530,152	12,150,694	20,827,176
1992	721,047	235,307	304	6,543,600	11,997,270	19,497,528
1991	1,113,160	143,105	272	9,906,511	14,590,589	25,753,637
1990	1,168,809	139,358	881	9,031,215	11,364,721	21,704,984
1989	2,444,211	150,965	556	7,984,172	20,559,164	31,139,068
1988	1,586,675	321,993	255	20,229,826	20,087,660	42,226,409

source: U.S. EPA (2000b)

Table 3-5. Environmental Releases (in pounds) for Manganese Compounds in the United States, 1988–1998.

Year	On-Site Releases				Off-Site Releases	Total On- & Off-site Releases
	Air Emissions	Surface Water Discharges	Underground Injection	Releases to Land		
1998	1,566,352	4,471,582	7,755,610	52,820,578	45,269,882	111,884,004
1997	1,549,505	4,202,876	14,412,830	50,141,026	47,233,186	117,539,423
1996	1,828,684	2,119,241	15,630	40,334,426	33,543,677	77,841,658
1995	2,928,644	1,627,184	3,590	41,832,058	25,994,951	72,386,427
1994	3,060,424	857,825	5,930	38,228,464	25,840,954	67,993,597
1993	2,324,442	685,737	8,740	47,763,821	22,780,860	73,563,600
1992	2,079,044	733,728	22,569	63,490,137	17,297,544	83,623,022
1991	1,531,832	709,557	15,327	66,559,047	27,250,630	96,066,393
1990	2,276,084	721,787	2,842	83,331,787	35,789,554	122,122,054
1989	1,847,528	907,866	1,005,518	85,191,013	33,004,908	121,956,833
1988	1,801,463	681,469	6,816,070	84,227,842	20,670,921	114,197,765

source: U.S. EPA (2000b).

Although the TRI can be useful in giving a general idea of release trends, the data are far from exhaustive and have significant limitations. For example, only industries which meet TRI criteria (at least 10 full-time employees and manufacture and processing of quantities exceeding 25,000 lbs/yr, or use of more than 10,000 lbs/yr) are required to report releases. These reporting criteria do not account for releases from smaller industries. Threshold manufacture and processing quantities also changed from 1988 to 1990 (dropping from 75,000 lbs/yr in 1988 to 50,000 lbs/yr in 1989 to its current 25,000 lbs/yr in 1990), creating possibly misleading data trends. Finally, the TRI data are meant to reflect releases and should not be used to estimate general exposure to a chemical (U.S. EPA, 2000c, d).

In summary, manganese and many of its compounds are naturally occurring and found at low levels in soil, water, air, and food. Furthermore, manganese compounds are produced in the United States from manganese ore and are in widespread use. Most ferromanganese is used in steel production, while other manganese compounds are used in a variety of applications from fertilizers and industrial products to water treatment. Recent statistics regarding import for consumption indicate production and use are substantial (Table 3-1). Manganese and its compounds are also TRI chemicals (Tables 3-4 and 3-5). Industrial releases have been reported since 1988 in all 50 States. Off-site releases constitute a considerable amount of total releases, with releases to land being the most significant on-site releases.

4.0 EXPOSURE FROM DRINKING WATER

4.1 Introduction

This chapter examines the occurrence of manganese in drinking water. No complete national database exists regarding the occurrence of unregulated or regulated contaminants in drinking water from public water systems (PWSs) collected under the Safe Drinking Water Act (SDWA). In this chapter, existing federal and State data that have been screened for quality, completeness, and representativeness are aggregated and analyzed. Populations served by PWSs exposed to manganese are also estimated, and the occurrence data are examined for special trends. To augment the incomplete national drinking water data and aid in the evaluation of occurrence, information on the use and environmental release, as well as ambient occurrence of manganese, is also reviewed.

4.2 Ambient Occurrence

To understand the presence of a chemical in the environment, an examination of ambient occurrence is useful. In a drinking water context, ambient water is source water existing in surface waters and aquifers before treatment. The most comprehensive and nationally consistent data describing ambient water quality in the United States are being produced through the United States Geological Survey's (USGS) National Ambient Water Quality Assessment (NAWQA) program. NAWQA, however, is a relatively young program and complete national data are not yet available from the entire array of sites across the nation.

Data Sources and Methods

The USGS instituted the NAWQA program in 1991 to examine water quality status and trends in the United States. NAWQA is designed and implemented in such a manner to allow consistency and comparison among representative study basins located around the country, facilitating interpretation of natural and anthropogenic factors affecting water quality (Leahy and Thompson, 1994).

The NAWQA program consists of 59 significant watersheds and aquifers referred to as "study units." The study units represent approximately two-thirds of the overall water usage in the United States and a similar proportion of the population served by public water systems. Approximately one-half of the nation's land area is represented (Leahy and Thompson, 1994).

To facilitate management and make the program cost-effective, approximately one-third of the study units at a time engage in intensive assessment for a period of 3 to 5 years. This is followed by a period of less intensive research and monitoring that lasts between 5 and 7 years. This way, all 59 study units rotate through intensive assessment over a ten-year period (Leahy and Thompson, 1994). The first round of intensive monitoring (1991–96) targeted 20 watersheds and the second round monitored 16 basins beginning in 1994.

Manganese is an analyte for both surface and ground water NAWQA studies, with a Minimum Reporting Level (MRL) of 0.001 mg/L. Manganese occurrence in bed sediments and aquatic biota tissue is also assessed, with MRLs of 4 mg/kg and 0.1 mg/kg, respectively. Additional information on analytical methods used in the NAWQA study units, including minimum reporting levels, are described by Gilliom and others (1998).

Manganese data from the first two rounds of intensive NAWQA monitoring have undergone USGS quality assurance checks and are available to the public through their NAWQA Data Warehouse (USGS, 2001). EPA has analyzed these data after further data quality review and occurrence results are presented below. The descriptive statistics generated from the manganese NAWQA data broadly characterize the frequency of manganese detections by sample and by site. Furthermore, detection frequencies above a Health Reference Level (HRL) of 0.3 mg/L are also presented for all samples, and by site. The HRL is a preliminary health effect level used for this analysis (see Section 4.3 for further discussion of the HRL and its development). The median and 99th percentile concentrations are included as well to characterize the spread of manganese concentration values in ambient waters sampled by the NAWQA program.

Results

Typical of many inorganic contaminants, manganese occurrence in ambient surface and ground waters is high (Table 4-1). This is to be expected, considering that manganese constitutes approximately 0.1% of the earth's crust (of the heavy metals, it is surpassed in abundance only by iron), and the element and its compounds are used in many products. Significantly, potassium permanganate is used in wastewater and drinking water treatment.

Detection frequencies are consistently greater for surface water than for ground water, possibly because surface waters are more likely to act as sinks for anthropogenic releases of manganese. Median concentrations are also generally higher for surface water (median concentration for all sites is 0.016 mg/L in surface water and 0.005 mg/L in ground water). However, manganese detection frequencies > HRL are consistently higher in ground water, and 99th percentile ground water concentrations are as much as eight times larger than corresponding 99th percentile surface water concentrations. Locally high concentrations in ground water, higher than any seen in surface water, are not surprising given the possibility of long contact times between ground water and rocks enriched in manganese at a given location. Contact times between surface waters and naturally occurring manganese are orders of magnitude shorter, hence concentrations are lower. Furthermore, surface waters subject to large anthropogenic inputs of manganese are more easily diluted by waters integrated from other parts of the watershed, where manganese concentrations may be lower.

Table 4-1 illustrates that low-level manganese occurrence is ubiquitous. Surface water detection frequencies by site are greater than 95% for all land use categories. Median concentrations and HRL exceedances (by site) are greater in urban and agricultural basins compared to basins characterized as mixed land use or forest/rangeland. This distribution of manganese occurrence is probably influenced by the wide use of manganese compounds in both

industry and agriculture. Mixed land use basins are generally larger than either urban or agricultural basins, and the lower occurrence in these basins may reflect some dilution of the contaminant. The 99th percentile concentrations for surface water range from 0.4 mg/L to 0.8 mg/L. The frequency of detections exceeding the MRL and HRL by site for all sites are approximately 96.9% and 10.2%, respectively. These figures indicate that, although manganese is nearly ubiquitous in surface water, detections at levels of public health concern are relatively low.

For ground water, detections by site are higher in urban and forest/rangeland areas than in mixed or agricultural lands. Over 80% of urban and forest/rangeland sites reported detections, while approximately 63 to 64% of mixed and agricultural land use sites detected manganese. The finding that ground water manganese occurrence is higher in forest/rangeland areas than in either mixed or agricultural sites may result from natural variation in manganese occurrence in soil and rock. Urban areas have the highest median and 99th percentile concentrations (0.015 mg/L and 5.6 mg/L, respectively), as well as the highest detection frequencies (by site: 85.3%) and HRL exceedances (both by sample and by site: 17.2% and 21%, respectively) of manganese in groundwater. These results suggest that urban releases of manganese and manganese compounds can leach to ground water.

Detection frequencies and HRL exceedances by site for all ground water sites are approximately 70.1% and 13.8%, respectively. Again, these figures suggest that, while manganese occurrence in ground water is high, detections at levels of public health concern are relatively low.

Manganese was detected at 100% of NAWQA stream bed sediment sampling sites. The median and 99th percentile concentrations in bed sediments are 1.1 mg/kg (dry weight) and 9.4 mg/kg (dry weight), respectively. The occurrence of manganese in stream sediments is pertinent to drinking water concerns because, though many manganese compounds are either insoluble or have low solubility and are transported in water as suspended sediment, some desorption of the compound from sediments into water will occur through equilibrium reactions, although in very low concentrations.

In aquatic biota tissue, detections are also 100% of all samples and sites (Table 4-2). However, concentration percentiles for tissues are substantially lower than for bed sediments: the median for biotic tissue is 0.01 mg/kg (dry weight) and the 99th percentile is 2.9 mg/kg (dry weight). Significant manganese concentrations in aquatic biota tissues would imply a potential for bioaccumulation. Although manganese was detected in aquatic biota tissues at 100% of samples and sites, low concentration percentiles suggest that the element does not bioaccumulate appreciably.

4.3 Drinking Water Occurrence

National Inorganic and Radionuclide Survey (NIRS)

In the mid-1980s, EPA designed and conducted the National Inorganic and Radionuclide Survey (NIRS) to collect national occurrence data on a select set of radionuclides and inorganic chemicals being considered for National Primary Drinking Water Regulations. The NIRS database includes 36 inorganic compounds (IOC) (including 10 regulated IOCs), 2 regulated radionuclides, and 4 unregulated radionuclides. Manganese was one of the 36 IOCs monitored.

The NIRS provides contaminant occurrence data from 989 community PWSs served by ground water. The NIRS does not include surface water systems. The selection of this group of PWSs was designed so that the contaminant occurrence results are statistically representative of national occurrence. Most of the NIRS data are from smaller systems (based on population-served) and each of these statistically randomly selected PWSs was sampled a single time between 1984 and 1986.

The NIRS data were collected from PWSs in 49 States. Data were not available for the State of Hawaii. In addition to being statistically representative of national occurrence, NIRS data are designed to be divisible into strata based on system size (population served by the PWS). Uniform detection limits were employed, thus avoiding computational (statistical) problems that sometimes result from multiple laboratory analytical detection limits. Therefore, the NIRS data can be used directly for national contaminant occurrence analyses with very few, if any, data quality, completeness, or representativeness issues.

Supplemental IOC Data

One limitation of the NIRS study is a lack of occurrence data for surface water systems. To provide perspective on the occurrence of manganese in surface water PWSs relative to ground water PWSs, SDWA compliance monitoring data that were available to EPA were reviewed from States with occurrence data for both kinds of systems.

The State ground water and surface water PWS occurrence data for manganese used in this analysis were submitted by States for an independent review of the occurrence of regulated contaminants in PWSs at various times for different programs (U.S. EPA, 1999a). In the U.S. EPA (1999a) review, occurrence data from a total of 14 States were noted. However, because several States contained data that were incomplete or unusable for various reasons, only 12 of the 14 States were used for a general overview analysis. From these 12 States, 8 were selected for use in a national analysis because they provided the best data quality and completeness and a balanced national cross-section of occurrence data. These eight were Alabama, California, Illinois, Michigan, Montana, New Jersey, New Mexico, and Oregon.

Table 4-1. Manganese Detections and Concentrations in Streams and Ground Water.

	Detection frequency > MRL*		Detection frequency >HRL*		Concentration percentiles (all samples; mg/L)	
	% samples	% sites	% samples	% sites	median	99th
Surface Water						
urban	99.1 %	99.6 %	4.6 %	13.0 %	0.036	0.7
mixed	92.4 %	98.5 %	1.3 %	6.4 %	0.012	0.4
agricultural	96.3 %	97.2 %	3.7 %	12.3 %	0.019	0.7
forest/rangeland	90.9 %	96.4 %	5.0 %	6.6 %	0.011	0.8
all sites	94.0 %	96.9 %	3.0 %	10.2 %	0.016	0.7
Ground Water						
urban	74.7 %	85.3 %	17.2 %	21.0 %	0.015	5.6
mixed	56.9 %	62.9 %	8.9 %	9.0 %	0.002	1.3
agricultural	61.4 %	64.0 %	11.9 %	12.8 %	0.004	1.6
forest/rangeland	75.3 %	81.3 %	10.9 %	13.8 %	0.012	2.9
all sites	64.1 %	70.1 %	12.8 %	13.8 %	0.005	2.9

** The Minimum Reporting Level (MRL) for manganese in water is 0.001 mg/L and the Health Reference Level (HRL) is 0.3 mg/L. The HRL is a preliminary health effect level used for this investigation.*

Table 4-2. Manganese Detections and Concentrations in Bed Sediments and Aquatic Biota Tissues (all sites).

	Detection frequency > MRL*		Concentration percentiles (all samples; mg/kg dry weight)	
	% samples	% sites	median	99th
sediments	100 %	100 %	1.1	9.4
aquatic biota tissues	100 %	100 %	0.01	2.9

** The Minimum Reporting Levels (MRLs) for manganese in sediments and biota tissues are 4 µg/g and 0.1 µg/g, respectively.*

Only the Alabama, California, Illinois, New Jersey, and Oregon State data sets contained occurrence data for manganese. The data represent more than 37,000 analytical results from about 4,000 PWSs mostly during the period from approximately 1993 to 1997, though some earlier data are also included. The number of sample results and PWSs vary by State.

Data Management

The data used in the State analyses were limited to only those data with confirmed water source and sampling type information. Only standard SDWA compliance samples were used; "special" samples, "investigation" samples (investigating a contaminant problem that would bias results), or samples of unknown type were not used in the analyses. Various quality control and review checks were made of the results, including follow-up questions to the States providing the data. Many of the most intractable data quality problems encountered occurred with older data. These problematic data were, in some cases, simply eliminated from the analysis. For example, when the number of data with problems were insignificant relative to the total number of observations, they were dropped from the analysis (for further details see U.S. EPA, 1999a).

Occurrence Analysis

The summary descriptive statistics presented in Table 4-3 for manganese are derived from analysis of the NIRS data. Included are the total number of samples, the percent samples with detections, the 99th percentile concentration of all samples, the 99th percentile concentration of samples with detections, and the median concentration of samples with detections. The percentages of PWSs and population served indicate the proportion of PWSs and PWS population served whose analytical results showed a detection(s) of the contaminant (simple detection, > MRL) at any time during the monitoring period; or a detection(s) greater than half the Health Reference Level (HRL); or a detection(s) greater than the HRL. The HRL used for this analysis is 0.30 mg/L.

The HRL was derived for contaminants not considered to be "linear" carcinogens by the oral route of exposure. EPA derived the HRL using an RfD approach as follows: HRL = (RfD × 70 kg)/2 L × RSC,

where:

RfD = Reference Dose; an estimated dose (mg/kg-day) to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime. It can be derived from a NOAEL, LOAEL, or benchmark dose, with uncertainty factors generally applied to reflect limitations of the data used;

70 kg = The assumed body weight of an adult;

2 L = The assumed daily water consumption of an adult;

RSC = The relative source contribution, or the level of exposure believed to result from drinking water when compared to other sources (e.g., air), and is assumed to be 20% unless noted otherwise.

EPA used only the best available peer reviewed data and analyses in evaluating adverse health effects. Health effects information is available for manganese in the Integrated Risk Information System (IRIS). IRIS is an electronic EPA data base containing reviewed information (both inside and outside of the Agency) on human health effects that may result from exposure to various chemicals in the environment. These chemical files contain descriptive and quantitative information on RfDs for chronic noncarcinogenic health effects and hazard identification, as well as slope factors and unit risks for carcinogenic effects.

In Table 4-3, national occurrence is estimated by extrapolating the summary statistics for manganese to national numbers for systems, and population served by systems, from the *Water Industry Baseline Handbook, Second Edition* (U.S. EPA, 2000e). From the handbook, the total number of ground water community water systems (CWSs) plus ground water non-transient, non-community water systems (NTNCWSs) is 59,440, and the total population served by ground water CWSs plus ground water NTNCWSs is 85,681,696 persons (see Table 4-3). To arrive at the national occurrence estimate for the HRL, the national estimate for ground water PWSs (or population served by ground water PWSs) is simply multiplied by the percentage for the given summary statistic [i.e., the national estimate for the total number of ground water PWSs with detections at the HRL of 0.30 mg/L (40,388) is the product of the percentage of ground water PWSs with detections (68%) and the national estimate for the total number of ground water PWSs (59,440)].

In Table 4-4, occurrence data on manganese directly submitted by the States of Alabama, California, Illinois, New Jersey, and Oregon for *A Review of Contaminant Occurrence in Public Water Systems* (U.S. EPA, 1999a) were used to augment the NIRS study which lacked surface water data. Included in the table are the same summary statistics as shown in Table 4-3, with additional information describing the relative distribution of manganese occurrence between ground water and surface water PWSs in the 5 States.

The State data analysis was focused on occurrence at the system level because a PWS with a known contaminant problem usually has to sample more frequently than a PWS that has never detected the contaminant. The results of a simple computation of the percentage of samples with detections (or other statistics) can be skewed by the more frequent sampling results reported by the contaminated site. The system level of analysis is conservative. For example, a system need only have a single sample with an analytical result greater than the MRL, i.e., a detection, to be counted as a system with a result "greater than the MRL."

When computing basic occurrence statistics, such as the number or percent of samples or systems with detections of a given contaminant, the value (or concentration) of the MRL can have important consequences. For example, the lower the reporting limit, the greater the number of detections (Ryker and Williamson, 1999). As a simplifying assumption, a value of half the

Table 4-3. Manganese Occurrence in Ground Water PWS of NIRS Survey.

Frequency Factors	Health Reference Level = 0.3 mg/L	National System & Population Numbers[1]
Total number of samples/systems	989	59,440
99th percentile concentration (all samples)	0.63 mg/L	--
Minimum Reporting Level (MRL)	0.001 mg/L	--
99th percentile concentration of detections	0.72 mg/L	--
Median concentration of detections	0.01 mg/L	--
Total population	1,482,153	85,681,696
Occurrence by Samples/System		**National Extrapolation HRL = 0.3 mg/L**
% Ground water PWSs with detections (> MRL) Range of sampled States	67.9% 8.3–100%	40,388 NA
% Ground water PWSs > ½ HRL Range of sampled States	6.1% 0–31.6%	3,606 NA
% Ground water PWSs > HRL Range of sampled States	3.2% 0–21.0%	1,923 NA
Occurrence by Population Served		
% Ground water PWS population served with detections Range of sampled States	55.4% 0.3–100%	47,502,000 NA
% Ground water PWS population served > ½ HRL Range of sampled States	4.6% 0–89.2%	3,940,000 NA
% Ground water PWS population served > HRL Range of sampled States	2.6% 0–89.2%	2,256,000 NA

[1] *Total PWS and population numbers are from EPA March 2000 Water Industry Baseline Handbook.*

MRL = minimum reporting level
PWS = public water system
NA = not applicable

HRL = health reference level
– = no data

MRL is often used as an estimate of the concentration of a contaminant in samples/systems whose results are less than the MRL. However, for these occurrence data this is not straightforward. This is in part related to State data management differences as well as real differences in analytical methods, laboratories, and other factors.

The situation can cause confusion when examining descriptive statistics for occurrence. Because a simple meaningful summary statistic is not available to describe the various reported MRLs, and to avoid confusion, MRLs are not reported in the summary table (Table 4-4).

Additional Drinking Water Data From 1996 AWWA Survey

To augment the SDWA drinking water data analysis described above, results from a 1996 American Water Works Association (AWWA) survey are reviewed. The survey, called WATER:/STATS, is a cooperative project of AWWA and AWWA Research Foundation. The WATER:/STATS survey database stores results from the 1996 WATER:/STATS survey of water utilities in the United States and Canada in terms of facilities, scale of operation, and major inputs and outputs. A total of 794 AWWA member utilities responded to the survey with ground water and/or surface water information. However, the actual number of respondents for each data category varies because not all participants in the survey responded to every question.

4.4 Results

The NIRS data in Table 4-3 show that approximately 68% of ground water PWSs (an estimate of approximately 40,000 systems nationally) had detections of manganese, affecting about 55% of the ground water PWS population served (approximately 47.5 million people nationally). At an HRL of 0.30 mg/L, approximately 6.1% of the NIRS PWSs had detections > ½ HRL (about 3,600 ground water PWSs nationally), affecting approximately 4.6% of the population served (estimated at 3.9 million people nationally). The percentage of NIRS PWSs with detections > HRL of 0.30 mg/L was approximately 3.2% (about 1,900 ground water PWSs nationally), affecting 2.6% of the population served (estimated at approximately 2.3 million people nationally) (Table 4-3).

Drinking water data for manganese from the supplemental individual States vary among States (Table 4-4). Manganese has not been required for monitoring under SDWA, though these States had obviously conducted some monitoring. The number of systems with manganese data for Illinois and Oregon is far less than the number of PWSs in these States. Hence, the extent to which these data are representative is unclear. Alabama, California, and New Jersey have substantial amounts of data and PWSs represented. Because the NIRS data only represent manganese occurrence in ground water PWSs, the supplemental State data sets provide some perspective on surface water PWS occurrence. For example, the median concentration of detections for the States ranged from 0.02 mg/L to 0.15 mg/L, higher than the NIRS data (0.01 mg/L). For detections by PWSs, 3 of the 5 States (California, Illinois, and Oregon) had higher ground water PWS detections.

Table 4-4. Occurrence Summary of Ground and Surface Water Systems by State for Manganese.

Frequency Factors	Alabama	California	Illinois	New Jersey	Oregon
Total number of samples	1,343	31,998	344	3,196	172
Number of ground water samples	934	29,923	275	2,795	90
Number of surface water samples	409	2,075	69	401	82
% Samples with detections	30.2%	16.5%	44.2%	39.7%	39.5%
% Ground water samples with detections	28.1%	17.5%	50.2%	40.6%	61.1%
% Surface water samples with detections	35.0%	1.9%	20.3%	33.7%	15.9%
99th percentile concentration (all samples)	0.13 mg/L	0.71 mg/L	0.96 mg/L	0.42 mg/L	1.6 mg/L
Minimum reporting level (MRL)	Variable[1]	Variable[1]	Variable[1]	Variable[1]	Variable[1]
99th percentile concentration of detections	0.56 mg/L	1.52 mg/L	57 mg/L	0.89 mg/L	6.7 mg/L
Median concentration of detections	0.02 mg/L	0.15 mg/L	0.04 mg/L	0.02 mg/L	0.05 mg/L
Total number of PWSs	434	2,516	227	1,179	84
Number of ground water PWSs	365	2,293	160	1,147	54
Number of surface water PWSs	69	223	67	32	30
Total population served	3,662,222	45,388,246	1,995,394	7,472,565	1,306,283
Ground water population	1,820,214	27,840,774	724,635	2,386,396	301,440
Surface water population	1,837,743	30,675,992	1,270,179	3,687,076	1,117,782
Occurrence by System					
% PWSs with detections (> MRL)	46.5%	28.2%	41.4%	53.5%	46.4%
% Ground water PWSs with detections	41.6%	29.8%	50.6%	52.3%	55.6%
% Surface water PWSs with detections	72.5%	11.7%	19.4%	96.9%	30.0%
Health Reference Level (HRL) = 0.3 mg/L					
% PWSs > ½ HRL	1.8%	17.2%	9.3%	5.8%	13.1%
% Ground water PWSs > ½ HRL	1.4%	18.5%	11.9%	5.7%	20.4%
% Surface water PWSs > ½ HRL	4.4%	3.6%	3.0%	9.4%	0.0%
% PWSs > HRL	0.9%	10.1%	4.4%	2.5%	6.0%
% Ground water PWSs > HRL	0.6%	10.9%	5.0%	2.5%	9.3%
% Surface water PWSs > HRL	2.9%	1.8%	3.0%	3.1%	0.0%
Occurrence by Population Served					
% PWS population served with detections	71.9%	49.3%	36.5%	85.7%	58.0%
% Ground water PWS population with detections	50.9%	66.2%	66.3%	70.4%	41.8%
% Surface water PWS population with detections	73.4%	10.5%	19.5%	100.0%	56.8%
Health Reference Level (HRL) = 0.3 mg/L					
% PWS population > ½ HRL	5.9%	34.8%	16.5%	15.3%	4.6%
% Ground water PWS population > ½ HRL	0.8%	52.6%	29.1%	10.4%	19.9%
% Surface water PWS population > ½ HRL	0.7%	4.4%	9.4%	23.3%	0.0%
% PWS population > HRL	2.4%	27.2%	14.7%	9.1%	3.2%
% Ground water PWS population > HRL	0.1%	42.8%	24.2%	4.9%	14.0%
% Surface water PWS population > HRL	0.6%	4.2%	9.4%	14.5%	0.0%

[1] *See text for details*
MRL = minimum reporting level
PWS = public water system
HRL = health reference level

For simple detections, the supplemental State data show a range from 30% to 56% of ground water PWSs (Table 4-4). These figures are lower than the NIRS ground water PWS results: 68% > MRL (Table 4-3). The supplemental State data show considerably greater percentages of simple detections for surface water PWSs, with higher variability as well: 12%–97% >MRL. Comparisons made between data for simple detections need to be viewed with caution because of differences in MRLs between the State data sets and the NIRS study, and among the States themselves (see Section 3).

The supplemental State data sets indicate ground water PWS detections > HRL of 0.30 mg/L between 0.6% and 11% (Table 4-4). Again, this range brackets the NIRS national average of PWS > HRL of 0.30 mg/L (3.2%) (Table 4-4). Notably, surface water PWSs showed fewer exceedances of the HRL than ground water PWSs at this higher concentration; ranging from 0% to 3.1%.

Reviewing manganese occurrence by PWS population served shows that from 0.1%–43% of the States' ground water PWS populations were served by systems with detections > HRL of 0.30 mg/L (Table 4-4). Comparatively, 2.6% of the NIRS ground water PWS population served experienced detections > HRL of 0.30 mg/L (Table 4-3). Populations served by surface water PWSs with detections > HRL of 0.30 mg/L ranged from 0%–14.5% among the five supplemental States. Population figures for the supplemental States are incomplete and are only reported for those systems in the database that have reported their population data. For manganese, approximately 80% of the PWSs reporting occurrence data for these 5 States also reported population data.

Occurrence in AWWA PWSs

The AWWA sponsored 1996 WaterStats Survey showed manganese occurrence above levels at which health effects are expected to be realized to be relatively similar to that reported in the NIRS data and the supplemental State data. Approximately 11% of the participating ground water PWSs (serving about 5.1 million people) had maximum detections of manganese in raw water greater than the HRL of 0.30 mg/L. The 99th percentile of concentration and the median concentration were 9.0 mg/L and 0.09 mg/L, respectively. Surface water PWSs showed comparable results with approximately 12.8% of survey respondents (serving about to 10.5 million people) having maximum detections of manganese in raw water greater than the HRL of 0.30 mg/L. The 99th percentile of concentration and the median concentration in raw surface waters were 3.08 mg/L and 0.092 mg/L, respectively.

In finished ground water samples, approximately 3% of survey respondents (serving close to 1.7 million people) had maximum detections of manganese greater than the HRL of 0.30 mg/L. The 99th percentile concentration and the median concentration were 0.80 mg/L and 0.021 mg/L, respectively. For finished surface water samples, approximately 1.5% of survey respondents (about 1.7 million people) reported maximum detections greater than the HRL of 0.30 mg/L. The 99th percentile concentration and the median concentration in finished surface water samples were 0.64 mg/L and 0.013 mg/L, respectively.

4.5 Conclusion

Manganese and its compounds are TRI chemicals. Industrial releases have been recorded since 1988 in all 50 States. Off-site releases constitute a considerable amount of total releases, with releases to land being the most significant on-site releases.

Low-level manganese occurrence in ambient waters and bed sediments monitored by the USGS NAWQA program is ubiquitous, with detections approaching 100% of surface water sites and greater than 62% of ground water sites. Stream bed sediments and aquatic biota tissues show detections of 100% by sample and by site. Urban basins generally have more surface and ground water manganese detections greater than the HRL than basins in other land use categories, and higher median and 99th percentile concentrations. Although manganese detection frequencies are high in ambient waters, stream bed sediments, and aquatic biota tissue, manganese occurrence at levels of public health concern is low.

Manganese has been detected in ground water PWS samples collected through the NIRS study. Occurrence estimates are relatively high with approximately 68% of all samples showing detections affecting about 55% of the national population served. The 99th percentile concentration of all samples is 0.63 mg/L. Exceedances of the HRL at 0.30 mg/L affect 2.6% of the ground water PWS population served, or approximately 2.3 million people nationally.

Additional SDWA data from the States of Alabama, California, Illinois, New Jersey, and Oregon, including both ground water and surface water PWSs, were examined through independent analyses and also show substantial levels of manganese occurrence. These data provide perspective on the NIRS estimates that only include data for ground water systems. The supplemental State data show ground water systems reported higher manganese detections in 3 of the 5 States (California, Illinois, and Oregon). If national data for surface water systems were available, the occurrence and exposure estimates would be substantially greater than from NIRS alone.

5.0 EXPOSURE FROM ENVIRONMENTAL MEDIA OTHER THAN WATER

5.1 Food

5.1.1 Concentrations of Manganese in Food

Table 5-1 summarizes mean manganese concentrations in 234 foods analyzed by the Food and Drug Administration (FDA). Nuts and grains contain the highest manganese concentrations, with values as high as 40 to 50 mg/kg reported. Fruits, vegetables, fish, poultry, meat, and eggs tend to have intermediate concentrations. Manganese levels in milk tend to be low, with concentrations of 10 and 30 micrograms per liter (μg/L) reported for human and cow's milk, respectively. In contrast, values of 50 to 300 μg/L have been reported for infant formula (Collipp et al., 1983 as cited in U.S. EPA, 1996a).

Manganese has been detected in the muscle of fresh bluefin tuna (*Thunnus thynnus*). Hellou et al. (1992) as reported in ATSDR (2000), analyzed concentrations in 14 tuna samples using inductively coupled plasma mass spectrometry. The level of manganese varied from 0.16 to 0.31 micrograms per gram (μg/g) dry weight, with a mean value of 0.22 μg/g dry weight.

Black tea samples from the United Kingdom (UK) were found to have mean manganese concentrations of 4.6 mg/L, 40% of which was bioavailable (Powell et al., 1998).

The issue of bioavailability is important to consider when assessing manganese levels in foods, and is discussed further in the next section. For instance, the actual absorption of manganese from ingested tea is limited by the presence of polyphenolic compounds (tannins) in the tea which bind manganese (Freeland-Graves and Llanes, 1994). This explains the low bioavailabilty of manganese in tea. Likewise, the relatively high levels of manganese in fruits, nuts, grains, and vegetables, as well as in soy-based infant formula (discussed in Section 5.1.2), are limited in their bioavailability by the presence of phytic acids, oxalic acids, and fiber in these foods (U.S. EPA, 1996a). In addition, high levels of calcium or magnesium ingestion may inhibit manganese absorption, while persons with diets that are deficient in iron may experience increased manganese absorption (U.S. EPA, 1996a).

