FINAL DRAFT
ECAO-CIN-D008
~~September~~. 1993

United States
Environmental Protection
Agency

EPA

Research and Development

DRINKING WATER CRITERIA DOCUMENT FOR MANGANESE

Prepared for

Office of Water

Prepared by

Environmental Criteria and Assessment Office
Office of Health and Environmental Assessment
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Cincinnati, OH 45268

DISCLAIMER

This report is an external draft for review purposes only and does not constitute Agency policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

FOREWORD

Section 1412 (b) (3) (A) of the Safe Drinking Water Act, as amended in 1986, requires the Administrator of the Environmental Protection Agency to publish maximum contaminant level goals (MCLGs) and promulgate National Primary Drinking Water Regulations for each contaminant, which, in the judgement of the Administrator, may have an adverse effect on public health and which is known or anticipated to occur in public water systems. The MCLG is nonenforceable and is set at a level at which no known or anticipated adverse health effects in humans occur and which allows for an adequate margin of safety. Factors considered in setting the MCLG include health effects data and sources of exposure other than drinking water.

This document provides the health effects basis to be considered in establishing the MCLG. To achieve this objective, data on pharmacokinetics, human exposure, acute and chronic toxicity to animals and humans, epidemiology and mechanisms of toxicity are evaluated. Specific emphasis is placed on literature data providing dose-response information. Thus, while the literature search and evaluation performed in support of this document has been comprehensive, only the reports considered most pertinent in the derivation of the MCLG are cited in the document. The comprehensive literature data base in support of this document includes information published up to 1992; however, more recent data may have been added during the review process.

When adequate health effects data exist, Health Advisory values for less than lifetime exposures (1-day, 10-day and longer-term, ~10% of an individual's lifetime) are included in this document. These values are not used in setting the MCLG, but serve as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur.

This document was prepared for the Office of Water by the Office of Health and Environmental Assessment (Environmental Criteria and Assessment Office, Cincinnati, Ohio) to provide the scientific support for the human health-based risk assessment used in the determination of the drinking water MCLG. For more information, contact the Human Risk Assessment Branch of the Office of Water at (202)260-7571.

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CNS	Central nervous system
DWEL	Drinking water equivalent level
GI	Gastrointestinal
HA	Health Advisory
i.m.	Intramuscular
i.t.	Intratracheal
i.v.	Intravenous
LC_{50}	Concentration lethal to 50% of recipients
LD_{50}	Dose lethal to 50% of recipients
LOAEL	Lowest-observed-adverse-effect-level
MAO	Monoamine oxidase
NOAEL	No-observed-adverse-effect level
RfD	Reference dose
RSC	Relative source contribution
s.c.	Subcutaneous

I. SUMMARY

Manganese is a gray-pink metal that is too brittle to be used unless alloyed. It exists in 11 oxidation states with the compounds containing Mn^{2+}, Mn^{4+} and Mn^{7+} being the most economically and environmentally important.

Manganese is absorbed from the GI tract after ingestion and is distributed primarily to the liver, kidney, endocrine glands and brain. The absorption of manganese is low, averaging 3-9% in adults. Bile is the main route of excretion of manganese and represents the principal regulatory mechanism. The metabolism of manganese is controlled by homeostatic mechanisms at the level of excretion as well as absorption, which respond very efficiently to increases in manganese concentration. However, prolonged exposure to excess manganese lessens the efficiency of the homeostatic mechanism. The biologic half-life ranges from 2-5 weeks and depends upon body stores of manganese. In both humans and animals, the biologic half-life decreases with increased exposure. Retention in the brain appears to be longer than in other parts of the body.

Manganese is an essential element, being required by mammals and birds for both normal growth and maintenance of health. However, manganese deficiency is practically nonexistent in humans as it is widely available in the diet. Manganese is also considered to be of low toxicity because of efficient homeostatic controls that regulate the absorption and excretion of manganese. However, high levels of manganese can result in poisoning, particularly by the inhalation route of exposure.

The CNS is the primary system affected by chronic exposure to high levels of manganese. The human neurobehavioral deficits (e.g., tremor, gait disorders) resulting from manganese poisoning can be reproduced in other primates but not in rodents. Parenteral administration of manganese to monkeys results in extrapyramidal symptoms and histologic lesions in the brain, which resemble those seen in human manganism. However, by the oral route there has been only one limited study using primates that employed only one dose level. Studies of rodents orally exposed to manganese report neurochemical, but not behavioral effects as seen in humans. Therefore, these studies are of questionable relevance with respect to human health risk assessment.

In chronic manganese toxicity, several neurotransmitter systems in the brain appear to be affected. The primary effect is on the levels of monoamines, especially dopamine, but the precise mechanism of this effect is not understood.

Studies on occupationally exposed humans, although supporting the association of neurotoxic effects with inhalation exposure to manganese, have not provided a clear dose-response relationship. Most human studies have related to inhalation exposure and have found that exposure to levels >5 mg/m^3 have been associated with neurotoxic effects.

A Japanese study of health effects resulting from the ingestion of manganese-contaminated drinking water for several months found neurotoxic signs and symptoms occurring at drinking water concentrations estimated to be roughly 28 mg Mn/L. In contrast to what has been shown in laboratory animals, children were less affected than

adults by this exposure. The elderly were most severely affected. An epidemiologic study performed in Greece has shown that a lifetime consumption of drinking water containing naturally high levels of manganese (up to 2 mg/L) leads to increased manganese retention as demonstrated by the concentration of manganese in hair. At levels of about 2 mg/L, the authors suggested that some neurologic impairment may be apparent in people over 50 years of age. These two studies provide the basis for the establishment of separate risk assessments for manganese in food and water.

There are no epidemiologic studies investigating the relationship between manganese exposure and carcinogenic, mutagenic or teratogenic effects in humans.

The National Toxicology Program (NTP, 1992) conducted a 2-year feeding bioassay of manganese sulfate in B6C3F1 mice and F344 rats. No evidence of carcinogenicity was seen in mice. In rats there was equivocal evidence based on an increased incidence of thyroid follicular cell tumors, but only at very high doses of manganese. The relevance of these tumors to human carcinogenesis is questionable.

Existing guidelines recommend a maximum concentration of 0.05 mg/L for manganese in freshwater to prevent undesirable taste and discoloration. For the protection of consumers of marine mollusks, a criterion for manganese of 0.1 mg/L for marine waters has been recommended. The rationale for this criterion has not been specified, but it is partially based on the observation that manganese can bioaccumulate in marine mollusks.

There are insufficient data to calculate separate 1-day and 10-day HAs for manganese in drinking water. The HA values of 1 mg/L are based on the RfD for manganese in water. Shorter-term exposure to higher levels of manganese is generally not of great concern because of the efficient homeostatic mechanisms in manganese metabolism and the taste and odor properties of manganese.

While there are limited data on the toxicity of ingested manganese in humans, there are several studies demonstrating levels of manganese in the diet that are safe and adequate for chronic human consumption. An RfD (food) of 0.14 mg Mn/kg/day (verified by the RfD/RfC Workgroup in June 1990) has been calculated based on these safe and adequate levels. It is also noted that some diets, particularly vegetarian diets, may contain higher levels of manganese. While the intake of manganese from these diets may exceed the RfD (equivalent to 10 mg Mn/day), the bioavailability of manganese from vegetable sources is substantially decreased by dietary components such as fiber and phytates. Therefore, these intakes are considered to be safe as well. It is emphasized that this RfD was calculated for total dietary manganese, which under normal circumstances accounts for virtually all manganese intake.

The two studies of humans ingesting significant quantities of manganese in drinking water led to the development of a separate drinking water RfD. In September 1992, the RfD/RfC Workgroup verified a drinking water RfD of 0.005 mg/kg/day. Assuming a body weight of 70 kg and a drinking water consumption of 2 L/day, this is roughly equivalent to water concentration of 200 μg Mn/L. Because the studies used to support the drinking water RfD assumed that there was an additional dietary

contribution of manganese, the RfD assumes the same. Therefore, no relative source contribution needs to be factored in and the concentration of 200 μg Mn/L may be used directly in the setting of drinking water standards.

II. PHYSICAL AND CHEMICAL PROPERTIES

Introduction

Manganese is a brittle, gray-pink metal with an atomic weight of 54.938. It is too brittle to be used unless alloyed. The CAS registry number is 7439-96-5. Manganese has only one stable natural isotope, 55Mn. Its melting point is 1244°C and the boiling point is 1962°C. It can exist in 11 oxidation states, with valences of 2+, 4+ and 7+ being the most common. The four allotropic forms of manganese are alpha, beta, gamma and delta, with the alpha form being stable below 710°C. The gamma form decomposes to alpha at normal temperatures. Manganese has a density of 7.43 at 20°C and a vapor pressure of 1 mm Hg at 1292°C. Pure electrolytic manganese is not hydrolyzed at normal temperatures. It does decompose slowly in cold water and more rapidly when heated.

The principal use of manganese is in the manufacture of iron, steel and other alloys, which accounts for about 95% of the U.S. demand. A minor use of manganese is in pyrotechnics and fireworks. Manganese compounds are used as feed additives and fertilizers, colorants in brick and tile manufacture, components in dry cell battery manufacture, precursors in chemical manufacture and processing, and fuel additives (U.S EPA, 1984). Table II-1 gives estimated production capacities for several manganese compounds.

The manganese compounds most economically and environmentally important are those that contain Mn^{2+}, Mn^{4+} and Mn^{7+}. The 2+ compounds are stable in acid solution but are readily oxidized in alkaline medium. The most important Mn^{4+} compound

TABLE II-1

Estimated U.S. Production, Capacity and Use of Selected Manganese Compounds*

Product	Formula	Estimated U.S. Production Capacity (metric tons/year	Use
Electrolytic manganese	MnO_2	18,000	Dry-cell batteries; ferrites
High purity manganese oxide	MnO	9,000	High-quality ferrites; ceramics; intermediate for high purity Mn (II) salts
60% manganese oxide	MnO	36,000	Fertilizer; feed additive, intermediate for electrolytic manganese metal and dioxide
Manganese sulfate	$MnSO_4$	68,000	Feed additive; fertilizer; intermediate for many products
Manganese chloride	$MnCl_2$	3,000	Metallurgy, MMT synthesis; brick colorant; dye; dry-cell batteries
Potassium permanganate	$KMnO_4$	14,000	Oxidant; catalyst; intermediate; water and air purifier
Methylcyclopentadienyl manganese tricarbonyl (MMT)	$CH_3C_5H_4Mn(CO)_3$	500-1,000	Fuel additive

*Source: Adapted from Reidies, 1981

is the oxide, MnO_2, also known as pyrolusite. Manganese colors glass an amethyst color and is responsible for the color of true amethyst (U.S. EPA, 1984). Several important compounds of manganese are described in the following text and in Table II-2.

Manganese (I) Compound

Methylcyclopentadienyl Manganese Tricarbonyl. $CH_3C_5H_4Mn(CO)_3$ or MMT is a light amber liquid that is added to fuels to prevent engine knock and as a smoke suppressant. It has a specific gravity of 1.39 at 20°C and is insoluble in water (U.S. EPA, 1984).

Manganese (II) Compounds

Manganous Carbonate. $MnCO_3$ is a naturally-occurring compound, but it is produced commercially by precipitating it out of manganese sulfate solutions. It is used in the production of ferrite, animal feeds, ceramics and as a source of acid soluble manganese (U.S. EPA, 1984). $MnCO_3$ is a rose-colored rhombic compound that turns light brown when exposed to air. It has a density of 3.125 at 20°C. It is soluble in water and dilute acid.