5.1.2 Intake of Manganese From Food

General Population

Manganese is an essential nutrient. It is very unevenly distributed in foods. Although manganese is rich in tea, whole grains, legumes, and nuts, it is found in negligible amounts in meats, dairy products, sweets, refined grains, and most fruits. Thus, many individuals who do not consume whole grains, nuts, certain fruits (pineapple), green leafy vegetables, and tea will consume a "low manganese" diet - less than 2 mg per day (Davis et al., 1992). In addition, women tend to consume less food than men; hence their intakes of individual nutrients, including manganese, are often lower than those of men (Pennington et al., 1989).

The Food and Nutrition Board set an adequate intake level (AI) for manganese at 2.3 mg/day for men and 1.8 mg/day for women (Food and Nutrition Board, 2002; Trumbo et al., 2001). The current recommendations for infants and children are 0.003 to 0.6 mg/day and 1.2 to 1.9 mg/day, respectively (Food and Nutrition Board, 2002). An adequate intake level is defined as "a recommended intake value based on observed or experimentally determined approximations or estimates of nutrient intake by a group (or groups) of healthy people that are assumed to be adequate - used when an RDA cannot be determined." Some nutritionists feel that this level may be too low. Freeland-Graves et al. (1987), as cited in U.S. EPA (1996a), have suggested a range of 3.5 to 7 mg/day for adults based on a review of human studies.

Dietary habits have evolved in recent years to include a larger proportion of meats and refined foods in conjunction with a lower intake of whole grains (Freeland-Graves, 1994; U.S. EPA, 1996a). The net result of such dietary changes includes a lower intake of manganese. A significant number of adult Americans, particularly women, may consume suboptimal amounts of manganese (ATSDR, 2000; Pennington et al., 1986). On the other hand, it is not known whether infants may ingest more than the AI for their age group as a result of the high manganese content of prepared infant foods and formulas.

Table 5-1. Manganese Concentrations in Selected Foods[a]

TYPE OF FOOD	RANGE OF MEAN CONCENTRATIONS (mg/kg)
Nuts and nut products	18.21–46.83
Grains and grain products	0.42–40.70
Legumes	2.24–6.73
Fruits	0.20–10.38
Fruit juices and drinks	0.05–11.47
Vegetables and vegetable products	0.42–6.64
Desserts	0.04–7.98
Infant foods	0.17–4.83
Meat, poultry, fish and eggs	0.10–3.99
Mixed dishes	0.69–2.98
Condiments, fats, and sweeteners	0.04–1.45
Beverages (including tea)	0.00–2.09
Soups	0.19–0.65
Milk and milk products	0.02–0.49

[a] Adapted from ATSDR (2000) and Pennington et al. (1986).

Based on various surveys, the Food and Nutrition Board (2002) concluded that the average manganese intake of adults eating western-type and vegetarian diets ranged from 0.7 to 10.9 mg/day (Food and Nutrition Board, 2002), and the median intakes for women and men ranged from 1.6 to 2.3 mg/day (Food and Nutrition Board, 2002). The total dietary manganese intake among individuals may vary greatly depending upon dietary habits. Individual intake estimates for Canadian adult male blue-collar workers (n = 28) and garage mechanics (n = 37), as determined by analysis of dietary records, ranged from 1.0 to 14 mg/day (Loranger and Zayed, 1995). The mean values in this study for manganese intake by blue-collar workers and mechanics were 3.7 and 2.9 mg/day, respectively. It should be noted that FDA's Total Diet Study menus used to measure the levels of several nutritional elements including manganese from 1982 to 1986 in Pennington et al. (1989) reflect "typical" American diets and contain less manganese than the diets consumed by Canadian males.

The Food and Nutrition Board also set a tolerable upper intake level (UI) for manganese at 11 mg per day for adults, based on the upper range of manganese intake for adults (see review by Greger, 1999). An UI is defined as "the highest level of daily nutrient intake that is likely to pose no risk of adverse health effects for almost all individuals in the general population. As intake increases above the UL, the risk of adverse effects may increase." For shorter duration, Davis and Greger (1992) reported that women given daily supplements of 15 mg manganese for 90 days experienced no adverse effects other than a significant increase in lymphocyte manganese-dependent superoxide dismutase (Greger, 1998, 1999; Food and Nutrition Board, 2002).

Based on a conservative range for manganese intake of 2 to 10 mg/day, U.S. EPA (1996a) estimated a dietary manganese intake of 28.6 to 126 micrograms per kilogram per day (μg/kg-day). For children, assuming a manganese intake of 1.28 μg/calorie (U.S. EPA, 1984; ATSDR, 2000) and a caloric intake of 1,000 calories/day for a 10 kg child, the estimated average daily intake would be 128 μg/kg-day.

Groups with Potential for High Manganese Intake from Food

Groups with potential for high intake of dietary manganese include vegetarians, heavy tea drinkers, and infants. Vegetarians may consume a larger proportion of manganese-rich nuts, grains, and legumes in their diet than the general population (U.S. EPA, 1996a). Manganese intake by North American vegetarians has been estimated to be as high as 10 mg Mn/day (Gibson, 1994). However, many components of vegetarian diets, including phytates, tannins, oxalates, and fiber, inhibit manganese uptake from the gastrointestinal tract. Consequently, the bioavailability of manganese in vegetarian diets is uncertain. Johnson et al. (1991) studied the absorption of radiolabelled manganese from various plant foods in adult men and women, and reported that mean fractional absorption values from lettuce and spinach were 5.20 and 3.81%, respectively. Mean fractional absorption from sunflower seeds was significantly less (1.71%), while that from wheat was 2.16%. All percent absorption values from plant food were significantly less than mean values from $MnCl_2$ dissolved in water, which ranged from 7.74 to 10.24%.

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). Consumption of three cups of tea per day would therefore have the potential to double manganese intake for some individuals. Again, however, it is likely that the high level of tannins in tea will result in reduced manganese absorption (Freeland-Graves and Llanes, 1994).

Infants may ingest high levels of manganese from infant formulas or prepared baby foods, although manganese absorption in infants is influenced by several variables, and the degree to which absorption levels may be a health concern is unknown. Infant formulas contain 50 to 300 μg/L manganese (Collipp et al., 1983 as cited in U.S. EPA, 1996a), compared to human milk which contains 7 to 15 μg/L manganese (U.S. EPA, 1996a). Assuming an intake of 742 milliliters (mL) of breast milk/day (U.S. EPA, 1996a), a breast-fed infant would have an estimated daily manganese intake of 5.2 to 11.1 μg/day. An infant consuming the same volume of infant formula would have an estimated daily manganese intake of 37.1 to 223 μg/day. Assuming an average weight of 6 kg for an infant of age 6 months, the weight-adjusted average daily intake would range from 0.87 to 1.85 μg/kg-day for breast-fed infants. The corresponding weight-adjusted intake for a formula-fed infant would be 6.2 to 37.2 μg/kg-day. Generally, solid foods are introduced at the age of 4 months. Once solid foods are introduced, the dietary intake of manganese increases so substantially that the contribution of Mn intake from milk becomes less significant.

In assessing infant exposure to manganese, however, one must also consider constituents of infant formula and of breast milk which may affect manganese bioavailability. For instance, formula made from soy protein contains high levels of phytic acids and vegetable proteins which probably decrease the manganese bioavailability. If the formula is also iron-fortified, manganese bioavailability may be further decreased, although studies on the inhibitory influences of iron have produced conflicting results (Freeland-Graves, 1994). Davidsson et al. (1989a) measured absorption of radiolabelled manganese in adult humans given human milk, cow's milk, or soy formula and found that fractional manganese absorption from human milk (8.2%) was significantly higher than absorption from cow's milk (2.4%) and soy formula (0.7%). Manganese in infant formula is in the divalent state, the absorption of which cannot be regulated by the lactoferrin receptors in the gut; breast milk manganese is in the trivalent form bound to lactoferrin, and its absorption is thus regulated (U.S. EPA, 1996a). Davidsson et al. (1989a) suggested that the lactoferrin in human milk as well as the higher calcium content in cow's milk contributed to the difference in absorption. Dorner et al. (1989) observed similar differences in fractional manganese retention in infants as those observed by Davidsson et al. (1989a) in adults. In the infant study, a higher percentage of manganese was retained from ingested breast milk (41%) than from cow's-milk formula (~19%). Therefore, many factors probably control manganese absorption from infant formula, and firm conclusions are difficult to make in the absence of more direct data. Keen et al. (1986) demonstrated that fractional manganese uptake from human breast milk and cow's milk were relatively high (~80% and ~89 %, respectively), whereas uptake from soy formula was lowest (~60%) in rat pups.

It should be noted that Davidsson et al. (1989a) performed their studies in adults; manganese body burden in infants may be additionally influenced by the fact that the biliary excretion system, which is the primary route of manganese excretion, is not completely developed in neonates (Lönnerdal, 1994). Studies in rats have further demonstrated that young animals absorb significantly more manganese in the gut than do mature animals (Lönnerdal et al. 1987). Also, animal studies have shown that manganese crosses the blood-brain barrier in neonates at a rate 4 times higher than that in adults (Mena, 1974). However, the relevance of these studies to humans is unknown, and few direct absorption data for manganese in human infants are available. In this context, it is noteworthy that Collipp et al. (1983) reported hair manganese levels that increased significantly from birth (0.19 μg/g) to 6 weeks (0.865 μg/g) and 4 months (0.685 μg/g) of age in infants given formula, while infants given breast milk exhibited no significant increase (0.330 μg/g at 4 months). This study also reported that the average hair manganese level in children exhibiting learning disabilities was significantly increased (0.434 μg/g) compared to those that exhibited normal learning ability (0.268 μg/g).

5.2 Air

5.2.1 Concentration of Manganese in Air

General Population

Table 5-2 summarizes nationally aggregated data collected between 1953 and 1982 for manganese concentrations in ambient air of nonurban, urban, and source-dominated locations. Average manganese concentrations for nonurban areas ranged from a high value of 60 nanograms per cubic meter (ng/m^3) determined in 1953–1957 to a low of 5 ng/m^3 in 1982. Average concentrations for urban areas ranged from 110 to 33 ng/m^3 over the same period. Average levels in source-dominated locations varied widely, ranging from a high reading of 8,300 ng/m^3 during the 1965–1967 measurement period, to concentrations of 130 to 140 ng/m^3 in 1982. Although differences in sample collection and analytical methods complicate interpretation, these data suggest that manganese concentrations in ambient air decreased over the time period of record (U.S. EPA, 1984). This change has been attributed to installation of emissions controls in the metals industry (ATSDR, 2000). More recently, U.S. EPA (1990) has proposed an average annual background concentration of 40 ng/m^3 for urban areas, based on data for 24-hour average concentrations in 102 cities across the U.S.

Multiple local studies have estimated airborne manganese concentrations. A series of Canadian studies evaluated total airborne manganese concentrations in the home and workplace (Sierra et al., 1995; Zayed et al., 1994, 1996). Table 5-3 summarizes the results of these studies. Concentrations of manganese were determined by use of personal sampling devices. Mean levels of manganese measured in homes ranged from 7 to 12 ng/m^3. Mean workplace concentrations ranged from 12 to 44 ng/m^3 for non-automotive workers (primarily office workers) and taxi drivers. Automotive workers, such as auto mechanics, experienced mean workplace levels ranging from 250 to 448 ng/m^3. Sample sizes for these studies ranged from 9 to 35 individuals.

Table 5-2. Average Concentrations of Manganese in Ambient Air Sampled from 1953–1982[a].

SAMPLING LOCATION/YEAR	CONCENTRATION (ng/m³)		
	1953–1957	1965–1967	1982
Nonurban	60	12	5
Urban	110	73	33
Source-dominated	No data	250–8,300	130–140

[a]Source: ATSDR (2000) and U.S. EPA (1984).

Table 5-3. Manganese Levels in Air of Canadian Urban Locations as Determined by Personal Exposure Monitoring.

OCCUPATION	LOCATION	DURATION	N	MEAN (ng/m³)	RANGE (ng/m³)	REFERENCE
Garage worker	Work	5 days	10	250	9–2,067	Zayed et al. (1994)
Garage worker	Home	2 days	10	7	4–27	
Taxi driver	Work	5 days	10	24	6–69	
Taxi driver	Home	2 days	10	11	4–22	
Auto Mechanic	Work	4 weeks	35	448	10–6,673	Sierra et al. (1995)
Auto Mechanic	Home	4 weeks	35	12	6–63	
Nonautomotive	Work	4 weeks	30	44	11–1,862	
Nonautomotive	Home	4 weeks	30	8	5–87	
Office worker	Work	7 days	23	12	2–44	Zayed et al. (1996)
Taxi driver	Work	7 days	9	28	8–73	

Automotive fuels in Canada and the U.S. contain the antiknock agent methylcyclopentadienyl manganese tricarbonyl (MMT). The allowable level of MMT in Canadian gasoline is 0.062 grams per gallon (g/gal), which is double the allowable limit of 0.031 g/gal in the U.S. (Davis, 1998). Combustion of MMT releases manganese to the atmosphere in the form of manganese oxides, phosphates, and sulfates (see Section 3.2 above), and these compounds may constitute a significant source of manganese contamination in urban environments. In Canada, a car exhaust study determined that 4 to 41% of Mn in gasoline is emitted from the tailpipe,

depending on the vehicle and driving cycle (Ardeleanu et al., 1999). The fraction not emitted to the atmosphere appears to remain in the engine (Ardeleanu et al., 1999).

Levels of unburned MMT in air resulting from emission of residual MMT in vehicular exhaust or evaporative emissions (e.g., at gas stations) are expected to be low. Although data are limited, Zayed et al. (1999a) reported concentrations ranging from 0.4 ng/m^3 to 12 ng/m^3 when measured in five different microenvironments in Montreal, Canada. The highest average concentration of MMT in ambient air was measured at gas stations.

Use of MMT in gasoline has resulted in public health concerns related to the potential health effects of increased manganese exposure. As a result, determination of the extent to which MMT contributes to environmental levels of manganese (and ultimately to human exposure) has been an area of active research. Several studies in Montreal, Canada have examined manganese concentrations in ambient air in relation to motor vehicle traffic (Table 5-4). Loranger et al. (1994a) found ambient manganese concentrations to be significantly correlated with traffic density. Areas of intermediate and high traffic densities had ambient manganese concentrations above the natural background level in Montreal of 40 ng/m^3 (Loranger and Zayed, 1994; Loranger et al., 1994a).

Loranger et al. (1995) summarized modeling and empirical data relating atmospheric manganese concentrations to combustion of gasoline containing varying concentrations of MMT (Table 5-5). Estimated increases predicted by studies listed in the table but conducted prior to 1990 were characterized by Loranger et al. (1995) as being of limited use due to insufficient information on methodology. Based on an estimated background level of 40 ng/m^3 (calculated by taking the average of data from 102 U.S. cities), U.S. EPA (1990) predicted that the potential increase in ambient background manganese from the use of MMT would be 0.05P, where P is the fraction of total manganese in fuel that is emitted in vehicular exhaust.

Canadian studies have addressed the fraction of total manganese concentration in air associated with particulates of respirable size. Zayed et al. (1996) reported respirable manganese (MnR) and total manganese (MnT) concentrations determined by personal exposure monitoring of taxi drivers and office workers. Mean concentrations of MnR were 10 and 15 ng/m^3 for office workers and taxi drivers, respectively. Mean concentrations of MnT were 12 and 28 ng/m^3 for the same respective groups. Loranger and Zayed (1997a) measured concentrations of MnR and MnT at two sites in Montreal with different vehicle traffic densities. MnR and MnT concentrations adjacent to a heavily traveled (> 100,000 vehicles/day) road were 24 and 50 ng/m^3, respectively. Values for MnR and MnT at a site with lower traffic density (10,000 to 15,000 vehicles/day) were 15 and 27 ng/m^3, respectively. Zayed et al. (1999a) measured mean concentrations of respirable manganese ranging from 18 to 53 ng/m^3 in five microenvironments in Montreal. The overall mean concentrations of respirable and total manganese were 36 ± 7 ng/m^3 and 103 ± 32 ng/m^3, respectively. These data indicate that approximately 35 to 90% of total manganese in urban air is respirable.

Table 5-4. Ambient Air Concentrations of Manganese in Relation to Traffic Density, Montreal, Canada 1981–1994.

TRAFFIC DENSITY (vehicles/day)	Mn (ng/m^3)	REFERENCE
< 15,000	< 40 (50% of samples)	Loranger et al. (1994a)
> 15,000	> 40 (50% of samples)	Loranger et al. (1994a)
4,900	26	Loranger et al. (1994b)
75,000	36	Loranger et al. (1994b)
< 15,000	20	Loranger and Zayed (1994)
< 30,000	50	Loranger and Zayed (1994)
> 100,000	60	Loranger and Zayed (1994)
117,585	54	Loranger et al. (1995a)
117,585	29–37	Loranger et al. (1995a)

Source: Zayed et al. (1999b)

Table 5-5. Estimated Atmospheric Mn Concentration in Relation to the Combustion of MMT in Gasoline.

Mn concentration in gasoline		Estimated concentration from MMT source ng/m^3	Ambient air concentration from all sources ng/m^3	Reference
mg/L	g/gal			
132.0	0.5	--	200–800	(Mena, 1974)
33.0	0.125	335	1,200–1,500[a]	(Piver, 1974)
33.0	0.125	--	2–250[b]	(Moran, 1975)
33.0	0.125	--	20–3,400[c]	(U.S. EPA, 1975)
33.0	0.125	--	70–720[d]	(U.S. EPA, 1975)
33.0	0.125	--	730–10,000[e]	(U.S. EPA, 1975)
33.0	0.125	--	120–3,630[f]	(U.S. EPA, 1975)
26.4	0.100	20–200[g]	< 1,000[h]	(Ter Haal et al., 1975)
18.0	0.068	25[i]	< 500[h]	(Cooper, 1984)
17.0	0.064	20–200	--	(Abbott, 1987)
16.5	0.063	70–140	90–3,800[j]	(HWC, 1978)
16.5	0.063	20	--	(Pierson et al., 1978)
8.3	0.031	17	--	(Ethyl Corp., 1990)
8.3	0.031	150[k]	55[l]	(U.S. EPA, 1990)
8.3	0.031	10–20	50–60[m]	(U.S. EPA, 1991b)
10.0	0.038	< 1–3[n]	34	(Loranger et al., 1995)
		2–29[o]	--	

Source: Table adapted from Loranger et al. (1995).
[a] Annual average.
[b] 24-hour average.
[c] EPA model: 24-hour average; beside highway (1–500 m), 20% emission at the tailpipe.
[d] Ethyl corp. model: 24-hour average, beside highway (1–500 m), 20% emission at the tailpipe.
[e] EPA model: hourly peak, beside highway (1-500 m), 20% emission at the tailpipe.
[f] Ethyl corp. model: hourly peak, beside highway (1-500 m), 20% emission at the tailpipe.
[g] Median value = 0.05, near roadway.
[h] Median value.
[i] Beside highways.
[j] Maximum monthly average.
[k] 30% emission at the tailpipe, mid-size car (20 mi/US gal).
[l] Urban annual average background concentration = 0.04 μg m^{-3}.
[m] SCREAM model, background concentration = 0.04 μg m^{-3}.
[n] CALINE4 and ISCLT models: > 250 m beside expressway.
[o] CALINE4 model: < 250 m beside expressway.
-- = no data

Personal exposures (expressed as concentration in air) to airborne manganese were measured before and after the introduction of MMT into 20% of the diesel fuel used in London (Pfeifer et al., 1999). Concentrations of manganese encountered by office workers and taxi drivers (10 subjects/occupation) were measured during 2-week periods in both 1995 (before MMT introduction) and 1996 (after MMT introduction). Manganese concentrations reported for office workers ranged from 2 to 239 ng/m^3 and from 4 to 147 ng/m^3 in 1995 and 1996, respectively. Taxi drivers experienced exposure to concentrations of 4 to 44 ng/m^3 and 9 to 36 ng/m^3 in 1995 and 1996, respectively. Thus, neither occupational group experienced apparent exposure to increased Mn after the introduction of MMT to gasoline. The greater exposure of office workers to airborne manganese when compared to taxi drivers was an unexpected result. The higher intake by office workers was attributed to manganese enrichment (approximately 10-fold greater than in the general environment) of the particulate matter in subway tunnels. When combined with elevated levels of particulates, manganese concentrations were estimated to be two orders of magnitude higher in the underground microenvironment. While these results differed from previous studies where, regardless of MMT use, taxi driver exposures to airborne manganese were higher than office workers' exposures (Lynam et al., 1994; Zayed et al., 1994; Riveros-Rosas et al., 1997), they are consistent with findings cited in Lynam et al. (1999) which indicated that subway system commuters in Toronto, Canada had higher manganese exposures than non-subway users.

The Particle Total Exposure Assessment Methodology (PTEAM) study provided information on levels of airborne manganese in Riverside, CA [findings summarized in Davis (1998)]. This study was conducted over a 7-week period in Fall 1990, and utilized personal and stationary monitors to measure indoor and outdoor concentrations of manganese. Study directors used a stratified sampling plan to select 178 individuals over the age of 10 to represent the general population of the region. Each individual was monitored over two 12-hour periods. Personal exposure measurements of manganese associated with PM_{10} (particulate matter of diameter 10 μm or less) indicated that approximately half of the population in Riverside experienced daily exposure to concentrations exceeding 35 ng/m^3. Approximately 1% of the population experienced personal exposures to manganese concentrations above 220 ng/m^3.

Another study measured concentrations of manganese associated with PM in Toronto, Canada during 1995–1996 (Pellizzari et al., 1999). Residential indoor, outdoor, and personal air samples were collected over 3-day periods. Table 5-6 lists the mean 3-day PM-associated manganese concentrations by sample type. Average concentrations for manganese associated with either PM_{10} or $PM_{2.5}$ (PM of diameter 2.5 μm or less) were higher in personal monitor samples than in indoor or outdoor air.

Clayton et al. (1999) simulated annual exposures to manganese using 3-day personal exposure measurements reported by Pellizari et al. (1999). The mean manganese exposure concentration for non-occupationally exposed populations was predicted to be 9.2 ng/m^3. Approximately 0.4% and 7.6% of the exposed population were estimated to have annual exposure concentrations greater than 25 ng/m^3 and 15 ng/m^3, respectively (Clayton et al., 1999).

Table 5-6. Mean Manganese Exposures from 3-day Indoor, Outdoor and Personal Air Samples.

Sample	PM_{10}-associated Mn (ng/m^3)[a]	$PM_{2.5}$-associated Mn (ng/m^3)
Personal	35.8	13.1
Indoor Air	8.0	5.5
Outdoor Air	17.5	9.7

Source: Pellizzari et al.(1999).
[a] Estimated from Figure 4 in Pellizarri et al. (1999).

Populations with Potential for High Exposure

Workers in certain occupations may be exposed to significantly higher manganese concentrations than the general population. Historically, the production of manganese fumes or manganese-containing dusts in the ferromanganese, iron and steel, dry cell battery manufacturing, welding, and mining industries may result in workplace concentrations as much as 10,000-fold higher than average ambient levels in air (ATSDR, 2000). ATSDR (2000) has noted that data for current occupational levels of manganese exposure are not available. However, to be in compliance with Occupational Safety and Health Administration (OSHA) regulations, manganese levels in the workplace should not exceed the OSHA time-weighted average Permissible Exposure Limit (PEL) of 1 mg/m^3.

5.2.2 Intake of Manganese in Air

General Population

U.S. EPA (1990) has calculated an average annual atmospheric manganese background concentration of 40 ng/m^3 for urban areas, based on data for 24-hour average concentrations in 102 cities across the U.S. (U.S. EPA, 1990). Assuming an intake of 15.2 cubic meters per day (m^3/day) (U.S. EPA, 1996d), the average estimated daily intake for a 70 kg adult would be 8.7 ng/kg-day. The corresponding average daily intake for a 10 kg child would be 35 ng/kg-day if an inhalation rate of 8.7 m^3/day (U.S. EPA, 1996d) is assumed. Alternatively, assuming a range of ambient concentrations from 2 to 220 ng/m^3 for rural and urban populations, and an inhalation rate of 15.2 m^3/day, the estimated daily intake range for a 70 kg adult would be 0.43 to 47.8 ng/kg-day. The daily intake for a 10 kg child would range from 1.74 to 122 ng/kg-day. These calculated adult intakes are in general agreement with intakes calculated by others. Loranger and Zayed (1997a) predicted a total manganese dose for adults of 1 to 50 ng/kg-day predicted for two urban sites in Montreal, Canada, using Monte Carlo simulation. Zayed et al. (1999a) calculated intakes of 5 to 15 ng/kg-day based on measurements of respirable manganese concentrations at five sites in Montreal.

Populations with Potential for High Exposure

Historically, workers in occupational settings such as manganese mining or ferromanganese smelting have experienced the potential for high levels of manganese exposure. Published estimates of current occupational exposure levels were not available in the materials reviewed for this document. However, assuming a maximal legal concentration of 1 mg/m^3 and inhalation of 10 m^3 of air over the course of a work day, adults exposed to manganese in some occupational settings may have a daily intake as high as 143,000 ng/kg-day (ATSDR, 2000).

5.3 Soil

5.3.1 Concentration of Manganese in 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 mg/kg, with a geometric mean concentration of 330 mg/kg (Shacklette and Boerngen, 1984). The estimated arithmetic mean concentration is 550 mg/kg. 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).

5.3.2 Intake of Manganese in Soil

General Population

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 of soil/day, the average manganese intake of a 70-kg adult would be 0.0014 to 5 μg/kg-day. The corresponding intake for a 10-kg child consuming 100 mg of soil/day would be 0.02 to 70 μg/kg-day.

Populations with Potential for High Exposure

No highly exposed populations were identified with respect to soil intake.

5.4 Other Media

No published reports identify other sources of manganese exposure.

5.5 Summary of Exposure to Manganese in Media Other Than Water

Table 5-7 summarizes information on exposure to manganese in media other than water. Inspection of data in this table reveals that ingestion of food contributes a major proportion of manganese exposure. This observation is consistent with the findings of Loranger and Zayed

(1995, 1997b), who estimated that food contributed 95 to 99% of the multimedia dose of manganese in Canadian studies. The contribution of soil as a source of manganese was not evaluated in the 1995 study (Loranger and Zayed, 1995). However, as evident from Table 5-7, soil ingestion has the potential to contribute substantially to intake in areas with naturally high or anthropogenically enriched concentrations of soil manganese.

EPA has derived an oral reference dose (RfD) for manganese of 0.14 mg/kg-day and an inhalation reference concentration (RfC) of 5×10^{-5} mg/m^3 (see Section 8.1). These values can be converted to daily doses (assuming a 70 kg adult inhaling 15.2 m^3/day of air) of 10 mg and 7.6×10^{-4} mg manganese, respectively. Thus, the level of safe exposure determined for the inhalation route is five orders of magnitude less than that determined for the oral route, reflecting the much greater toxicity observed for inhaled versus ingested manganese. For exposure to manganese from drinking water, EPA recommends applying an additional modifying factor of three to the above RfD, yielding 0.047 mg/kg-day (U.S. EPA, 1996a). This recommendation derives from concern raised by the Kondakis study (1989) (see Sections 7.1.3 and 8.1) about the potential for higher absorption of manganese from water, and also from consideration of potentially higher absorption in fasting individuals and neonates, the latter of which may have higher absorption rates and lower excretion rates of manganese than mature individuals (U.S. EPA, 1996a).

For drinking water, a National Secondary Drinking Water Regulation (or secondary Maximum Contaminant Levels, s-MCL) for manganese also exists (0.05 mg/L) to prevent clothes staining and taste problems. Secondary standards are non-enforceable guidelines regulating contaminants that may cause aesthetic effects (such as color, taste or odor) or cosmetic effects (such as skin or tooth discoloration) in drinking water. EPA recommends s-MCLs to water systems but does not require systems to comply.

Table 5-7. Summary of Human Exposure to Manganese in Media Other than Water (General Population).

PARAMETER	EXPOSURE MEDIUM					
	Food		Air		Soil	
	Adult	Child	Adult	Child	Adult	Child
Concentration in Medium	0.04–47 mg/kg		40 ng/m^3		< 2–7,000 mg/kg	
Estimated Average Daily Intake (µg/kg-day)	28.6–126	0.87–37.2 (infant) 128 (child)	0.0087	0.034	0.0014–5.0	0.02–70

6.0 TOXICOKINETICS

The absorption, distribution, metabolism and excretion of manganese in the body are reviewed, discussed, and summarized in Greger (1999), U.S. EPA (1984), Kies (1987), U.S. EPA (1993), 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 controlled by several dose-dependent processes: biliary excretion, intestinal absorption, and intestinal elimination. 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. Absorption of manganese via inhalation, intratracheal instillation, or intravenus infusions bypasses the control processes by the gastrointestinal tract. After absorption to the blood system by these alternate routes, 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 these other routes 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).

6.1 Absorption

Human Studies

The following sections discuss absorption of manganese following oral exposure only. 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).

Past manganese intake and iron, phosphorus, and calcium intake affect manganese absorption in humans. Further, phytate, fiber, and polyphenols (tannins) in vegetable diet tend to decrease manganese absorption (Greger, 1999; Greger and Snedeker, 1980). Manganese speciation and the route of exposure also affect its absorption (Andersen et al., 1999; Tjalve et al., 1996).

Mena et al. (1969) investigated gastrointestinal absorption of manganese in 11 healthy, fasted human subjects. The subjects received 100 μCi of $^{54}MnCl_2$ with 0.200 mg stable $^{55}MnCl_2$ (0.087 mg Mn) as a carrier. After 2 weeks of daily whole body counts, the absorption of ^{54}Mn was calculated to average approximately 3%. Comparable absorption values were found for healthy manganese miners and ex-miners with chronic manganese poisoning. However, enterohepatic circulation was not taken into account in this study. These values may therefore underestimate absorption (U.S. EPA, 1993).

Thomson et al. (1971) reported a higher absorption rate of $^{54}MnCl_2$ in segments of jejunum and duodenum using a double-lumen tube. The mean absorption rate in eight subjects was 27 ± 3%.

Schwartz et al. (1986) studied the absorption and retention of manganese over a 7-week period in seven healthy male volunteers aged 22–32 years. Volunteers consumed 3,100–4,400 kcal/day which provided levels of manganese ranging from 12.0 to 17.7 mg Mn/day. The authors noted that this level of manganese intake was higher than the 2 to 5 mg/day reported as being safe and adequate by the Food and Nutrition Board of the National Research Council (NRC, 1989). Assuming an adult body weight of 70 kg, this intake corresponds to 0.17 to 0.25 mg/kg-day. During weeks 2 to 4, manganese absorption was -2.0 ± 4.9% of the intake. During weeks 5 to 7, the reported absorption was 7.6 ± 6.3%. Despite the high level of intake, net retention of manganese was not observed in these individuals. Fecal loss accounted for nearly all of the ingested manganese, and in some cases exceeded the intake. A portion of this loss likely represents biliary secretion of previously absorbed manganese.

Sandström et al. (1986) administered 450 mL of infant formula containing 0.050 mg Mn/L to eight healthy subjects, aged 20 to 38 years. The average absorption for seven of the subjects was 8.4 ± 4.7%. The eighth subject was diagnosed with iron deficiency anemia, and absorbed 45.5%. Six additional subjects received 2.5 mg of manganese (as sulfate) in a multi-element preparation. The mean absorption for the second group of subjects was 8.9 ± 3.2%.

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%. This range corresponds to a 20-fold difference between the highest and lowest values. The mean value was 5.9±4.8%. Retention at day 10 ranged from 0.6–9.2%, with a mean value of 2.9±1.8% when measured in 14 healthy individuals. These results suggest substantial variation in absorption between individuals.

In addition, Davidsson et al. (1989a) studied manganese absorption from human milk, cow's milk, and infant formulas in human adults using extrinsic labeling of the foods with ^{54}Mn or ^{52}Mn and measurements of whole-body retention. The fractional manganese absorption from human milk (8.2%±2.9%) was significantly different when compared with cow's milk (2.4%±1.7%) or soy formula (0.7%±0.2%). The total amount of absorbed manganese, however, was significantly higher from the cow's milk formula as compared with human milk.

Several studies have reported a greater retention of manganese in the neonate than in adults. In a study of the nutritional requirements for manganese in pre-term infants, Zlotkin and Buchanan (1986) showed that 99% of the manganese given intravenously for 6 days was retained. Mena (1969) observed that healthy adults absorb 3% of ingested manganese. Lonnerdäl 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 and Lonnerdäl (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.

Dorner et al. (1989) studied retention of manganese in breast-fed infants compared to pre-term (~34-36 weeks gestational age) or full-term (2-17 weeks postgestational age) infants fed cow's milk formulas. This study is unique in that it analyzed potential differences in infant development on the intake and retention of manganese from different dietary sources. The authors observed that full-term breast-fed infants retain approximately 41% of ingested manganese from breast milk (containing 6.2 μg Mn/L). Manganese intake in the formula-fed infants (14.2 μg/kg, full-term and 15.0 μg/kg, pre-term) was high relative to that of breast-fed infants (1.06 μg/kg). Formula-fed infants also retained a higher absolute amount of manganese from their diet compared to breast-fed infants (0.06, 2.8, and 0.43 μg/kg retained in pre-term formula-fed, full-term formula-fed, and breast-fed, respectively). These data indicate that the percentage of manganese retained between the different food sources is not comparable; a higher percentage of ingested manganese from breast milk is retained by the infant. Nevertheless, formula-fed babies retain a larger total amount of manganese, due to the greater amount of manganese present in the formula (77-99 μg/L). The data also indicate that pre-term infants had an active excretory capacity for manganese obtained from formula, as compared to full-term infants.

Because human breast milk contains low levels of manganese (4-10 μg/L; Arnaud and Favier, 1995; Collipp et al. 1983; Dorner et al. 1989), it is suggested that the neonates' propensity to retain greater amounts of manganese was an adaptive mechanism to insure that sufficient amounts were available to the developing animal. Regardless of the mechanism (e.g., increased uptake and/or decreased elimination), results from human and animal studies suggest increased manganese retention in the neonate. Neurological development in the rat is incomplete at birth, suggesting that there may be differential susceptibility to excess levels of manganese during this critical developmental period. Although much of the nervous system is complete at birth in humans, there is evidence that some discrete neurological functions undergo further development after birth. The developmental stage in humans that is exactly comparable to the pre-weanling age in rats is unclear. Although results from animal data suggest that elimination rates reach adult levels by the age of weaning, the comparable period in human development at which manganese uptake and elimination reaches that of an adult is unknown.

Factors that Affect Absorption in Humans

Bioavailability of ingested manganese is an important issue in assessing the health hazard of manganese. Multiple factors have been reported to affect the absorption of manganese by humans, including chemical form, age, dose, route of exposure, and presence or deficiency of other dietary components (Greger, 1999; Greger and Snedeker, 1980). Thomson et al. (1971) and Gibbons et al. (1976) reported that the divalent form of manganese is absorbed most efficiently. However, the efficiency of absorption also varies for different manganese salts. In this regard, Bales et al. (1987) reported that manganese chloride was more efficiently absorbed than the sulfate or acetate salts.

Presence of other dietary components may influence the absorption of manganese. Calcium, for example, may inhibit the absorption of manganese. McDermott and Kies (1987)

suggested that this inhibition results from the influence of calcium on GI tract pH. Manganese is more readily absorbed as the Mn(II) form. As the pH rises, conversion to the less absorbable Mn(III) and Mn(IV) forms is favored, and uptake is decreased. Alternatively, calcium and manganese may compete for common absorption sites. The extent to which calcium effects on absorption influence net manganese balance is uncertain. However, Spencer et al. (1979) did not observe any significant effect of dietary calcium levels (from 200–800 mg/day) on manganese balance in healthy males.

A strong association between dietary iron and manganese uptake has been noted in several human studies. Thomson et al. (1971) observed that iron deficiency increased manganese absorption. Davis and Greger (1992) reported that women consuming increased levels of non-heme iron experienced decreased levels of serum and urinary manganese. Finley et al. (1994) observed that serum sodium ferritin concentration was negatively associated with manganese absorption in young women consuming a manganese-adequate diet.

Finley (1999) demonstrated that iron status (assessed as serum concentrations of sodium ferritin) may also affect manganese absorption and retention. Absorption (determined by regression of whole body ^{54}Mn counts) was assessed in women aged 20 to 45 years who were categorized as having high (upper 10% of normal range, mean values 68 to 69 µg/L) or low (lower 10% of normal range, mean values 8.7 to 8.9 µg/L) serum ferritin levels. Absorption was determined under conditions of high (9.5 mg Mn/day) or low (0.7 mg Mn/day) dietary manganese intake. Within a diet group, individuals with low ferritin absorbed 3- to 5-fold more manganese (as a percentage of dose) than individuals with high ferritin. Manganese absorption was greatest in women with low serum ferritin concentrations consuming the low manganese diet. The level of dietary manganese had no significant effect on absorption in women with high ferritin concentrations.

Phytate, a component of plant protein, may also interfere with manganese absorption. Davies and Nightingale (1975) observed a decrease in manganese retention in the presence of phytate. This result was attributed to the formation of a stable complex between manganese and phytate in the intestinal tract. Bales et al. (1987) reported that cellulose, pectin, and phytate reduced the plasma uptake of manganese in human subjects. These data suggest that the presence of these components contributes to the decreased bioavailability of manganese from vegetarian diets. However, Schwartz et al. (1986) found no significant correlation between phytate intake and manganese absorption in healthy males.

Ruoff (1995) conducted a literature review to determine the relative bioavailability of manganese from water versus food. The calculated ratio following evaluation of a wide variety of exposure scenarios in non-fasted subjects was 1.4. However, the difference in absorption between the two media was not statistically significant. The ratio for fasted subjects was 2.0, indicating that the absorption from drinking water is twice that from foods when the water is consumed in the absence of partially digested foods in the gastrointestinal tract. A study that directly measured the absorption of radiolabelled manganese from various manganese-rich plant foods given to adult men and women after an overnight fast reported a significantly greater

percent absorption of $MnCl_2$ from water compared to manganese absorption from lettuce, spinach, sunflower seeds, or wheat (Johnson et al., 1991). In addition, different diets may have different levels of constituents that affect manganese absorption. The greater levels of phytates, tannins, oxalates, and fiber in vegetarian diets, for instance, are expected to have an inhibitory effect on manganese uptake from the gastrointestinal tract. Johnson et al. (1991) reported mean percent absorption values from lettuce and spinach of 5.20 and 3.81%, respectively, and from sunflower seeds and wheat of 1.71 and 2.16%, respectively. Mean percent absorption values from $MnCl_2$ dissolved in water only (controls) ranged from 7.74 to 10.24%.

Animal Studies

There are studies using ^{54}Mn-labeled manganese to estimate absorption by animals. However, these studies measured the apparent absorption, not true absorption., because feeding radioactive isotopes of manganese does not eliminate the problem that absorbed manganese is very rapidly excreted through bile into the feces (Malecki et al., 1996). Thus, it is impossible to separate non-absorbed manganese from secreted manganese without elaborate study designs. When investigators used elaborate methodology in which ^{54}Mn bound to albumin was injected intraportally, true manganese absorption was calculated to be 8.2%, and 37% of the absorbed manganese was excreted into the gut (Davis et al., 1993).

Greenberg et al. (1943) administered a single oral dose containing 0.1 mg of ^{54}Mn-labeled manganese (as chloride) to rats, and estimated that 3–4% was absorbed from the intestine. Pollack et al. (1965) administered a single oral dose of ^{54}Mn as chloride with 5 µmol (0.27 mg Mn) stable carrier to fasted rats and reported 2.5–3.5% absorption 6 hours after administration. In separate studies, Rabar (1976) and Kostial et al. (1978) administered a single oral dose of ^{54}Mn as chloride, carrier free, to post-weaning non-fasted rats and reported 0.05% absorption 6 days after administration. This low absorption value may reflect either loss of absorbed manganese through fecal excretion, or the fact that the rats were not fasted (U.S. EPA, 1984).

Cikrt and Vostal (1969) showed that manganese is likely to be absorbed from both the small and large intestine in rats. Factors reported to influence manganese absorption in animals include dose, chemical form, and age. With respect to dose, Garcia-Aranda et al. (1983) studied the intestinal uptake of manganese in adult rats and concluded that saturation of the absorptive process occurred at higher levels of intake. Keen et al. (1986) observed that when suckling rats were fed 0.5 mL of infant formula containing 5 or 25 mg Mn/mL, retention of manganese decreased at the higher concentration.

Tissue levels of manganese may be influenced by the form of manganese administered in the diet. Komura and Sakamoto (1991) administered manganese in soluble (manganese acetate or manganese chloride) and relatively insoluble (manganese dioxide or manganese carbonate) forms to male ddY mice. Weight gain was reduced in animals receiving the more soluble forms. Manganese levels in the liver and kidney appeared to be higher in animals fed manganese acetate or manganese carbonate. The statistical significance of these apparent differences was not determined.

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 (≥80%) in pups less than 15 days old. In older pups (16–19 days old), the average retention was 40%. Keen et al. (1986) also administered infant formula to rat pups. Soy formula typically contains a much higher level of Mn than does human milk. The amount of manganese retained in 14-day old rat pups was 25 times higher in animals given soy formula when compared with pups receiving human milk.

Chan et al. (1987) demonstrated that developmental stage has a significant influence on the absorption of manganese. Manganese absorption decreased in rat pups from age 9 days to 20 days. The observed decrease in manganese absorption was correlated with a switch in the site of maximal absorption. The duodenum was more active in manganese uptake in younger rats, while the jejunum became more important as the animals matured.

Little is known about the factors that determine the bioavailability of ingested manganese in animals. Chan et al. (1982, 1987) reported differences in the concentration and chemical form of manganese found in different milk sources. Human milk contained only 0.008 ± 0.003 mg Mn/L, while bovine milk, infant formula and rat milk contained 0.030 ± 0.005, 0.073 ± 0.004, and 0.148 ± 0.018 mg Mn/L, respectively. However, absorption of manganese by suckling rats from these four types of milk was comparable, suggesting that total concentration may not always be a reliable indicator of bioavailable manganese. Chan et al. (1982) determined that the chemical form of manganese in infant formula is very different from that in human or cows' milk. Human and cow's milk contain two and three manganese-binding proteins, respectively. All manganese in milk from these sources is protein bound, while the manganese in infant formulas is in the form of soluble salts. The degree to which the association of manganese with protein influences absorption is unknown, but is likely to be important.

Lönnerdal et al. (1987) reported that age, manganese intake and dietary factors affect manganese absorption and retention in rats. Retention is very high during the neonatal period and decreases considerably with age. Decreased absorption with age apparently results from a combination of decreased intestinal absorption and increased excretion in the bile. In young rat pups, the bioavailability of manganese from various milk sources varied, with greater absorption occurring from human and cow's milk formula than from soy formula. These differences were less pronounced in older pups.

Several studies have explored the interrelationship among manganese, cobalt, and iron uptake. Thomson et al. (1971) reported that iron and cobalt compete with manganese for the same absorption sites. Competition was proposed to occur during uptake from the lumen into mucosal cells and in the transfer from mucosa into other compartments. Rehnberg et al. (1982) administered dietary Mn_3O_4 (450, 1,150, or 4,000 mg/kg 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. Gruden (1984) demonstrated that 3-week-old rat pups

given a high concentration of iron (0.103 mg Fe/L) in cow's milk absorbed 50% less manganese than pups receiving the control milk (0.005 mg Fe/mL). This difference was not observed in rats tested at 8, 11, 14, or 17 days of age, suggesting that the inhibition of manganese absorption by iron has a rapid onset during the third week of life.

6.2 Distribution

Human Studies

Manganese is a normal component of human tissues and fluids. Information about the distribution of manganese in humans is generally derived from post-mortem analyses of various organs and tissues. The patterns observed in these analyses reflect the body and organ burden of a lifetime intake of manganese. Cotzias (1958) and WHO (1981) reported a total of 12-20 mg manganese in a normal 70 kg man. Sumino et al. (1975) reported an average of 8 mg among 15 male and 15 female cadavers with an average weight of 55 kg.

The highest concentrations of manganese in the body of persons without excessive exposure are found in the liver, kidney, pancreas, and adrenal glands. Intermediate concentrations occur in the brain, heart and lungs (Table 6-1) (ATSDR, 2000). 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).

Manganese levels have been determined in human serum and blood. Serum concentrations in healthy male and female subjects in Wisconsin were 1.06 μg/L and 0.86 μg/L, respectively (Greger et al., 1990; Davis and Greger, 1992). Blood and serum levels of manganese in healthy subjects living in the Lombardy region of Italy were 8.8 ± 0.2 μg/L and 0.6 ± 0.014 μg/L, respectively (Minoia et al., 1990).

A variety of factors have been reported to influence manganese levels in blood and blood fractions. Hagenfeldt et al. (1973) found variations in plasma manganese concentrations in women and suggested that the variation may be due to hormonal changes. Horiuchi et al. (1967) and Zhernakova (1967) found no difference in the concentration of manganese in the blood of men and women. Slight seasonal (lower during summer and autumn) and diurnal (lower during the night) variations in blood manganese concentrations have also been reported (U.S. EPA, 1984).

Three studies have addressed manganese distribution within human organs. Perry et al. (1973) investigated manganese concentrations in different sections of the liver and found little variation. Larsen et al. (1979) and Smeyers-Verbeke et al. (1976) studied the regional distribution of manganese in the brain and reported the highest concentrations in the basal ganglia.

Table 6-1. Normal Manganese Levels in Human and Animal Tissues.

Tissue	Tissue concentrations (μg Mn/g wet weight)			
	Humans		Rats	Rabbits
	A	B	C	D
Liver	1.68	1.2	2.6–2.9	2.1
Pancreas	1.21	0.77	--	1.6
Adrenals	0.20	0.69	2.9	0.67
Kidney	0.93	0.56	0.9–1.0	1.2
Brain	0.34	0.30*	0.4	0.36
Lung	0.34	0.22	--	0.01
Heart	0.23	0.21	--	0.28
Testes	0.19	0.20	0.4	0.36
Ovary	0.19	0.19	--	0.60
Muscle	0.09	0.09	--	0.13
Spleen	0.22	0.08	0.3	0.22
Fat	--	0.07	--	--
Bone (rib)	--	0.06	--	--
Pituitary	--	--	0.5	2.4

Adapted from ATSDR (2000)
A Tipton and Cook (1963)
B Sumino et al. (1975)
C Rehnberg et al. (1982)
D Fore and Morton (1952)
* Average of cerebrum and cerebellum
-- No data

Studies by Schroeder et al. (1966) and Widdowson et al. (1972) indicate that placental transfer of manganese occurs in humans. Manganese levels in fetal and newborn tissues were reported to be similar to adult levels, with the exception of higher concentrations observed in fetal bone.

Animal Studies

Knowledge of manganese distribution patterns in animals was initially derived from parenteral exposure studies which facilitated the use of radioactive manganese as a tracer. The

distributions of parentally (injected) and orally administered manganese are very different. Cellular uptake of manganese is affected by the way in which manganese is transported in the plasma. Injected manganese (and probably inhaled manganese as well), which is transported by transferrin, is more apt to accumulate in the brain and cause toxicity than orally administered manganese, which is transported from the gut to the liver by albumin (Andersen et al., 1999; Davis et al., 1993). Davis et al. (1993) demonstrated that the distribution pattern of albumin-bound, but not transferrin-bound, intraportally-injected manganese was similar to that of orally-administered manganese.

Kato (1963) and Maynard and Cotzias (1955) suggested that mitochondria-rich tissues such as liver, kidney, and pancreas contain higher levels of manganese. Distribution studies in mice, rats, and monkeys have subsequently identified liver, kidney, and endocrine glands as primary sites of manganese accumulation following parenteral exposure. Kato (1963), for example, investigated distribution in mice using radiolabeled manganese. High levels of radioactive manganese were found in the liver, kidneys, and endocrine glands, with lesser amounts detected in brain and bone. Dastur et al. (1969) administered an intraperitoneal dose of radioactive manganese to rats, and subsequently found the highest concentrations of labeled manganese in suprarenal, pituitary, liver, and kidney tissue. In general, these results are in agreement with the patterns of manganese distribution observed in human tissues.

Dastur et al. (1971) observed a similar pattern of distribution in monkeys exposed to manganese by intraperitoneal injection. The highest concentrations of manganese were found in the liver, kidney and endocrine glands, as observed in rodents. Following treatment, manganese levels in the central nervous system decreased more slowly than levels in other tissues. Suzuki et al. (1975) injected monkeys subcutaneously with manganese, and subsequently found increased tissue concentrations of manganese in endocrine and exocrine glands (thyroids, parotids, and gall bladder) and in the nuclei of cerebral basal ganglia. Newland et al. (1989) noted substantial accumulation in the pituitary gland of *Macaca fascicularis* and *Cebus apella* monkeys at low cumulative doses.

Several studies have addressed regional distribution of manganese in the brain following parenteral exposure. Newland and Weiss (1992) investigated distribution of manganese in the brain of monkeys. Three Cebus monkeys received multiple intravenous doses of 5 or 10 mg/kg of manganese chloride over the course of 450 days. Magnetic resonance imaging revealed darkening of the globus pallidus and substantia nigra. This result is consistent with accumulation of manganese in these regions.

Scheuhammer and Cherian (1981) reported the distribution of manganese in male rat brain tissue with and without intraperitoneal exposure to 3 mg Mn/kg as manganese chloride. In unexposed rats, the highest concentrations of manganese were found in the hypothalamus, colliculi, olfactory bulbs, and midbrain. In treated rats, all brain regions showed an increase in manganese concentration, and the highest manganese concentrations were observed in the corpus striatum and corpus callosum.

Autissier et al. (1982) reported that rats given a daily intraperitoneal dose of 10 mg/kg-day manganese chloride for 4 months showed significant increases in the accumulation of manganese in the brain. This dose was equivalent to 4.4 mg Mn/kg-day. The study showed a 359% increase in the concentration of manganese in the brain stem, a 243% increase in the corpus striatum, and a 138% increase in the hypothalamus.

The tissue distribution of manganese appears to be affected by co-exposure to other metals. Shukla and Chandra (1987) exposed young male rats to lead (5 mg/L in drinking water) and/or manganese (1 or 4 mg/kg, by intraperitoneal injection) for 30 days. Exposure to individual metals resulted in accumulation in all brain regions. Co-exposure to lead and manganese resulted in increased levels of both metals, particularly in the corpus striatum. Administration of manganese alone led to dose-dependent increased levels in liver, kidney and testis. Co-exposure to lead further increased manganese accumulation in liver. The authors concluded that the interaction of metals can alter tissue distribution of manganese, and that adverse health effects may result from co-exposure to even low levels of metals.

The chemical form in which manganese is injected may influence the subsequent tissue distribution of manganese. Gianutsos et al. (1985) demonstrated that blood and brain levels of manganese in mice are increased following intraperitoneal injection of manganese chloride, manganese oxide, or methylcyclopentadienyl manganese tricarbonyl (MMT). However, $MnCl_2$ administration resulted in more rapid accumulation and ultimately higher levels of blood and brain manganese. It was suggested that the differences seen among the three manganese compounds result from the oxide and MMT forms being more hydrophobic. Hydrophobicity may cause formation of a depot at the site of injection that retards absorption. Gianutsos et al. (1985) also demonstrated that the exit of manganese from the brain is a slower process than its entry, resulting in a long retention period and potential accumulation. A single injection of 0.4 mEq Mn/kg resulted in a significant increase (> 2-fold) in brain levels within 1–4 hours. The increased levels were maintained for at least 21 days. Brain manganese levels were especially sensitive to a repeated dose regimen. Much greater accumulation occurred when the dose was divided into 10 injections given every other day as compared with a single injection. This observation may help explain the slow onset of manganese neurotoxicity: acute exposure results in other organs serving as the primary target, while chronic exposure results in gradually increasing brain levels with subsequent neurotoxicity.

Distribution of manganese has also been investigated in oral exposure studies. Chan et al. (1981) administered 278 mg/L manganese chloride in drinking water to rats for two years. At the termination of the study, these investigators found a 31% increase in manganese concentration in the brain and a 45% increase in the liver relative to control values. Assuming a body weight for rats of 0.35 kg and water consumption of 0.049 L/day, the average daily dose of manganese in this experiment was equivalent to 17 mg/kg-day.

Some oral exposure data suggest that developmental stage may influence the distribution of manganese. The brain, for example, may be a site for preferential accumulation of manganese in neonates. Kostial et al. (1978) observed that rat pups showed a greater accumulation of

manganese in the brain, but not in the liver, than did their mothers. The data of Rehnberg et al. (1980, 1981, 1982) indicate that the neonatal brain reaches higher concentrations of manganese than other tissues. The authors suggested that this pattern reflects a response to a nutritional need for manganese in the developing brain.

Kontur and Fechter (1985) demonstrated placental transfer of manganese in Long-Evans rats exposed via drinking water throughout gestation. Transfer was limited, with only 0.4% of the administered manganese accumulating in a single fetus. Neonatal pups of exposed dams had significantly increased levels of manganese in the forebrain. However, the increase was not associated with any overt signs of toxicity.

Komura and Sakamoto (1993) investigated the subcellular distribution of Mn and the binding characteristics of Mn to brain protein in male mice following administration of different forms of manganese. Four different manganese compounds ($MnCl_2 \cdot 4H_2O$, $Mn(CH_3COO)_2 \cdot 4H_2O$, $MnCO_3$, or MnO_2) were administered in the diet at a concentration of 2,000 mg Mn/kg for 12 months. Each treatment group included 6 male mice. The control group received a diet containing approximately 130 mg Mn/kg (form not specified). Assuming a food factor of 0.13, the control and treatment dietary levels correspond to approximately average daily doses of 17 and 260 mg Mn/kg-day, respectively. Cerebral cortex concentrations of Mn were significantly higher in mice receiving the relatively insoluble compounds $MnCO_3$ and MnO_2 than in controls. The subcellular distribution of manganese in the striatum and the gel chromatographic profiles of manganese were similar for all tested manganese compounds.

Roels et al. (1997) reported that repeated gavage dosing of rats (once weekly for 4 weeks) with 24.3 mg Mn/kg (5% of the dose, or 1.22 mg/kg, was assumed to be absorbed by the study authors) as $MnCl_2$ resulted in significantly increased concentrations of the metal in blood (68%) and brain cortex (22%) compared to saline controls but did not significantly increase striatal or cortex Mn concentrations. Similar administration of MnO_2 at the same dose level did not result in significant increases of Mn in blood or any brain tissue. Further studies indicated that Mn from $MnCl_2$ was absorbed much more rapidly and reached a higher peak concentration in the bloodstream of the dosed rats than did MnO_2. The peak Mn blood level following gavage dosing of $MnCl_2$ was roughly twice that of the oxide and was reported 1 hour post-dosing, while that of MnO_2 was not reported until 144 hours post-dosing (Roels et al. 1997). These data indicate that administered manganese can be distributed into the brain and the kinetics of uptake and partitioning depend on the chemical form of the manganese compound.

6.3 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). This reaction may be enhanced by the high affinity of the iron-transporting protein transferrin for Mn(III).

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.

6.4 Excretion

The primary route for elimination of manganese is to the feces through bile, as demonstrated in several animal studies (Weigand et al., 1986; Davis et al., 1993; Malecki et al., 1996). Fecal manganese concentration reflects both unabsorbed manganese and biliary secretion of absorbed manganese.

Human Studies

The primary route for elimination of manganese is via the feces. Fecal manganese concentration reflects both unabsorbed manganese and biliary secretion of absorbed manganese.

Price et al. (1970) determined the excretion pattern for preadolescent girls consuming 2.13 to 2.43 mg Mn/day. Approximately 1.66 to 2.23 mg Mn/day was excreted in the feces. In contrast, only 0.01 to 0.02 mg/day was excreted in the urine. Results from other studies confirm the importance of the fecal pathway for excretion. WHO (1981) and Newberne (1973) reported that human excretion of manganese in urine, sweat, and milk is minimal. The normal level of manganese found in urine of humans has been reported to be 1–8 μg/L, but values as high as 21 μg/L have also been reported (U.S. EPA, 1984). Greger et al. (1990) reported urinary excretion levels of 7.0 and 9.3 nmol Mn/g creatine/day (0.38 and 0.51 μg Mn/g creatinine/day) for healthy men and women, respectively. Urinary excretion of manganese was not responsive to oral intake levels of manganese (Davis and Greger, 1992).

A number of studies have addressed the kinetics of manganese excretion. Humans who ingested tracer levels of radioactive manganese excreted the tracer with whole-body retention half-times of 13 to 37 days (Mena et al., 1969; Davidsson et al., 1989b; Sandström et al., 1986). Sandström et al. (1986) gave volunteers a single oral dose of radioactive manganese and reported a mean biologic half-life value of 13 days (range 6–30 days) for 14 subjects monitored on post-exposure days 5–20, and a mean half-life of 34 days (range 26–54 days) for 6 subjects monitored on post-exposure days 20–50. Two additional subjects received manganese intravenously and experienced a much slower turnover.

Mahoney and Small (1968) investigated the clearance of intravenously injected $MnCl_2$ by humans. These investigators observed a biphasic clearance pattern, with a rapid phase that lasted 4 days and a slow phase that lasted 39 days. Schroeder et al. (1966) reported a whole body turnover rate in healthy adults of about 40 days, with a total body manganese content of about 20 mg.

Cotzias et al. (1968) injected manganese intravenously and reported values for biological half-time of 37.5 days in healthy subjects, 15 days in healthy miners, and 28 days in subjects with chronic manganese poisoning. These researchers also found that clearance by healthy subjects averaged 25 days from the liver, 54 days from the head, and 57 days from the thigh, as measured by external counting with a collimator. In healthy miners, liver clearance averaged 13 days; head clearance averaged 37 days; and thigh clearance averaged 39 days. Subjects with chronic manganese poisoning cleared manganese from the liver in 26 days, from the head in 62 days, and from the thigh in 48 days.

Finley (1999) demonstrated that iron status (assessed as serum concentrations of sodium ferritin) may affect manganese excretion. Biological half-life (determined by regression of whole body ^{54}Mn counts) was assessed in women aged 20 to 45 years who were categorized as having high (upper 10% of normal range, mean values 68 to 69 µg/L) or low (lower 10% of normal range, mean values 8.7 to 8.9 µg/L) serum ferritin levels. Biological half-life was determined under conditions of high (9.5 mg Mn/day) or low (0.7 mg Mn/day) dietary manganese intake. Subjects with low ferritin status consuming the low manganese diet had a mean biological half-life that was more than twice the value determined for high ferritin status subjects consuming the same diet (36.6 days versus 17.0 days). There was no effect of ferritin status on mean half-life for subjects consuming the high manganese diet (13.0 and 11.8 days for low and high ferritin status groups, respectively).

Animal Studies

No studies of excretion following oral administration of manganese in animals were identified.

Greenberg and Campbell (1940) reported that 90.7% of a 1 mg intraperitoneal dose of radiolabeled manganese (^{54}Mn) was found in rat feces within 3 days. In a subsequent study, Greenberg et al. (1943) found that 27.1% of a 0.01 mg intraperitoneal dose of radiolabeled manganese and 37.3% of a 0.1 mg dose were collected in rat bile within 48 hours. Tichy et al. (1973) administered a 0.6 µg dose of manganese chloride to rats and reported that 27% was excreted into the bile within 24 hours.

Klaassen (1974) demonstrated that bile is the main route of manganese excretion, and that biliary excretion represents a major homeostatic mechanism for manganese levels in the body. This investigator administered intravenous doses of 0.3, 1.0, 3.0, or 10.0 mg Mn/kg to rats, rabbits and dogs. Urinary excretion was low. As the dose increased, the excretion of manganese into the bile also increased. The concentration of manganese in bile was 100 to 200 times higher

than in plasma at the three lower doses. However, at the 10 mg dose there was no further increase in excretion of manganese into the bile. A maximum excretion rate of 8.5 μg Mn/min/kg was attained, suggesting that a saturable active transport mechanism may exist (U.S. EPA, 1984).

Britton and Cotzias (1966) and Suzuki (1974) found that an increase in dietary intake of manganese decreased biologic half-times. Studies also indicate that the biologic half-time of manganese in the brain of rats, mice and monkeys is longer than the half-life in the body (Suzuki, 1974; Dastur et al., 1969, 1971).

In developmental studies of manganese excretion, neonatal mice, rats, and kittens were found to rapidly accumulate manganese without excreting it during the first 18 days of life (U.S. EPA, 1984). In contrast, when lactating rats and cats were given excessive doses of manganese in drinking water (> 280 mg/L), their offspring initiated excretion before the 16th day of life.

Although human and animal evidence indicates that most manganese is excreted to the feces in bile, alternative routes for manganese excretion also exist. Experiments conducted by Bertinchamps and Cotzias (1958), Kato (1963), and Papavasiliou et al. (1966) demonstrated direct excretion of manganese through the intestinal wall. This route is most evident in the presence of biliary obstruction or following high doses of manganese. Bertinchamps et al. (1966) and Cikrt (1973) reported that in rats excretion of manganese occurred through the intestinal wall into the duodenum, jejunum and terminal ileum. Burnett et al. (1952) demonstrated that manganese excretion by dogs also occurs via the pancreatic juice. Other potential sources of fecal manganese include intestinal secretions and the manganese present in sloughed off intestinal microvillus cells. The fraction of total excretion attributable to these alternative pathways has not been reported, but is expected to be relatively small when compared to biliary secretion.

7.0 HAZARD IDENTIFICATION

7.1 Human Effects

7.1.1 Case Reports

General Population

A number of investigators reported the toxicity of total parenteral (TPN) manganese in humans, especially on changes in brain MRI scans (Ejima et al., 1992; Fell et al., 1996; Mirowitz and Westrich, 1992). These studies emphasize the difference in the effect of oral and parenteral manganese. When administered parenterally, manganese bypasses the typical excretory mechanisms in the gastrointestinal tract and liver and accumulates in the brain (Mirowitz and Westrich, 1992).

In addition, there are a limited number of case reports describing the outcome of exposure following accidental or intentional ingestion of manganese from potassium permanganate, a strong oxidizing agent. Unspecified toxic effects were reported following ingestion of 2.4 mg/kg-day potassium permanganate (0.83 mg Mn/kg-day) by a woman of unknown age and health status. This information was reported in a 1933 French study cited in NIOSH (1984), and was not available for review. Dagli et al. (1973) described a case in which oral ingestion of a 300 mg dose of potassium permanganate (104 mg Mn) resulted in extensive damage to the distal stomach and pyloric stenosis. Mahomedy et al. (1975) described two cases of methemoglobinemia following ingestion of an unspecified amount of potassium permanganate which had been prescribed by African tribal healers. Development of methemoglobinemia likely reflects the chemical oxidation of heme iron.

Holzgraefe et al. (1986) reported neurological effects in an adult man who ingested approximately 1.8 mg/kg-day of potassium permanganate (0.62 mg Mn) for 4 weeks. A syndrome similar to Parkinson's disease developed after about 9 months. However, data in this study are reported to be insufficient to establish causation (U.S. EPA, 1993). Bleich et al. (1999) published a 14-year follow-up of this case report. Most of the symptoms originally noted (including rigor, muscle pain, hypersomnia, increased libido, sweating, fatigue, and anxiety) had improved, and the study authors noted that there appeared to be no evidence for progression of the parkinsonian syndrome as described by others (Huang et al., 1998).

Additional case reports suggest the potential for manganese toxicity following oral exposure, but are difficult to assess quantitatively. One report involved a 59-year-old male who was admitted to the hospital with classical symptoms of manganese poisoning, including dementia and a generalized extrapyramidal syndrome (Banta and Markesbery, 1977). The patient's serum, hair, urine, feces, and brain were found to have manganese "elevated beyond toxic levels." No source of manganese exposure was identified for this individual. Exposure may have resulted from the use of large quantities of vitamin and mineral supplements for 4 to 5 years. No quantitative data were provided in this report.