Manganous Chloride. $MnCl_2$ can exist in both the anhydrous form and as a hydrate with 6, 4 or 2 water molecules. The anhydrous form is a pink, cubic crystalline structure, also known as scacchite. It has a density of 2.977 at 25°C. The hydrate form ($\cdot 4H_2O$) is a rose colored monoclinic crystalline structure. It has a density of 2.01 at

TABLE II-2

Physical Properties of Some Manganese Compounds*

Name and CAS Registry Number	Valence	Chemical Formula	Molecular Weight	Specific Gravity or Density	Melting Point (°C)	Boiling Point (°C)	Solubility
Methylcyclopentadienyl manganese tricarbonyl (MMT) [12108-13-3]	+1	$CH_3C_5H_4Mn(CO)_3$	218.09	1.39	1.5	233	Insoluble in H_2O. Soluble in most organic solvents
Manganous carbonate* [598-62-9]	+2	$MnCO_3$	114.95	3.125	decomposes	NS	65 mg/L (25°C) Soluble in dilute acid Insoluble in NH_3 and alcohol
Manganous chloride [7773-27-01-5]	+2	$MnCl_2$	125.84	NS	650	1190	Soluble in alcohol, insuluble in ether and NH_3, 622 g/L (10°C), 1238 g/L (100°C)
Manganous acetate [15243-27-3]	+2	$Mn(C_2H_3O_2)_2 \cdot 4H_2O$	245.08	1.589	NS	NS	Soluble in cold H_2O and alcohol
Manganous acetate [638-38-0]	+2	$Mn(C_2H_3O_2)_2$	173.02	1.74	NS	NS	Soluble in alcohol Decomposes in water
Manganese ethylenebisdithiocarbamate (Maneb) [12427-38-2]	+2	$CH_2NHCS_2)_2Mn$	265.24	NS	NS	NS	Moderately soluble in H_2O
Manganous oxide [1344-43-0]	+2	MnO	70.94	5.43-5.46	1945	NS	Insoluble in H_2O
Manganous phosphate [NS]	+2	$Mn_3(PO_4)_2$	259.78	NS	NS	NS	NS
Manganous sulfate [7785-87-7]	+2	$MnSO_4 \cdot H_2O$	169.01	2.95	stable 57-117	NS	NS
Manganous difluoride [7782-64-1]	+2	MnF_2	92.93	3.98	856	NS	6.6 g/L (40°C), 4.8 g/L (100°C) Soluble in acid Insoluble in alcohol and ether
Manganous trifluoride [7782-53-1]	+2	MnF_3	111.93	3.54	decomposes 600	NS	Soluble in acid Decomposes in H_2O
Manganese borate [12228-91-0]	+2	$MnB_4O_7 \cdot 8H_2O$	354.17	NS	NS	NS	Insoluble in H_2O or alcohol Soluble in dilute acids
Manganese formate	NS	$Mn(CHO_2)_2 \cdot 2H_2O$	181.00	1.953	decomposes	NS	Soluble in H_2O
Manganese glycerophosphate	+2	$MnC_3H_7O_6P$	225.00	NS	NS	NS	Slightly soluble in cold H_2O Soluble in acid, citric acid Insoluble in alcohol

TABLE II-2 (cont.)

Name and CAS Registry Number	Valence	Chemical Formula	Molecular Weight	Specific Gravity or Density	Melting Point (°C)	Boiling Point (°C)	Solubility
Manganous hydroxide	+2	$Mn(OH)_2$	88.95	3.258 (13°C)	decomposes	NS	Slightly soluble in cold H_2O Soluble in acid, NH_4 salts Insoluble in alkaline solution
Manganous nitrate	+2	$Mn(NO_3)_2 \cdot 4H_2O$	215.01	1.82	25.8	129.4	Soluble in water Very soluble in alcohol
Manganous sulfide	+2	MnS	87.00	3.99	decomposes	NS	Soluble in cold H_2O Soluble in dilute acid and alcohol Insoluble in $(NH_4)_2S$
Manganese dioxide [1313-13-9]	+4	MnO_2	86.94	5.026	535	NS	Insoluble in H_2O Insoluble in HNO_3 and acetone Soluble in HCl
Potassium permanganate [7722-64-7]	+7	$KMnO_4$	158.03	2.7	decomposes 240	NS	28.3 g/L (0°C); 250 g/L (65°C) Decomposes in alcohol Soluble in water, acetic acid; slightly soluble in methyl alcohol and acetone

[a]Source: U.S. EPA, 1984; Weast, 1980; Windholz, 1976

[b]"Manganese" refers to the divalent form of manganese and is often used interchangeably with "manganese" when the valence is +2.

NS = Not specified

20°C. Manganous chloride is used as a starting material for other manganese compounds. The anhydrous form is used as a flux in magnesium metallurgy.

Manganese Ethylenebisdithiocarbamate. $(CH_2NHCS_2)_2Mn$ is a yellow powder used as a fungicide. It is sold under the name of "Maneb." It is produced by treating a solution of manganous chloride containing sodium hydroxide and ethylenediamine with carbon disulfide and neutralizing the resulting solution with acetic acid (U.S. EPA, 1984).

Manganous Acetate. $Mn(C_2H_3O_2)_2 \cdot 4H_2O$ is a pale red transparent crystal. Manganous acetate is used as a mordant in dyeing and as a drier for paints and varnishes. It has a density of 1.589 at 20°C. There is also an anhydrous form of manganous acetate, which is a brown crystalline substance with a density of 1.74 at 20°C (U.S. EPA, 1984).

Manganous Oxide. MnO is a naturally-occurring compound, known as manganosite. Manganous oxide has a green cubic crystalline structure with a density of 5.43-5.46 at 20°C. It is insoluble in both hot and cold water. It is produced by reducing higher oxides with either carbon monoxide or coke or by the thermal decomposition of manganous carbonate. It can be used as a good starting material for other manganous salts, in ferrites, in welding, and as a nutrient in agricultural fertilizers (U.S. EPA, 1984).

Manganous Phosphate. $Mn_3(PO_4)_2$ is produced by reacting manganous carbonate with phosphoric acid. Manganous phosphate is used as an ingredient of proprietary solutions for phosphating iron and steel (U.S. EPA, 1984).

Manganous Sulfate. $MnSO_4 \cdot H_2O$ is a pale pink monoclinic crystalline structure. It has a density of 2.95 at 20°C. The sulfate can be produced by reacting any manganese compound with sulfuric acid. The monohydrate form loses all water when heated to 400-450°C. It is a co-product of the manufacture of hydroquinone. Pure manganous sulfate is used as a reagent. The majority of manganous sulfate is used as fertilizer and as a nutritional supplement in animal feeds (U.S. EPA, 1984).

Manganous Soaps. Manganese (II) salts of fatty acids (2-ethyl hexoate, linoleate, naphthenate, oleate, resinate, stearate and tallate) are used as catalysts for the oxidation and polymerization of oils and as paint driers (U.S. EPA, 1984).

Other manganese (II) compounds include manganese borate ($MnB_4O_7 \cdot 8H_2O$), which is a brownish-white powder that is insoluble in water and alcohol, yet it decomposes on long exposure to water. It is used in drying varnishes and oils, as a drier for linseed oil and also in the leather industry. Manganous difluoride (MnF_2) is a pink, quadratic prism structure or a reddish powder. It has a density of 3.98 at 20°C and has varying solubilities in water (depending on water temperature). It is made from manganese carbonate and hydrogen fluoride. Manganous trifluoride (MnF_3) is a red mass of monoclinic crystals with a density of 3.54 at 20°C. It can be easily hydrolyzed by water. It is used primarily as a fluorinating agent in organic chemistry. Manganese

formate [$Mn(CHO_2)_2 \cdot 2H_2O$] is a rhombic crystal with a density of 1.953 at 20°C. Manganese glycerophosphate ($MnC_3H_7O_6P$) is a white or slightly red powder. Manganous hydroxide [$Mn(OH)_2$] is a whitish-pink trigonal crystal with a density of 3.258 at 13°C. It is also known as pyrochaoite. Manganous nitrate [$Mn(NO_3)_2 \cdot 4H_2O$] is a colorless or rose-colored monoclinic crystal with a density of 1.82 at 20°C. It is used as an intermediate in the manufacture of reagent grade MnO_2 and also in the preparation of porcelain colorants. Manganous sulfide (MnS) is a green cubic crystal or a pink amorphous structure. It has a density of 3.99 at 20°C. It is very highly soluble in cold water and soluble in dilute acid (Weast, 1980; Windholz, 1976).

Manganese (IV) Compounds

Manganese Dioxide. MnO_2 is also known as pyrolusite. It is the most important Mn(IV) compound and the most important commercial compound of manganese. Pyrolusite is the principal ore of manganese. More than 90% is used in the production of ferromanganese and other manganese metals and alloys. The other 10% is used to produce dry cell batteries, chemicals and as an oxidant in the production of some dyes. It is generally a black crystal or a brown-black powder with a density of 5.026 at 20°C. Pyrolusite is insoluble in water (U.S. EPA, 1984).

Manganese (VII) Compounds

Potassium Permanganate. $KMnO_4$ is a deep purple or bronze-like, odorless crystal structure. It is stable in acid and soluble in water with a density of 2.7 at 20°C. It is used in the organic chemical industry; in the alkaline pickling process; in bleaching resins, waxes, fats, oils, straw, cotton, silk and other fibers; in dyeing wood brown; with

formaldehyde solution to expel formaldehyde gas for disinfecting; and for water purification and odor abatement in various industrial wastes (U.S. EPA, 1984).

Summary

Manganese is a brittle, gray-pink metal principally used for alloying with other metals to impart hardness. It exists in 11 oxidation states with the compounds Mn^{2+}, Mn^{4+} and Mn^{7+} being the most economically and environmentally important. Most of the Mn^{2+} compounds, including manganous carbonate, manganous chloride and manganous acetate, are soluble in water. The most common Mn^{4+} compound, which is also the most important commercial compound of manganese, is manganese dioxide and it is insoluble in water. The Mn^{7+} compound, potassium permanganate, is soluble in water.

III. TOXICOKINETICS

The absorption, distribution, metabolism and excretion of manganese in the body has been reviewed by the U.S. EPA (1984). A symposium conducted in 1986 by the American Chemical Society resulted in a publication entitled Nutritional Bioavailability of Manganese (Kies, 1987). This volume contains a lot of additional information on the toxicokinetics of manganese.

Absorption

Gastrointestinal. Cikrt and Vostal (1969) showed that manganese is likely to be absorbed from the small as well as the large intestine. It is absorbed most efficiently in the divalent form (Gibbons et al., 1976). Different manganese salts are absorbed with varying efficiencies, manganese chloride being better absorbed than the sulfate or acetate (Bales et al., 1987).

Mena et al. (1969) reported findings on GI absorption of manganese in 11 healthy, fasted human subjects. The subjects were given 100 μCi of $^{54}MnCl_2$ with 200 μg stable $^{55}MnCl_2$ as a carrier. After 2 weeks of daily whole body counts, the absorption of ^{54}Mn was calculated to average ~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; therefore, these values could be an underestimate of absorption. Thomson et al. (1971) reported a much higher

absorption rate of $^{54}MnCl_2$ in segments of jejunum and duodenum using a double-lumen tube. In eight subjects, the mean absorption rate was 27±3%.

Schwartz et al. (1986) studied the absorption and retention of manganese over a 7-week period in seven healthy male volunteers, 22-32 years of age. Relatively high caloric diets (3100-4400 kcal/day) were consumed, providing high levels of manganese: 12.0-17.7 mg/day. The authors noted that these levels were high compared with the level of 2-5 mg/day reported as being safe and adequate by the Food and Nutrition Board of the National Research Council. During weeks 2-4, manganese absorption was -2.0±4.9% of the intake and during weeks 5-7 an absorption of 7.6±6.3% was measured. No explanation was offered for the difference in absorption between these two time points. Despite the high intakes, there was no net retention of manganese in these individuals; fecal loss accounted for almost all of the ingested manganese and in some cases was greater than the intake.

Sandström et al. (1986) administered 450 mL of infant formula containing 50 µg Mn/L to eight healthy subjects, aged 20-38 years. The absorption from seven of the subjects was 8.4±4.7% while one subject with an iron deficiency anemia absorbed 45.5% Six additional subjects were administered 2.5 mg of manganese (as sulfate) in a multi-element preparation with an absorption of 8.9±3.2%.

In humans administered a dose of radiolabeled manganese in an infant formula, the mean absorption was 5.9 ± 4.8%, but the range was 0.8-16%, a 20-fold difference

(Davidsson et al., 1989). Retention at day 10 was 2.9±1.8%, but the range was 0.6-9.2%, again indicating substantial differences between individuals.

Calcium has been suggested to inhibit the absorption of manganese. McDermott and Kies (1987) have postulated that this inhibition may be due to an effect by calcium on GI tract pH. Manganese is more readily absorbed in the +2 valence state and as the pH rises, oxidation to the +3 and +4 states is favored. Thus, calcium may inhibit manganese absorption by increasing the alkalinity of the GI tract. Alternatively, calcium and manganese may compete for common absorption sites. In contrast to these findings by McDermott and Kies (1987), Spencer et al. (1979) did not observe any effect of dietary calcium levels (from 200-800 mg/day) on manganese balance in healthy males.

Dietary phytate, a component of plant protein, was found to decrease the retention of manganese, possibly as a result of the formation of a complex between manganese and phytate, which is stable in the intestinal tract (Davies and Nightingale, 1975). Bales et al. (1987) reported that cellulose, pectin and phytate were all found to reduce the plasma uptake of manganese in human subjects. This may contribute to the decreased bioavailability of manganese from vegetarian diets. Schwartz et al. (1986) reported that while no significant correlation was found between phytate intake and manganese absorption in healthy males, phytate excretion was significantly correlated with manganese excretion.

Animal studies lead to similar estimates of absorption values. 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 μmoles 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 postweaning nonfasted rats and reported 0.05% absorption 6 days after administration. This low absorption value may be due to the result of the loss of absorbed manganese through fecal excretion or to the fact that the rats were not fasted (U.S. EPA, 1984).

Keen et al. (1986) point out that while others have suggested that a relatively constant percentage of manganese is absorbed from the intestine, this is only true up to a point. In suckling rats fed 0.5 mL of infant formulas containing 5 or 25 mg Mn/mL, the percentage of manganese retained was decreased at the higher level. Saturation of the absorptive process was also reported by Garcia-Aranda et al. (1983) who studied the intestinal uptake of manganese in adult rats.

Keen et al. (1986) demonstrated that there is 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 5 μg ^{54}Mn/mL. Manganese retention was highest ($\geq$80%) in pups $\leq$15 days old. In older pups (16-19 days old), the average retention was 40%. Infant formulas were also administered to rat pups. Soy formula

contains a much higher level of Mn than does human milk with the amount of manganese retained in 14-day-old rat pups being 25 times higher from soy formula compared with human milk. Chan et al. (1987) also demonstrated that the developmental stage of the rat has a big influence on the absorption of manganese. From age 9 days to 20 days there is a decline in the amount of manganese absorbed, which is correlated with a switch in the site of maximal absorption. The duodenum is more active in manganese uptake in younger rats while the jejunum becomes more important as the animals mature.

Chan et al. (1987) also reported a large variation in the concentration of manganese from different milk sources. Human milk contained only 8±3 μg Mn/L while bovine milk, infant formula and rat milk contained 30±5, 73±4 and 148±18 μg Mn/L, respectively. However, these absolute quantities may not reflect the actual amount of bioavailable manganese as indicated by the comparable absorption of manganese from these four types of milk in suckling rats. In an earlier study, Chan et al. (1982) determined that the chemical form of manganese in infant formula is very different from that in human or cow milk. Human and cow milk contain two and three manganese-binding proteins, respectively. All manganese in these milks is protein bound while the manganese in infant formulas is in the form of soluble salts. The degree to which the chemical form of manganese affects bioavailability is not known.

Lönnerdal et al. (1987) also reported that age, manganese intake and dietary factors all affect manganese absorption and retention. Retention is very high during the neonatal period and decreases considerably with age because of both decreased

absorption and increased excretion in the bile. In young rat pups, the bioavailability of managanese from various milk sources varied, with greater absorption occurring from human milk and cow's milk formula than from soy formula. These differences were not as pronounced in older pups.

Manganese appears to be absorbed in the +2 valence state and competes with iron and cobalt for the same absorption sites (Thomson et al., 1971). Animal studies have demonstrated an effect of iron deficiency on manganese uptake. Rehnberg et al. (1982) administered dietary Mn_3O_4 (450, 1150 or 4000 ppm Mn) to young rats. Using basal diets either sufficient or deficient in iron, it was shown that iron deficiency promotes the intestinal absorption of manganese. Conversely, manganese absorption is inhibited by large amounts of dietary iron. Gruden (1984) demonstrated that 3-week-old rat pups given a high concentration of iron in cow's milk (103 μg/mL) absorbed 50% less manganese than pups receiving the control milk (0.5 μg Fe/mL). This difference was not seen in rats tested at 8, 11, 14 or 17 days of age, suggesting that the inhibition of manganese absorption by iron develops quickly in rats in the third week of life.

Respiratory. Although there appear to be no quantitative data on absorption rates following inhalation of manganese, the Task Group on Metal Accumulation (TGMA, 1973) considered some basic principles that may be applied to inhaled metals. Small particles (<1 μm) reach the alveolar lining and are likely to be absorbed directly into the blood. Of the inhaled metal initially deposited in the lung, a portion is thought to be removed by mucociliary clearance and swallowed, consequently entering the GI

absorption process. The single study of respiratory absorption of manganese, performed by Mena et al. (1969), was reviewed in U.S. EPA (1984) and was noted to be lacking in complete experimental data.

Distribution

Studies of the distribution of manganese in humans are generally based upon post-mortem analyses of various organs and tissues. They reflect the body and organ burden of a lifetime intake of manganese. Both Cotzias (1958) and WHO (1981) reported a total of 12-20 mg manganese in a normal 70 kg man, while 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 undue exposure have been found in the liver, kidney and endocrine glands with lesser concentrations found in the brain, heart and lungs. Perry et al. (1973) found little variation in manganese concentration from one part of the liver to another. Regional studies of the distribution of manganese in the brain by Larsen et al. (1979) and Smeyers-Verbeke et al. (1976) have reported the highest concentration in the basal ganglia.

Animal study results have generally shown agreement with the pattern of tissue distribution revealed in human studies (U.S. EPA, 1984). In mice, Kato (1963) reported a high uptake of radioactive manganese by the liver, kidneys and endocrine glands and a lesser amount in brain and bone. This study and a study by Maynard and Cotzias (1955) found that tissues rich in mitochondria (for example, liver, kidney and pancreas) contained higher levels of manganese. Similarly in mice, Mouri (1973) reported that the

highest concentrations of manganese occurred in the kidney, liver, pancreas and brain both 8 and 15 days after inhalation of manganese. In rats, after an intraperitoneal dose of radioactive manganese, Dastur et al. (1969) found the highest concentrations in the suprarenal, pituitary, liver and kidney tissue. Scheuhammer and Cherian (1981) reported findings on the distribution of manganese in male rat brain tissue with and without intraperitoneal exposure to 3 mg Mn/kg as $MnCl_2$. 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; the highest concentrations were found in the corpus striatum and corpus callosum. In monkeys exposed intraperitoneally to manganese, Dastur et al. (1971) found the highest concentrations to be in liver, kidney and endocrine glands. In monkeys injected subcutaneously with manganese, increased concentrations were found in the tissues of the endocrine and exocrine glands (thyroids, parotids and gall bladder) and in the nuclei of cerebral basal ganglia (Suzuki et al., 1975).

The distribution of manganese in the body appears to differ depending on the route of administration. Autissier et al. (1982) reported that rats given a daily intraperitoneal dose of 10 mg/kg manganese chloride for 4 months showed significant increases in the accumulation of manganese in the brain. The study showed a 359% increase in the concentration of manganese in the brain stem, 243% in the corpus striatum, and 138% in the hypothalamus. In rats given drinking water containing 278 ppm $MnCl_2$ for 2 years, Chan et al. (1981) found a 31% increase in manganese concentrations in the brain and a 45% increase in liver relative to control values.

The form in which manganese is administered may also have an effect on its subsequent tissue distribution. Gianutsos et al. (1985) demonstrated in mice that blood and brain levels of manganese are increased following i.p. injection of manganese chloride ($MnCl_2$), manganese oxide (Mn_3O_4), or methylcyclopentadienyl manganese tricarbonyl (MMT). However, $MnCl_2$ administration resulted in faster and higher levels of blood and brain manganese. It was suggested that the differences seen among the three manganese compounds are due to the oxide and MMT forms being more hydrophobic. This may result in a depot being formed at the site of injection so that absorption is retarded. It was 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 and the high levels were maintained for at least 21 days. Brain manganese levels were especially sensitive to repeated treatment with a much greater accumulation resulting from the dose being divided into 10 injections given every other day as compared with a single injection. This may be related to the slow onset of manganese neurotoxicity; an acute exposure may result in other organs serving as the primary target while a chronic exposure results in gradually increasing brain levels with subsequent neurotoxicity.

The tissue distribution of manganese is also 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, i.p.) for 30 days. They reported that exposure to the metals individually resulted in accumulation in all brain regions, but

co-exposure to lead and manganese further increased levels of both metals, especially 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. It was concluded that the interaction of metals can alter tissue distribution and that adverse health effects may result from co-exposures to even low levels of metals.

Human studies by Schroeder et al. (1966) and Widdowson et al. (1972) confirm that placental transfer of manganese takes place. While most manganese levels in the fetus and newborn were reported to be similar to adult levels, fetal bone manganese concentration was reported to be higher than in the adult. In animal studies, neonatal mice, rats and kittens were found to very rapidly accumulate manganese without excreting it in the first 18 days of life (U.S. EPA, 1984). However, 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.

Kontur and Fechter (1985) demonstrated placental transfer of manganese in Long Evans rats exposed throughout gestation. However, the transfer was limited with only 0.4% of manganese accumulating in a single fetus. Neonatal rats of exposed dams did have significantly increased levels of manganese in the forebrain, but this was not associated with any toxicity.

In another study, rat pups showed a greater accumulation of manganese in the brain, but not in the liver, than did their mothers (Kostial et al., 1978). Rehnberg et al. (1980, 1981, 1982) reported similar results showing that the neonatal brain reaches higher concentrations of manganese than other tissues. This could be a response to a nutritional need. The relationship to toxicity is unclear.

Normal values in humans reported for the concentration of manganese in whole blood range from 7-12 μg/L (U.S. EPA, 1984). In most cases, manganese blood levels in exposed and unexposed workers have not differed significantly. This is supported by a study by Tsalev et al. (1977) that found workers exposed to ~1 mg Mn dust/m^3 air for 1-10 years had blood levels of manganese averaging 11-16 μg/L compared with a mean of 10 μg/L in unexposed workers.

Roels et al. (1987b) found that workers exposed to an average of ~1 mg/m^3 Mn dust (range = 0.07-8.61 mg/m^3) for 1-19 years had a blood manganese concentration of 0.1-3.59 μg/100 mL (arithmetic mean = 1.36) while a group of control workers had levels ranging from 0.04-1.31 μg/100 mL (mean = 0.57). Levels of manganese in the urine ranged from 0.06-140.6 (geometric mean = 1.56) μg/g creatinine in exposed workers while levels ranged from 0.01-5.04 (mean = 0.15) μg/g creatinine in controls. On a group basis, a correlation does exist between blood manganese and past exposure and also between urine manganese and airborne manganese levels. However, no relationship was found between blood and urine manganese concentrations and neither level

correlated on an individual basis with the current level of airborne manganese or the duration of manganese exposure.

Hagenfeldt et al. (1973) found variations in plasma manganese concentrations in women and suggested 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 night) variations in blood manganese concentrations have also been reported (U.S. EPA, 1984).

The concentration of manganese in blood and urine has not proven to be a reliable indicator of exposure (Roels et al., 1987b; U.S. EPA, 1984). In addition, only a single study by Horiuchi et al. (1970) showed a positive correlation between manganese blood and urine levels and the finding of neurologic symptoms and signs. Jindrichova (1969) recommended the determination of manganese in feces for evaluation of exposure. Since biliary excretion is the major route of elimination, the amount in the feces seems to be a reliable measure of exposure.