Manganese intoxication was described in a 62 year-old male who received total parenteral nutrition that provided 2.2 mg of manganese (form not stated) daily for 23 months (Ejima et al., 1992). This level corresponds to a dose of approximately 0.023 mg Mn/kg-day for a 70 kg adult. The patient's whole blood manganese concentration was elevated. The patient exhibited dysarthria, mild rigidity, hypokinesia with masked face, a halting gait, and severely impaired postural reflexes, and the diagnosis of this condition was parkinsonism. Assuming an average absorption of roughly 5% of an oral dose, the intravenous dose of 2.2 mg Mn/day would be approximately equivalent to an oral intake of 40 mg Mn/day (U.S. EPA, 1993).

Sensitive Populations

Individuals with impaired liver function or bile flow may represent potentially sensitive subpopulations for manganese exposure. For example, Hauser et al. (1994) reported changes in brain MRI scans in liver failure patients which were identical to those observed in cases of manganese intoxication. The patients (n=3) examined exhibited bilateral signal hyperintensity in the globus palladi and substantia nigrae in T1-weighted MRI and increased blood manganese levels but had no history of increased exposure to manganese. Hauser et al. (1994) postulated that impaired elimination of normal dietary manganese could result in manganese intoxication. Devenyi et al. (1994) described a case study of an 8 year-old girl with Alagille's syndrome, an autosomal dominant disorder characterized by neonatal cholestasis, intrahepatic bile duct paucity, and end-stage liver disease. The patient exhibited a stable peripheral neuropathy, and for a period of 2 months exhibited episodic, dystonic posturing, and cramping of her hands and arms. Whole blood manganese level was elevated (27 μg/L, in contrast to a normal range of 4 to 14 μg/L), and cranial T1-weighted magnetic resonance imaging (MRI) revealed symmetric, hyperintense globus pallidi and subthalamic nuclei. These findings were interpreted as indications of manganese toxicity. Following liver transplantation, the patient's manganese levels returned to normal, her neurological symptoms improved, and MRI results appeared normal. The interpretation of this series of events was that: 1) progressive liver dysfunction resulted in inadequate excretion of manganese into the bile, 2) subsequent accumulation of manganese resulted in neurotoxicity, and 3) liver transplantation restored biliary excretion and alleviated the symptoms.

Manganese has been identified as a possible etiologic agent in the occurrence of neurological symptoms associated with hepatic encephalopathy (a brain disorder associated with chronic liver damage). Medical evidence supporting an etiologic role has been summarized by Layrargues et al. (1998). Patients with chronic liver disorders such as cirrhosis experience a high incidence of extrapyramidal symptoms resembling those observed in cases of occupational manganism. Manganese concentration increases in the blood and brain of patients with chronic liver disease and these changes are accompanied by pallidal hyperintensity on T1-weighted MRI. Autopsy data from ten patients who died in hepatic coma indicate that manganese levels are 2- to 7-fold higher in the globus pallidus of cirrhotic patients when compared to the general population. Liver transplantation normalizes the pallidal MR signals and results in the disappearance of extrapyramidal symptoms. Conversely, transjugular intrahepatic portosystemic shunting (a procedure which increases the systemic availability of manganese) intensifies the pallidal MR signal and results in deterioration of neurological function.

7.1.2 Short-term Studies

General Population

Kawamura et al. (1941) reported health effects resulting from the ingestion of manganese-contaminated well water by 25 individuals. The source of contamination was identified as leachate from approximately 400 dry cell batteries buried near the drinking water well. Chemical analysis also revealed high levels of zinc in the well water. The length of exposure to manganese was estimated to be 2 to 3 months. The concentration of manganese in the well was approximately 14 mg Mn/L (as Mn_3O_4) when analyzed 7 weeks after the first case appeared. This level corresponds to a dose of approximately 28 mg Mn/day (assuming a daily water intake of 2 L), or 0.5 mg Mn/kg-day (for a 60 kg adult). When reanalyzed 1 month later, the manganese concentration had decreased by about 50%. Based on these measurements, retrospective extrapolation suggests that the initial exposure level may have been 28 mg Mn/L or higher. Assuming a daily water intake of 2 L, and an additional manganese intake from food of at least 2 mg/day, this represents a dose of at least 58 mg Mn/day. This intake of manganese is about 10 to 20 times the level considered to be safe and adequate by the Food and Nutrition Board of the National Research Council (NRC, 1989). Assuming a body weight of 60 kg for an adult, this intake level corresponds to a dose of 0.93 mg Mn/kg-day from drinking water. No information on dietary intake was available.

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 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 control autopsies. Extreme macroscopic and microscopic changes were seen in the brain tissue, especially in the globus pallidus. The authors also reported elevated levels of zinc in the well water, but 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 batteries that caused the contamination were removed from the site. This pattern contrasts with the longer latency period and irreversible damage caused by inhalation exposure to manganese.

These observations may represent differences in the pharmacokinetics of ingested versus inhaled manganese, but there is little information to support this conclusion. Although these individuals were clearly exposed to high levels of manganese, it is possible that additional factors contributed to the observed effects (U.S. EPA, 1993; ATSDR 2000).

Sensitive Populations

Study data for sensitive populations were not identified in the materials reviewed for preparation of this document.

7.1.3 Long-Term and Epidemiological Studies

General Populations

Kondakis et al. (1989) conducted an epidemiologic study of manganese in drinking water in northwest Greece. Three areas with different levels of manganese in the drinking water supply were chosen for this study. Area A had manganese concentrations of 3.6 to 14.6 μg/L, Area B had concentrations of 81.6 to 252.6 μg/L, and Area C had concentrations of 1,800 to 2,300 μg/L. The total population in the study areas ranged from 3,200 to 4,350 people. The study included only individuals over the age of 50 drawn from a random sample of 10% of all households. The sample sizes were 62, 49, and 77 for areas A, B, and C, respectively. The study authors reported that "all areas were similar with respect to social and dietary characteristics," but few details were provided. Kondakis et al. (1989) determined whole blood and hair manganese concentrations in samples collected from study participants. The mean concentration of manganese in hair was 3.51, 4.49 and 10.99 μg/g dry weight for areas A, B and C, respectively. Concentrations in hair differed significantly between areas C and A ($p < 0.001$). No significant differences in whole blood manganese levels were observed among the three areas. However, manganese concentration in blood is not considered to be a reliable indicator of manganese exposure (U.S. EPA, 1993).

Kondakis et al. (1989) also administered a neurological examination which evaluated the presence and severity of 33 symptoms (e.g., weakness/fatigue, gait disturbances, tremors, dystonia) in all subjects. The results of the neurological examination were expressed as a composite score. A higher neurological score indicated an increased frequency and/or severity of the 33 evaluated symptoms. Results for the three geographic areas are summarized in Table 7-1. Mean scores for both sexes combined were 2.7 (range 0–21) for Area A; 3.9 (range 0–43) for Area B; and 5.2 (range 0–29) for Area C. The authors indicated that the difference in mean scores for Area C versus Area A was statistically significant (Mann-Whitney Test, $z = 3.16$, $p = 0.002$, for both sexes combined), suggesting neurologic impairment in people living in Area C. In a subsequent analysis, logistic regression indicated a significant difference between areas A and C when both age and sex were taken into account (Kondakis, 1990).

Table 7-1. Mean Neurological Scores of Residents in Three Areas of Northwest Greece with Different Levels of Manganese in Drinking Water (range is given in parentheses).

Subject	Area A (3.6–14.6 μg Mn/L)	Area B (81.6–252.6 μg Mn/L)	Area C (1,800–2,300 μg Mn/L)
Males	2.4 (0–21)	1.6 (0–6)	4.9 (0–29)
Females	3.0 (0–18)	5.7 (0–43)	5.5 (0–21)
Both	2.7 (0–21)	3.9 (0–43)	5.2 (0–29)

Source: Kondakis et al. (1989)

Limitations to the Kondakis study have been noted by ATSDR (2000). These include: 1) lack of clearly detailed descriptions of neurological signs and symptoms that reportedly increased following manganese exposure, and 2) failure to describe procedures for avoiding bias when evaluating subjective neurological scoring parameters. An additional shortcoming of this study is the lack of quantitative exposure data (U.S. EPA, 1996a). The individuals examined by Kondakis et al. (1989) also consumed manganese in their diet. The initial estimate of dietary intake was 10 to 15 mg/day based on high intake of vegetables (Kondakis, 1990). This figure was subsequently revised to an estimate of 5 to 6 mg Mn/day (Kondakis, 1993), but data were not provided to substantiate this estimate. Lack of dietary intake and water consumption data prevents determination of a quantitative dose-response relationship for manganese toxicity in this study. Nevertheless, this study raises concern for adverse neurological effects at estimated doses that are not far from the range of essentiality (U.S. EPA, 1996a).

Although conclusive evidence is lacking, some investigators have linked increased intake of manganese with violent behavior. Gottschalk et al. (1991) found significant increases in the level of manganese in the hair of convicted felons (1.62 ± 0.173 ppm in prisoners compared with 0.35 ± 0.020 ppm in controls). The study authors suggested that "a combination of cofactors, such as the abuse of alcohol or other chemical substances, as well as psychosocial factors, acting in concert with mild manganese toxicity may promote violent behavior." The number of potential variables indicates that caution should be exercised in interpretation of these data.

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 mg/kg), and the fruits and vegetables grown in the region are reported to contain elevated concentrations of manganese. 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. The affinity of manganese for pigmented tissue may result in variation of manganese concentration with hair color.

Goldsmith et al. (1990) investigated a Parkinson's disease cluster within southern Israel. The prevalence of the disease was increased among persons 50 to 59 years old, suggesting an early onset of the disease. 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. However, several factors limit the use of this study for evaluation of the human health effects of excess manganese exposure. Lack of environmental concentration data prevented reliable estimation of exposure rates. Potentially confounding factors included 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), a piperidine derivative which causes irreversible symptoms of parkinsonism in humans.

Vierrege et al. (1995) investigated the neurological impact of chronic manganese exposure via drinking water in a cross-sectional study of two proband cohorts in rural northern Germany. The study population was drawn from the county Herzogtum Lauernburg in the northernmost province of Germany. This region is characterized by agricultural and forestry activities but no steel or mining industry. Many of the residents of this area draw their drinking water from wells, and by law, the well water is routinely monitored for chemicals and bacteria. A survey was conducted in 1991 and was combined with a cross-sectional investigation of a randomly selected group of right-handed residents aged 40 years or older who had used their wells as the primarily source of drinking water for a minimum of 10 years (range 10 to 40 years). Complete documentation of manganese monitoring results for six years prior to the investigation was required for study eligibility. Participants were assigned to two groups on the basis of manganese concentration in their well water. Group A included individuals who continually ingested well

water containing between 0.300 and 2.160 mg Mn/L. Group B included individuals whose well water manganese concentration had never exceeded 0.050 mg/L. Detailed information on medical history, employment history, diet, alcohol consumption, drug use and smoking was collected by interview. Individuals in Group A were matched to individuals in Group B with respect to age, sex, nutritional habits, and drug intake. Criteria for exclusion from the study included history of employment in the steel industry, adherence to dietary restrictions, history of CNS-relevant drug use, diabetes mellitus, history of stroke, or treatment for psychiatric disorders. Conditions that could affect performance on the neurological assessment tests (neurorthopedic impairment of hand-finger function or poor vision) were also grounds for exclusion from the study.

A total of 164 eligible subjects was identified. Of these, 49 subjects were excluded for failure to meet the health or water monitoring criteria. Group A included 41 subjects (21 male and 20 female) with a mean age (± standard deviation) of 57.5 ± 10.3 years (range 41 to 84 years). Group B included 74 subjects with a mean age of 56.9 ± 11.8 years (range 41 to 86) years. No dietary differences were evident between the two groups. Neurological status was assessed by experienced personnel blinded to the group status of the subjects. Each participant was evaluated for neurotoxicological symptoms by use of a modified German version of a standardized symptoms list. Signs of parkinsonism were evaluated by the Columbia University Rating Scale (CURS). Fine motor ability (each hand) was assessed using a conventional apparatus ("Motorische Leistungsserie," MLS) and application of aiming, steadiness, line pursuit, and tapping tests. Manganese status was evaluated by determination of manganese in blood. The concentration of manganese in hair or nails was not determined.

The results of neurological evaluations are summarized in Table 7-2. There were no significant differences between groups for the mean item scores on the standardized symptoms list or the CURS. MLS test results were obtained for 36 group A subjects (18 male and 18 female, mean age 56.4 ± 8.4 years, range 41 to 72 years) and 67 Group B subjects (35 men and 32 women, mean age 55.1 ± 9.9 years, range 41 to 72 years). Results of participants older than 72 years were not included in the statistical analysis of MLS data because normative information from the general population have an upper age limit of 72 years. No significant differences were observed between groups for any test when results were standardized to age-corrected values. Mean blood manganese concentrations were 8.5 ± 2.3 µg/L and 7.7 ± 2.0 µg/L for groups A and B, respectively. The blood manganese values did not differ significantly and both fell within the normal range for the general (non-occupationally exposed) population. Separate analyses for possible confounding factors did not reveal differences in clinical or instrumental test outcomes related to high or low consumption of alcohol, mineral water, coffee, tea, tobacco, vegetables, or fruit. Where cases of parkinsonism (n = 3) were encountered in this study, they occurred in the low exposure group (Group B) and were considered to be typical Parkinson's disease and thus unrelated to manganese exposure. The authors of this study concluded that there was no evidence of an association between consumption of high concentrations of manganese in well water and neurological impairment (including those suggestive of parkinsonism).

Table 7-2. Mean Neurological Scores of Residents in Germany Exposed to Different Levels of Manganese in Well Water.

Assessment	Measure	Exposure Group	
		Group A (High)	Group B (Low)
Neurotoxicological Symptom Questionnaire	Item Number	3.2 ± 3.0[a]	3.9 ± 3.1
CURS Parkinsonism	Item Number	1.2 ± 1.0	1.7 ± 2.0
MLS Aiming	Duration (sec)	104.8 ± 9.1[b]	102.9 ± 10.0
MLS Steadiness	Errors (number)	103.9 ± 103.9	103.1 ± 7.9
	Duration of errors (sec)	100.8 ± 10.6	100.2 ± 10.5
MLS Line Pursuit	Errors (number)	106.4 ± 7.6	106.6 ± 8.0
	Duration of errors (sec)	102.3 ± 8.1	103.1 ± 10.6
	Total duration (sec)	104.3 ± 12.6	100.7 ± 15.5
MLS Tapping	Rate (number)	103.1 ± 7.2	103.9 ± 10.5

[a] Mean ± standard deviation
[b] MLS test results are for right hand

Three potential limitations related to the ecologic design of this investigation were noted by Vieregge et al. (1995). First, the investigators could not control for possible migration of subjects with manganese-induced neurological disorders from the study area prior to the investigation. However, Vieregge et al. (1995) stated on the basis of inquiries and general experience in the region that a migration effect was unlikely to be significant. Second, although possible confounding by several dietary items or groups was evaluated and found to be non-evident, confounding effects of nutrition (particularly in subjects working outside their home residence) could not be completely excluded. Finally, blood manganese levels are thought to primarily reflect current body burden of manganese rather than exposure.

Iwami et al. (1994) reported that the incidence of motor neuron disease (MND) in a small town in Japan was positively correlated with a significantly increased manganese concentration in local rice and a low magnesium concentration in the drinking water. This study, however, did not provide good estimates of overall exposure to manganese in either the control population or the population with MND.

Adverse neurological effects (decreased performance in school and in neurobehavioral exams of the WHO core test battery) were reported in 11- to 13-year-old children who were exposed to excess manganese through ingestion of well water and from wheat fertilized with sewage water (He et al. 1994; Zhang et al. 1995). The exposed group consisted of 92 children pair-matched to 92 controls from a nearby area. The groups were matched for age, sex, grade, family income level, and parental education level; further, both groups lived on farms. The average manganese concentration of the drinking water of the exposed group was 0.241 mg/L compared to the control level of 0.04 mg/L. Although the study authors had drinking water data from a 3-year period, it was not clear how long the children were exposed prior to the study. Further, the exposure data were not well-characterized; therefore it was not possible to establish a cause-effect link between ingestion of excess manganese and preclinical neurological effects in children.

Sensitive Populations

Study data for sensitive populations were not identified in the materials reviewed for preparation of this document.

7.1.4 Beneficial Effects

Manganese is a naturally-occurring element that is required for normal physiological functioning in all animal species (U.S. EPA, 1996a). Manganese plays a role in bone mineralization, metabolic regulation, protein and energy metabolism, protection of cells from oxidative stress, and synthesis of mucopolysaccharides (ATSDR, 2000). Many of these roles are achieved by participation of manganese as a catalytic or regulatory factor for enzymes, including hydrolases, dehydrogenases, kinases, decarboxylases and transferases. In addition, manganese is a structural component of the metalloenzymes mitochondrial superoxide dismutase, pyruvate carboxylase, and liver arginase. Additional information on the biochemical and nutritional roles of manganese in human health is available in Wedler (1994) and Keen et al. (1999). At present, the optimal levels of oral manganese exposure have not been well defined for humans (Greger, 1999).

Overt signs of manganese deficiency have been demonstrated in multiple animal species (Keen et al., 1999). Biochemical effects observed in manganese-deficient animals include alterations in carbohydrate, protein, and lipid metabolism. Physiological outcomes associated with deficiency include impaired growth (Smith, 1944), skeletal abnormalities (Amdur et al., 1944; Strause et al., 1986), impaired reproductive function in females, and testicular degeneration in males (Boyer et al., 1942). The molecular basis for these effects has not been established with certainty, but may be related to the participation of manganese in numerous enzymatic reactions. In addition, the effect of manganese deficiency on mitochondrial superoxide dismutase activity has functional consequences. Manganese-deficient rats experience more oxidation of mitochondrial membranes of the heart and more formation of conjugated dienes than manganese-adequate rats (Malecki and Greger, 1996). In another study, Gong and Amemiya (1996) observed ultrastructural changes suggestive of oxidative damage in the retinas of rats fed a manganese-deficient diet for 12 to 30 months

Manganese is ubiquitous in human foods, and outright manganese deficiency has not been observed in the general population. However, observations reported by Doisy (1973) and Friedman et al. (1987) indicate that manganese is an essential element for humans. Doisy (1973) reported a decreased level of clotting proteins, decreased serum cholesterol, reddening of black hair, retarded growth of hair and nails, and scaly dermatitis in a subject inadvertently deprived of manganese. Friedman et al. (1987) administered a manganese-deficient diet to seven men for 39 days. Five of the seven subjects exhibited dermatitis at the end of the manganese-deficient period. The development of dermatitis was attributed to decreased activity of manganese-requiring enzymes that are required for skin maintenance. The symptoms cleared rapidly when manganese was restored to the diet.

7.2 Animal Studies

This section presents the results of manganese toxicity studies in animals. The first four subsections provide study results by duration of exposure. In general, acute studies are those which address exposure durations of 24 hours or less. Short-term studies have exposure durations greater than 24 hours but less than approximately 90 days. The exposure duration of subchronic studies is typically 90 days, and chronic studies are those in which exposure is longer than 90 days. Some studies may fall into more than one exposure category since they measure impacts over several exposure periods. The discussion of acute, short-term, subchronic and chronic studies summarizes observed toxicological effects on all body systems. The remaining subsections of Section 7.2 provide toxicological data related to specific organ systems and types of endpoints, including neurotoxicity, developmental and reproductive toxicity, and carcinogenicity.

7.2.1 Acute Toxicity

Oral Exposure

LD_{50} values determined for selected manganese compounds are summarized in Table 7-3. Oral LD_{50} values among the water soluble manganese compounds ranged from 400 to 475 mg Mn/kg for manganese chloride, and from 379 to 810 mg Mn/kg for potassium permanganate. An LD_{50} of 836 mg Mn/kg was reported for manganese acetate.

Age may be a factor in susceptibility to acute manganese toxicity. Kostial et al. (1978) found that $MnCl_2$ produced the greatest oral toxicity in the youngest and oldest groups. 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.

Table 7-3. LD_{50} Values for Manganese Compounds.

Compound	Species	Route	LD_{50} (mg Mn/kg)	Reference
Manganese acetate	rat	oral	836	Smyth et al. (1969)
Manganese chloride	rat	oral	425	Shigan and Vitvickaja (1971)
	rat	oral	475	Kostial et al. (1978)
	rat	oral	410	Holbrook et al. (1975)
	mouse	oral	450	Shigan and Vitvickaja (1971)
	guinea pig	oral	400	Shigan and Vitvickaja (1971)
	rat	i.p.	38	Franz (1962); Holbrook et al. (1975)
	mouse	i.p.	56	Franz (1962); Holbrook et al. (1975)
	mouse	i.v.	16	Larsen and Grant (1997)
Manganese dioxide	rat	oral	2,197	Holbrook et al. (1975)
Manganese sulfate	mouse	i.p.	44	Bienvenu et al. (1963)
Manganese sulfate, tetrahydrate	mouse	i.p.	64	Yamamoto and Suzuki (1969)
Manganese nitrate	mouse	i.p.	56	Yamamoto and Suzuki (1969)
Methylcyclo-pentadienyl manganese tricarbonyl (MMT)	rat	oral	10	Hanzlik et al. (1980)
	rat	oral	12	Hinderer (1979)
	rat	oral	12	Hysell et al. (1974)
	mouse	oral	48	Hinderer (1979)
Potassium permanganate	mouse	oral	750	Shigan and Vitvickaja (1971)
	rat	oral	379	Smyth et al. (1969)
	rat	oral	750	Shigan and Vitvickaja (1971)
	guinea pig	oral	810	Shigan and Vitvickaja (1971)

i.p. = intraperitoneal
i.v. = intravenous

Parenteral Exposure

Manganese compounds administered by parenteral routes generally result in mortality at lower doses. LD_{50} values for the intraperitoneal route ranged from 14 to 64 mg Mn/kg. Franz (1962) and Bienvenu et al. (1963) conducted comparative intraperitoneal toxicity studies, and found that manganese is less toxic than many other metals. Jonderko (1965) found increased serum calcium and decreased inorganic phosphorous in rabbits exposed intramuscularly to 3.5 mg Mn/kg. Details on the compound and the duration of exposure were not available.

Baxter et al. (1965) measured physiological parameters in manganese-treated rats weighing 100 to 550 g. Measurements were made 1 to 72 hours after subcutaneous administration of 5 to 150 mg of manganese as $MnCl_2$ in saline. Levels of hemoglobin, hematocrit, and mean corpuscular volume were significantly increased in rats receiving 150 mg Mn/kg. A measurable response in these parameters occurred at 50 mg Mn/kg, while the peak increase in these parameters occurred at 12 and 18 hours after dosing. The maximum response occurred at 170 to 300 mg Mn/kg. Necrotic changes were noted in hepatic tissue 18 hours after a single dose of 170 mg Mn/kg.

Pancreatic endocrine function is affected by acute manganese exposure. Baly et al. (1985) injected rats intraperitoneally with 40 mg Mn/kg. Manganese injection resulted in a decrease in plasma insulin levels, an increase in plasma glucose levels, and a transitory increase in glucagon concentration.

Larsen and Grant (1997) administered a single intravenous dose of 150, 200, 300, or 400 µmol/kg manganese chloride 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 LD_{50} value of 300 µmol/kg (16 mg Mn/kg).

7.2.2 Short-Term Studies

Oral Exposure

Matrone et al. (1959) orally administered 2,000 ppm manganese as $MnSO_4 \cdot H_2O$ to 6-month-old anemic rabbits for 6 weeks. The investigators also administered 125 ppm Mn as $MnSO_4 \cdot H_2O$ to anemic newborn pigs for 27 days. In each case, the investigators observed decreased hemoglobin content in the blood of treated animals. Hemoglobin depression in baby pigs fed up to 2,000 ppm manganese was overcome by a dietary supplement of 400 ppm iron.

Kimura et al. (1978) provided rats with diets supplemented with 564 mg/kg of manganese as $MnCl_2$ for 3 weeks. Assuming a food consumption factor of 0.05 above the dietary background, 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.

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 β-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 α-amylase activity in the livers of treated rats.

Hietanen et al. (1981) also 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 0.049 L/day (U.S. EPA, 1986d), this corresponds to an exposure of 0.7 mg Mn/kg-day. Changes in the activity of several enzymes, including aryl hydrocarbon hydroxylase, ethoxycoumarin *O*-deethylase, and epoxide hydrase, were observed at 1 week but not at 6 weeks. Enzyme activities were increased in the liver, and decreased in the intestines and kidney.

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 $B6C3F_1$ 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.

Parenteral Exposure

Singh et al. (1974; 1975) administered 6 mg Mn/kg-day (as $MnSO_4 \cdot 4H_2O$) intraperitoneally to male IRTC rats for 25 days. Histopathological analysis of the livers revealed mild congestion of central veins and sinusoids, and some focal necrosis in treated animals.

Scheuhammer and Cherian (1983) reported toxic effects in the pancreas resulting from intraperitoneally injected manganese. The exposure duration was 30 days. Adverse effects included a pancreatitis-like reaction. The authors suggested that this reaction was potentiated by

the presence of manganese in the peritoneal cavity, and would not occur as readily with manganese administered by the oral route.

Khandelwal et al. (1984) administered 6 mg Mn/kg-day (as $MnCl_2 \cdot 4H_2O$) intraperitoneally to male IRTC rats for 28 days. Activity of succinic dehydrogenase and cytochrome oxidase in liver tissue were decreased after 28 days of manganese treatment.

Khan et al. (1997) administered 16 mg/kg-day $MnCl_2 \cdot 4H_2O$ in saline intravenously to male beagle dogs (3/group). Treatment duration was up to 4 hours/day for 4 days. Two of the three dosed animals were in moribund condition, and were sacrificed for ethical reasons (one on day 3 and one on day 4). The third treated dog died on day 4. Symptoms prior to death included vomiting, diarrhea, tremors, lethargy, reduced food intake, reduced blood pressure with reflex tachycardia, and severe hepatotoxicity.

7.2.3 Subchronic Studies

Oral Exposure

Mitochondria-rich organs, such as the liver and pancreas, are hypothesized to be most affected by excess manganese exposure. 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 0.049 L/day and an average body weight of 0.35 kg (U.S. EPA, 1986d), 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.

Carter et al. (1980) exposed young, iron-deficient rats to 400 to 3,550 ppm Mn as Mn_3O_4 for 32 weeks (route not specified). Manganese treatment resulted in decreased hemoglobin levels.

Leung et al. (1982) administered 1,000, 10,000, or 20,000 mg $MnCl_2 \cdot 4H_2O$/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.

In a 13-week study, NTP (1993) administered diets containing 0, 1,600, 3,130, 6,250, 12,500, or 25,000 mg/kg manganese sulfate monohydrate above basal levels to F344 rats (10/sex/dose). The concentration of manganese in the control diets was approximately 92 mg/kg. 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 ≥6,250 mg/kg manganese sulfate experienced decreased body weight gain. Absolute and relative liver weights were decreased in males receiving diets with ≥1,600 mg/kg, 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 ≥6,250 mg/kg in the diet and females in the three highest dose groups. The low dose of 1,600 mg/kg (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 0, 3,130, 6,250, 12,500, 25,000, or 50,000 mg/kg manganese sulfate monohydrate above basal levels to B6C3F_1 mice (10/sex/dose). The concentration of manganese in the control diets was approximately 92 mg/kg. 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. All groups of male mice and female mice in the highest dose group exhibited significantly decreased body weight gain. Relative and absolute liver weights were decreased in males in the highest dose group. Both sexes receiving the 50,000 mg Mn/kg diet exhibited decreased hematocrit and hemoglobin concentration. The NTP report suggests that these findings may indicate microcytic anemia, which may have resulted from a sequestration or deficiency of iron. Males receiving ≥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 mg/kg-day (107 mg Mn/kg-day), based on significantly decreased body weight gain in male mice.

Komura and Sakamoto (1991) supplemented mouse diets with different chemical forms of manganese. 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 $MnCl_2 \cdot 4H_2O$, $Mn(CH_3COO)_2 \cdot 4H_2O$, $MnCO_3$, 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 $Mn(CH_3COO)_2 \cdot 4H_2O$ or $MnCl_2$. Motor activity was reduced in the $MnCO_3$ group. Tissue manganese concentrations in groups receiving supplemental

manganese was 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.

Parenteral Exposure

Suzuki et al. (1975) administered 250, 500, or 1,000 mg of MnO_2 in saline to monkeys (*Macaca mullata*) by subcutaneous injection. Injections were given once a week for 9 weeks. The study authors reported a body weight of 4 kg for monkeys used in the study. 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.

7.2.4 Neurotoxicity

Occupational studies of miners, industrial workers, and agricultural workers have established injury to the central nervous system as the chief health effect associated with inhalation exposure to manganese. High level exposure by this pathway typically results in a suite of neurological effects collectively termed manganism. Chronic manganism associated with inhalation exposure is characterized by an extrapyramidal syndrome with symptoms that are somewhat similar to those observed in Parkinson's disease. One characteristic difference is the "cock-walk"of the manganism patient, in which the patient walks on his toes with his spine erect and elbows flexed. Further, manganism patients do not often exhibit the "resting tremor" that Parkinson's patients do, and they have a propensity for losing their balance and falling backwards. The clinical course of manganism occurs in three phases: an initial phase of subjective and nonspecific symptoms; an intermediate phase of evolving neurological symptoms related to speech, dexterity, facial expression, and movement; and an established phase characterized by persistent, often irreversible neurological deficits (Chang, 1996). While MRI scans of the brains of humans and non-human primates exposed to excess manganese indicate that the metal deposits in the globus pallidus and to a lesser extent in the substantia nigra, degenerative lesions are limited to the globus pallidus (Calne et al. 1994). An important question in the evaluation of health effects associated with manganese in drinking water is whether similar neurotoxicological effects occur following exposure by the oral route.

Oral Exposure

Table 7-4 summarizes studies of the neurotoxic effects of manganese exposure. A single study exists for evaluation of manganese exposure in primates by the oral route. Gupta et al. (1980) administered 25 mg $MnCl_2 \cdot 4H_2O$/kg orally to four male rhesus monkeys daily for 18 months. This level is equivalent to an average daily dose of 6.9 mg Mn/kg-day. Animals were maintained on monkey pellets, two bananas/day, and tap water. The monkeys developed muscular weakness and rigidity of the lower limbs. Histological analysis revealed degenerated neurons in the substantia nigra and scanty neuromelanin granules in some of the pigmented cells.

Bonilla and Diez-Ewald (1974) noted that chronic exposure of rats to manganese chloride produces a marked decrease in brain biogenic amines, particularly dopamine.

Singh et al. (1979) administered manganese (16 mg/kg in a 10% sucrose solution) alone or in combination with ethanol to groups of 20 male albino rats for 30 days. Exposure to manganese alone led to a 72% increase in manganese concentration in the brain (3.13 μg/g dry weight versus 1.82 μg/g for controls). This outcome was not altered by ethanol exposure. There were no morphologic changes in the brain tissue of any group. Significant alterations in activity were reported for several brain enzymes. Manganese exposure resulted in significant increases in monoamine oxidase ($p < 0.001$), adenosine triphosphatase ($p < 0.001$), ribonuclease ($p < 0.001$), and glutamate-oxaloacetate transaminase (= aspartate aminotransferase, or AST; $p < 0.001$). Significant decreases were observed for succinic dehydrogenase ($p < 0.02$ and deoxyribonuclease ($p < 0.001$). Concurrent exposure to ethanol resulted in a synergistic effect with some enzymes and an antagonistic effect with others. No mechanism was proposed to explain the pattern observed in the presence of ethanol.

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 manganism.

Gray and Laskey (1980) found that dietary exposure to 1,100 mg/kg manganese (as Mn_3O_4) in rats for 2 months produced only reduced reactive locomotor activity. Assuming a body weight of 0.35 kg, this level of exposure corresponds to an average daily dose of 55 mg Mn/kg-day. Deskin et al. (1980) studied neurochemical alteration induced by manganese chloride in neonatal CD rats. Rats were intubated with daily doses of 1, 10, or 20 mg Mn/kg-day from birth to 24 days old. Neurochemical components were subsequently analyzed in the hypothalamus and corpus striatum. Administration of 10 and 20 mg Mn/kg-day resulted in significantly elevated manganese concentrations in both regions, but neurochemical alterations were observed only in the hypothalamus. These alterations included a decrease in dopamine concentration and turnover. The highest dose of manganese also resulted in a significant decrease in hypothalamic tyrosine hydroxylase activity, and an increase in monoamine oxidase activity. Visible signs of toxicity were not observed in any group.