Alternatively, hair concentrations of manganese may be a more reliable indicator of environmental exposure. In such an analysis, caution must be exercised to account for differences that could be attributed to age, sex, race, hair color and hair treatment. (Sky-Peck, 1990). With proper control groups to be used for comparisons, hair concentrations of manganese may be reflective of increased exposures as demonstrated

in the epidemiologic study of manganese in drinking water by Kondakis et al. (1989). This study is decribed in Chapter 6.

Metabolism

Manganese is an essential element for many species, including mammals. Although the daily requirement of manganese for development and growth has not been adequately studied, it was accepted that diets containing 50 mg/kg manganese are adequate for most laboratory animals (NAS, 1978; Rogers, 1979). Assuming a food consumption equivalent to 5% of body weight, this corresponds to a requirement for about 2.5 mg Mn/kg bw/day. Manganese requirements for humans have not been fully determined. However, the Food and Nutrition Board of the National Research Council (NRC, 1989) estimated an "adequate and safe" intake of manganese to be 2-5 mg/day for adults, or about 0.03-0.07 mg Mn/kg bw/day, assuming a reference body weight of 70 kg. The dietary requirement for manganese in rats then, may be about 2 orders of magnitude higher than the estimated safe and adequate intake for humans.

Manganese is a constituent of the enzymes pyruvate carboxylase and superoxide dismutase, and is required for the activation of many enzymes. Most of the glycosyl transferases, which synthesize polysaccharides and glycoproteins, require manganese for normal activity (Leach, 1971, 1976). Experimental evidence suggests that an impairment in glycosaminoglycan metabolism is associated with symptoms of manganese deficiency (Leach and Lilburn, 1978). Manganese has been shown to stimulate the

synthesis of chondroitin sulfate, contained in cartilage and connective tissue (Piscator, 1979).

Manganese is removed from the blood very efficiently by the liver after binding to an α_2-macroglobulin in the portal blood. Some manganese becomes bound to transferrin. The metabolism of manganese is controlled by homeostatic mechanisms at the levels of excretion and absorption. These mechanisms respond very efficiently to increases in manganese concentration (U.S. EPA, 1984).

Normal manganese metabolism varies with the potential for interaction with other metals in the body and the age of the individual. Iron deficiency has been shown to enhance the absorption of manganese in both humans and animals (U.S. EPA, 1984). Studies have found that manganese competes with iron and cobalt in the process of uptake from the lumen into the mucosal cells and in the transfer across the mucosa into the body (U.S. EPA, 1984).

Excretion

Manganese is excreted almost exclusively in the feces of humans and animals. Both the WHO (1981) and Newberne (1973) have reported that human excretion of manganese in urine, sweat and milk is minimal.

Price et al. (1970) reported that for preadolescent girls consuming 2.13-2.43 mg Mn/day, 1.66-2.23 mg/day was excreted in the feces and 0.01-0.02 mg/day was excreted in the urine.

Although the kidney is not an important route of excretion for inorganic species, some manganese is found in the urine. 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).

Tanaka and Lieben (1969) found a rough correlation in humans between mean urine levels and the average concentration of manganese in workroom air; however, the correlation was poor in individual cases. Similarly, both Horiuchi et al. (1967) and Chandra et al. (1981b) reported an association between mean urinary manganese levels and increased levels of manganese in air.

In early animal studies of manganese excretion, Greenberg and Campbell (1940) reported that 90.7% of a 1 mg intraperitoneal dose of manganese (^{54}Mn) was found in rat feces within 3 days. In a subsequent study using rats, Greenberg et al. (1943) found that 27.1% of a 0.01 mg intraperitoneal dose of manganese (^{54}Mn) and 37.3% of a 0.1 mg dose were collected in bile within 48 hours. Later studies have confirmed that bile is the main route of excretion of manganese and represents the principal regulatory mechanism. Tichy et al. (1973) administered a dose of 0.6 μg manganese chloride to rats and reported that 27% was excreted into bile within 24 hours. Klaassen (1974) administered increasing

doses (0.3, 1.0, 3.0, 10.0 mg/kg bw) of manganese to rats, rabbits and dogs. The concentration of manganese in bile was 100-200 times higher than in plasma at the three lower doses. As the dose increased, the excretion of manganese into the bile was found to increase. However, after the 10.0 mg dose there was no further increase in excretion of manganese into the bile and a maximum excretion rate of 8.5 μg/min/kg was attained, indicating that a saturable active transport mechanism may exist (U.S. EPA, 1984). Klaassen (1974) also reported urinary excretion to be low.

Experiments in animals by Bertinchamps and Cotzias (1958), Kato (1963) and Papavasiliou et al. (1966) have shown that manganese is also excreted through the intestinal wall. This has been found to be particularly true in the presence of biliary obstruction or with overloading of manganese. Bertinchamps et al. (1966) and Cikrt (1973) have also reported that in rats the excretion of manganese through the intestinal wall into the duodenum, jejunum and terminal ileum may take place. In dogs, Burnett et al. (1952) have shown manganese to be excreted with the pancreatic juice.

In human studies of the biologic half-time of manganese in the body, Mahoney and Small (1968) reported a biphasic clearance of intravenously injected $MnCl_2$, the rapid phase being 4 days and the slow phase lasting 39 days. Sandström et al. (1986) reported biologic half-life values of 13±8 days and 34±8 days in 14 healthy subjects given manganese orally. Two subjects were also administered manganese intravenously and had a much slower turnover. 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 a biologic half-time of 37.5 days in healthy subjects, 15 days in healthy miners and 28 days in those with chronic manganese poisoning. The study also found that, in healthy subjects, clearance from the liver averaged 25 days; from the head 54 days; and from the thigh 57 days. In healthy miners, liver clearance averaged 13 days; head, 37 days; and thigh, 39 days. Those 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.

The clearance of manganese in primates was studied by Newland et al. (1987). Following a 30-minute inhalation of trace amounts of $^{54}MnCl_2$ aerosol by two female macaque monkeys, radioactivity was monitored for over a year in the chest, head and feces. Levels of radioactivity in the chest remained elevated throughout the experiment. Three half-times, ranging from 0.2-187 days, were needed to describe the clearance of manganese from the chest. Fecal excretion of manganese was described by two half-times of <1 day and 50-60 days. Head levels peaked 40 days after exposure and remained elevated for over a year. Clearance of manganese from the head was described by a single half-time of ~245 days. This slow clearance was attributed both to the slow disappearance of manganese from the head and to replenishment from other tissues, particularly the lung. A third monkey was administered a subcutaneous dose of $^{54}MnCl_2$ and clearance from the head was 4.5 times faster. This study demonstrates

that lung deposits can prolong elevated brain levels and this may account for the occurrence and progression of manganism after inhalation exposure has ended.

In animal studies, both 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 that in the body (Suzuki, 1974; Dastur et al., 1969, 1971).

Homeostasis

As pointed out by Rehnberg et al. (1980), the normal human adult effectively maintains tissue manganese at stable levels despite large variations in manganese intake. Although some investigators maintain that this homeostatic mechanism is based on controlled excretion, a critical review of the evidence reveals that regulation of manganese levels also occurs at the level of absorption (U.S. EPA, 1984).

Summary

Manganese is absorbed from the GI tract after being ingested. Human and animal studies estimate that ~3-9% of the ingested manganese is absorbed with values being higher for suckling animals (Mena et al., 1969; Greenberg et al., 1943; Sandstrom et al., 1986; Keen et al., 1986). A portion of inhaled manganese may be swallowed and subsequently absorbed from the GI tract. There are no definitive data, however, on absorption rates following the inhalation of manganese.

A total of 12-20 mg has been reported to be the normal body burden of manganese in a 70 kg man (WHO, 1981), with the highest concentrations occurring in the liver, kidney and endocrine glands of both humans and animals (WHO, 1981; Kato, 1963). In animals, the distribution of excess manganese in the body appears to differ depending on the route of administration. Intraperitoneally administrated manganese has been shown to increase the accumulation of manganese in the rat brain more than that orally administered (Autissier et al., 1982; Rehnberg et al., 1982; Chan et al., 1981).

Studies have confirmed that the placental transfer of manganese takes place, and have concluded also that the neonatal brain may be at a higher risk of accumulating excess manganese than are other tissues (Schroeder et al., 1966; Kostial et al., 1978).

Normal human values for manganese in whole blood range from 7-12 μg/L, and in most cases do not differ for exposed and nonexposed individuals (U.S. EPA, 1984). Thus, the level of manganese in blood is not a good indicator of manganese exposure. Concentrations in hair are considered to be more reliable (Sky-Peck, 1990).

Manganese is an essential element that is a constituent and activator of many enzymes. There are no well-defined occurrences of manganese deficiency in humans, but deficiency has been demonstrated in laboratory mice, rats, rabbits and guinea pigs (U.S. EPA, 1984). The main manifestations of manganese deficiency are those associated with skeletal abnormalities, impaired growth, ataxia of the newborn, and defects in lipid and carbohydrate metabolism.

Under normal circumstances of exposure, manganese is efficiently controlled in the body by homeostatic mechanisms. Excess manganese exposure may be most toxic to the brain, where CNS effects are related to alterations in levels of brain monoamines. The appropriateness of using rodents to model the CNS effects observed in humans has been questioned. Pigmented brain tissue, which more readily accumulates manganese, is more characteristic of primates than of rodents (U.S. EPA, 1984).

Bile is the main route of manganese excretion and represents the principal regulatory mechanism. Minimal excretion has been reported to occur in urine, sweat and milk (Klaassen, 1974). Manganese is also excreted through the intestinal wall, especially in the presence of biliary obstruction or overloading of manganese.

Increased manganese intake has been shown to decrease biologic half-times. A biologic half-time of 37.5 days has been reported for healthy subjects and 28 days for subjects with chronic manganese poisoning (Cotzias et al., 1968). Brain biologic half-times appear to be longer than in the rest of the body (Suzuki, 1974).

V. HEALTH EFFECTS IN ANIMALS

General Toxicity

Acute Toxicity. Information on the LD_{50} and LD_{10} values will be presented for oral, parenteral and s.c. exposures, while LC_{50} and LC_{10} values will be presented for inhalation exposure for various manganese compounds. The toxicity of manganese varies with the chemical form, with the insoluble oxide being less toxic than the soluble forms. This information was obtained primarily from a review of U.S. EPA (1984) and NIOSH (1984).

Oral -- Oral LD_{50} values observed in animal experiments are presented in Table V-1 and range from 10 mg Mn/kg for exposure to MMT (methylcyclopentadienyl manganese tricarbonyl) in rats to 2197 mg Mn/kg bw for exposure to manganese dioxide in rats. Manganese toxicity may vary not only with route of exposure and chemical compound, but also with the age, sex and species of animal. For example, studies by Hinderer (1979) indicate that female rats and mice are more sensitive to MMT by the oral route of exposure than male rats and mice. In addition, rats were reported to be more sensitive to MMT oral exposure than mice (Hinderer, 1979). Kostial et al. (1978) found that $MnCl_2$ produced the greatest oral toxicity in the oldest and youngest groups. Rothand and Adleman (1975) suggest that for the older rats, increased susceptibility to manganese toxicity may be due to a decrease in adaptive responsiveness, which is characteristic of the aging process. Increased sensitivity among the younger rats may be the result of higher intestinal absorption and body retention of manganese.

TABLE V-1

Oral LD_{50} Values for Manganese Compounds

Compound	Species	LD_{50} (mg Mn/kg bw)	Reference
Methylcyclopentadienyl manganese tricarbonyl (MMT)	rat	10	Hanzlik et al., 1980
	rat	12	Hinderer, 1979
	rat	12	Hysell et al., 1974
	mouse	48	Hinderer, 1979
Cyclopentadienyl manganese tricarbonyl	rat	22	Penney et al., 1985
Manganese chloride	rat	425	Sigan and Vitvickaja, 1971
	rat	475	Kostial et al, 1978
	rat	410	Holbrook et al., 1975
	mouse	450	Sigan and Vitvickaja, 1971
	guinea pig	400	Sigan and Vitvickaja, 1971
Manganese acetate	rat	836	Smyth et al., 1969
Manganese dioxide	rat	2197	Holbrook et al., 1975
Potassium permanganate	mouse	750	Sigan and Vitvickaja, 1971
	rat	379	Smyth et al., 1969
	rat	750	Sigan and Vitvickaja, 1971
	guinea pig	810	Sigan and Vitvickaja, 1971

Parenteral -- Generally, parenteral routes produce mortality at lower doses than do oral exposures. Parenteral LD_{50} values are presented in Table V-2 and range from 14-64 mg Mn/kg bw. In comparative intraperitoneal toxicity studies, Franz (1962) and Bienvenu et al. (1963) have shown that manganese is less toxic than many other metals.

Baxter et al. (1965) measured a number of physiologic parameters in rats (100-550 g) 1-72 hours after s.c. administration of 5-150 mg of manganese as $MnCl_2$, diluted in normal saline. Levels of hemoglobin, hematocrit and mean corpuscular volume were significantly increased in rats receiving 15 mg Mn/100 g bw. The peak increase in these parameters occurred at 12 and 18 hours after dosing. The maximum response occurred at 170-300 mg Mn/kg. A measurable response occurred at 50 mg Mn/kg. Necrotic changes were noted in hepatic tissue 18 hours after a single dose of 170 mg Mn/kg.

Subchronic and Chronic Toxicity. Epidemiologic studies of chronic manganese intoxication in exposed workers indicate that the CNS is the major target, and that the pulmonary system may also be affected. To a lesser extent hematologic, cardiovascular and digestive system effects may also occur. This chapter will cover the effects of chronic exposure to manganese on systemic toxicity and carcinogenic, mutagenic, reproductive and teratogenic effects in animals.

In humans, the overt CNS effects of manganese exposure result from an extrapyramidal neurologic dysfunction. Some of these signs resemble those associated

TABLE V-2

Parenteral LD_{50} Values for Manganese Compounds

Compound	Species	Route of Administration	LD_{50} (mg/kg bw)	Reference
Cyclopentadienyl manganese tricarbonyl	rat	i.p.	14	Penney et al., 1985
Manganese chloride	rat	i.p.	38	Franz, 1962; Holbrook et al., 1975
	mouse	i.p.	53	Franz, 1962; 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

with Parkinsonism and include muscular rigidity and lack of coordination. Other reported signs more closely resemble some forms of dystonia. Barbeau (1984) summarized the similarities and differences between manganism and Parkinsonism and suggested that manganism, rather than a model of Parkinsonism, is a mixture of extrapyramidal bradykinesia and dystonia.

Studies conducted to model this disease in small laboratory animals are open to some question since one must rely upon analogous, not homologous behaviors. Also, as discussed in Chapter III, the accumulation and neurotoxicity of manganese may differ for rodents as compared with primates. Among other differences, primate brain tissue contains more pigmented areas that favor manganese accumulation than rodent brain tissue. Moreover, the overt neurologic impairment in primates is often preceded or accompanied by psychologic symptoms, such as irritability and emotional lability, that are not evident in rodents.

There may also be significant species differences in the requirements for manganese as an essential element. The NRC (1989) has determined a safe and adequate intake of 2-5 mg Mn/day for adults. Assuming a body weight of 70 kg, this range is equivalent to about 0.03-0.07 mg Mn/kg/day for humans. Rodents require greater intakes of manganese: 50 mg/kg diet for rats and 45 mg/kg diet for mice (National Research Council, cited in NTP, 1992). Assuming a food consumption of 5% of body weight per day for rats and 13% for mice (U.S. EPA, 1986b), these dietary concentrations are equivalent to 2.5 mg Mn/kg bw/day for rats and 5.85 mg Mn/kg bw/day for mice, about 100 times higher than the requirement for humans.

Studies using monkeys show results consistent with the hypothesis that chronic manganese exposure results primarily in disturbances of the CNS. The U.S. EPA (1984) reported that there are insufficient data to determine an accurate dose-response relationship for the neurologic effects of chronic inhalation exposure to manganese.

Oral Exposure. In a 14-day study, NTP (1992) administered diets containing 0, 3130, 6250, 12,500, 25,000 or 50,000 ppm manganese sulfate monohydrate (~33% manganese) to groups (5/sex/dose) of B6C3F1 mice and F344 rats. All rats survived the exposure period. High-dose males had a final mean body weight that was 13% lower, and a mean body weight gain that was 57% lower than controls. High-dose females had a final mean body weight that was 7% lower, and a mean body weight gain that was 20% lower than controls. These groups also exhibited diarrhea during the second week of the study. No other effects attributed to manganese exposure were reported in any group of mice.

Neurotoxic Effects -- Studies of rodents exposed to manganese by drinking water or food have not been able to produce the characteristic signs of extrapyramidal neurologic disease seen in humans. For example, Gray and Laskey (1980) found that dietary exposure to 1100 ppm manganese (as Mn_3O_4) in rats for 2 months produced only reduced reactive locomotor activity (RLA).

Accurate dose-response relationships based upon neurobehavioral endpoints, which are characteristic of chronic manganese exposure in humans, are not available from animal studies. Neurochemical responses, however, may offer useful ancillary

information. Such studies have been based largely upon the supposition 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 effects reported, for example on the level of dopamine as affected by manganese exposure, are not consistent from one study to another. While manganese exposure is generally considered to result in decreased dopamine levels, some studies report increases, while others report effects that change over time.

Singh et al. (1979) administered manganese (16 mg/kg bw in a 10% sucrose solution) alone or in combination with ethanol to groups of 20 male albino rats for 30 days. The manganese exposure alone led to a 72% increase in manganese concentration in the brain (3.13 μg/g dry weight vs. 1.82 μg/g for controls). This was not affected by ethanol exposure. There were no morphologic changes in the brain tissue of any group; however, significant alterations were reported for several brain enzymes. Manganese exposure resulted in significant increases in monoamine oxidase ($p<0.001$), adenosine triphosphatase ($p<0.001$), ribonuclease ($p<0.001$), glutamate-oxaloacetate transaminase ($p<0.01$). Significant decreases were reported for succinic dehydrogenase ($p<0.02$) and deoxyribonuclease ($p<0.001$). Several other enzymes were not affected. Concurrent exposure to ethanol resulted in a synergistic effect with some enzymes and an antagonistic effect with others. The authors were not able to suggest a definitive role for ethanol ingestion with regard to simultaneous manganese exposure.

Deskin et al. (1980) studied neurochemical alteration induced by manganese chloride in neonatal CD rats. Rats were intubated daily with 1, 10 or 20 μg Mn/g from birth to 24 days old. Neurochemical components were then analyzed in the hypothalamic area and corpus striatum. Manganese administration (10 and 20 μg/g) resulted in a significant elevation of manganese in both regions of the brain, but neurochemical alterations were observed only in the hypothalamic area. These alterations included a decrease in dopamine concentration and turnover. The highest dose also resulted in a significant decrease in hypothalamic tyrosine hydroxylase activity and an increase in monoamine oxidase activity. There were no visible signs of toxicity in any group. A subsequent study by Deskin et al. (1981) using the same protocol (but doses of 10, 15 or 20 μg/g) reported a significant elevation in serotonin levels in the hypothalamus, but not the striatum, following exposure to 20 μg/g.

Kontur and Fechter (1988) intubated neonatal Long-Evans rats daily with 0, 25 or 50 μg/g manganese chloride ($MnC1_2 \cdot 4H_20$) for 14 or 21 days. The level of manganese in the brain was increased at both 14 and 21 days, but was greater at 14 days. However, monoamine and metabolite levels were not altered by manganese treatment in any region at either age. The authors suggest that the different results reported by different laboratories may be because of species or strain differences, the dosing regimen or vehicle, the route of administration, or the time points chosen for testing.

Whether neurochemical indices, such as changes in the level of dopamine, can serve as a direct toxic assay may be debated. Silbergeld (1982) suggests that the earliest detectable expressions of neurotoxicity for many substances, including

manganese, are likely to be behavioral and that altered behavior represents a functionally significant outcome. If the mechanism is assumed to be biochemical or morphologic aberrations, then behavioral indices may be used as a measure of adverse effects.

Chandra et al. (1979a) found elevated levels of striatal dopamine, norepinephrine and homovanillic acid with a concomitant increase in spontaneous locomotor activity at 60 and 90 days of age in mice exposed to manganese from birth. While suckling, the mice were exposed by their lactating dams, which were exposed to $MnCl_2$ (5 mg/mL) in their drinking water. The mice were weaned at 25 days and subsequently received drinking water exposures to manganese that were determined, on average, to be 30 μg Mn/day for 60 days, 36 μg Mn/day through the 90th day, 75 μg Mn/day through the 120th day and 90 μg Mn/day for $\leq$150 and 180 days. Exposure past 90 days did not influence motor activity. Chandra et al. (1979a) suggest that the hyperactivity observed in mice may be an early behavioral effect of excess manganese exposure and resultant dopamine and norepinephrine elevations, comparable with the early psychotic phase in humans exposed to manganese. Although in this experiment the levels of brain biogenic amines were comparable with controls after 90 days of exposure, other investigators have noted that continued exposure to manganese produces a marked decrease in brain biogenic amines, especially dopamine (Bonilla and Diez-Ewald, 1974). In a later experiment using rats, Chandra and Shukla (1981) did find initial increases in dopamine, norepinephrine and homovanillic acid followed by a period of normal levels, and after 300 days, a decrease in all levels. In addition, accompanying behavioral studies found an initial increase in spontaneous locomotor activity followed by a decrease during later periods of manganese exposure (Ali et al., 1981).

Chandra and Shukla (1981) suggested that 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 dyskinesias in humans.

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 were given a daily dose of 150 mg Mn/kg bw (as $MnCl_2$) by gavage for $\leq$44 days of age. At 15-22 days of age there was a large but transient increase (7-40 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 only 3 times the control level. Histologic analysis revealed no abnormalities in the brains of rats exposed to manganese. Axonal growth and the axon-myelin relation were also found to be normal. Another group of rats was treated for only 15 days at which time half were sacrificed and half were maintained untreated until 60 days of age. These rats were analyzed for brain content of dopamine and its metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), and serotonin (5-HT) and its major metabolite, 5-hydroxyindolacetic acid (5-HIAA). Of these, only HVA levels in the hypothalamus and striatum were affected by manganese treatment. However, the significantly decreased HVA levels were seen only at the 15-day sacrifice. The rats that were treated 15 days and then maintained without manganese treatment until 60 days of age were not different from controls. The investigators concluded that divalent manganese has a very low degree of toxicity for the developing nervous system in rats but that a longer-term exposure to more active manganese compounds may cause severe damage to certain

neurologic pathways. They also emphasize that rodents may not be appropriate for comparison with primates, as their unpublished studies with monkeys exposed to manganese oxide reveal severe motor disturbances.

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 ($MnC1_2 \cdot 4H_20$) for 60, 100, 165 or 265 days. There were no clinical signs of poisoning. Following 60 days of exposure, manganese concentration in the striatum was estimated to be 1.3-2.0 μg/g compared with control levels of 0.4-0.5 μg/g. 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 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.

Lai et al. (1981a) exposed female Wistar rats to 1 mg $MnCl_2 \cdot 4H_2O$ per mL drinking water. Exposure was initiated at mating. Pups were exposed *in utero*, then by maternal milk and, after weaning, by drinking water. The rats were exposed to manganese either for 2 or for 24-28 months. The brains were dissected and then analyzed. Levels of glutamic acid decarboxylase (GAD), choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) from treated rats were compared with the concentrations of these enzymes in controls. GAD, ChAT and AChE are the

neurochemical markers for the GABA and cholinergic systems, which have also been implicated in manganese toxicity (Sitaramayya et al., 1974; Bonilla, 1978a,b). The effects of chronic manganese exposure on the activities of GAD, ChAT and AChE were not apparent in 2-month-old rats. Life-long exposure (over 2 years) to manganese produced effects that tended to counteract age-related decreases in GAD, ChAT and AChE. Leung et al. (1981) examined the same groups of rats and focused on monoamine oxidase (MAO) activity. MAO is a key enzyme in brain amine metabolism. Leung et al. (1981) reported that the only effect of manganese exposure on 2-month-old rats was a small decrease in the neurotransmitter amine, 5-hydroxytryptamine (5-HT) (serotonin) in the cerebellum. No significant changes in the levels of dopamine appeared in young rats. In rats 24-28 months old, no significant differences were found in manganese-exposed rats as compared with controls.