Deskin et al. (1981) intubated rats with daily doses of 10, 15 or 20 mg $MnCl_2 \cdot 4H_2O$/kg from birth to 24 days old. The authors reported a significant elevation of serotonin levels in the hypothalamus, but not the striatum, following exposure to 20 mg Mn/kg.

Table 7-4. Neurological Effects of Oral Exposure to Manganese.

Species	Compound	Route	Dose	Duration	CNS Effects			Reference
					Behavioral	Histological	Biochemical	
Mouse	$MnCl_2$	drinking water	3 µg $MnCl_2$/mL	6 months	+	NS	+	Chandra et al. (1979)
Mouse	$MnCl_2$	diet	1% $MnCl_2$ (1 month), 4% $MnCl_2$ (5 months)	6 months	NS	NS	+	Gianutsos and Murray (1982)
Mouse	MnO_2	diet	1 mg MnO_2/g	7.5 months	-	NS	NS	Morganti et al. (1985)
Rat	$MnCl_2$	drinking water	5 mg $MnCl_2$/mL	7 months	NS	NS	+	Bonilla and Diez-Ewald (1974)
Rat	$MnCl_2$	gavage	1, 10, 20 mg Mn/kg-day	Birth–24 days old	NS	NS	+	Deskin et al. (1980)
Rat	$MnCl_2 \bullet 4H_2O$	gavage	10, 15, 20 mg Mn/kg-day	Birth–24 days old	NS	NS	+	Deskin et al. (1981)
Rat	$MnCl_2 \bullet 4H_2O$	drinking water	1 mg $MnCl_2 \bullet 4H_2O$/mL	12 months	+	NS	+	Chandra and Shukla (1981)
Rat	$MnCl_2 \bullet 4H_2O$	drinking water	1 mg $MnCl_2 \bullet 4H_2O$/mL	28 months	NS	NS	+	Leung et al. (1981)
Rat	$MnCl_2 \bullet 4H_2O$	drinking water	1 mg $MnCl_2 \bullet 4H_2O$/mL	over 2 years	NS	NS	+	Lai et al. (1981)
Rat	$MnCl_2 \bullet 4H_2O$	drinking water	1 mg $MnCl_2 \bullet 4H_2O$/mL	4 months	NS	-	+	Lai et al. (1982a)

Table 7-4 (continued)

Species	Compound	Route	Dose	Duration	CNS Effects			Reference
					Behavioral	Histological	Biochemical	
Rat	$MnCl_2$	gavage	150 mg Mn/kg	42 days	+	NS	+	Kristensson et al. (1986)
Rat	$MnCl_2 \bullet 4H_2O$	drinking water	1 mg $MnCl_2 \bullet 4H_2O$/mL	65 weeks	+	NS	NS	Nachtman et al. (1986)
Rat	$MnCl_2 \bullet 4H_2O$	drinking water	4,360 mg Mn/L	60–265 days	NS	NS	+	Eriksson et al. (1987)
Rat	$MnCl_2$	gavage	25, 50 mg $MnCl_2 \bullet 4H_2O$ /kg-day	14 or 21 days	NS	NS	+	Kontur and Fechter (1988)
Rat	$MnCl_2 \bullet 4H_2O$	gavage	0.357 Mn mg/kg-day	30 days	+	NS	NS	Oner and Senturk (1995)
Rat	Not specified	10% sucrose	16 Mn mg/kg	30 days	NS	NS	+	Singh et al. (1979)
Monkey	$MnCl_2 \bullet 4H_2O$	diet	25 mg $MnCl_2 \bullet 4H_2O$/kg	18 months	+	+	NS	Gupta et al. (1980)

Notes: NS = Not studied
Source: U.S. EPA, 1993

Chandra and Shukla (1981) exposed male albino rats to 1,000 mg/L $MnCl_2 \cdot 4H_2O$ (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.

Lai et al. (1981) exposed female Wistar rats to 1,000 mg/L $MnCl_2 \cdot 4H_20$ (280 mg Mn/L) in drinking water. Exposure was initiated at mating. Pups were exposed *in utero* by administration of manganese in drinking water to dams via maternal milk during nursing, and by inclusion in drinking water after weaning. Groups of rats were exposed to manganese for over 2 years and were either 2 months or 24 to 28 months of age at examination. Assuming a body weight of 0.35 kg and water consumption of 0.049 L/day, the average daily dose for rats at adulthood was approximately 39 mg/kg-day. The brains were dissected and analyzed for activity of glutamic acid decarboxylase (GAD), choline acetyltransferase (ChAT), and acetylcholinesterase (AChE). GAD, ChAT, and AChE are neurochemical markers for the GABA and cholinergic systems, and had previously been implicated in manganese toxicity (Sitaramayya et al., 1974; Bonilla, 1978a, b). Adverse effects of chronic manganese exposure on the activity of GAD, ChAT, or AChE were not apparent in 2-month-old rats. The study authors reported that lifetime exposure to manganese produced effects that counteracted age-related decreases in GAD, ChAT, and AChE.

Leung et al. (1981) analyzed the same groups of rats used by Lai et al. (1981) for monoamine oxidase (MAO) activity. MAO is a key enzyme in oxidative degradation of neurotransmitter amines. The only effect observed following exposure of 2-month-old rats to manganese was a small decrease in the neurotransmitter serotonin in the cerebellum. No significant differences were observed in manganese-treated 24- to 28-month-old rats.

Lai et al. (1982a) examined the effects of manganese exposure on male Wistar rats. The rats were initially exposed to manganese *in utero*. Following weaning, the rats were exposed to 1,000 mg $MnCl_2 \cdot 4H_2O$/L (280 mg Mn/L) in drinking water for either 70 to 90 days or 100 to 120 days after birth. Assuming an adult weight of 0.35 kg, and water consumption of 0.049 L/day, this level corresponds to a dose of approximately 39 mg/kg-day. Levels of dopamine, noradrenaline, serotonin, and choline were determined. A significant decrease was seen in the uptake of dopamine by synaptosomes isolated from the hypothalamus, striatum and midbrain in 70- to 90-day-old rats. No effect was observed in the 100- to 120-day-old rats. Choline levels

were higher in 70- to 90-day-old-exposed rats and lower in 100- to 120-day-old-exposed rats when compared with controls. The authors suggested that this finding may reflect involvement of both the dopaminergic and cholinergic systems in manganese toxicity. They concluded that, although the rat may not serve as an ideal model for understanding the neurotoxic effects of manganese, neurochemical effects are discernible when analyses are made at the appropriate period.

Lai et al. (1982b) investigated the effect of manganese exposure on the developmental profile of acetylcholinesterase activity in different regions of the brain. Female Wistar rats were exposed to manganese chloride tetrahydrate provided in drinking water at a concentration of 1,000 mg/L. Exposure was initiated at conception. Male offspring were weaned onto drinking water containing 1,000 mg/L manganese chloride tetrahydrate and exposed for up to 60 days. Enzyme activity in the cerebral cortex, striatum, midbrain, pons and medulla, hypothalamus, and cerebellum was determined at 5, 12, 20, 30, and 60 days after birth. The developmental profile of the enzyme differed in the various regions. Activity was detected earlier in the more caudal regions, except in the cerebellum where there was no increase. Exposure to manganese from conception did not influence the developmental profile of acetylcholinesterase activity.

Gianutsos and Murray (1982) studied changes in the concentrations of dopamine and GABA in mice exposed to $MnCl_2$ in the diet. A 1% concentration of $MnCl_2$ was administered in the diet to an unspecified number of male CD-1 mice for 1 month. This level of exposure corresponds to 568 mg Mn/kg-day. The concentration was increased to 4% for an additional 5 months. During this period, the average daily dose was 2,272 mg Mn/kg-day. Dopamine content in the striatum and in the olfactory tubule at 6 months was reduced compared with controls ($p < 0.05$). GABA content of the striatum was increased ($p < 0.05$). Apparent increases in the substantia nigra area and a decrease in the cerebellum were not statistically significant. No changes in neurotransmitter levels were observed when assays were conducted after 1–2 months of exposure.

Morganti et al. (1985) conducted a behavioral study using male ICR strain Swiss mice. The mice were fed powdered Charles River's RMH 300 diet that contained 1,000 mg MnO_2/kg. This dietary concentration corresponds to approximately 632 mg Mn/kg. The mice consumed 5 g of food daily. Assuming a body weight of 0.03 kg (U.S. EPA, 1986d), this level of exposure corresponds to an average daily dose of 105 mg/kg-day. Neurobehavioral evaluation began after 16 weeks of feeding and continued at 2-week intervals for 30 weeks. The endpoints evaluated were open field and exploratory behavior, passive avoidance learning, and rotarod performance (a measurement of balance and coordination). Multivariate analysis of variance (2 treatments and 8 samples by week of exposure) was used to test for intergroup differences. No significant behavioral differences were apparent in any treatment group. In contrast, Morganti et al. (1985) observed significant effects in mice exposed to manganese by inhalation for 7 hours/day, 5 days/week, at levels greater than 50 mg Mn/m^3. The duration of exposure was 16 to 32 weeks. This level of inhalation exposure was considered by the authors to be comparable to the oral exposure.

Ali et al. (1985) studied the effect of dietary protein on manganese neurotoxicity. Rats received either a normal diet (21% casein) or a low protein diet (10% casein). Half of each dietary group served as a control while the other half received $MnCl_2 \cdot 4H_2O$ (3,000 mg Mn/L) in the drinking water for 90 days. Assuming an adult body weight of 0.35 kg and water consumption of 0.049 L/day, this corresponds to an average daily dose of 420 mg/kg-day. The low-protein diet was associated with decreased levels of brain dopamine (DA), norepinephrine (NE), and serotonin. Manganese exposure resulted in a marked increase in DA and NE levels, which were more pronounced in the low-protein group. A significant decrease in serotonin levels following manganese exposure occurred only in the low-protein group. Weaned F_1 pups of treated rats exhibited the same pattern of effects. The study authors concluded that protein deficiency can increase vulnerability of rats to the neurotoxic effects of manganese.

Nachtman et al. (1986) studied the behavioral effects of chronic manganese exposure. Male Sprague-Dawley rats were administered 0 or 1 mg $MnCl_2 \cdot 4H_2O$/mL in drinking water for 65 weeks. Assuming a body weight of 0.35 kg and water consumption of 0.049 L/day, this corresponds to an average daily dose of 39 mg Mn/kg-day. The treatment did not result in any change in body weight. The manganese-exposed rats exhibited a significant increase in locomotor activity during weeks 5 to 7. However, the effects were transient, and by 8 weeks the activities had returned to control levels. Treated rats examined at 14 and 29 weeks were found to be more responsive to the effects of *d*-amphetamine (a locomotor stimulant that works primarily by releasing dopamine) than were controls. There was no difference between manganese-treated rats and controls at 41 or 65 weeks. The investigators concluded that manganese exposure may result in a transient increase in dopaminergic function, as evidenced by increased spontaneous and *d*-amphetamine-stimulated locomotor activity.

Kristensson et al. (1986) studied the effect of manganese on the developing nervous system of young rats. Starting at 3 days of age, Sprague-Dawley rats received a daily dose of 150 mg Mn/kg-day (as $MnCl_2$) by gavage. The treatment continued until the rats reached 44 days of age. At days 15 to 22 there was a large but transient increase (7- to 40-fold) of manganese in the brain, and the rats displayed a rigid and unsteady gait. By 44 days, the rats appeared normal and brain manganese levels had declined to approximately 3 times the control level. Histological analysis revealed no abnormalities in the brains of manganese-exposed rats. Axonal growth and the axon-myelin relation were normal. A second group of rats was treated for 15 days. At this time point, half the rats were sacrificed and half were maintained untreated until sacrifice at 60 days of age. The rats were subsequently analyzed for brain content of dopamine and its metabolites, including 2,4-dihydroxyphenylacetic acid and homovanillic acid (HVA), and serotonin and its major metabolite 5-hydroxyindolacetic acid. Only HVA levels in the hypothalamus and striatum were affected by manganese treatment. Significantly decreased HVA levels were seen at the 15-day sacrifice. Similar decreases in rats treated for 15 days and allowed to recover until 60 days of age were not observed. The investigators concluded that divalent manganese has a very low degree of toxicity for the developing nervous system in rats, but that longer-term exposure to more active manganese compounds may cause severe damage to certain neurologic pathways. In addition, the investigators emphasized that rodents may not be

appropriate for comparison with primates. Unpublished studies, where monkeys exposed to manganese oxide developed severe motor disturbances, were cited as the basis for this conclusion.

Eriksson et al. (1987) studied the effect of long-term manganese exposure on biogenic amine levels in rat brain. Starting at 20 days of age, groups of male Sprague-Dawley rats were provided with drinking water containing 10 g/L manganese chloride ($MnCl_2 \cdot 4H_20$) for 60, 100, 165, or 265 days. This concentration corresponds to 2,777 mg Mn/L. Assuming an adult body weight of 0.35 kg and water consumption of 0.049 L/day, this level of exposure results in an average daily dose of approximately 390 mg Mn/kg-day. There were no clinical signs of toxicity. Following 60 days of exposure, manganese concentration in the striatum was estimated to be 1.3 to 2.0 mg/kg, in contrast to control levels of 0.4 to 0.5 mg/kg. Levels of dopamine, 3,4-dihydroxyphenylacetic acid, homovanillic acid, serotonin and 5-hydroxyindoleacetic acid were determined in discrete regions of the caudate-putamen. Rats exposed for 60 and 165 days showed significantly increased levels of dopamine (DA) and 3,4-dihydroxyphenylacetic acid, but these alterations were not seen in rats exposed for 100 or 265 days. This suggests an increased synthesis and turnover of dopamine that is reversible, even with continuous manganese exposure. This study identifies a LOAEL of 390 mg Mn/kg-day based on increased levels of dopamine at 60 days.

Kontur and Fechter (1988) intubated neonatal Long-Evans rats daily with 0, 25, or 50 mg/kg-day manganese chloride ($MnC1_2 \cdot 4H_20$) for 14 or 21 days. These doses correspond to 6.9 and 13.9 mg Mn/kg-day. The level of manganese in the brain was increased at both 14 and 21 days, but was greater at 14 days. Monoamine and metabolite levels were not altered in any brain region by manganese treatment. The study authors suggested that the different results reported by different laboratories may be attributable to species or strain differences, the dosing regimen or vehicle, the route of administration, or the time points chosen for testing. These data suggest a NOAEL of 6.9 mg Mn/kg-day for this study, based on the absence of effect on monoamine levels.

These collective studies suggest that preclinical neurological effects are possible in the human following oral exposure; however, there are dissimilarities in the spectrum of responses between rodent and primate models of toxicity that preclude a determination of the oral dose range that might be expected to induce these preclinical effects. Further, conflicting data concerning responses in humans and confounding factors in the limited human epidemiological studies prevent determination of any dose-response effect in humans exposed to manganese excesses via ingestion.

Parenteral Exposure

Although deficiencies exist in experimental design (U.S. EPA, 1984), primate studies by parenteral routes of exposure have reported extrapyramidal signs and histologic lesions similar to those described in humans. Mella (1924) treated four rhesus monkeys with $MnCl_2$ for 18 months. Two monkeys served as controls. The treated monkeys received gradually increasing doses of $MnCl_2$ by intraperitoneal injection on alternate days. The doses started at 5 mg and reached a maximum of 25 mg per injection. The monkeys exhibited uncontrolled, involuntary movements

(chorea) followed by rigidity, disturbances of motility, fine hand tremors, and finally, contracture of the hands. Histological changes were reported in the putamen, the caudate, and the globus pallidus.

Suzuki et al. (1975) exposed monkeys subcutaneously to 39.5, 79.0 or 158.0 mg Mn/kg as MnO_2 once a week for 9 weeks and found the latency of neurologic signs (tremors, excitability, choreiform movement, loss of equilibrium, and contracture of hands) inversely related to cumulative dose. Signs appeared earlier when higher doses were administered, but the severity of symptoms was not completely dose-related. The estimated daily doses in this experiment were 5.6, 11, and 23 mg Mn/kg-day.

Olanow et al. (1996) reported damage to the globus pallidus and substantia nigra in monkeys that were dosed intravenously with doses as low as 4.4 mg Mn/kg/week (for 7 weeks). The brain damage was accompanied by neuromuscular toxicity including bradykinesia, rigidity, facial grimacing, and abnormal posturing of the limbs. Newland and Weiss (1992) administered repeated daily intravenous doses of manganese to Cebus monkeys so that the monkeys received cumulative doses of 5 or 10 mg/kg for 450 days. The dosings were separated by at least one week. The authors observed that single intravenous doses of 5 or 10 mg Mn/kg-day resulted in a significant increase in the number of incomplete responses of dosed monkeys to a spring-loaded test device that measured physical exertion through a rowing motion. The increase in incomplete responses occurred within a few days after dosing began. Further, action tremor was observed in the monkeys who had received cumulative doses of 40 mg/kg or higher; however, dystonia was never observed.

Eriksson et al. (1992) subcutaneously injected two monkeys with 0.4 g doses of MnO_2 (0.253 g Mn) in water. Eleven doses were administered over 4 months, followed by a final dose at 12 months. Both animals developed an unsteady gait and exhibited hypoactive behavior. PET scans indicated that degeneration of dopaminergic nerve endings occurred following Mn intoxication.

Additional studies have examined the neurotoxic effects of manganese administered by parenteral routes in non-primate species. Mustafa and Chandra (1971) and Chandra (1972) reported paralysis of the hind limbs in rabbits administered 169 mg Mn/kg (as MnO_2) intratracheally. The paralysis developed after a period of 18 to 24 months. Examination of the of affected animals brains showed widespread neuronal loss and neuronal degeneration in the cerebral cortex, caudate nucleus, putamen, substantia nigra and cerebellar cortex. These findings are reminiscent of the characteristic histopathologic and neurologic consequences of manganism found in exposed workers (U.S. EPA, 1993). A marked decrease in brain catecholamine levels and related enzyme activity was also noted.

Histopathologic evaluations of exposed rats by Chandra and Srivastava (1970), Chandra et al. (1979) and Shukla and Chandra (1976) found scattered neuronal degeneration in the cerebral and cerebellar cortex. Daily intraperitoneal administration of 2 to 4 mg Mn/kg for $\leq$120 days appeared to be the threshold for the appearance of microscopic lesions. These studies also

demonstrated an association between the maximum number of degenerated neurons and maximum manganese concentration in the brain.

Scheuhammer (1983) treated male Sprague-Dawley rats intraperitoneally for 30 days with either 3.0 mg Mn/kg or an equal volume of 0.9% NaCl. Assuming an average adult body weight of 0.35 kg, this treatment corresponds to an average daily dose of 8.6 mg Mn/kg-day. Following sacrifice, the pancreas was removed, fixed in 10% buffered formalin, and subsequently processed for light microscopy. Significant pathological changes were observed in pancreatic tissue from manganese-exposed rats. The changes were characterized by a pancreatitis-like reaction consisting of expanded interacinar spaces, a thickened connective tissue capsule with invaginations of fibrotic connective tissue septa extending into the body of the gland, the presence of an inflammatory infiltrate of neutrophils, lymphocytes, macrophages, and the separation of groups of acini from the body of the pancreas with occasional destruction of acinar cells. Other peritoneal organs did not exhibit pathological changes. This study suggests that intraperitoneally injected Mn(II) exerts a selective toxicity on pancreatic tissue. Therefore, the study author cautioned against use of intraperitoneal injection as the route of administration for chronic Mn neurotoxicity studies.

Brouillet et al. (1993) administered 0, 0.5, 1, or 2 μmol of $MnCl_2$ in deionized water to male rats by a single intrastriatal injection. Assuming a body weight of 0.35 kg for an adult rat, these doses correspond to 0, 0.077, 0.171, and 0.314 mg Mn/kg. Each treatment group contained 9 to 10 rats. The lowest dose produced a significant reduction in dopamine, but had no effect on the other neurochemical markers examined. Doses of 0.171 and 0.314 mg Mn/kg produced a reduction in dopamine levels, changes in neurochemical markers, and indications of impaired oxidative metabolism.

7.2.5 Developmental/Reproductive Toxicity

Developmental Studies

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. Studies by He et al. (1994) and Zhang et al. (1995) suggest that oral exposures to excess manganese can possibly result in increased neurological deficits measured as poorer performance in school and on standardized neurobehavioral exams. These studies also suffer from a lack of adequate exposure data and the potential presence of confounding factors, such as exposure to other potential neurotoxicants and possible inhalation exposure to manganese.

Developmental studies conducted in animals are summarized in Table 7-5. These studies suggest that manganese is a potential developmental toxicant, but additional studies that are better controlled are necessary in order to determine how potent it is.

Several studies have reported developmental effects in animal models following oral administration of manganese. Järvinen and Ahlström (1975) exposed female rats to 4, 24, 54, 154, 504, or 1,004 mg Mn/kg (as manganese sulfate heptahydrate) in the diet for 8 weeks after weaning and during pregnancy. No signs of embryotoxicity or fetotoxicity were observed. Increases in the whole body content of manganese in fetuses and in liver manganese content of the dams were reported at dietary levels above 154 mg Mn/kg. No increase in liver manganese content was observed in non-pregnant females. Chandra and Shukla (1978) administered bolus doses of 1 mg Mn/kg-day to neonatal rats for 60 days. Neuronal degeneration and increased monoamine oxidase were reported on days 15 and 30 of the study, but no clinical or behavioral signs of manganese neurotoxicity were reported.

Several studies have measured changes in brain chemistry in neonatal rats following oral exposure to manganese. Deskin et al. (1980, 1981) dosed rat pups via gavage with $MnCl_2$ in 5% sucrose for 24 days starting on the first postnatal day. The administered doses in the earlier study were 0, 1, 10, and 20 mg Mn/kg-day. Decreased dopamine levels in the hypothalamus were reported at the two highest doses, and decreased tyrosine hydroxylase levels and increased monoamine oxidase activity (perhaps due to increased levels of the enzyme) were reported in the hypothalamus at the highest dose. No other changes in brain chemistry were reported in the hypothalamus, and no other brain section was affected. In the latter study, doses of 0, 10, 15, and 20 mg Mn/kg-day were administered. Hypothalamic serotonin was observed to be increased at the highest dose; the level of this transmitter was unaffected in the striatum. Lai et al. (1984) reported small decreases in choline acetyltransferase activity in the cerebellum and midbrain of 2-month-old rats that had been exposed to 40 mg Mn/kg-day from conception, throughout gestation, and throughout life. Other neuronal enzymes (e.g., glutamic acid decarboxylase, acetylcholinesterase) were unaffected.

Kristensson et al. (1986) dosed 3-day old male rat pups with 150 mg Mn/kg-day (in water) for 41 days. The authors reported a transient ataxia (days 15-22), which was resolved by the end of the dosing period, in the pups. Manganese levels in the blood and brain (brain levels were increased 7-40 fold) were elevated significantly over controls in 15- and 20-day old pups; brain levels had decreased to approximately 3-fold over control levels in 43-day old pups. Homovanillic acid (metabolite of dopamine) concentrations were decreased in the striatum and hypothalamus, but not in other brain regions; no other monoamines or their metabolites were affected.

Table 7-5. Developmental Effects of Exposure to Manganese.

Compound	Species	Route	Dose (mg Mn/kg-day)	Effect	Reference
$MnSO_4 \bullet 7H_20$	Rat (female)	Oral (diet)	0 4 24 54 154 504 1,004	Increased manganese concentration in fetus and maternal liver; no indications of embryo- or fetotoxicity	Järvinen and Ahlström (1975)
$MnCl_2$	Rat	Bolus (in water)	1	Neuronal degeneration; increased monoamine oxidase; no indications (clinical or behavioral) of neurotoxicity	Chandra and Shukla (1978)
$MnCl_2$	Rat	Oral (gavage)	0 1 10 20	Decreased dopamine; decreased tyrosine hydroxylase; increased monoamine oxidase activity (all changes in hypothalamus only)	Deskin et al. (1980)
Mn_3O_4	Mouse (Male)	Oral (diet)	1,050	Decreased preputial gland, seminal vesicle, and testes growth	Gray and Laskey (1980)
$MnCl_2$	Rat	Oral (gavage)	0 10 15 20	Increased hypothalamic serotonin	Deskin et al. (1981)
Mn_3O_4	Rat	Oral (diet)	0 350 1,050 3,500	Decreased serum testosterone; decreased sperm count; decreased testes weight; prevented normal decrease in serum FSH	Laskey et al. (1982)
$MnCl_2$	Rat	Oral (drinking water)	240	Delayed air righting reflex; delayed age of eye opening; delayed development of auditory startle	Ali et al. (1983)
Mn_3O_4	Rat	Oral (drinking water)	40	Decreased chloline acetyltransferase activity in cerebellum and midbrain	Lai et al. (1984)
$MnCl_2$	Rat	Oral (drinking water)	0 68 136 232	Decreased water consumption and decrease in weight gain in two highest dose groups; no changes in catecholamine or startle response in the exposed pups	Kontur and Fechter (1985)

Table 7-5. Developmental Effects of Exposure to Manganese (continued).

Compound	Species	Route	Dose (mg Mn/kg-day)	Effect	Reference
Mn_3O_4	Rat	Oral (gavage)	0 71 214	Decreased serum testosterone following 7 days of hCG induction	Laskey et al. (1985)
$MnCl_2$	Rat (male)	Oral (drinking water)	150	Transient ataxia; decreased striatal and hypothalamic homovanillic acid concentrations	Kristensson et al. (1986)
$MnCl_2 \bullet 4H_20$	Mouse	Subcutaneous injection	0 0.56 1.1 2.2 4.4	Decreased weight gain/food consumption; increased late resorptions; reduced fetal body weight; increased incidence of morphological defects	Sanchez et al. (1993)
$MnCl_2$	Rat (female) Rabbit (female)	Oral (gavage)	0 11 22 33	Delayed skeletal and internal organ development and increased external malformations in rat pups delivered by Caesarean section. No effects in rabbit	Szakmáry et al. (1995)
$MnCl_2$	Mouse	Subcutaneous injection	50	Late resorptions; postimplantation loss; skeletal anomalies; reduced fetal body weight	Colomina et al. (1995)
$MnCl_2$	Rat	Intravenous	0 0.27×10^{-3} 1.1×10^{-3} 2.2×10^{-3}	Increased incidence of skeletal malformations including angulated or irregularly shaped clavicle, femur, fibula, humerus, ilium, radius, scapula, tibia, and/or ulna	Treinen and Blazak (1995)
$MnCl_2$	Mouse	Intravenous	0.3 1.6	Increased fetal weight at low dose; decreased fetal weight at high dose; fetal skeletal abnormalities at high dose	Grant et al. (1997)
$MnCl_2$	Rat	Oral (drinking water)	0 350 1,420	Thinning of cerebral cortex; absence of convincing brain histopathological or behavioral evidence from perinatal manganese exposure on the brain	Pappas et al. (1997)
$MnCl_2$	Rat	Oral (drinking water)	11 22	Decreased body weight gain; increased response to auditory stimulus	Dorman et al. (2000)

A few studies have measured reproductive endpoints in the developing rodent. Manganese administered to pre-weanling male mice at a dose of 1,050 mg Mn/kg-day beginning on postnatal day 15 resulted in the decreased growth of reproductive organs (preputial gland, seminal vesicle, and testes) measured on days 58, 73, and 90 but did not affect body growth or liver or kidney weights (Gray and Laskey, 1980). Laskey et al. (1982) administered dietary manganese at doses of 0, 350, 1,050, and 3,500 mg Mn/kg-day to male and female rats fed a diet either adequate or deficient in iron. Males and females were mated during days 90-100 of the study; testes weights of male offspring fed the iron-deficient diet were decreased as compared to controls at day 40 at the highest two doses and at day 100 at the intermediate dose. While 40-day-old weanling rats did not exhibit any treatment-related hormonal changes, exposed rats showed a dose-related decrease in serum testosterone at 60-100 days of age (when age-related increases were expected), and no increase in serum luteinizing hormone was observed. The normal decrease in serum follicle stimulating hormone (FSH) from 60 to 100 days was prevented by manganese exposure. Epididymal sperm count was decreased by the treatment only when given with the iron-deficient diet.

In an additional study measuring the effects of manganese exposure on the developing reproductive system, Laskey et al. (1985) administered 0, 71, and 214 mg Mn (as Mn_3O_4)/kg-day via gavage to pre-weanling rats on postnatal days 1-21. The study authors measured serum levels of FSH, LH, and testosterone in the pups at 21 or 28 days of age. Manganese exposure did not affect endogenous or stimulated serum levels of FSH or LH, nor did it affect endogenous or acute human chorionic gonadotropin (hCG)-induced serum testosterone at 2 hours. Serum testosterone was decreased following 7 days of hCG induction, however. The delayed decrease in testosterone was hypothesized by the study authors to be a result of an unknown manganese-induced effect on the Leydig cell.

Ali et al. (1983) evaluated potential changes in developmental endpoints in rat pups after administering excess manganese in drinking water to pregnant dams fed a normal or low-protein diet. Manganese exposure was started 90 days prior to mating and continued throughout gestation and nursing. The offspring of dams who had ingested 240 mg Mn/kg-day exhibited delayed air righting reflexes. Significant delays in the age of eye opening and the development of auditory startle were reported in pups from dams ingesting protein-deficient diets. No decreases in body weight or brain weight were reported in the offspring of rats fed normal-protein diets.

Kontur and Fechter (1985) exposed pregnant Long-Evans rats to 0, 5,000, 10,000, or 20,000 mg/L of manganese chloride in drinking water throughout the gestation period. Rats in the 10,000 and 20,000 mg/L groups showed reduced water intake and a significant decrease in weight gain. A significant decrease in birth weight was observed in the 20,000 mg/L group. At one day of age, pups from the 10,000 and 20,000 mg/L groups had increased manganese levels in the forebrain, although there was no difference in the extent of accumulation between the two groups. The increased manganese levels were not associated with any changes in catecholamine function or startle response in the exposed pups. The authors concluded that manganese is not particularly toxic to developing rats, perhaps as a result of limited placental transfer.

The developmental effects of manganese have also been evaluated following parenteral administration. Sanchez et al. (1993) investigated the embryotoxic and teratogenic potential of manganese during organogenesis. Pregnant Swiss mice received daily subcutaneous injections of 0, 2, 4, 8 or 16 mg/kg-day of $MnCl_2 \cdot 4H_2O$ on days 6 to 15 of gestation. These doses correspond to 0, 0.56, 1.1, 2.2, or 4.4 mg Mn/kg-day, respectively. Dams were sacrificed on gestational day 18. Significant reductions in weight gain and food consumption were reported in dams receiving 8 mg/kg-day and above, and treatment-related deaths were reported at 16 mg/kg-day. A significant increase in the number of late resorptions was observed at doses of 4 mg/kg-day and higher, and reduced fetal body weight and an increased incidence of morphological defects were reported at doses of 2.2 mg Mn/kg-day and higher. No difference was seen in the incidence of individual or total malformations in treated groups when compared with controls. A NOAEL of 1.1 mg/kg-day was identified by the study authors for maternal toxicity. A NOAEL of 0.56 mg/kg-day was identified for embryo/fetal toxicity

Pappas et al. (1997) assessed behavioral, neurohistological, and neurochemical endpoints in rats exposed to manganese from conception to weaning. The investigators administered 0, 2,000, or 10,000 mg Mn/L as manganese chloride in drinking water to female rats (10/group) and their litters from conception until postnatal day (PND) 30. The average daily consumption of manganese during gestation was 350 and 1,420 mg/kg-day, respectively, for the two manganese treatment groups. No effects were observed on pregnancy or birth parameters and no physical abnormalities were evident in the offspring of treated dams. The findings reflect a lack of effects on reproductive capability. Fifty male pups from each treatment group were subsampled for behavioral tests (10 to 22 per group), histopathology (6 to 8 per group) and neurochemical analyses (6 to 8 per group). The rats exposed to 10,000 mg Mn/L showed a 2.5-fold increase in brain cortical Mn levels. They also experienced reduced weight gain during PND 9 to 32, and were hyperactive at PND 17. Behavioral tests were conducted on pups from all groups at PND 17, 90 or 95. No significant differences in performance were noted for the radial arm maze, elevated plus apparatus, or Morris water maze behavioral tests. Both doses resulted in thinning of the cerebral cortex. The observed thinning may have been a consequence of either perinatal malnutrition or a direct effect on cortical development. Brain monoamine levels and choline acetyltransferase activity were unaffected by manganese exposure. Tyrosine hydroxylase immunohistochemistry indicated that dopamine neurons of the substantia nigra were intact. Glial fibrillary acidic protein immunoreactivity, an indicator of neuronal damage, was not increased in cortex, caudate nucleus or hippocampus. The authors emphasized that the most noteworthy result of this study was the absence of convincing histopathological and behavioral evidence for persistent effects of perinatal manganese exposure on the brain.

Grant et al. (1997) failed to observe any effects of manganese exposure on weight gain, gross malformations, or skeletal malformations in the offspring of pregnant rats dosed via gavage with 22 mg Mn/kg-day on gestational days 6-17. Another study indicates a lack of persistent developmental effects from oral manganese exposure during gestation. Szakmáry et al. (1995) reported the developmental effects of manganese administered via gavage to pregnant rats throughout gestation and to pregnant rabbits through organogenesis (gestation day 6-20) at doses of 0, 11, 22, and 33 mg/kg-day. No developmental effects in the rabbit were observed. The

highest dose resulted in retardation of development of the skeleton and internal organs of the rat, as well as a significant increase in external malformations (e.g., clubfoot) in pups delivered by caesarean section. These effects, however, were not observed in 100-day-old offspring of dams that had been similarly dosed, indicating that the developmental effects were self-correcting. Manganese treatment did not affect the following endpoints in either the pup group that was surgically delivered or the group born live: ears, teeth, eyes, forward motion, clinging ability, body posture, correction reflex, or negative geotaxis reflex.

In a more recent study, Dorman et al. (2000) dosed neonatal CD rats with 11 or 22 mg Mn (in water)/kg-day for 21 days from birth to weaning. The high dose resulted in decreased body weight gain in the pups and affected brain neurochemistry. Manganese treatment induced a significant increase in the amplitude of response to an auditory stimulus but did not affect motor activity, performance in a passive avoidance task, or brain histopathology.

Colomina et al. (1995) conducted a study to determine which gestation day is most critical for developmental toxicity of manganese in mice. The investigators administered a 50 mg/kg dose of manganese chloride by subcutaneous injection once during the period between gestation days 9 and 12. Late resorptions, post-implantation loss, and skeletal anomalies increased in all treatment groups. Significant reductions in fetal body weight occurred following exposure on gestation day 9 or 10, indicating these days were most critical.

Treinen and Blazak (1995, abstract only) dosed female Sprague-Dawley rats (15/group) intravenously with 0, 5, 20, or 40 nmol/kg $MnCl_2$ on days 6 to 17 of gestation. These doses correspond to approximately 0, 0.27, 1.1, or 2.2 μg Mn/kg-day. Treatment resulted in an increased incidence of skeletal malformations (doses which elicited effects were not reported). The observed malformations included angulated or irregularly shaped clavicle, femur, fibula, humerus, ilium, radius, scapula, tibia, and/or ulna.

Grant et al. (1997) administered 6 or 30 μmol $MnCl_2$/kg-day to female mice (24/group) by intravenous injection on gestation days 6 to 17. These doses correspond to approximately 0.3 and 1.6 mg Mn/kg-day. The experiment was terminated on gestation day 20. No significant differences were noted in manganese-treated mice for number of corpora lutea, implantation sites, pre- or post-implantation losses, or number of viable fetuses per litter. Fetal weight was significantly increased ($p < 0.05$) at the lower dose, and significantly decreased ($p < 0.05$) at the 1.6 mg/kg-day dose. Skeletal abnormalities were noted in the fetuses of dams receiving the higher intravenous dose. In contrast, no increase in skeletal abnormalities was observed in the fetuses of mice administered 400 μmol of $MnCl_2$ (approximately 22 mg Mn/kg-day) by oral gavage daily from days 6 to 17 of gestation.

One *in vitro* developmental study was located. Hanna et al. (1996, abstract only) cultured two-stage mouse embryos in media containing varying concentrations of essential and non-essential minerals, including manganese. Embryos were incubated in culture media containing 0.05–200 μM manganese for 72 hours. Both essential and nonessential minerals were embryotoxic at relatively low doses.

Reproductive Studies

Some inhalation data from occupational exposure studies suggest that male reproductive dysfunction is a primary endpoint of manganese toxicity. Toxicity is manifested in symptoms including loss of libido and impotence (U.S. EPA, 1996a). Some evidence indicates that the hypothalamus and pituitary are sites of manganese accumulation (see Section 6.2), suggesting disturbance of the hypothalamic-pituitary-gonadal axis hormones as a potential mechanism for reproductive effects. No human reproductive data for oral manganese exposure are available in the current literature. Reproductive studies in animals orally exposed to manganese are described below. Results of these studies are summarized in Table 7-6.

Chandra and colleagues consistently reported degenerative changes in the seminiferous tubules in the testes after parenteral exposure of rats and rabbits to manganese (Chandra, 1971; Shukla and Chandra, 1977; Imam and Chandra, 1975; Chandra et al., 1973, 1975). However, similar changes were not observed in subchronic or chronic studies in mice or rats (NTP, 1993).

Gray and Laskey (1980) exposed male mice to 1,100 mg Mn/kg as Mn_3O_4 in a casein diet from gestation day 15 to 90 days of age. Assuming a food consumption factor of 0.13 (U.S. EPA, 1986d), the estimated daily dose at the termination of the study would be approximately 143 mg/kg-day. Sexual development was retarded, as indicated by decreased weight of testes, seminal vesicles and preputial glands. Reproductive performance was not evaluated.

Laskey et al. (1982) exposed Long-Evans rats to 0, 400, 1,100 or 3,550 mg Mn/kg (as Mn_3O_4) in the diet from day 2 of mother's gestation to 224 days of age. Assuming a food consumption factor of 0.05 (U.S. EPA, 1986d), the average daily dose at the termination of the study was 0, 20, 55, or 177 mg Mn/kg-day. The investigators observed a dose-related decrease in serum testosterone concentration (without a concomitant increase in serum luteinizing hormone concentration), and reduced fertility at the highest dose. Testes weight, number of ovulations, resorption and preimplantation deaths, litter size, and fetal weights were unaffected by manganese exposure.

Laskey et al. (1985) conducted studies to assess the effect of manganese on hypothalamic, pituitary and testicular function. Long-Evans rat pups (8/litter) were dosed by gavage from day 1 to day 21 with a 50% sucrose solution containing particulate Mn_3O_4. The average daily dose of manganese was calculated to be 0, 71 or 214 mg Mn/kg-day. Assessments of the hypothalamic, pituitary, or testicular functions were determined by measuring the endogenous or stimulated serum concentrations of follicle-stimulating hormone, luteinizing hormone, and/or testosterone at 21 or 28 days of age. Body, testes, and seminal vesicles weight and tissue concentrations of Mn were also evaluated. Effects attributed to manganese included slight decreases in body and testes weights, and a reduction in serum testosterone. There was no indication of hypothalamic or pituitary dysfunction. The authors suggested that the decrease in testosterone level resulted from manganese-induced damage of Leydig cells.

Table 7-6. Reproductive Effects of Exposure to Manganese.

Compound	Species	Route	Dose	Effect	Reference
Mn_3O_4	Mouse	Oral (diet)	143 mg Mn/kg-day	Decreased weight of testes, seminal vesicles and preputial glands after 90 days.	Gray and Laskey (1980)
Mn_3O_4	Rat	Oral (diet)	20 mg Mn/kg-day 55 177	Dose-related decrease in serum testosterone concentration. Reduced fertility at 3550 ppm after 224 days.	Laskey et al. (1982)
Mn_3O_4	Rat	Oral (gavage)	71 mg Mn/kg-day 214	Decreased body and testes weights. Reduction in serum testosterone.	Laskey et al. (1985)
$MnCl_2$	Rat	i.p.	8 mg/kg-day	Degenerative changes in approx. 50% of seminiferous tubules after 150 and 180 days.	Chandra (1971)
$MnCl_2 \bullet 4H_20$	Rat	i.p.	15 mg/kg-day	Increased Mn in testes; decreased nonprotein sulfhydryls and decreased activity of glucose-6-phosphate dehydrogenase and glutathione reductase after 15–45 days.	Shukla and Chandra (1977)
$MnCl_2 \bullet 4H_20$	Rabbit	i.v.	3.5 mg/kg	Inhibition of succinic dehydrogenase in seminiferous tubules after 5 days. Morphologic changes were not apparent.	Imam and Chandra (1975)
$MnSO_4$	Rat	i.p.	6 mg Mn/kg	Increased Mn in testes after 25–30 days. Degenerative changes in 10% of seminiferous tubules.	Chandra et al. (1975)
MnO_2	Rabbit	i.t.	250 mg/kg single dose	Destruction and calcification of the seminiferous tubules at 8 months. Infertile females.	Chandra et al. (1973)

i.p. = intraperitoneal; i.v. = intravenous; i.t. = intratracheal

Studies exist, however, that report no adverse reproductive effects in female rats following oral manganese exposure. Pappas et al. (1997) dosed pregnant rats with up to 620 mg Mn/kg-day (as $MnCl_2$) throughout gestation. No treatment-related effects were reported in dam health, litter size, or sex ratios of the pups. The study did not include more extensive analysis of female reproductive organs. Grant et al. (1997) administered 22 mg Mn/kg-day (as $MnCl_2$) via gavage to pregnant dams on gestation days 6-17. No treatment-related effects were reported in dams as measured by mortality, clinical signs, food and water intake, or body weights.

7.2.6 Chronic Toxicity

NTP (1993) investigated the chronic toxicity of manganese in a 2-year oral exposure study. Concentrations of 0, 1,500, 5,000 or 15,000 mg/kg manganese sulfate monohydrate were administered in the diet to male and female F344 rats (70/sex). These dietary concentrations resulted in doses ranging from 30 to 331 mg Mn/kg-day for males, and 26 to 270 mg Mn/kg-day for females. Ten rats/group were sacrificed at 9 and 15 months. Survival of males in the high-dose group was significantly decreased starting at week 93 of the study, and death was attributed to advanced renal disease associated with manganese exposure. Food consumption was similar for all groups. However, by the end of the study, high-dose males exhibited a mean body weight that was 10% lower than controls. No clinical findings or effects on hematologic or clinical chemistry parameters were attributed to manganese exposure in any group. Tissue concentrations of manganese were elevated in the livers of mid- and high-dose males, concurrent with a decrease in hepatic iron concentrations. Renal disease in high-dose males was the only pathological effect noted. No increases in tumor incidence were attributed to manganese exposure.

The chronic oral toxicity of manganese was evaluated in mice in a concurrent study conducted by NTP (1993). Concentrations of 0, 1,500, 5,000, or 15,000 mg/kg manganese sulfate monohydrate were administered in the diet to $B6C3F_1$ mice (70/sex) in a 2-year oral exposure study. These dietary concentrations were reported to be equivalent to doses ranging from 63 mg Mn/kg-day to 722 mg Mn/kg-day for male mice, and from 77 mg Mn/kg-day to 905 mg Mn/kg-day for female mice. Interim sacrifices of 11 mice/group were made at 9 and 15 months. No clinical findings or effects on survival were observed in any group of mice. Mean body weights of males were not affected. Female mice had a dose-related decrease in mean body weight after week 37. The final mean body weights for the low-, mid- and high-dose females were 6%, 9% and 13% lower than controls, respectively. No differences were seen in food consumption for any group. No effects were reported on hematologic parameters. Tissue concentrations of manganese were significantly elevated in the livers of all exposed females, and in high-dose males. Elevated manganese concentration was associated with decreased hepatic iron.

7.2.7 Carcinogenicity

The carcinogenicity of ingested manganese was evaluated in concurrent 2-year oral exposure studies conducted in mice and rats by NTP (1993). An overview of these studies is provided below. No other studies of manganese carcinogenicity via the oral route were identified.

Groups of rats were exposed to dietary levels of manganese sulfate monohydrate that resulted in intakes ranging from 30 to 331 mg Mn/kg-day for males and 26 to 270 mg Mn/kg-day for females. At the termination of the study, no manganese-related increase in any tumor type was observed (NTP, 1993).

In a parallel study, NTP (1993) administered 0, 1,500, 5,000, and 15,000 mg/kg manganese sulfate monohydrate in the diet to $B6C3F_1$ mice (70/sex) for 2 years. These dietary concentrations resulted in intakes ranging from 63 to 722 mg Mn/kg-day for males and from 77 to 905 mg Mn/kg-day for females. The estimated manganese intake in the high-dose mice was approximately 107 times greater than the recommended dietary allowance. Incidence of thyroid follicular cell hyperplasia was significantly greater in high-dose male and female mice than in controls. The incidence of follicular cell adenomas is summarized in Table 7-7. In males, tumors were observed only at the highest dose (6% incidence). The highest incidence of tumors in females was also observed at the highest dose. No significant differences in tumor incidence relative to the controls were observed for either sex. The follicular cell tumors were seen only at the termination of the study (729 days), and their number was only slightly increased relative to the historical control range in female $B3C6F_1$ mice (0 to 9% historical range versus 10% tumor incidence in high-dose females). Hence, the relevance of these findings to human carcinogenesis is questionable. The issues of concern are: 1) the large intake of manganese required to elicit a response seen only at the end of the study, and 2) tumor frequencies that are not significantly different from historical controls. While NTP (1993) has concluded that the marginal increase in thyroid adenomas of the mice was equivocal evidence of carcinogenicity, others have questioned the relevance of these data to human carcinogenicity (U.S. EPA, 1993).

Table 7-7. Follicular Cell Tumor Incidence in $B6C3F_1$ Mice.

Sex	Concentration of $MnSO_4 \cdot H_2O$ in Diet			
	Control	Low	Medium	High
Males	0/50	0/49	0/51	3/50
Females	2/50	1/50	0/49	5/51

Source: NTP (1992)

Other studies reporting positive results for carcinogenicity are summarized in Table 7-8. 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 $MnSO_4$/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 $MnSO_4$/kg. These doses corresponded 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. Table 7-9 summarizes the results of this study. The percentage of mice with tumors was elevated at the highest dose level, but the difference was not significant (Fisher Exact test) when compared with the vehicle controls. An apparent increase in the average number of pulmonary adenomas per mouse was noted both at the

Table 7-8. Summary of Carcinogenicity Studies Reporting Positive Findings for Selected Manganese Compounds [a].

Compound	Species	Route	Dose	Duration (weeks intermit-tent)	Results	Reference
Manganese chloride	Mouse	i.p.	0.1 mL of 1%	26	41% - Lymphosarcomas	DiPaolo (1964)
	Mouse	s.c.	0.1 mL of 1%	26	67% - Lymphosarcomas	
			0% (control)		24% - Lymphosarcomas	
Manganese sulfate	Mouse	i.p.	660 mg/kg	8	67% - Lung adenomas	Stoner et al. (1976)
			0 mg/kg		31–37% - Lung ademomas	
Manganese acety-lacetonate (MAA)	Rat	i.m.	1,200 mg/kg[b]	26	40% (males) Fibrosarcomas 24% (females) Fibrosarcomas	Furst (1978)
			0 mg/kg		4 % (control males and females)	

i.p. = intraperitoneal; s.c. = subcutaneous; i.m. = intramuscular

[a] Source: U.S. EPA (1984)

Table 7-9. Pulmonary Tumors in Strain A Mice Treated with Manganese Sulfate [a].

Total Dose			Mortality	Mice with Lung Tumors (%)	Average Number Tumors/Mouse [b]	Value [c]
Group	mg $MnSO_4$/kg	mg Mn/kg				
Untreated control	0	0	1/20	6/19 (31)	0.28 ± 0.07	NA
Solvent control (0.85% NaCl)	0	0	1/20	7/19 (37)	0.42 ± 0.10	NA
Treated	132	42.9	1/20	7/19 (37)	0.47 ± 0.11	NS
Treated	330	107.2	0/20	7/20 (35)	0.65 ± 0.15	NS
Treated	660	214.4	2/20	12/18 (67)	1.20 ± 0.49	0.05[d]
20 mg urethane [e]	0	0	2/20	18/18 (100)	21.6 ± 2.81	NR

[a] Source: Stoner et al. (1976)
[b] X ± S.E.
[c] Student t-test
[d] Fisher Exact Test p = 0.068
[e] Single intraperitoneal injection
NA = Not applicable; NS = Not significant; NR = Not reported

middle and high doses, but the increase was significant only at the high dose (660 mg $MnSO_4$/kg) (Student's t-test, $p < 0.5$). Although these study results are suggestive of carcinogenic activity, they do not conclusively meet the positive response criteria (increase in the mean number of tumors per mouse and an observable dose-response relationship) for the interpretation of lung tumor data in this mouse strain (Shimken and Stoner, 1975).

Furst (1978) injected F344 rats intramuscularly with manganese acetylacetonate and observed an increased incidence of fibrosarcomas at the injection site, but did not observe increased tumor incidence at other sites.

7.3 Other Key Data

7.3.1 Mutagenicity/Genotoxicity

In Vivo Studies

No studies or reports were identified which describe mutagenic or genotoxic effects in humans following oral exposure to manganese. Table 7-10 summarizes the results of the most

Table 7-10. Genotoxicity of Manganese *In Vivo*.

Species (test system)	Compound	End Point	Route	Results	Reference
Nonmammalian systems:					
Drosophila melanogaster	$MnSO_4$	Sex-linked recessive lethal	Feeding Injection	-	Valencia et al. (1985)
Drosophila melanogaster	$MnSO_4$	Sex-linked recessive lethal	Feeding Injection	-	NTP (1993)
Drosophila melanogaster	$MnCl_2$	Somatic mutation	Soaking larvae	-	Rasmuson (1985)
Mammalian systems:					
Albino rat (bone marrow cells) (spermatogonial cells)	$MnCl_2$	Chromosomal aberrations	Oral	-	Dikshith and Chandra (1978)
Albino mouse	$MnSO_4$ $KMnO_4$	Chromosomal aberrations Chromosomal aberrations	Oral Oral	+ +	Joardar and Sharma (1990)

recent *in vivo* mutagenicity and genotoxicity studies in animals. Results from additional studies are noted in the text below.

Studies of genotoxicity in animals have shown mixed results. The bone marrow cells of rats receiving a 50 mg/kg oral dose of manganese (as manganese chloride) showed an increased incidence of chromosomal aberrations (30.9%) compared with those of control animals (8.5%) (Mandzgaladze, 1966; Mandzgaladze and Vasakidze, 1966). However, Dikshith and Chandra (1978) administered repeated oral doses of manganese chloride (0.014 mg/kg-day) to male rats for 180 days and did not observe significant chromosomal damage in bone marrow or spermatogonial cells.

Joardar and Sharma (1990) administered oral doses of manganese sulfate (approximately 102, 202, and 610 mg/kg) and potassium permanganate (65, 130, and 380 mg/kg) to male Swiss albino mice for three weeks. Both compounds were clastogenic, with manganese sulfate being more potent. The frequencies of chromosomal aberrations in bone marrow cells and micronuclei were significantly increased by both salts. There was also a statistically significant, dose-dependent enhancement of sperm-head abnormalities. A LOAEL of 23 mg Mn/kg-day was identified for this effect by ATSDR (2000).

The divalent manganese ion (Mn II) interacts with DNA and chromosomes (Kennedy and Bryant, 1986; Yamaguchi et al., 1986). In cultured mammalian cells, both $MnCl_2$ and $KMnO_4$ produced chromosome aberrations, including breaks, exchanges and fragments (Umeda and Nishimura, 1979). DNA-strand breaks have also been induced by manganese in Chinese hamster ovary calls and human diploid fibroblasts (Hamilton-Koch et al., 1986; Snyder, 1988). Tests for induction of chromosomal aberrations and sister chromatid exchanges in cultured Chinese hamster ovary cells were positive for manganese sulfate monohydrate in the absence of S9 metabolic activation. In the presence of S9, only the sister chromatid exchange test was positive (NTP, 1993).

Tests for mutagenicity in *Drosophila melanogaster* have given negative results. Manganese sulfate monohydrate did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila* treated by feeding or injection (Valencia et al., 1985, as reported in NTP, 1993). Treatment of *D. melanogaster* with manganese chloride by soaking did not induce somatic mutation (Rasmuson, 1985).

In Vitro Assays

Table 7-11 summarizes the results of the most recent *in vitro* mutagenicity and genotoxicity studies. Additional results from early studies are included in the text below.

Manganese chloride was mutagenic in *Escherichia coli* (Demerec et al., 1951; Durham and Wyss, 1957; Zakour and Glickman, 1984), *Photobacterium fischeri* (Ulitzer and Barak, 1988) and *Serretia marcescens* (Kaplan, 1962). Both positive (Nishioka, 1975) and negative (Kanematsu et al., 1980) results have been reported for the *Bacillus subtilis* recombination assay. Positive (Pagano and Zeiger, 1992; Wong, 1988) and negative results (Wong, 1988) have also been reported for manganese chloride in the *Salmonella typhimurium* reversion assay. Assays in mammalian cell lines were positive for gene mutation in mouse lymphoma cells (Oberley et al., 1982) and enhancement of transformation in Syrian hamster embryo cells (Casto et al., 1979). An assay for DNA damage in human lymphocytes gave negative results with metabolic activation, and positive results without activation (De Meo et al., 1991).

Manganese sulfate gave positive results in the T4 bacteriophage mutation test (Orgel and Orgel, 1965), and the *B. subtilis* recombination assay with S9 activation (Nishioka, 1975). Pagano and Zeiger (1992) obtained positive results for mutagenicity in *S. typhimurium* strain TA97. In contrast, manganese sulfate monohydrate was not mutagenic in *S. typhimurium* strains TA98, TA100, TA1535, or TA1537, either with or without exogenous metabolic (S9) activation when assayed by Mortelmans et al. (1986). Results for strain TA97 were negative when assayed with S9 activation, and equivocal when assayed without metabolic activation. Assays in eukaryotic test systems were positive for mutagenicity in *S. cerevisiae* (Singh, 1984) and chromosomal aberrations in Chinese hamster ovary (CHO) cells (NTP, 1993). Manganese sulfate gave negative results when assayed for induction of sister chromatid exchange in CHO cells (NTP, 1993).

Table 7-11. Genotoxicity of Manganese *In Vitro*.

Species (test system)	Compound	End Point	Strain	Results		Reference
				With S9 Activation	Without S9 Activation	
Prokaryotic organisms:						
Salmonella typhimurium	$MnCl_2$	Gene mutation	TA98 TA102 TA1535 TA1537	- - - -	- - - +	Wong (1988)
Salmonella typhimurium	$MnSO_4 \cdot H_2O$	Gene mutation	TA97 TA98 TA100 TA1535 TA1537	- - - - -	-/+ - - - -	Mortelmans et al. (1986)
Salmonella typhimurium	$MnSO_4$	Gene mutation	TA97	ND	+	Pagano and Zeiger (1992)
Salmonella typhimurium	$MnCl_2$	Gene mutation	TA100 TA102	ND ND	- +	DeMeo et al. (1991)
Photobacterium fischeri (bioluminescence test)	$MnCl_2$	Gene mutation (restored luminescence)	Pf-13 (dark mutant)	ND	+	Ulitzur and Barak (1988)
Escherichia coli	$MnCl_2$	Gene mutation	KMBL 3835	ND	+	Zakour and Glickman (1984)
Bacteriophage (*E. coli lysis*)	$MnSO_4$	Gene mutation	T4	ND	+	Orgel and Orgel (1965)
Bacillus subtilis (recombination assay)	$MnCl_2$ $Mn(NO_3)_2$ $MnSO_4$ $Mn(CH_3COO)_2$ $KMnO_4$	Inhibition of growth in recombination deficient mutant (Rec^-) compared to wild type (Rec^+)	M45 (Rec^-)	ND + 	+ + + -	Nishioka (1975)

Table 7-11 (continued)

Species (test system)	Compound	End Point	Strain	Results		Reference
				With S9 Activation	Without S9 Activation	
B. subtilis (recombination assay)	$MnCl_2$ $Mn(NO_3)_2$ $Mn(CH_3COO)_2$	Inhibition of growth in recombination deficient mutant (Rec^-) compared to wild type (Rec^+)	M45 (Rec^-)	ND -	- -	Kanematsu et al. (1980)
Eukaryotic organisms:						
Fungi: *Saccharomyces cerevisiae*	$MnSO_4$	Gene conversion, reverse mutation	D7	ND	+	Singh (1984)
Mammalian cells: Mouse lymphoma cells	$MnCl_2$	Gene mutation	L5178Y $TK_{+/-}$	ND	+	Oberley et al. (1982)
Mammalian cells: Syrian hamster embryo cells	$MnCl_2$	Enhancement of SA7 transformation		ND	+	Casto et al. (1979)
Mammalian cells: Human lymphocytes (Single-cell gel assay)	$MnCl_2$	DNA damage		-	+	DeMeo et al. (1991)
Mammalian cells: Chinese hamster ovary cells	$MnSO_4$	Chromosomal aberrations Sister chromatid exchange		- +	+ +	NTP (1993)

Notes:
- = negative results
+ = positive results
-/+ = equivocal results
ND = no data available
DNA = deoxyribonucleic acid
$MnSO_4 \cdot H_2O$ = manganese (II) sulfate monohydrate
$Mn(CH_3COO)_2$ = manganous acetate
$MnCl_2$ = manganous chloride
$Mn(NO_3)_2$ = manganous nitrate
$MnSO_4$ = manganous sulfate
Rec = recombination
Source: Modified from ATSDR (2000)

Comparatively little data are available that describes the genotoxic potential of other manganese compounds. Manganese oxide (Mn_3O_4) was not mutagenic in *S. typhimurium* or *S. cerevisiae* (Simmon and Ligon, 1977). Data obtained for manganese nitrate ($Mn(NO_3)_2$) in the *B. subtilis* recombination assay were inconsistent between studies (Nishioka, 1975; Kanematsu et al., 1980). Manganese acetate ($Mn(CH_3OO)_2$) was mutagenic in the *B. subtilis* recombination assay without exogenous metabolic activation, and gave negative results with activation (Nishioka, 1975; Kanematsu et al., 1980).

7.3.2 Immunotoxicity

Immunotoxicity and lymphoreticular effects do not appear to be significant outcomes of oral exposure to manganese. A single report describes effects in this category following oral exposure. NTP (1993) administered diets containing 0, 1,600, 3,130, 6,250, 12,500, or 25,000 mg/kg manganese sulfate monohydrate to F344 rats (10/sex/dose) in a 13-week study. Based on measured feed consumption, the study authors determined that the mean intake of manganese sulfate monohydrate ranged from 110 to 1,700 mg/kg-day (equal to about 36 to 553 mg Mn/kg-day) for males, and from 115 to 2,000 mg/kg-day (equal to about 37 to 621 mg Mn/kg-day) for females. Increased neutrophil counts were noted at 32 mg Mn/kg-day in male rats. Decreased leukocyte counts were noted at 155 mg Mn/kg-day in female rats.

Studies in animals exposed to manganese chloride by intraperitoneal or intramuscular injection suggest that manganese can affect several immunological cell types (ATSDR, 2000). Observed effects include stimulation of macrophage and natural killer cell activity in mice (Rogers et al., 1983; Smialowicz et al., 1985, 1987). Other effects include alteration of the responsiveness of lymphoid cells to mitogens and inhibited antibody production in response to a T-cell antigen (Hart, 1978; Lawrence, 1981; Srisuchart et al., 1987). The significance of these findings for human immune function is presently unknown.

7.3.3 Hormonal Disruption

No reports describing hormonal disruption associated with manganese exposure were located.

7.3.4 Physiological or Mechanistic Studies

Biochemical and Physiological Role

Manganese is a naturally-occurring element that is required for normal physiological functioning in all animal species (U.S. EPA, 1996a). It plays a role in bone mineralization, metabolic regulation, protein and energy metabolism, protection of cells from oxidative stress, and synthesis of mucopolysaccharides (ATSDR, 2000). Many of these roles are achieved by participation of manganese as a catalytic or regulatory factor for enzymes, including hydrolases, dehydrogenases, kinases, decarboxylases and transferases. In addition, manganese is a structural component of the metalloenzymes mitochondrial superoxide dismutase, pyruvate carboxylase, and

liver arginase. Studies conducted to determine the biochemical and nutritional roles of manganese in human health are reviewed in greater detail by Wedler (1994) and Keen et al. (1999).

The frequency of occurrence and consequences of manganese deficiency are issues of some debate (Keen et al., 1999). However, observations reported by Doisy (1973) and Friedman et al. (1987) suggest that manganese is an essential element for humans. Doisy (1973) reported decreased levels of clotting proteins, decreased serum cholesterol, reddening of black hair, retarded growth of hair and nails, and scaly dermatitis in a subject inadvertently deprived of manganese. Friedman et al. (1987) administered a manganese-deficient diet to seven men for 39 days. Five of the seven subjects exhibited dermatitis at the end of the manganese-deficient period. The development of dermatitis was attributed to decreased activity of manganese-requiring enzymes that are required for skin maintenance. The symptoms cleared rapidly when manganese was restored to the diet.

Manganese deficiency has been experimentally induced in multiple animal species. Outcomes associated with manganese deficiency in animals include impaired growth (Smith, 1944), skeletal abnormalities (Amdur et al., 1944; Strause et al., 1986), impaired reproductive function in females and testicular degeneration in males (Boyer et al., 1942), ataxia (Hurley et al., 1961), altered metabolism of carbohydrates (Baly et al., 1988; Hurley et al., 1984) and lipids (Abrams et al., 1976), and decreased cholesterol synthesis and excretion (Davis et al., 1990; Kawano et al., 1987). The biochemical basis for these effects has not been established with certainty, but it may be related to the participation of manganese in numerous enzymatic reactions.

Low serum manganese levels are associated with several disease states, including epilepsy, exocrine pancreatic insufficiency, multiple sclerosis, cataracts, and osteoporosis (Freeland-Graves and Llanes, 1994). In addition, the metabolic disorders phenylketonuria and maple syrup urine disease, genetic disorders of amino acid metabolism, are associated with poor manganese status (U.S. EPA, 1996a).

Mechanisms of Neurotoxicity

The central nervous system (CNS) has been identified as the major target of manganese toxicity (U.S. EPA, 1993; ATSDR, 2000). The blood-brain barrier (BBB) is a major regulator of the (CNS) milieu, and the rate and extent of manganese transfer across the BBB may be a determinant of manganese neurotoxicity (Aschner and Aschner, 1991). The mechanism by which manganese crosses the BBB to gain access to neuronal tissue has not been fully elucidated, but may be a function of binding to transferrin (Aschner and Aschner, 1991). In the portal circulation, manganese as Mn(II) initially binds to alpha-2-macroglobulin, and this complex cannot cross the BBB. The Mn(II)-alpha-2-macroglobulin complex is transported by the bloodstream to the liver (Tanaka, 1982), where a small fraction of the circulating Mn(II) may be oxidized to Mn(III). The iron-transporting protein transferrin has been shown to also bind Mn(III), and may be responsible for its transport into the brain. The observation that some of the regions of the brain that accumulate manganese (e.g., globus pallidus, striatum, and substantia

nigra) receive neuronal input from the transferrin-rich nucleus accumbens and the caudate-putamen supports this argument. Both of these regions are rich in transferrin receptors.

Additional evidence for the transferrin transport hypothesis was provided by an experiment in which rats were given a 6-hour intravenous administration of ferric-hydroxide dextran complex (Aschner and Aschner, 1990). The uptake of radiolabeled manganese into the brain was significantly ($p < 0.05$) inhibited following the administration of the iron complex as compared with rats administered iron-free dextran. It was concluded that iron homeostasis may play an important role in the regulation of manganese transport across the BBB, since both metals are transported by transferrin and may be competing for binding sites.

Once manganese has crossed the BBB, several neurotransmitter systems in the brain appear to be potential targets for manganese toxicity. The primary targets appear to be the monoamines, including dopamine, noradrenaline and serotonin (Neff et al., 1969; Mustafa and Chandra, 1971). The amino acid neurotransmitter γ-amino butyric acid (GABA) may also be affected (Gianutsos and Murray, 1982). Effects on neurotransmitters may be both specific and highly localized. Manganese neurotoxicity, for example, is reportedly associated with a selective depletion of dopamine in the striatum, a site of manganese accumulation (Neff et al., 1969; Bernheimer et al., 1973).