In a related study, Lai et al. (1982a) examined male Wistar rats exposed to the same drinking water regimen (1 mg $MnCl_2 \cdot 4H_2O$/mL) for either 70-90 days or 100-120 days after birth. In addition, the rats had been exposed *in utero*. 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, but not in the 100- to 120-day-old rats. This finding agrees with Chandra and Shukla (1981). The study also found that choline levels were significantly higher in 70- to 90-day-old exposed rats and significantly lower in 100- to 120-day-old exposed rats when compared with controls. The authors suggest that this finding may be related to involvement of both the dopaminergic and cholinergic systems in manganese toxicity. In rats exposed to the same regimen for $\leq$60 days (plus

in utero exposure), no effects were found in acetylcholinesterase activity in the brain (Lai et al., 1982b). They conclude that, although the rat may not serve as an ideal model for the neurotoxic effects of manganese, neurochemical effects are discernible when analyses are made at the appropriate period (Lai et al., 1982a). The significance for human exposure remains unclear.

Changes in the concentrations of dopamine and GABA were studied using mice exposed to $MnCl_2$ in the diet. Gianutsos and Murrary (1982) fed a 1% concentration of $MnCl_2$ in the diet to an unspecified number of male CD-1 mice for 1 month and then raised the concentration to 4% for 5 additional months. Dopamine content in the striatum and in the olfactory tubule at 6 months was reduced ($p<0.05$) compared with controls. GABA content of the striatum was increased ($p<0.05$) but neither the observed increase in the substantia nigra area nor the decrease in the cerebellum reached statistical significance. No changes in neurotransmitter levels were observed after only 1-2 months of 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 mg Mn/mL) in the drinking water for 90 days. The low protein diet resulted in decreased levels of brain dopamine (DA), norepinephrine (NE) and 5-hydroxytryptamine (5-HT). Manganese exposure resulted in a marked increase in DA and NE levels, which were more pronounced in the low protein group. There was a significant decrease in 5-HT levels because of manganese treatment, but only in the low protein group. Weaned F_1

pups of treated rats exhibited the same effects. It was concluded that protein undernutrition can increase vulnerability of rats to the neurotoxic effects of manganese.

Behavioral effects of chronic manganese exposure were studied by Nachtman et al. (1986). Male Sprague-Dawley rats were administered 0 or 1 mg $MnCl_2 \cdot 4H_2O$/mL in drinking water for 65 weeks. The treatment did not result in any change in body weight. The manganese-exposed rats did exhibit a significant increase in locomotor activity in weeks 5-7 but at 8 weeks 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 groups at 41 or 65 weeks. The investigators concluded that manganese exposure may result in a transient increase in dopaminergic function, evidenced by increased spontaneous and d-amphetamine-stimulated locomotor activity.

In a behavioral study by Morganti et al. (1985), male Swiss mice (ICR strain) were fed a powdered form of diet (Charles River's RMH 300) that contained 1 mg powdered MnO_2/g of diet. The authors stated that these mice consumed 5 g of food daily. Sampling began after 16 weeks of feeding and continued at 2-week intervals for $\leq$30 weeks. Evaluated were open field and exploratory behavior, passive avoidance learning and rotarod performance. Multivariate analysis of variance (2 treatments and 8 samples by weeks of exposure) was used to test for intergroup differences. No significant behavioral differences involving treatment appeared. This is in contrast to the inhalation exposures 7 hours/day, 5 days/week for 16-32 weeks at levels >50 mg Mn/m^3 (estimated to be comparable with the oral dose) for which significant effects related to

duration of exposure were found as well as significant uptake of manganese (Morganti et al., 1985).

The only report of neurobehavioral toxicity in primates from orally administered manganese is by Gupta et al. (1980). They administered 25 mg $MnCl_2 \cdot 4H_2O$/kg (6.9 mg Mn/kg) orally to four male rhesus monkeys daily for 18 months. Animals were maintained on monkey pellets, two bananas/day and tap water. The monkeys developed muscular weakness and rigidity of the lower limbs. There were no biochemical data. Histologic analysis compared with controls showed degenerated neurons in the substantia nigra and scanty neuromelanin granules in some of the pigmented cells. This study is of limited use because only one dose level was studied.

Studies of the neurotoxic effects of excess manganese exposure are summarized in Tables V-3 and V-4. Few studies have examined both behavioral and neurochemical effects of oral exposure.

Digestive System Effects -- Mitochondria-rich organs, such as the liver and pancreas, are hypothesized to be most affected by excess manganese exposure. Wasserman and Wasserman (1977) reported ultrastructural changes of the liver cells in rats exposed to 200 ppm $MnCl_2$ in their drinking water for 10 weeks. Increased metabolic activity was inferred from an increased amount of rough endoplasmic reticulum, the occurrence of multiple rough endoplasmic cisternae and prominent Golgi apparatuses, and large Golgi vesicles filled with osmiophilic particles in the biliary area

TABLE V-3

Neurotoxic Effects of Manganese in Experimental Animals: Oral and Inhalation Studies*

Species	Compound	Route	Dose	Duration	CNS Abnormality			Reference
					Behavioral	Histological	Biochemical	
Mice	$MnCl_2$	drinking water	3 ug $MnCl_2$/mL	6	+	NS	+	Chandra et al., 1979a
Mice	$MnCl_2$	diet	1% $MnCl_2$ (1 month), 4% MnCl2 (5 months)	6	NS	NS	+	Gianutsos and Murray, 1982
Mice	MnO_2	diet	1 mg MnO_2/g	7.5	-	NS	NS	Morganti et al., 1985
Rat	$MnCl_2$	drinking water	5 mg $MnCl_2$/mL	7	NS	NS	+	Bonilla and Diez-Ewald, 1974
Rat	$MnCl_2$	gavage	150 mg Mn/kg bw	42 days	+	NS	+	Kristensson et al., 1986
Rat	$MnCl_2 \cdot 4H_2O$	drinking water	1 mg $MnCl_2 \cdot 4H_2O$/mL	12	+	NS	+	Chandra and Shukla, 1981
Rat	$MnCl_2 \cdot 4H_2O$	drinking water	1 mg $MnCl_2 \cdot 4H_2O$/mL	28	NS	NS	+	Lai et al., 1981a
Rat	$MnCl_2 \cdot 4H_2O$	drinking water	1 mg $MnCl_2 \cdot 4H_2O$/mL	28	NS	NS	+	Leung et al., 1981
Rat	$MnCl_2 \cdot 4H_2O$	drinking water	1 mg $MnCl_2 \cdot 4H_2O$/mL	4	NS	NS	+	Lai et al., 1982a
Rat	$MnCl_2 \cdot 4H_2O$	drinking water	1 mg $MnCl_2 \cdot 4H_2O$/mL	65 weeks	+	NS	NS	Nachtman et al., 1986
Monkey	$MnCl_2 \cdot 4H_2O$	diet	25 mg $MnCl_2 \cdot 4H_2O$/kg	18	+	+	NS	Gupta et al., 1980
Monkey	Mn_3O_4	inhalation	72 ug Mn/m^3 3602 ug Mn/m^3	12 6	- -	NS NS	NS NS	Coulston and Griffin, 1977
Monkey	Mn_3O_4	inhalation	11.6 ug Mn/m^3 112.5 ug Mn/m^3 1152 ug Mn/m^3	9	-	NS	NS	Ulrich et al., 1979a,b,c
Monkey	MnO_2	inhalation	30 mg Mn/m^3	24	-	NS	NS	Bird et al., 1984

*Source: U.S. EPA, 1984

NS = Not studied

TABLE V-4

Neurotoxic Effects in Manganese in Experimental Animals: Parenteral Studies[a]

Species	Compound	Route	Dose (mg Mn/kg)		Duration (months)	CNS Abnormality			Reference
			Single	Total		Behavioral	Histological	Biochemical	
Rat	$MnCl_2 \cdot 4H_2O$	i.p.	2.2	535	8	+	NS	NS	Roussel and Renaud, 1977
Rat	$MnCl_2 \cdot 4H_2O$	i.p.	2.2	401	6	-	+	NS	Chandra and Srivastava, 1970
Rat	$MnCl_2 \cdot 4H_2O$	i.p.	2.2	268	4	NS	NS	+	Sitaramayya et al., 1974
Rat	$MnCl_2 \cdot 4H_2O$	i.p.	4.2	189	1.5	NS	NS	+	Shukla and Chandra, 1976
Rat	$MnCl_2 \cdot 4H_2O$	i.p.	4.0	120	1	-	+	+	Chandra et al., 1979b
Rat	$MnCl_2 \cdot 4H_2O$	i.p.	4.2	63	1	-	+	+	Shukla and Chandra, 1976
Rabbit	MnO_2	i.t.	169.0	169	24	+	+	+	Chandra, 1972
Monkey	MnO_2	i.m.	158,276[b]	434	12	+	NS	NS	Pentschew et al., 1963
					14	+	+	NS	
Monkey	MnO_2	s.c.	36.1[c]	72.2	3	+	-	+	Neff et al., 1969
Monkey	MnO_2	s.c.	39.5[d]	355	2	+	-	NS	Suzuki et al., 1975
			79.0	711	2	+	-	NS	
			158.0	1422	2	+	-	NS	

[a]Source: U.S. EPA, 1984

[b]Assumed body weight of rhesus monkey is 8.0 kg (U.S. EPA, 1980)

[c]Assumed body weight of squirrel monkey is 1.0 kg

[d]Body weight of monkey reported by authors to be 4.0 kg

NS = Not studied

of the liver cell. The authors suggested that the increased metabolic activity may be due to biochemical processes related to the essentiality of manganese, in addition to the maintenance of homeostasis of manganese during increased exposure. The authors also suggested that other liver effects observed, such as 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 be direct toxic phenomena or the consequence of the biologic effect exerted by manganese on other tissues.

Kimura et al. (1978) fed rats diets supplemented with 564 ppm of manganese as $MnCl_2$ for 3 weeks and found no significant difference in liver serotonin levels between control and manganese-treated rats. In addition, MAO activity in the liver and L-amino-acid decarboxylase activity in the liver remained unaltered. Structural changes of the liver cells were not examined.

Shukla et al. (1978) administered 16 mg $MnCl_2 \cdot 4H_2O$/kg bw in drinking water (dose calculated by investigators) to rats for 30 days and reported significantly decreased liver activity of succinic dehydrogenase and alcohol dehydrogenase compared with controls. Significantly increased activities of MAO, adenosine triphosphatase, arginase, glutamate-pyruvate transaminase, ribonuclease and glucose-6-phosphatase were also reported in the liver of rats exposed to manganese compared with controls. The level of a-amylase was significantly increased while the level of β-amylase was significantly decreased in the serum of exposed 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. Changes in several enzyme activities (e.g., arylhydrocarbon hydroxylase, ethoxycoumarin 0-deethylase and epoxide hydrase) were observed at 1 week but not at 6 weeks. The activities were increased in the liver and decreased in the intestines and kidney. Studies of the effects of excess manganese exposure on the liver are summarized in Table V-5.

Hematologic Effects -- Decreased hemoglobin content has been reported in the blood of 6-month-old anemic rabbits orally exposed to 2000 ppm Mn as $MnSO_4 \cdot H_2O$ for 6 weeks, anemic newborn pigs orally exposed to 125 ppm Mn as $MnSO_4 \cdot H_2O$ for 27 days (Matrone et al., 1959), and young, iron-deficient rats exposed for 32 weeks to 400-3550 ppm Mn as Mn_3O_4 (Carter et al., 1980). However, the hemoglobin depression in baby pigs fed as much as 2000 ppm manganese was overcome by a dietary supplement of 400 ppm iron (Matrone et al., 1959). Hartman et al. (1955) found that as little as 45 ppm manganese provided in a milk diet to young lambs resulted in a decrease in the concentrations of hemoglobin and serum iron. In a second experiment, anemic lambs fed 1000 ppm manganese in the diet had depressed serum iron and hemoglobin formation. It was determined that manganese interferes with iron absorption rather than affecting hematopoiesis.