A resemblance exists between the symptoms of manganism and Parkinsonism, a condition characterized by loss of dopaminergic neurons in the substantia nigra and globus pallidus. In addition, several clinical features of manganism respond favorably to therapy with L-dopa in a manner similar to patients with Parkinson's disease (Mena et al., 1970) although long-term response of manganism patients to L-dopa has not been observed (ATSDR, 2000; Calne et al. 1994). However, despite some similarities in symptoms, a comparative study of a 52-year-old worker exposed to manganese in an ore crushing plant and a patient with Parkinson's disease did not reveal any similarity in neuropathology (Yamada et al., 1986). Barbeau (1984), Calne et al. (1994), and Pal et al. (1999) have summarized the similarities and differences between manganism and Parkinsonism. These researchers have noted that manganism characteristically occurs in phases of increasing severity and that sufferers exhibit dystonia (disordered tonicity of muscles), symptoms of extrapyramidal dysfunction such as bradykinesia (extreme slowness of movements and reflexes), monotonic speech, and an expressionless or even grimacing face. Although the altered gait and fine tremor are common to both Parkinsonism and manganism, the syndromes are different in that manganism patients sometimes have psychiatric disturbances early in the onset of the syndrome, have a tendency to fall backwards, do not have the Lewy bodies in the substantia nigra that are commonly found in Parkinson's patients. Further, fluorodopa positron emission tomography (PET) scans are normal in manganism patients but not in individuals with Parkinson's disease (ATSDR, 2000).

Mapping studies by Yamada et al. (1986) indicate that most of the neuronal degeneration attributed to manganese exposure lies close to monoamine cell bodies and pathways. Histopathology in manganese-exposed primates shows more widespread deposition of the metal, with intense signaling observed in both the globus pallidus and substantia nigra using MRI

(Newland and Weiss, 1992). Studies in humans indicate that excess manganese in the brain deposits primarily in the globus pallidus (Fell et al. 1996; Kafritsa et al. 1998; Ono et al. 1995) and damage to the human brain from manganese deposition may be limited to that region. In a study that supports these findings, the globus pallidus exhibited atrophy in an autopsy performed on a worker with inhalation-related manganese poisoning (Yamada et al., 1986).

Although there is consensus that the monoaminergic systems, particularly the dopaminergic system, are affected by excess exposure to manganese, the precise mechanism of action remains obscure. One hypothesis proposes that oxidation of dopamine plays a key role in manganese neurotoxicity. Manganese (III) has been shown to oxidize dopamine to its cyclized *O*-quinone (cDAoQ) (Archibald and Tyree, 1987). This irreversible process ultimately results in decreased dopamine levels. The formation of cDAoQ may subsequently initiate the generation of reactive oxygen species, leading to oxidative stress and cell death (Segura-Aguilar and Lind, 1989).

An alternative hypothesis for manganese toxicity proposes an effect on brain cytochrome P-450 activity. Liccione and Maines (1989) demonstrated a high sensitivity of rat striatal mitochondria to manganese-induced increases in cytochrome P-450 activity. These authors speculated that the increase in mixed function oxidase activity may trigger an increase in the formation of active oxygen species (e.g., superoxide anions) that exert a harmful effect on dopaminergic pathways.

Other mechanistic studies have identified tyrosine hydroxylase (TOH), the rate limiting enzyme in dopamine synthesis, as a potential target in manganese-induced neurochemical effects. Bonilla (1980) and Chandra and Shukla (1981) found that changes in TOH activity in the presence of manganese closely paralleled dopamine levels. Qato and Maines (1985) determined that alterations in the activity of TOH and other monooxygenases may be related to manganese-induced alterations in brain heme metabolism.

Manganese toxicity may be selectively associated with adverse effects on mitochondria. Maynard and Cotzias (1955) originally proposed the mitochondrion as the target organelle for manganese cytotoxicity, with adverse effects expressed primarily as disruption of Ca(II) homeostasis. Mn(II) preferentially accumulates in the mitochondria in regions of the brain associated with neurological symptoms and manganism. Once inside the mitochondria, Mn(II) disrupts oxidative phosphorylation. The fundamental role of mitochondrial energy metabolism in manganese neurotoxicity has been highlighted by the studies of Aschner and Aschner (1990) and Gavin et al. (1992), as cited in U.S. EPA (1996a) and ATSDR (2000).

The results of Brouillet et al. (1993) confirm that manganese impairs mitochondrial oxidative metabolism. In addition, their findings indicate that manganese neurotoxicity involves an *N*-methyl-D-aspartate receptor-mediated process similar to that observed for some other mitochondrial toxicants. Manganese may thus produce neuronal degeneration by an excitotoxic process secondary to its ability to disrupt oxidative energy metabolism.

7.3.5 Structure-Activity Relationship

Information on structure-activity relationships is not available for manganese.

7.4 Hazard Characterization

7.4.1 Synthesis and Evaluation of Major Noncancer Effects

Manganese is an ubiquitous element that is essential for normal physiological functioning in all animal species. The biochemical basis for this requirement is most likely the participation of manganese as a structural component or catalytic cofactor for many enzymes. The Adequate Intake levels for manganese range from 0.003 to 0.6 mg/day for infants from birth to 6 months, 0.6 mg/day for infants from 7 months to 1 year, 1.2 mg/day for children aged 1-3 years, 1.5 to 1.9 mg/day for children aged 4-13 years, and from 1.6 to 2.3 mg/day for adolescents and adults (Food and Nutrition Board, 2002). Although outright manganese deficiency has not been observed in the general population, sub-optimal intake may be of concern for some individuals.

In contrast to the beneficial effects of manganese as a nutrient, excess exposure to manganese may be associated with toxic effects. At present, the optimal level of oral exposure to manganese is not well defined (Greger, 1999).

Ingested manganese appears to be primarily absorbed in the Mn(II) form, and may compete with iron and cobalt for common absorption sites. Absorption varies among individuals and is also influenced by dietary factors. Absorption of 3 to 10% of ingested dietary manganese is considered to be representative of the general population (U.S. EPA, 1996a). Iron deficiency enhances the absorption of manganese in animals (U.S. EPA, 1984). Uptake of dietary manganese may be reduced in the presence of other dietary components such as calcium and phytate.

Once absorbed, manganese has the potential to accumulate in mitochondria-rich tissues, including liver, pancreas, and kidney. Lesser amounts accumulate in brain and bone. Manganese is efficiently removed from the blood by the liver and released into bile. Biliary secretion represents the major pathway for manganese transport to the intestine, and studies in humans indicate that manganese is primarily excreted in the feces. The rate of excretion responds efficiently to increased manganese intake. The rate of biliary secretion acts in concert with absorptive processes to establish homeostatic control of manganese levels in the body. As long as physiological systems are not overwhelmed, humans appear to exert efficient homeostatic control over manganese levels, so that levels in the body are kept relatively constant despite moderate variations in intake. Manganese is also reabsorbed in the intestine through enterohepatic circulation (Schroeder et al. 1966).

While it is apparent that exposure to excess manganese can result in increased tissue levels, the interrelationships between oral exposure levels, tissue accumulation, and health effects in humans are not completely understood. Epidemiological studies of workers exposed by

inhalation to manganese dusts and fumes have identified the central nervous system (CNS) as the primary target for chronic manganese toxicity by the inhalation route (U.S. EPA, 1993). Both Mn(III) and Mn(II) have been associated with the neurotoxic effects of manganese. While some researchers note the similarities in CNS effects occurring following manganese exposure and in Parkinson's disease (dystonia, rigidity, bradykinesia), there are significant differences in the two diseases. For example, manganism patients exhibit a less-frequent resting tremor than do Parkinson's patients, extrapyramidal symptoms including fixed expression or a facial grimace, active tremor (particularly in the upper body), a "cock-walk" in which the patient walks on the toes with the back stiff and the elbows flexed, a propensity to fall backwards (especially when pushed), and a failure to respond to dopaminomimetics (Barbeau, 1984; Calne et al., 1994; Pal et al., 1999).

Several investigators have proposed a link between elevated oral manganese intake by humans and neurological symptoms resembling manganism (Kawamura et al., 1941; Kilburn, 1987; Kondakis et al., 1989; Goldsmith et al., 1990). Results from these studies are described in detail in Section 7.1. In each case, the data from these studies were insufficient to establish that manganese was the causative factor (ATSDR, 2000). The evidence for a similar pattern of neurotoxicity in humans following oral exposure is therefore considered equivocal.

Numerous studies have investigated manganese neurotoxicity in rodent models. However, the utility of rodent studies for evaluating the potential neurotoxic effects of manganese in humans has been questioned. Although biochemical and behavioral evidence of neurological effects has been observed, signs of impaired motor function resembling those seen in humans are usually not detected. In particular, studies of rodents exposed to manganese by drinking water or food have been unable to produce the characteristic signs of extrapyramidal neurologic disease seen in humans. In contrast, chronic administration of manganese to monkeys by oral (one study) or parenteral routes (two studies) has resulted in neurological signs consistent with chronic manganism. The failure to reproduce these signs in rodent studies may result from differences in manganese accumulation and distribution between rodents and primates. The dietary requirement for manganese in rodents, for example, is estimated to be 100 times higher than in humans. In addition, neurotoxic effects in humans are associated with manganese accumulation in neuromelanin-rich regions of the brain, and the homologous regions in rats and mice lack this pigment. Although primates are likely to be better models of the neurological manifestations of manganese intoxication than rodent species, sufficient data from well-designed oral studies are not currently available.

An additional drawback to animal studies of manganese neurotoxicity is the inability to identify certain psychological or neurobehavioral signs. Overt neurological impairment in humans is often preceded by psychological symptoms such as irritability and emotional lability. Since accurate dose-response relationships based on neurobehavioral endpoints are generally not available from animal studies, neurochemical responses have been examined as alternative indicators of neurotoxicity. Such studies have been conducted on the assumption that since the toxic manifestations of chronic manganese exposure resemble Parkinsonism, altered biogenic amine metabolism in the CNS may be one of the underlying mechanisms. However the patterns

of neurochemical response reported following manganese exposure are not consistent among studies. Although manganese exposure is generally thought to result in decreased dopamine concentrations, some studies report increased or fluctuating levels. The effect of manganese on dopamine levels, for example, appears to be age-dependent. Neonatal rats and mice exposed to manganese from birth to 15 or 30 days of age have an increased levels of dopamine and norepinephrine in the brain (Chandra et al., 1979; Cotzias et al., 1976; Shukla et al., 1980). Further, temporal changes in dopamine neurochemistry have been observed with prolonged or continuous manganese treatment and it is not established how these time-related changes affect manganese-induced neurotoxicity.

Route of administration is also an issue of concern in evaluating the results of animal studies. Scheuhammer (1983), for example, determined that intraperitoneal injection is not the route of choice for studies of manganese exposure that are longer than 30 days in duration, especially for investigations of neurotoxicity. Intraperitoneally administered manganese appears to have a selectively toxic effect on the pancreas. This effect may make it difficult to distinguish between subtle neurochemical changes resulting directly from manganese exposure, and changes that are secondary to cellular damage in the pancreas. In addition, U.S. EPA (1984) noted that results from parenteral studies are of limited value in predicting the reproductive hazards of ingested manganese. At least one study exists, however, that shows the differential uptake and distribution of manganese administered via injection compared to oral dosing. Roels et al. (1997) investigated the uptake and distribution of manganese (as either MnO_2 or $MnCl_2$) in rats following intra peritoneal injection or gavage dosing. Manganese concentrations were not increased in the blood or brain following administration of 4 weekly doses of 1.22 mg Mn/kg of the dioxide via gavage; following i.p. dosing, manganese concentrations were significantly increased in the blood, striatum, cerebellum and cortex. Steady-state blood manganese concentrations were increased to similar levels by both gavage and i.p. dosing of $MnCl_2$. Gavage dosing of the dichloride significantly increased the cortex manganese concentrations, but not that of the other two regions. Intra peritoneal dosing of the compound increased the manganese levels in the striatum and cortex, but not the cerebellum. These data indicate that depending on the compound, injection administration of manganese results in higher blood and brain concentrations of the metal than does gavage administration.

Toxic effects of oral manganese exposure have also been reported in the hematopoietic, cardiovascular, reproductive, and digestive systems in animals. Hematological and biochemical outcomes vary depending on age and iron status, with young or iron-deficient animals more likely to exhibit adverse effects. Other effects observed following manganese exposure include reduced body weight and reduced liver weight. Animal studies suggest that manganese is not a potent developmental toxicant.

Infants have been identified as a potentially sensitive subpopulation for excess manganese exposure. This determination reflects evidence for higher levels of manganese retention in the brains of neonates than in adults, although the relationship between manganese accumulation in the neonatal brain and toxicity remains unclear (U.S. EPA, 1993). Additional concerns include evidence for greater extent of manganese transport across the blood-brain barrier, the high

concentration of manganese in some infant formulas, and evidence suggestive of a possible link between manganese exposure and learning disabilities. Although a causal relationship has not been established for elevated manganese intake and learning disabilities, a need for further research in this area has been noted (U.S. EPA, 1993).

Other potentially sensitive subpopulations for manganese exposure have been identified. In general, these are groups who may have greater potential for increased body burdens due to increased absorption or altered clearance mechanisms. The list includes pregnant women, elderly persons, iron- or calcium-deficient individuals, and individuals with impaired liver function.

7.4.2 Synthesis and Evaluation of Carcinogenic Effects

The carcinogenic potential of ingested manganese has not been systematically evaluated in epidemiological studies.

Data from animal studies are also limited. Currently, one of the few adequately designed investigations is the 2-year oral exposure study conducted by the National Toxicology Program (NTP, 1993). Groups of F344 rats (70/sex) were provided with diets containing 0, 1,500, 5,000, or 15,000 ppm manganese sulfate monohydrate. These dietary concentrations were reported to be equivalent to an intake ranging from 30 to 331 mg Mn/kg-day for males, and 26 to 270 mg Mn/kg-day for females. No increase in any tumor type could be attributed to manganese exposure.

In a concurrent study, $B6C3F_1$ mice were administered 0, 1,500, 5,000, or 15,000 mg/kg manganese sulfate monohydrate (NTP, 1993). These dietary concentrations were reported to be equivalent to an intake ranging from 63 to 722 mg Mn/kg-day for males and 77 to 905 mg Mn/kg-day for females. Compared to controls, the incidences of thyroid follicular cell hyperplasia were significantly greater in high-dose males and in females at all dose levels. The incidence of follicular cell adenomas in high-dose males (6%) was slightly greater than the range of historical incidence in NTP studies of follicular cell adenomas in male $B6C3F_1$ mice (0–4%). In high-dose females, the incidence of follicular cell adenomas (10%) was also slightly above the historical control range (0–9%). Follicular cell tumors were seen only at the termination of the study (729 days). NTP (1993) reported that the manganese intakes in the high-dose mice were 107 times greater than the recommended dietary level. While NTP (1993) concluded that these data provided "equivocal evidence of carcinogenic activity in mice," U.S. EPA (1993) questioned the relevance of these findings to human carcinogenesis. The basis for concern was 1) the large dose of manganese required to elicit a response observed only at the end of the study, and 2) tumor frequencies that were not statistically different from historical controls.

Three additional studies address the carcinogenicity of manganese. DiPaolo (1964) found that a larger percentage of DBA/1 mice exposed subcutaneously and intraperitoneally to manganese chloride developed lymphosarcomas when compared to controls. A comprehensive evaluation of these data was not possible, however, because they were published in an abstract form which lacked sufficient experimental detail. Stoner et al. (1976) found a higher frequency of

lung tumors in strain A/Strong mice administered manganese sulfate intraperitoneally as compared to controls. Although these results are suggestive of carcinogenic activity, they fail to meet the positive response criteria for the interpretation of lung tumor data in this strain of 1) an increase in the mean number of tumors per mouse, and 2) an observable dose-response relationship (Shimkin and Stoner, 1975). In the third study, Furst (1978) injected F344 rats intramuscularly with manganese acetylacetonate. An increased incidence of fibrosarcomas was observed at the injection site. Increased tumor incidence was not observed at other sites. When evaluated as a group, these studies do not provide convincing evidence for carcinogenicity of manganese.

Both negative and positive results have been obtained in assays for the genotoxic effects of manganese. Mutagenicity assays in multiple tester strains of *Salmonella typhimurium* gave predominately negative results for manganese sulfate monohydrate and manganese chloride when tested with or without exogenous metabolic activation by S9 fraction (Wong, 1988; DeMeo et al., 1991; Pagano and Zeiger, 1992; NTP, 1993). Neither compound induced mutations in *Drosophila melanogaster* as evaluated by sex-linked recessive lethal or somatic mutation assays (Rasmuson, 1985; Valencia et al., 1985; NTP, 1993). Dikshith and Chandra (1978) did not observe increased incidence of chromosomal aberrations in rat bone marrow or spermatogonial cells following oral administration of manganese chloride.

In addition to the negative results described above, positive results for manganese compounds have been obtained in some assays for genotoxicity. Manganese sulfate induced sister chromatid exchange and chromosomal aberrations *in vitro* in Chinese hamster ovary cells, and induced chromosomal aberrations *in vivo* in albino mice following oral administration (Joardar and Sharma, 1990). Manganese compounds also induced or enhanced mutation, transformation, chromosomal aberrations, and DNA damage in some assays conducted in mammalian cell lines (Casto et al., 1979; Oberly et al., 1982; DeMeo et al., 1991; NTP, 1993), bacteria (Orgel and Orgel, 1965; Nishioka, 1975; Zakour and Glickman, 1984), and yeast (Singh, 1984). Although these results suggest that manganese may have genotoxic potential, there are presently no epidemiological or unequivocal animal data to suggest that manganese is carcinogenic.

7.4.3 Mode of Action and Implications in Cancer Assessment

The molecular mechanisms responsible for the toxicity of manganese have not been identified with certainty. Most effort has focused on identification of mechanisms mediating the toxic effects observed in the central nervous system. Multiple researchers have proposed that elevated levels of Mn(II) and Mn(III) trigger the production of free radicals, reactive oxygen species, and other cytotoxic metabolites in brain tissue. Generation of these reactive species is hypothesized to occur via the oxidation or turnover of intracellular catecholamines, impacts on mitochondrial metabolism, or stimulation of cytochrome P-450 activity. Manganese may also influence transport systems, enzyme activity and receptor function in the brain and other organs. At the present time, there is no evidence to link these proposed mechanisms of action to carcinogenic potential.

7.4.4 Weight of Evidence Evaluation for Carcinogenicity

The weight of evidence for manganese carcinogenicity is currently rated as Group D (Not Classifiable) using the criteria of the U.S. EPA Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1986a). The classification of Group D was verified on May 25, 1988 by the CRAVE Work Group of the U.S. EPA. The basis for this determination is the inadequacy of existing studies for assessment of manganese carcinogenicity (U.S. EPA, 1996a).

Manganese has not yet been evaluated using the criteria of the U.S. EPA Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1996b). However, evaluation of currently available data suggests that the appropriate descriptor for manganese would be "inadequate data for an assessment of human carcinogenic potential." This descriptor is appropriate when the available information is judged to be inadequate to perform an assessment of human carcinogenic potential. It is applied when there are conflicting data on carcinogenicity or in situations where there is a paucity of data.

7.4.5 Sensitive Populations

Sensitive populations are defined as those which will exhibit an enhanced or altered response to a chemical when compared with most persons exposed to the same concentration of chemical in the environment. Factors that can contribute to this altered response include genetic composition, age, developmental stage, health status, substance use history, and nutritional status. These factors may alter the function of detoxification and excretory processes, or compromise the function of target organs. In general, the elderly with declining organ function and infants and children with developing organs are expected to be more sensitive to toxic substances than healthy adults.

7.4.6 Potential Childhood Sensitivity

Neonates have been identified as a potentially sensitive subpopulation for manganese exposure. This determination reflects observations in human (Zlotkin and Buchanan, 1986) and animal (Keen et al., 1986; Kostial et al., 1978; Rehnberg, et al. 1980) studies that suggest that neonates retain higher levels of administered manganese than adults.

In adults, manganese concentrations are retained within a narrow range by the ability of excretion systems to match the intake of this element (Fechter, 1999). The process responsible for manganese excretion is generally believed to require a significant time period to mature into the adult pattern, with adult patterns of excretion developing at about the time of weaning (Fechter, 1999). During this period of development, the young organism might be susceptible to manganese toxicity if exposed to high levels in the diet or via environmental contamination.

Data with respect to fetal accumulation are not numerous, but appear to consistently demonstrate that manganese is transported across the placenta to a limited extent (Fechter, 1999). When all available data are examined, it appears that the fetus is relatively protected from

manganese accumulation when maternal exposure occurs at relatively low doses. Under conditions of high maternal exposure, manganese accumulation also appears to be limited (Fechter, 1999). The mechanism underlying this lack of accumulation is unknown, but may reflect increased maternal excretion, limited uptake across the placenta, or fetal elimination.

The greatest concern for developmental susceptibility has been generated by data which suggest the existence of a period prior to weaning when the neonate is unable to eliminate manganese. Fechter (1999) reassessed data in the published literature and concluded that the available literature does not support a toxicokinetic basis for accumulation in the fetal or neonatal organism [relative to the adult organism], under conditions of excess exposure to manganese. While the available data indicate that manganese does reach brain tissue, currently available evidence does not support a clear regional distribution.

Kaur et al. (1980) found that younger neonates and 19-day fetuses were more susceptible to manganese toxicity than older rats. Studies with ^{54}Mn indicated that manganese was localized to the liver and brain in younger animals, and there was more manganese per unit weight in younger animals when compared with older animals.

Collipp et al. (1983) found that hair manganese levels in newborn infants increased significantly from birth (0.19 μg/g) to 6 weeks of age (0.885 μg/g) and 4 months of age (0.685 μg/g) when the infants were given formula. In contrast, there was no significant increase in babies who were breast-fed (0.330 μg/g at 4 months). These results were attributed to the difference in manganese content between infant formula and breast milk. Human breast milk is relatively low in manganese (7 to 15 μg/L), while levels in infant formulas are 3 to 100 times higher. Collipp et al. (1983) further reported that the level of manganese in the hair of learning disabled children (0.434 μg/g) was significantly increased in comparison to samples from normal children (0.268 μg/g).

There is at least one study reporting different responses in manganese-treated neonatal animals compared to treated adults (Dorman et al., 2000). Pups were administered $MnCl_2$ in water at 11 or 22 mg Mn/kg for 21 days by mouth and were dosed starting after birth, postnatal day 1 (PND 1), until weaning, PND 21. At PND 21, the effect of manganese treatment on motor activity, learning and memory (passive avoidance task), evoked sensory response (acoustic startle reflex), brain neurochemistry, and brain pathology was evaluated. Manganese treatment at the highest dose was associated with decreased body weight gain in pups, although the authors indicated absolute brain weight was not significantly altered. There were no statistically significant effects on motor activity or performance in the passive avoidance task. However, manganese treatment induced a significant increase in amplitude of the acoustic startle reflex. Significant increases in striatal DA and DOPAC concentrations were also observed in the high-dose treated neonates. No pathological lesions were observed in the treated pups. No effects on body weight or behavior were observed in treated adult animals in this study. The authors indicated that these results suggest that neonatal rats are at greater risk than adults for manganese-induced neurotoxicity when compared under similar exposure conditions. This study, along with evidence for increased absorption and reduced elimination in the neonate, suggests that

the very young may be more susceptible to the harmful effects of manganese exposure due to differences in toxicokinetics.

Other investigators have reported an association between elevated hair levels of manganese and learning disabilities in children (Barlow and Kapel, 1979; Pihl and Parkes, 1977). Although no causal relationship has been established for learning disabilities and manganese intake, further research in this area is warranted (U.S. EPA, 1993). The studies by He et al. (1994) and Zhang et al. (1995) reported increased manganese levels in hair of school-age children exposed to excess levels of manganese in drinking water and food stuffs. These studies conflict with the Kawamura et al. (1941) study which showed that children were not adversely affected by ingesting excess levels of manganese. The more recent studies differ in design, however, because they measured early preclinical neurological effects of manganese overexposure. The older studies did not have the sensitivity to measure such effects; this may explain why children were not previously identified as a sensitive population. None of the studies in children provide adequate exposure levels or properly control for confounding factors; therefore, they are not strong enough to indicate that children are more sensitive than adults. They do confirm the need for additional studies to investigate the possibility that children may be more susceptible than adults to the effects of manganese overexposure.

High levels of manganese in infant formulas may also be of concern since Lönnerdal et al. (1987) reported increased absorption and retention of manganese in neonatal animals. Manganese has also been shown to cross the blood-brain barrier, with the rate of penetration in animal experiments being 4 times higher in neonates than in adults (Mena, 1974). Dieter et al. (1992) stated that "if there were a toxicological limit to manganese according to the principles of preventive health care, then it would have to be set at 0.2 mg/L of manganese in water for infants as a group at risk."

7.4.7 Other Potentially Sensitive Populations

U.S. EPA (1996a) has identified additional sensitive subpopulations for manganese exposure. In general, these are groups who may have greater potential for increased body burdens due to increased absorption or altered clearance mechanisms. The list includes pregnant women, elderly persons, iron- or calcium-deficient individuals, and individuals with impaired liver function.

8.0 DOSE-RESPONSE ASSESSMENT

8.1 Dose-Response for Noncancer Effects

8.1.1 RfD Determination

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 this can be seen in IRIS (USEPA, 1996a).

The reference dose (RfD) is based on the extensive information available for the dietary intake of manganese by human populations (U.S. EPA, 1996a). Freeland-Graves et al. (1987) reviewed human studies and proposed an estimated safe and adequate daily dietary intake of 3.5 to 7 mg for adults. WHO (1973) reviewed data on adult diets and concluded on the basis of manganese balance studies that 2 to 3 mg/day is an adequate daily intake and 8 to 9 mg/day is "perfectly safe."

Dose-Response Assessment and Method of Analysis

The current RfD for manganese was derived from information gathered in dietary surveys of manganese exposure. In various surveys, manganese intakes of adults eating western-type and vegetarian diets ranged from 0.7 to 10.9 mg per day (Freeland-Graves, 1994; Gibson, 1994 as cited by Food and Nutrition Board, 2002). Depending on individual diets, a normal intake may be well over 10 mg per day, especially from a vegetarian diet Based on this information, the U.S. EPA (1996a) considers a dietary intake of 10 mg/day to be safe for a 70 kg adult. Thus, the resulting dose of 0.14 mg/kg-day represents a NOAEL for chronic human consumption of manganese in the diet (U.S. EPA, 1996a).

Application of Uncertainty and Modifying Factors

U.S. EPA (1996a) has recommended use of an uncertainty factor of 1 for derivation of the manganese RfD. This recommendation 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 when concentration of manganese in the diet varies.

U.S. EPA (1996a) has recommended the use of a modifying factor of 3 when assessing exposure to manganese from drinking water. U.S. EPA (1996a) has outlined four reasons for this recommendation:

- 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.

These 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, 1996a). 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:

$$RfD = \frac{0.14 \text{ mg/kg-day}}{1 \times 3} = 0.047 \text{ mg/kg-day}$$

where:

0.14 mg/kg-day = Chronic NOAEL for dietary manganese.

1 = Uncertainty factor.

3 = Recommended uncertainty factor for exposure in drinking water

8.1.2 RfC Determination

The inorganic manganese compounds predominating in drinking water are non-volatile. Inhalation of manganese during use of drinking water for residential activities is therefore not expected to be a significant pathway of exposure or toxicity.

U.S. EPA (1996a) has derived an inhalation Reference Concentration (RfC) for manganese of 5×10^{-5} mg/m^3.

Choice of Principal Study and Critical Effect

The RfC for manganese (U.S. EPA, 1996a) was derived using data from two epidemiological studies of workers exposed to manganese dioxide dust in occupational studies (Roels et al., 1987; Roels et al., 1992). The critical effect was impairment of neurobehavioral function, as assessed by medical questionnaire, audio-verbal short-term memory, visual simple reaction time, hand steadiness, and eye-hand coordination.

Dose-Response Characterization and Method of Analysis

The toxicity data for manganese were evaluated using the conventional NOAEL/LOAEL approach. Neither of the principal studies identified a NOAEL. The LOAEL from the Roels et al. (1992) is derived from an occupational-lifetime integrated respirable dust (IRD) concentration of manganese dioxide (based on 8-hour time-weighted average [TWA] occupational exposures for various job classifications, multiplied by individual work histories in years). This LOAEL is expressed as mg Mn/m^3-year. The IRD concentrations ranged from 0.040 to 4.433 mg Mn/m^3-year, with a geometric mean of 0.793 mg Mn/m^3-year and a geometric standard deviation of 2.907. The geometric mean concentration (0.793 Mn/m^3-year) was divided by the average duration of manganese dioxide exposure (5.3 years) to obtain a LOAEL TWA of 0.15 mg Mn/m^3-year. The LOAEL (Human Equivalent Concentration, HEC) is 0.05 mg/m^3.

The LOAEL indentified in the Roels et al. (1987) study is based on an 8-hour TWA occupational exposure. The TWA of total airborne manganese dust ranged from 0.07 to 8.61 mg/m^3, and the median was 0.97 mg/m^3. The LOAEL(HEC) is 0.34 mg/m^3.

Application of Uncertainty and Modifying Factors

No modifying factor was used in derivation of the RfC. A composite uncertainty factor of 1,000 was used and reflects a factor of 10 for protection of sensitive individuals, a factor of 10 for use of a LOAEL, and a factor of 10 for database limitations. The factor of 10 for database limitations reflects an exposure period of less than chronic duration, lack of developmental data, and potential but unquantified differences in the toxicity of different forms of manganese.

8.2 Dose-Response for Cancer Effects

Manganese is currently classified as a Group D chemical—NOT CLASSIFIABLE as to HUMAN CARCINOGENICITY. This category is assigned to chemicals for which there is inadequate human and animal evidence of carcinogenicity, or for which no data are available. There are presently no human data to suggest an association of oral manganese exposure with increased cancer incidence. Data collected from a 2-year oral exposure study in rats did not reveal evidence for carcinogenic activity (NTP, 1993). Data collected from a 2-year oral exposure study in mice revealed an apparent increase in tumor incidence at the highest dose administered, but only near the end of the study (NTP, 1993). The observed increase was not significantly different from the historical control incidence. These results are considered to be equivocal. Based on the absence of any significant cancer response, a quantitative cancer dose-response assessment for manganese will not be conducted.

Manganese has not yet been evaluated using the criteria of the U.S. EPA Proposed Guidelines for Carcinogen Risk Assessment (U.S. EPA, 1999b). However, based on available data it is likely that the appropriate descriptor for manganese would be "Data are inadequate for assessment of human carcinogenic potential." This descriptor is appropriate when there is a paucity of data on carcinogenic effects, or when the data are conflicting.

9.0 RISK DETERMINATION AND CHARACTERIZATION OF RISK FROM DRINKING WATER

9.1 Regulatory Determination for Chemicals on the CCL

The Safe Drinking Water Act (SDWA), as amended in 1996, required the Environmental Protection Agency (EPA) to establish a list of contaminants to aid the Agency in regulatory priority setting for the drinking water program. EPA published a draft of the first Contaminant Candidate List (CCL) on October 6, 1997 (62 FR 52193, U.S. EPA, 1997). After review of and response to comments, the final CCL was published on March 2, 1998 (63 FR 10273, U.S. EPA, 1998). The CCL grouped contaminants into three major categories as follows:

Regulatory Determination Priorities - Chemicals or microbes with adequate data to support a regulatory determination,

Research Priorities - Chemicals or microbes requiring research for health effects, analytical methods, and/or treatment technologies,

Occurrence Priorities - Chemicals or microbes requiring additional data on occurrence in drinking water.

The March 2, 1998 CCL included one microbe and 19 chemicals in the regulatory determination priority category. More detailed assessments of the completeness of the health, treatment, occurrence and analytical method data led to a subsequent reduction of the regulatory determination priority chemicals to a list of 12 (one microbe and 11 chemicals) which was distributed to stakeholders in November 1999.