In a 13-week study, NTP (1992) administered diets containing 0, 1600, 3130, 6250, 12,500 or 25,000 ppm manganese sulfate monohydrate to groups (10/sex/dose) of F344 rats. Mean daily intake of manganese sulfate monohydrate ranged from 98 mg/kg/day (32 mg Mn/kg/day) for the low-dose to 1669 mg/kg/day (542 mg Mn/kg/day) for the high-dose males. For females, the range was 114 mg/kg/day (37

TABLE V-5

Liver Effects of Manganese Exposure in Animals[a]

Species, Strain, Sex	Route and Dose	Duration	Converted Dose[b] (mg Mn/kg bw/injection)	Effects	Reference
Rat, male	drinking water 200 ppm $MnCl_2$	10 weeks	12.2	Increased rough endoplasmic reticulum, proliferated smooth endoplasmic reticulum, prominent Golgi apparatuses, multiple rough endoplasmic cisternae	Wasserman and Wasserman 1977
Rat, Wistar, male	drinking water 0.5% Mn as $MnCl_2$	6 weeks	700	Increased enzyme activities seen at 1 week but not at 6 weeks	Hietanen et al., 1981
Rat, ITRC, male	gavage 10 mg/kg $MnCl_2 \cdot 4H_2O$ in 1 ml saline	15 days	2.8	Slight histologic alterations of liver	Chandra and Tandon, 1973
Rat, ITRC, male	drinking water 16 mg $MnCl_2 \cdot 4H_2O$/kg bw	30 days	4.4	Decreased liver activity of succinic dehydrogenase and alcohol dehydrogenase. Increased liver activity of MAO and several other enzymes	Shukla et al., 1978
Rat, Wistar, male	diet 564 ppm Mn as $MnCl_2$	3 weeks	28.2[c]	No effects seen in liver	Kimura et al., 1978
Rat, Wistar, female	drinking water 1, 10 or 20 mg $MnCl_2 \cdot 4H_2O$ per ml	from conception to 60 days old	38.9 389 778	Liver necrosis, ultrastructural alterations similar to human cholestasis	Leung et al., 1982
Rat, Sprague-Dawley, male	i.v. 10 mg Mn/kg bw	2 hours	10	No liver enzyme effects, significant Mn accumulation in liver.	Klaassen, 1974
Rat, ITRC, male	i.p. 6 mg Mn/kg/day as $MnSO_4 \cdot 4H_2O$	25 days	6	Decreased succinic dehydrogenase and lactate dehydrogenase. Mild congestion of central veins and adjacent sinusoids, some focal necrosis	Singh et al., 1974, 1975
Rat, ITRC, male	i.p. 6 mg/kg Mn as $MnCl_2 \cdot 4H_2O$	28 days	6	Succinic dehydrogenase and cytochrome oxidase decreased after 28 days	Khandelwal et al., 1984
Monkey, rhesus	i.m. (in olive oil) 2000 mg MnO_2 (2 months later)	sacrificed 14.5 months after 1st injection	158 (1st injection)	Mild changes/hemosiderosis of Kupffer cells	Pentschew et al., 1963

TABLE V-5 (cont.)

Species, Strain, Sex	Route and Dose	Duration	Converted Dose[b] (mg Mn/kg bw/injection)	Effects	Reference
Monkey, squirrel	s.c. 200 mg MnO_2 in 1 mL olive oil	2 or 3 injections within 5 months	126.4/injection[d]	Variable, mild cacuolar changes in liver cells	Neff et al., 1969
Monkey (Macaca mullata)	s.c. 0.25, 0.5 or 1.0 g MnO, in saline	injections once a week for 9 weeks	39.5[e] 79 158	Irregular arrangement of hepatic cords and lymphocytic infiltration	Suzuki et al., 1975

[a]Source: U.S. EPA, 1984

[b]The following default values have been used for dose conversions (U.S. EPA, 1980)

	Body Weight	Water (L/day)	Food (fraction of body weight)
Rat	0.35 kg	0.049	0.05
Mouse	0.03 kg	0.0057	0.13
Monkey	8.0 kg		

[c]Food consumption of 10% body weight (bw = 100 g) is used

[d]Weight of squirrel monkey assumed to be 1.0 kg

[e]Weight of monkey reported by authors to be 4.0 kg

NS = Not specified

mg Mn/kg/day) for the low-dose group and 1911 mg/kg/day (621 mg Mn/kg/day) for the high-dose group. No rats died during the study, and no clinical or histopathologic findings were attributed to manganese exposure. Decreased body weight gain was reported in males receiving ≥3130 ppm and females receiving ≥6250 ppm manganese sulfate. Absolute and relative liver weights were decreased in males receiving ≥1600 ppm and females in the highest dose group only. Hematologic effects were also reported: all groups of exposed males exhibited a significantly increased neutrophil count; lymphocyte counts were decreased in males receiving ≥6250 ppm and females in the three highest dose groups. Based on effects on liver weight and neutrophil counts in the male rats, the lowest dose of 1600 ppm (about 32 mg Mn/kg/day) is the LOAEL for this study.

In a concurrent 13-week study, NTP (1992) administered diets containing 0, 3130, 6250, 12,500, 25,000 or 50,000 ppm manganese sulfate monohydrate to groups (10/sex/dose) of B6C3F1 mice. Mean daily intake of manganese sulfate monohydrate ranged from 328 mg/kg/day (107 mg Mn/kg/day) for the low-dose to 8450 mg/kg/day (2746 mg Mn/kg/day) for the high-dose males. For females, the range was 390 mg/kg/day (127 mg Mn/kg/day) for the low-dose group and 7760 mg/kg/day (2522 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 statistically significantly decreased body weight gain. Relative and absolute liver weights were decreased in males in the highest dose group. Both sexes receiving 50,000 ppm 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; this finding was of questionable relevance to manganese exposure. No clinical findings were reported to be attributed to manganese exposure. The LOAEL for this study, based on significantly decreased body weight gain in male mice, was 3130 ppm (about 107 mg Mn/kg/day).

In discussing trace metals and hemoglobin metabolism, Garnica (1981) noted that although exposure to divalent forms of manganese may cause a decrease in hemoglobin levels, other chemical forms may not. This hypothesis does not explain the findings of all of the above studies, since exposure to trivalent Mn_3O_4 decreased hemoglobin levels in rats in the Carter et al. (1980) study and exposure to divalent $MnCl_2$ produced increased hemoglobin levels in rats in the Baxter et al. (1965) study. Conflicting results of hematopoietic studies may more likely result from differences in the age and iron status of the animal in addition to the route and duration of exposure. Carter et al. (1980) found that as the rat matures, hematologic and biologic values return to normal because of a reduction in iron excretion and lowering of the rate of erythropoiesis with maturation. Matrone et al. (1959) found that depressed hemoglobin regeneration was overcome by the addition of iron to the diet.

Cardiovascular System Effects -- Kimura et al. (1978) reported that rats exposed to 564 ppm manganese in the diet showed significantly increased blood serotonin levels, which resulted in decreased blood pressure.

Parenteral Exposure.

Neurotoxic Effects -- Intraperitoneal injection is not the most appropriate route of administration for studies of >30 days, especially those whose purpose is to investigate the neurotoxicity of chronically administered manganese. According to Scheuhammer (1983), intraperitoneally administered manganese appears to have a selectively toxic effect on the pancreas. This effect may then render any subsequent changes found in the brain, especially subtle biochemical changes, difficult to interpret since they may be secondary to cellular damage in the pancreas. The shortcomings of the use of rodents and intraperitoneal administration render several studies of chronic exposure to manganese and their reported CNS effects somewhat ambiguous. Histopathologic evaluations of exposed rats by Chandra and Srivastava (1970), Chandra et al. (1979b) and Shukla and Chandra (1976) found scattered neuronal degeneration in the cerebral and cerebellar cortex. Daily intraperitoneal administration of 2-4 mg Mn/kg for $\leq$120 days appeared to be the threshold for the appearance of microscopic lesions. Their studies also demonstrated that a maximum number of degenerated neurons is present when manganese concentration in the brain is at a maximum.

Two animal studies reported some of the characteristic histopathologic and neurologic consequences of manganism found in exposed workers. Mustafa and Chandra (1971) and Chandra (1972) reported paralysis of the hind limbs in rabbits intratracheally inoculated with 169 mg Mn/kg bw (as MnO_2). The paralysis developed after a period of 18-24 months. In addition, the brains showed widespread neuronal loss and neuronal degeneration in the cerebral cortex, caudate nucleus, putamen, substantia

nigra and cerebellar cortex, and a marked decrease in brain catecholamine levels and related enzyme activity.

Primates are considered to be better models of the neurologic manifestations of manganese intoxication than rodent species. Despite many deficiencies in experiments (U.S. EPA, 1984), the studies have consistently reported extrapyramidal signs and histologic lesions similar to those described in humans. 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 totally dose-related. In an early study by Mella (1924), four rhesus monkeys were treated with $MnCl_2$ for 18 months while two monkeys served as controls. The treated monkeys received intraperitoneal injections every other day with gradually increasing doses of $MnCl_2$ starting at 5 mg and reaching a maximum of 25 mg per injection. The monkeys developed choreic movements followed by rigidity, disturbances of motility, fine hand tremors, and finally, contracture of the hands. Histologic changes were reported in the putamen, the caudate, and the globus pallidus. Degenerative processes were also found in the liver. Other studies of the neurotoxic effects of excess manganese exposure are listed in Tables V-3 and V-4.

Digestive System Effects -- Scheuhammer and Cherian (1983) reported adverse effects in the pancreas resulting from intraperitoneally injected manganese. Toxic effects included a pancreatitis-like reaction, which the authors suggest is potentiated by the

presence of manganese in the peritoneal cavity and thus would not occur as readily with oral routes of exposure.

Pancreatic endocrine function is also affected by intraperitoneally injected manganese. In conjunction with increased hepatic glycogenolysis and gluconeogenesis, acute manganese exposure can affect carbohydrate metabolism in rats (Baly et al., 1985). Manganese injection (40 mg/kg bw) resulted in a decrease in plasma insulin levels, an increase in plasma glucose levels, and a transitory increase in glucagon concentration.

The liver removes manganese by biliary excretion. Klaassen (1974) reported that >99% of an i.v. dose of manganese was excreted by rats in the feces. Large doses of manganese, however, may result in cholestasis of the liver, similar to that seen in humans exposed to manganese (Witzleben, 1969). One researcher (Klaassen, 1974) has suggested that both manganese and bilirubin are necessary for cholestasis to occur. Table V-5 presents some of the liver effects of exposure to manganese observed in animals.

Hematologic Effects -- Animals injected with manganese have shown a variety of hematologic and biochemical responses. Chandra et al. (1973b) reported decreased serum alkaline phosphatase and inorganic phosphate and increased calcium levels in rats exposed intratracheally to 400 mg of MnO_2. The duration of exposure was not reported.

Jonderko (1965) found increased serum calcium and decreased inorganic phosphorous in rabbits exposed intramuscularly to 3.5 mg Mn/kg. These results agree with those of Chandra et al. (1973b). Details on the compound and the duration of exposure were not available.

Inhalation Exposure.

Neurotoxic Effects -- Studies of chronic inhalation exposure to manganous manganese oxide (Mn_3O_4) (the major residue produced by combustion of MMT) report no behavioral or histologic CNS abnormalities. Coulston and Griffin (1977) exposed eight rhesus monkeys to 72 μg Mn/m^3 (as Mn_3O_4) for 12 months or to 3602 μg Mn/m^3 (as Mn_3O_4) for 23 weeks and observed no overt neurotoxic effects. Ulrich et al. (1979a,b,c) observed no overt neurotoxic effects in rats and monkeys exposed to 11.6, 112.5 or 1152 μg Mn/m^3 (as Mn_3O_4) for 9 months. The Coulston and Griffin (1977) and Ulrich et al. (1979a,b,c) studies lack details of the clinical examinations, lack biochemical data and lack brain manganese data.