SDWA requires EPA to make regulatory determinations for no fewer than five contaminants in the regulatory determination priority category by August, 2001. In cases where the Agency determines that a regulation is necessary, the regulation should be proposed by August 2003 and promulgated by February 2005. The Agency is given the freedom to also determine that there is no need for a regulation if a chemical on the CCL fails to meet one of three criteria established by SDWA and described in Section 9.1.1.

9.1.1 Criteria for Regulatory Determination

These are the three criteria used to determine whether or not to regulate a chemical on the CCL:

The contaminant may have an adverse effect on the health of persons,

The contaminant is known to occur or there is a substantial likelihood that the contaminant will occur in public water systems with a frequency and at levels of public health concern,

In the sole judgment of the administrator, regulation of such contaminant presents a meaningful opportunity for health risk reduction for persons served by public water systems.

The findings for all criteria are used in making a determination to regulate a contaminant. As required by SDWA, a decision to regulate commits the EPA to publication of a Maximum Contaminant Level Goal (MCLG) and promulgation of a National Primary Drinking Water Regulation (NPDWR) for that contaminant. The Agency may determine that there is no need for a regulation when a contaminant fails to meet one of the criteria. A decision not to regulate is considered a final Agency action and is subject to judicial review. The Agency can choose to publish a Health Advisory (a nonregulatory action) or other guidance for any contaminant on the CCL independent of the regulatory determination.

9.1.2 National Drinking Water Advisory Council Recommendations

In March 2000, the EPA convened a Working Group under the National Drinking Water Advisory Council (NDWAC) to help develop an approach for making regulatory determinations. The Working Group developed a protocol for analyzing and presenting the available scientific data and recommended methods to identify and document the rationale supporting a regulatory determination decision. The NDWAC Working Group report was presented to and accepted by the entire NDWAC in July 2000.

Because of the intrinsic difference between microbial and chemical contaminants, the Working Group developed separate but similar protocols for microorganisms and chemicals. The approach for chemicals was based on an assessment of the impact of acute, chronic, and lifetime exposures, as well as a risk assessment that includes evaluation of occurrence, fate, and dose response. The NDWAC Protocol for chemicals is a semi-quantitative tool for addressing each of the three CCL criteria. The NDWAC requested that the Agency use good judgement in balancing the many factors that need to be considered in making a regulatory determination.

The EPA modified the semi-quantitative NDWAC suggestions for evaluating chemicals against the regulatory determination criteria and applied them in decision making. The quantitative and qualitative factors for manganese that were considered for each of the three criteria are presented in the sections that follow.

9.2 Health Effects

The first criterion asks if the contaminant may have an adverse effect on the health of persons. Because all chemicals have adverse effects at some level of exposure, the challenge is to define the dose at which adverse health effects are likely to occur, and estimate a dose at which

adverse health effects are either not likely to occur (threshold toxicant), or have a low probability for occurrence (non-threshold toxicant). The key elements that must be considered in evaluating the first criterion are the mode of action, the critical effect(s), the dose-response for critical effect(s), the RfD for threshold effects, and the slope factor for non-threshold effects.

A full description of the health effects associated with exposure to manganese is presented in Chapter 7 of this document and summarized below in Section 9.2.2. Chapter 8 and Section 9.2.3 present dose-response information.

9.2.1 Health Criterion Conclusion

The available toxicological data indicate that manganese has the potential to cause adverse health effects in humans and animals at high doses. The primary route of exposure to toxic levels of manganese is through the inhalation of manganese dust. An increased potential exists for inhalation and ingestion exposure to manganese as a result of the use of MMT in fuels. Zayed et al. (1999) measured airborne manganese concentrations (as MMT, respirable, and total manganese) in five different microenvironments around Montreal, Canada. The authors determined that the average daily exposure to respirable manganese was 0.010 μg/kg-day and had a low contribution to air, food, and water. Oral exposure to levels of toxicological concern is rare. In humans, neurological effects are the most likely manifestation of manganese toxicity. There is no information available regarding the carcinogenicity of manganese in humans, and animal studies have reported mixed results. Manganese is classified as Group D, or Not classifiable as to human carcinogenicity. The Reference Concentration (RfC) for manganese is 5×10^{-5} mg/m^3 (U.S. EPA, 1998a) which is derived using data from two epidemiological studies of workers exposed to manganese dioxide dust in an occupational setting (Roels et al., 1987; Roels et al., 1992). The critical effect was impairment of neurobehavioral function. The current RfD for manganese in food is 0.14 mg/kg-day; and for drinking water, 0.047 mg/kg-day. Despite the fact that it is possible for manganese to elicit some toxic effects at very high doses, the database is too uncertain, especially related to children and other sensitive populations. Based on the occurrence of adverse effects in humans and animals, the evaluation for Criterion #1 is *positive.*

9.2.2 Hazard Characterization and Mode of Action Implications

The primary health effect of manganese exposure is neurotoxicity, which is characterized at high doses by ataxia, increased anxiety, dementia, a "mask-like" face, general extrapyrimidal syndrome, or manganism, a syndrome similar to Parkinson's disease. The precise mechanisms of manganese neurotoxicity are not known, although the observed effects of manganese on the globus pallidus region of the brain suggest that a likely mechanism involves impairment of dopaminergic function. Preclinical adverse neurological effects have been reported at much lower doses than those resulting in manganism, however. Therefore, the possibility exists that any potential neurological effects resulting from environmental exposures to manganese would likely be more comparable to these subtle, though potentially significant, changes in neurological function.

Studies in humans and animals are mixed, but most animal studies indicate that children are a potentially sensitive subpopulation based on decreased excretion in the neonate (Lönnerdal, 1994). Additional potentially sensitive sub-populations include the elderly, pregnant women, iron-deficient individuals, and individuals with impaired liver function.

Because the primary route of elimination for manganese is biliary excretion, persons with impaired liver function may be especially susceptible to manganese toxicity (Layrargues et al., 1998). Persons in a state of iron deficiency may also experience greater susceptibility to manganese absorption and toxicity (Finley, 1999; Finley et al., 1994). In addition, infants and neonates, in which the capacity for excretion through the bile is not fully developed, may also be potentially susceptible to manganese toxicity (Lönnerdal, 1994). Although animal studies have indicated an increased potential in neonates for gastrointestinal absorption of manganese, as well as decreased excretion potential, the degree to which these findings apply to human infants is unknown. Dorman et al. (2000) have shown, however, that there is increased sensitivity for neurotoxic effects following manganese exposure in neonatal rats compared to adult rats. Because manganese is an essential nutrient in developing infants, however, the potential adverse effects from manganese deficiency may be of greater concern than potential toxicity from over-exposure.

An added complication is the fact that many inhibitors of manganese absorption, such as phytates and plant fiber, are common in the diet and may thus lower the actual absorption of ingested manganese. Also, manganese absorption from foods that are potentially high sources may be inhibited by other factors such as the presence of co-occurring plant proteins that bind manganese and decrease its bioavailability. Thus, although the manganese content in the soy-based formula is higher than manganese content in human milk, the actual absorption of manganese in the formula may not be substantially greater since it is prepared with soy milk, which is high in phytate and vegetable protein. Data exist, however, that argue against this possibility. For example, Keen et al. (1986) demonstrated in rat pups that manganese uptake from human breast milk and cow's milk was higher (~80% and ~89 %, respectively) than that from soy formula (~60%), but the absolute amount of manganese retained from soy was 25 times the amount retained from human milk. Dorner et al. (1989) also reported increased retention of manganese in full-term human infants fed cow's-milk formulas compared to breast-fed infants. Human milk and cow's milk contain different proteins that bind manganese. In some cases, the presence of these proteins may enhance manganese transport across the gut wall and hence increase absorption. If infant formula is prepared with contaminated water, then it is possible that the manganese will remain in a soluble form which may be more easily absorbed. More data are needed on the various factors affecting manganese absorption in infants before a confident determination can be made. Other instances in which high dietary levels of manganese may not necessarily correspond to high dose levels include vegetarian diets (many vegetables contain high manganese levels but also high fiber and phytate levels) and possibly tea drinkers (tea also contains high manganese levels accompanied by high levels of tannin, another inhibitor of manganese absorption).

Several studies have explored the level of manganese intake which may be considered safe in humans. The Food and Nutrition Board (2002) set an adequate intake level for manganese of 2.3 mg/day for men and 1.8 mg/day for women (Food and Nutrition Board, 2002; Trumbo et al., 2001). The Food and Nutrition Board also set a tolerable upper intake level of 11 mg Mn/day for adults based on the Greger (1999) review, which suggested that people eating western-type and vegetarian diets may have intakes as high as 10.9 mg/day (Food and Nutrition Board, 2002). Further, for short-term duration, Davis and Greger (1992) found that daily intake of 15 mg/day for 90 days resulted in no adverse effects in women; the only effect seen was an increase in superoxide dismutase activity.

No significant exposure-related neurological effects were seen in a cohort in Germany exposed for up to 40 years to manganese in their well water at levels as high as 2.160 mg/L (0.3 to 2.160 mg/L; Vierrege et al., 1995). On the other hand, a study in Greece which examined older populations chronically exposed to well water containing up to around 2 mg/L found effects on neurological function in the high-exposure group (Kondakis et al., 1989); however, this study did not adequately account for potential bias in subjective neurological test scores. Neither study reported the dietary or other sources of manganese intake.

9.2.3 Dose-Response Characterization and Implications in Risk Assessment

The dose-response relationship for neurological effects of manganese by ingestion is not well-characterized in animals or humans, but epidemiological data for humans indicate that intakes as high as 11 mg/day (0.16 mg/kg-day) may not cause adverse effects in adult humans. Additional evidence suggests a safe level as high as 15 mg/day (0.21 mg/kg-day for adult), based on a study in which women received daily supplements of 15 mg manganese for 90 days and exhibited only an increase in lymphocyte manganese-dependent superoxide dismutase, but no measured adverse effects (Davis and Greger, 1992). Characterizing dose-response in humans is complicated by the fact that manganese is an essential nutrient, and therefore some minimal level of intake is necessary for good health. There are many reports of toxicity to humans exposed to manganese by inhalation; much less is known, however, about oral intakes resulting in toxicity. 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).

A review of acute animal toxicity studies of manganese indicates that the manganese has low to moderate oral toxicity. For example, the oral LD_{50} values for manganese compounds in rats are in the range of 400 to 2,000 mg Mn/kg. Some animal studies have also reported developmental and reproductive effects at high doses for some manganese compounds, but most data from oral exposure suggest that manganese has a low developmental toxicity.

EPA has calculated an RfD for manganese. The RfD for manganese in food is 0.14 mg/kg-day, based on dietary surveys that have reported that, for an average 70 kg adult, having a daily manganese intake of 10 mg presents no adverse effect. For drinking water, EPA recommends to apply a modifying factor (MF) of 3 to yield a value of 0.047 mg/kg-day. This modifying factor is meant to address the concern raised by the epidemiology study (Kondakis et

al., 1989), and a potential higher absorption of manganese in water, especially when drinking fluids early in the morning, when the gut is empty. EPA has medium confidence in the RfD for manganese.

9.3 Occurrence in Public Water Systems

The second criterion asks if the contaminant is known to occur or if there is a substantial likelihood that the contaminant will occur in public water systems with a frequency and at levels of public health concern. In order to address this question, the following information was considered:

- Monitoring data from public water systems
- Ambient water concentrations and releases to the environment
- Environmental fate

Data on the occurrence of manganese in public drinking water systems were the most important determinants in evaluating the second criterion. EPA looked at the total number of systems that reported detections of manganese, as well those that reported concentrations of manganese above an estimated drinking water health reference level (HRL). For noncarcinogens the estimated HRL risk level was calculated from the RfD assuming that 20% of the total exposure would come from drinking water. For carcinogens, the HRL was the 10^{-6} risk level. The HRLs are benchmark values that were used in evaluating the occurrence data while the risk assessments for the contaminants were being developed.

The available monitoring data, including indications of whether or not the contamination is a national or a regional problem, are included in Chapters 4 of this document and are summarized below. Additional information on production, use, and environmental fate are found in Chapters 2 and 3.

9.3.1 Occurrence Criterion Conclusion

The available data for manganese production and use indicate a fairly stable trend for both. While release of manganese to surface water is variable within a wide range of values, release of manganese compounds to surface water is increasing. Releases of manganese and manganese compounds to land are generally decreasing, while releases of manganese to air are decreasing and air emissions of manganese compounds are stable (Tables 3-4 and 3-5). MMT in gasolines provides a relatively new environmental source of manganese exposure. 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. Data on the occurrence of manganese in air resulting from combustion of MMT and other sources are presented in Section 4.2. Pfeifer et al. (1999) determined that two occupational groups, office workers and taxi drivers were exposed to comparable concentrations of manganese both before

and after MMT was present in fuels. These data, however, are counter to other modeling data that indicate that taxi drivers are exposed to increased concentrations of manganese as a result of MMT use (Lynam et al., 1994; Zayed et al., 1994; Riveros-Rosas et al., 1997). Modeling data from five microenvironments in Canada indicate that with the currently acceptable levels of MMT allowed in fuel, little impact to air and surface water concentrations of manganese is expected from the use of MMT in fuels (Zayed et al., 1999). Monitoring data indicate that manganese is infrequently detected in public water supplies. When manganese is detected, it rarely exceeds the HRL or a value of one-half the HRL. Further, because manganese is an essential nutrient, the risks of over-exposure must be weighed against the risks of manganese deficiency. Based on these data, it is unlikely that manganese will occur in public water systems at frequencies or concentration levels that are of public health concern. Therefore, the evaluation for Criterion #2 is *negative*.

9.3.2 Monitoring Data

Drinking Water

Occurrence data for manganese in drinking water are presented and analyzed in Chapter 4 of this document. Estimates of exposed populations are derived in Section 4.3. The National Inorganic and Radionuclide Survey (NIRS) data represent 49 States. Data were not available for the State of Hawaii. Since NIRS data lack occurrence information for surface water systems, occurrence data on manganese exposure from the States of Alabama, California, Illinois, New Jersey, and Oregon were used to obtain information on surface water.

At a health reference level (HRL) of 0.3 mg/L, approximately 6.2% of the NIRS PWSs had detections greater than one-half the HRL (about 3,700 ground water PWSs nationally), affecting approximately 4.6% of the population served (estimated at 4.0 million people nationally).

The percentage of NIRS PWSs with detections greater than the HRL of 0.3 mg/L was approximately 3.6% (about 2,200 ground water PWSs nationally), affecting 2.7% of the population served (estimated at approximately 2.3 million people nationally).

The supplemental State data sets indicate that ground water PWS detections greater than the HRL of 0.3 mg/L are between 0.6% and 12%. Again, the NIRS national average is within this range, with 3.6% of PWSs greater than the HRL. Notably, surface water PWSs showed fewer exceedances of the HRL than ground water PWSs at this higher concentration, ranging from 0% to 3%. Extrapolating national population exposures from these limited data sets is not possible because exposure to manganese through surface water is not quantified beyond the five States shown. However, exposure estimates incorporating surface water sources would certainly be larger than the estimates provided here for groundwater sources.

Ambient Water

The National Ambient Water Quality Assessment (NAWQA) program was begun in 1991 by the United States Geological Survey (USGS) to monitor water quality in representative study basins located around the country. This program, which consists of 59 significant watersheds and aquifers, was described in Chapter 4 of this document in regard to its use for monitoring ambient levels of manganese in surface and ground waters. The Minimum Reporting Level (MRL) in water is 0.001 mg/L, while the MRLs in sediments and aquatic biota tissue are 4 mg/kg and 0.1 mg/kg, respectively.

The data indicate that manganese is ubiquitous in surface and ground waters, presumably as a result of its natural occurrence in the earth's crust. The frequency of detection above the HRL is generally higher in ground water than in surface water, but the median concentration in sites reporting a detection is higher in surface water (0.016 mg/L in surface water versus 0.005 mg/L in ground water). Overall, the data indicate that, while manganese is nearly ubiquitous in surface and ground water, detections at levels of concern to public health are relatively few.

Manganese has been universally detected in stream sediments and aquatic biota tissues at low levels. Manganese is not thought to bioaccumulate in tissues to any significant degree, and desorption from sediments into the water column is also limited by the insolubility of most manganese compounds.

9.3.3 Use and Fate Data

Manganese is a naturally occurring element and is commonly found in soil, water, air, and food, generally as a component of over 100 mineral compounds. Most manganese ore is imported to the United States, with the amount increasing from 308 thousand metric tons in 1984 to 535 thousand metric tons in 1999. Most of this ore is smelted to produce ferromanganese, which is used in steel production. Manganese compounds have a variety of other uses in industry and agriculture, as described in Table 3-3 of this document.

Examination of data from the Toxic Release Inventory (TRI), shown in Tables 3-4 and 3-5 of this document, indicates that releases of manganese to water varied between 89 thousand and 2.4 million pounds for the period 1988 to 1998. Data for manganese compounds reveal an increasing trend in surface water discharges, from 681 thousand to 4.5 million pounds for the same period.

Once released to the environment, manganese is readily deposited in the soil and taken up by plants, whereupon it may enter the food chain. Significant bioaccumulation is not expected to occur. Manganese is an essential nutrient in the diet, so some minimal intake is necessary for good health. Manganese particles may also become airborne, and some manganese compounds are soluble in water. Manganese compounds may also adsorb to sediment surfaces and precipitate out of solution.

Manganese, in the form of potassium permanganate, may be used in drinking water treatment for oxidation and disinfection purposes (ANSI/NSF, 2000), in addition to its use in industrial wastewater purification and odor abatement (ATSDR, 2000; U.S. EPA, 1984). The adsorption properties of some manganese compounds may cause them to be more prevalent in certain types of soils or sediments.

9.4 Risk Reduction

The third criterion asks if, in the sole judgment of the Administrator, regulation presents a meaningful opportunity for health risk reduction for persons served by public water systems. In order to evaluate this criterion, EPA looked at the total exposed population, as well as the population exposed above the estimated HRL. Estimates of the populations exposed and the levels to which they are exposed were derived from the monitoring results. These estimates are included in Chapter 4 of this document and summarized in Section 9.4.2 below.

In order to evaluate risk from exposure through drinking water, EPA considered the net environmental exposure in comparison to the exposure through drinking water. For example, if exposure to a contaminant occurs primarily through ambient air, regulation of emissions to air provides a more meaningful opportunity for EPA to reduce risk than regulation of the contaminant in drinking water. In making the regulatory determination, the available information on exposure through drinking water (Chapter 4) and information on exposure through other media (Chapter 5) were used to estimate the fraction that drinking water contributes to the total exposure. The EPA findings are discussed in Section 9.4.3 below.

In making its regulatory determination, EPA also evaluates effects on potential sensitive populations, including the fetus, infants and children. The sensitive population considerations are included in Section 9.4.4.

9.4.1 Risk Reduction Criterion Conclusion

Approximately 47.5 million people are served by ground water public water systems with detections greater than the MRL. More than 2.3 million of these individuals are served by systems with detections greater than the HRL. Manganese is an essential nutrient that is common and necessary in the diet. The estimated daily exposure to manganese from public water systems is far below the expected daily intake from the diet, and also far below the level determined to be safe and adequate. When average daily intakes from drinking water are compared with intakes from food, air and soil, drinking water accounts for a relatively small proportion of manganese intake. On the basis of these observations, the impact of regulating manganese concentrations in drinking water on health risk reduction is likely to be small. Therefore, the evaluation for Criterion #3 is negative.

9.4.2 Exposed Population Estimates

Estimates of exposed populations were derived in Chapter 4. National population estimates for manganese exposure were derived using summary statistics from the National Inorganic and Radionuclide Survey (NIRS), which lacked surface water data, with supplemental surface water occurrence data that had been separately submitted to EPA from five States. An estimated 47.5 million people in the U.S. are served by public water systems supplied from ground water with detections of manganese above the minimum reporting level (MRL). An estimated 4.0 million people (4.6% of the population) are served by ground water with levels above one-half the health reference level (HRL) of 0.3 mg/L, and an estimated 2.3 million people (2.7% of the population) are served by ground water with levels above the HRL. It should be noted that these estimates are based on very limited and outdated data. The possibility exists that the number of people served by ground water with Mn levels that are above the HRL could be higher than these estimates; however, the data are lacking at this time to develop a more timely assessment.

9.4.3 Relative Source Contribution

Relative source contribution analysis compared the magnitude of exposure expected via drinking water to the magnitude of exposure from intake of manganese from other media such as food, air, and soil. To perform this analysis, intake of manganese from drinking water must be estimated. Occurrence data for manganese are presented in Chapter 4 of this document. According to the NIRS data (Table 4-1), the median and 99th percentile concentrations for manganese in ground water public water supplies were above the MRL of 0.001 mg/L. This is not surprising considering the ubiquity with which manganese is present in the earth's crust.

Taking the median concentration of detections from the NIRS data (0.01 mg/L), and assuming a daily intake of 2 L of drinking water by a 70 kg adult, the average daily dose would be 0.02 mg/person-day or 2.8×10^{-4} mg/kg-day. The corresponding dose for a 10 kg child consuming 1 L/day of drinking water would be 0.01 mg/child-day or 1.0×10^{-3} mg/kg-day. These values are far below those expected from a normal diet ($2.9–12.6 \times 10^{-2}$ mg/kg-day for adults, 1.3×10^{-1} mg/kg-day for children, see Table 9-1 below), and are also less than the levels determined by the National Academy of Sciences to be safe and adequate. The NAS determined that a daily intake of 2.3 mg Mn is adequate for men and 1.8 mg is adequate for women, while the daily adult intake expected from drinking water containing 0.01 mg/L Mn is 0.02 mg Mn. The NAS also determined that a daily intake of 1.9 mg Mn is adequate for boys and 1.6 mg is adequate for girls, while the daily intake expected from drinking water containing 0.01 mg/L Mn is 0.01 mg for children. The NAS has proposed that the Adequate Intake (AI) for manganese is 1.8 to 2.3 mg/day for adults (Food and Nutrition Board, 2002).

Table 9-1. Comparison of Average Daily Intake from Drinking Water and Other Media[a]

Medium	Adult (μg/kg-day)	Child (μg/kg-day)
Drinking Water[b]	**0.29**	**1.0**
Food	**28.6-126**	**128.0 (0.87-37.2 for infants)**
Air	**0.0087**	**0.034**
Soil	**0.0014 - 5.0**	**0.02 - 70**

[a] See Chapter 5 for derivation of intakes from media other than water
[b] based on median values

9.4.4 Sensitive Populations

The sensitive populations identified for manganese include persons with impaired detoxification and excretory function, such as infants and the elderly. Individuals with damaged or impaired liver function may be particularly sensitive.

9.5 Regulatory Determination Decision

As stated in Section 9.1.1, a positive finding for all three criteria is required in order to make a determination to regulate a contaminant. While there is evidence that manganese may have adverse health effects in humans at high doses through inhalation, the evidence for adverse effects through oral exposure at low or moderate levels is less compelling. Because manganese is an essential nutrient, concern over potential toxic effects from high oral exposure must be balanced against concern for adverse effects from manganese deficiency should intake be too low. Manganese has been found to occur in an estimated 2,200 ground water public water systems representing more than 2.3 million people exposed (2.7% of the population) to levels at or above 0.3 mg/L. The Agency believes that a meaningful opportunity for health risk reduction does not exist for persons served by public water systems because the average dietary intake of manganese exceeds the contribution normally found in public drinking water systems. Thus, based on the evaluation of available data using the criteria described above, the regulatory determination is *"Do not regulate"*.

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APPENDIX A: Abbreviations and Acronyms

ACGIH	- American Conference of Governmental Industrial Hygienists
ATSDR	- Agency for Toxic Substances and Disease Registry
CAS	- Chemical Abstract Service
CCL	- Contaminant Candidate List
CERCLA	- Comprehensive Environmental Response, Compensation & Liability Act
CMR	- Chemical Monitoring Reform
CWS	- Community Water System
DWEL	- Drinking Water Equivalent Level
EPA	- Environmental Protection Agency
EPCRA	- Emergency Planning and Community Right-to-Know Act
GW	- ground water
HA	- Health Advisory
HAL	- Health Advisory Level
HRL	- Health Reference Level
IOC	- inorganic compound
IRIS	- Integrated Risk Information System
MRL	- Minimum Reporting Level
NAWQA	- National Water Quality Assessment Program
NCOD	- National Drinking Water Contaminant Occurrence Database
NIOSH	- National Institute for Occupational Safety and Health
NIRS	- National Inorganic and Radionuclide Survey
NPDES	- National Pollution Discharge Elimination System
NPDWR	- National Primary Drinking Water Regulation
NTIS	- National Technical Information Service
NTNCWS	- Non-Transient Non-Community Water System
ppm	- part per million
PWS	- Public Water System
RCRA	- Resource Conservation and Recovery Act
SARA Title III	- Superfund Amendments and Reauthorization Act
SDWA	- Safe Drinking Water Act
SDWIS	- Safe Drinking Water Information System
SDWIS FED	- the Federal Safe Drinking Water Information System
STORET	- Storage and Retrieval System
SW	- surface water
TRI	- Toxic Release Inventory
UCM	- Unregulated Contaminant Monitoring
UCMR	- Unregulated Contaminant Monitoring Regulation/Rule
UMRA	- Unfunded Mandates Reform Act of 1995
URCIS	- Unregulated Contaminant Monitoring Information System
U.S. EPA	- United States Environmental Protection Agency
USGS	- United States Geological Survey

µg/L	- micrograms per liter
mg/L	- milligrams per liter
> MCL	- percentage of systems with exceedances
> MRL	- percentage of systems with detections

APPENDIX B: Complete NIRS Data for Manganese

NIRS Data - Manganese Occurrence in Public Water Systems (HRL = 0.3 mg/L)

State	# Samples	# Samples > MRL	% Samples > MRL	# Detects > 1/2 HRL	% Detects > 1/2 HRL	# Detects > HRL	% Detects > HRL	Min Value (mg/L)	99% Value (mg/L)	Max Value (mg/L)	Min Detects (mg/L)	Median Detects (mg/L)
AK	8	7	87.50%	2	25.00%	1	12.50%	< 0.00	0.50	0.50	0.02	0.05
AL	8	4	50.00%		0.00%		0.00%	< 0.00	0.05	0.05	0.00	0.01
AR	9	6	66.67%		0.00%		0.00%	< 0.00	0.06	0.06	0.00	0.01
AZ	14	5	35.71%	1	7.14%	1	7.14%	< 0.00	0.58	0.58	0.00	0.00
CA	60	26	43.33%	2	3.33%	1	1.67%	< 0.00	0.65	0.65	0.00	0.01
CO	10	7	70.00%		0.00%		0.00%	< 0.00	0.13	0.13	0.00	0.00
CT	23	18	78.26%		0.00%		0.00%	< 0.00	0.09	0.09	0.00	0.01
DE	10	10	100.00%		0.00%		0.00%	0.00	0.08	0.08	0.00	0.01
FL	56	29	51.79%		0.00%		0.00%	< 0.00	0.03	0.03	0.00	0.00
GA	23	9	39.13%		0.00%		0.00%	< 0.00	0.05	0.05	0.00	0.02
IA	28	22	78.57%	5	17.86%	4	14.29%	< 0.00	1.34	1.34	0.00	0.01
ID	12	1	8.33%		0.00%		0.00%	< 0.00	0.13	0.13	0.13	0.13
IL	46	34	73.91%	1	2.17%	1	2.17%	< 0.00	0.36	0.36	0.00	0.01
IN	19	18	94.74%	2	10.53%	1	5.26%	< 0.00	0.33	0.33	0.01	0.03
KS	6	3	50.00%	1	16.67%	1	16.67%	< 0.00	0.83	0.83	0.01	0.07
KY	8	6	75.00%	2	25.00%	1	12.50%	< 0.00	0.50	0.50	0.00	0.02
LA	26	24	92.31%	3	11.54%		0.00%	< 0.00	0.25	0.25	0.00	0.01
MA	7	6	85.71%	1	14.29%		0.00%	< 0.00	0.19	0.19	0.00	0.00
MD	6	5	83.33%		0.00%		0.00%	< 0.00	0.05	0.05	0.00	0.02
ME	7	6	85.71%		0.00%		0.00%	< 0.00	0.04	0.04	0.00	0.01
MI	25	22	88.00%	2	8.00%		0.00%	< 0.00	0.20	0.20	0.00	0.02
MN	19	17	89.47%	6	31.58%	4	21.05%	< 0.00	0.63	0.63	0.01	0.09
MO	21	16	76.19%	3	14.29%	1	4.76%	< 0.00	1.22	1.22	0.00	0.00
MS	26	21	80.77%		0.00%		0.00%	< 0.00	0.09	0.09	0.00	0.01
MT	11	5	45.45%	1	9.09%	1	9.09%	< 0.00	0.33	0.33	0.00	0.07
NC	44	33	75.00%		0.00%		0.00%	< 0.00	0.09	0.09	0.00	0.01
ND	19	19	100.00%	3	15.79%	2	10.53%	0.00	0.63	0.63	0.00	0.01
NE	19	10	52.63%	3	15.79%	2	10.53%	< 0.00	1.24	1.24	0.00	0.05
NH	10	8	80.00%		0.00%		0.00%	< 0.00	0.11	0.11	0.01	0.05
NJ	6	2	33.33%		0.00%		0.00%	< 0.00	0.09	0.09	0.01	0.05
NM	7	5	71.43%	1	14.29%	1	14.29%	< 0.00	0.38	0.38	0.00	0.02
NV	2	1	50.00%		0.00%		0.00%	< 0.00	0.00	0.00	0.00	0.00
NY	57	32	56.14%	4	7.02%	2	3.51%	< 0.00	0.40	0.40	0.00	0.03
OH	25	19	76.00%		0.00%		0.00%	< 0.00	0.13	0.13	0.00	0.02
OK	12	6	50.00%		0.00%		0.00%	< 0.00	0.08	0.08	0.00	0.00
OR	8	5	62.50%	1	12.50%		0.00%	< 0.00	0.17	0.17	0.00	0.01
PA	36	28	77.78%	7	19.44%	4	11.11%	< 0.00	0.86	0.86	0.00	0.02
PR	1	1	100.00%		0.00%		0.00%	0.01	0.01	0.01	0.01	0.01
RI	1	1	100.00%		0.00%		0.00%	0.03	0.03	0.03	0.03	0.03
SC	18	11	61.11%		0.00%		0.00%	< 0.00	0.07	0.07	0.00	0.01
SD	8	7	87.50%	2	25.00%	1	12.50%	< 0.00	0.72	0.72	0.00	0.06
TN	9	8	88.89%		0.00%		0.00%	< 0.00	0.08	0.08	0.00	0.00
TX	74	51	68.92%		0.00%		0.00%	< 0.00	0.13	0.13	0.00	0.02
UT	10	4	40.00%		0.00%		0.00%	< 0.00	0.02	0.02	0.00	0.00
VA	30	25	83.33%		0.00%		0.00%	< 0.00	0.13	0.13	0.00	0.01
VT	12	8	66.67%	2	16.67%	2	16.67%	< 0.00	0.33	0.33	0.00	0.00
WA	52	31	59.62%	3	5.77%		0.00%	< 0.00	0.18	0.18	0.00	0.01
WI	30	24	80.00%	1	3.33%		0.00%	< 0.00	0.18	0.18	0.00	0.02
WV	8	3	37.50%	1	12.50%	1	12.50%	< 0.00	0.76	0.76	0.00	0.10
WY	3	3	100.00%		0.00%		0.00%	0.02	0.09	0.09	0.02	0.02
Total	989	672	67.95%	60	6.07%	32	3.24%	< 0.00	0.63	1.34	0.00	0.01

PWS= Public Water Systems; GW= Ground Water (PWS Source Water Type); SW= Surface Water (PWS Source Water Type); MRL= Minimum Reporting Limit (for laboratory analyses)
The Health Reference Level (HRL) is the estimated health effect level as provided by EPA for preliminary assessment for this work assignment.
"% > HRL" indicates the proportion of systems with any analytical results exceeding the concentration value of the HRL.
The Health Reference Level (HRL) used for Manganese is 0.28 mg/L. This is a draft value for working review only.
Manganese data were analyzed using two different HRLs and are, therefore, listed separately.