Neurologic and brain manganese measurements were made on rhesus monkeys after inhalation exposure to MnO_2. Bird et al. (1984) examined concentrations of dopamine in the caudate, putamen, globus pallidus and substantia nigra of the brains of four female rhesus monkeys exposed to 30 mg Mn/m^3 for 2 years. Exposures were for 6 hours/day, 5 days/week to dust <5 μ diameter. No behavioral or abnormal neurologic signs were noted, but dopamine concentrations in the caudate and globus pallidus of treated animals were statistically significantly ($p<0.01$) decreased.

Manganese concentrations were 60-80% greater in the basal ganglia of the brain in the treated animals.

Respiratory Effects -- The toxic effects of excess airborne manganese on the lung include a primary inflammatory reaction, and at high exposure levels, a high incidence of pneumonia. The severity of the effects increases when pathogens are present, possibly because the manganese increases susceptibility to infection (Bergström, 1977; Suzuki et al., 1978; Adkins et al., 1980a,b,c). The effects of manganese on the lung are reported to be the exclusive result of inhalation or intratracheal exposure. The evidence from animal studies indicates a lack of gross toxic effects at low levels of exposure; reversible respiratory symptoms have occurred in humans exposed to airborne particulates that contained manganese (Nogawa et al., 1973).

Hematopoietic Effects -- In rabbits exposed to MnO_2 by inhalation, Doi (1959) found increased levels of hemoglobin, erythrocytes, leukocytes and lymphocytes. Information on the duration and dose were not available.

Other Effects

Carcinogenicity. In a 2-year bioassay, groups of F344 rats (70/sex) were administered 0, 1500, 5000 or 15,000 ppm manganese sulfate monohydrate (NTP, 1992). These dietary concentrations were reported to be equivalent to an intake ranging from 91 mg/kg/day (30 mg Mn/kg/day) for low-dose males to 1019 mg/kg/day (331 mg Mn/kg/day) for high-dose males. For females, the range of intakes was from 81

mg/kg/day (26 mg Mn/kg/day) for the low-dose group to 833 mg/kg/day (270 mg Mn/kg/day) for the high-dose group. Interim sacrifices of 10 rats/group were made at 9 and 15 months. Survival of high-dose males was significantly decreased, starting at week 93 of the study, because of advanced renal disease associated with manganese exposure. Survival of females was not affected. Feed consumption was similar for all groups, but 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 and females, concurrent with a decrease in hepatic iron concentrations. The only pathologic finding was that of renal disease in high-dose males. No increases in any tumor type reported were attributed to manganese exposure in rats.

In a 2-year bioassay, groups of B6C3F1 mice (70/sex) were administered 0, 1500, 5000 or 15,000 ppm manganese sulfate monohydrate (NTP, 1992). These dietary concentrations were reported to be equivalent to an intake ranging from 194 mg/kg/day (63 mg Mn/kg/day) for low-dose males to 2222 mg/kg/day (722 mg Mn/kg/day) for high-dose males. For females, the range of intake was from 238 mg/kg/day (77 mg Mn/kg/day) for the low-dose group to 2785 mg/kg/day (905 mg Mn/kg/day) for the high-dose group. 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; however, 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 feed 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. This was associated with decreased hepatic iron.

Incidences of thyroid follicular cell hyperplasia were significantly greater in high-dose males and females than in controls. The incidence of follicular cell adenomas was 0/50, 0/49, 0/51 and 3/50 (6%) for control, low-, mid- and high-dose males, respectively. The historical control range for males was reported to be 0-4%. For females, the incidence of follicular cell adenomas was 2/50, 1/50, 0/49 and 5/51 (10%) for control, low-, mid- and high-dose groups, respectively. The historical control range for females was reported to be 0-9%. None of the reported incidences were statistically significantly increased over controls, nor were they dose-related in either sex. Also, the follicular cell tumors were seen only at the termination of the study (729 days) and only slightly increased relative to the historical control range in the highest dose groups. NTP (1992) reported that the manganese intakes in the high-dose mice was 107 times higher than the recommended dietary allowance. The relevance of these findings to human carcinogenesis is questionable, particularly because of the very large intakes of manganese required to elicit a response seen only at the end of the study, and at frequencies not statistically significantly different from historical controls. NTP also considers the marginal increase in thyroid adenomas of the mice to be equivocal evidence of carcinogenicity.

Few other data are available on the carcinogenicity of manganese by the oral route. Table V-6 presents those studies by other routes of exposure that have reported a positive finding and provides the dose at which possible carcinogenic activity was observed. DiPaolo (1964) subcutaneously or intraperitoneally injected DBA/1 mice with 0.1 mL of a 1% $MnCl_2$ aqueous solution twice weekly for 6 months. A larger percentage of the mice exposed subcutaneously (67%) and intraperitoneally (41%) to manganese developed lymphosarcomas compared with controls injected with water (24%). In addition, tumors appeared earlier in the exposed groups than in the control groups. The number of tumors other than lymphosarcomas (e.g., mammary adenocarcinomas, leukemias, injection site tumors), however, did not differ significantly between the exposed and control groups. A thorough evaluation of the results of this study was not possible because the results were published in abstract form and lacked sufficient detail.

Stoner et al. (1976) exposed Strain A/Strong mice of both sexes, 6-8 weeks old, intraperitoneally to 6, 15 or 30 mg $MnSO_4$/kg bw 3 times a week for a total of 22 injections. The total administered doses were 132, 330 and 660 mg $MnSO_4$/kg bw. The frequency of lung tumors in exposed mice was compared with that in controls. Table V-7 presents the results of the study, which showed that a slight but statistically significant increase in the number of pulmonary adenomas per mouse was associated with administration of the highest dose (660 mg $MnSO_4$/kg). Although the response was somewhat elevated at the other doses, it was not statistically significant. The study results are suggestive of carcinogenic activity but do not conclusively meet specific criteria for the interpretation of lung tumor data in this mouse strain as a positive response (Shimken and Stoner, 1975).

TABLE V-6

Summary of Carcinogenicity Studies Reporting Positive Findings for Selected Manganese Compounds[a]

Compound	Species	Route	Dose	Duration (weeks intermittent)	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 adfenomas	Stoner et al., 1976
			0 mg/kg		31-37% - Lung adenomas	
Manganese acetylacetonate (MAA)	rat	i.m.	1200 mg/kg[b]	26	40% (males) - Fibrosarcomas	Furst, 1978
					24% (females) - Fibrosarcomas	
			0 mg/kg		4% (males and females - Fibrosarcomas	

[a]Source: U.S. EPA, 1984

[b]As reported in NIOSH, 1984

TABLE V-7

Pulmonary Tumors in Strain A Mice Treated with Manganese Sulfate[a]

Group	Total Dose mg $MnSO_4$/kg	mg Mn/kg	Mortality	Mice with Lung Tumors (%)	Average Number Tumors/Mouse[b]	Value[c]
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

Furst (1978) exposed F344 rats intramuscularly or by gavage to manganese powder, MnO_2 and manganese (II) acetylacetonate (MAA). Swiss mice were exposed intramuscularly to manganese powder and MnO_2. Table V-8 presents the results of the study, which showed a statistically significant number of fibrosarcomas at the injection site in male (40%) and female (24%) rats exposed intramuscularly to MAA compared with controls (4% male, 4% female). No difference in tumor incidence was found between rats and mice exposed to manganese powder and MnO_2 and controls. The U.S. EPA (1984) noted that the study results regarding MAA, an organic manganese compound, cannot necessarily be extrapolated to pure manganese or inorganic manganese compounds.

Sunderman et al. (1974, 1976) exposed Fischer rats to 0.5-4.4 mg manganese dust intramuscularly and found that no tumors were induced at the injection site. Subsequent studies by Sunderman et al. (1980) suggest that manganese dust may even inhibit local tumor induction.

Witschi et al. (1981) exposed female A/J mice intraperitoneally to 80 mg/kg MMT and found that although cell proliferation was produced in the lungs, lung tumor incidence did not increase.

Mutagenicity. The available information supports a positive mutagenic role for manganese. The bone marrow cells of rats given manganese orally (as $MnCl_2$) at 50 mg/kg showed an unusual incidence of chromosome aberrations (30.9%) compared with those of control animals (8.5%) (Mandzgaladze, 1966; Mandzgaladze and

TABLE V-8

Carcinogenicity of Manganese Powder, Manganese Dioxide and Manganese Acetylacetonate in F344 Rats and Swiss Albino Mice[a]

Compound[b]	Species	Route	Treatment Schedule[c]	Total Dose	Tumor Type	Incidence	
						Males	Females
Triglyceride control	rat	i.m.	0.2 mL/month x 12 months	2.4 mL	Lymphomas/leukemia	1/25	3/25
					Fibrosarcomas[d]	1/25	1/25
Manganese powder	rat	i.m.	10 mg/month x 9 months	90 mg	Lymphomas/leukemia	3/25	5/25
					Fibrosarcomas	3/25	0/25
Manganese acetylacetonate	rat	i.m.	50 mg/month x 6 months	300 mg	Lymphomas/leukemia	2/25	2/25
					Fibrosarcomas	10/25[e]	6/25
Triglyceride control	rat	i.m.	0.2 mL/month x 12 months	2.4 mL	Lymphomas/leukemia	0/25	4/25
					Fibrosarcomas	0/25	0/25
Manganese dioxide	rat	i.m.	10 mg/month x 9 months	90 mg	Lymphomas/leukemia	0/25	3/25
					Fibrosarcomas	0/25	0/25
Triglyceride control	rat	oral	0.5 mL, twice monthly x 12 months	12.5 mL	Lymphomas/leukemia	3/25	3/25
					Fibrosarcomas	0/25	0/25
Manganese powder	rat	oral	10 mg, twice monthly x 12 months	240 mg	Lymphomas/leukemia	0/25	0/25
					Fibrosarcomas	0/25	0/25
Triglyceride control	mouse	i.m.	0.2 mL/injection x 3 injections	0.6 mL	Leukemia	NT	2/25
					Lymphomas	NT	1/25
Manganese powder	mouse	i.m.	10 mg (single injection)	10 mg	Leukemia	NT	6/25
					Lymphomas	NT	1/25
Triglyceride control	mouse	i.m.	0.2 mL/injection x 12 injections[h]	24 mL	Leukemia	NT	2/25
					Lymphomas	NT	0/25
Manganese dioxide	mouse	i.m.	3 mg/injection x 6 injections[h]	15 mg	Leukemia	NT	4/25
					Lymphomas	NT	1/25
Manganese dioxide	mouse	i.m.	5 mg/injection x 6 injections[h]	30 mg	Leukemia	NT	1/25
					Lymphomas	NT	2/25

[a]Source: Furst, 1978
[b]Compounds suspended in 0.2 mL (i.m.) or 0.05 mL (gavage) tricaprylin
[c]Duration of experiments was not stated, but was implied to be 2 years in the rat studies. The average weights of the treated and control mice ranged from 22-25 g at the start of the experiments to 33-39 g at the end of the experiments.
[d]Injection site fibrosarcoma
[e]Fischer Exact Test p = 0.002
[f]Fischer Exact Test p = 0.049
[g]Incidence includes rhabdomyosarcomas and 1 myxosarcoma
[h]Intervals between injections not stated
NT = Not tested

Vasakidze, 1966). Manganese dichloride has been reported to be mutagenic for *Escherichia coli* (Demerec et al., 1951; Durham and Wyss, 1957) and *Serretia marcescens* (Kaplan, 1962). Manganese oxide (Mn_3O_4) was not mutagenic in *Salmonella typhimurium* or *Saccharomyces cerevisiae* (Simmon and Ligon, 1977). Manganese sulfate monohydrate was not mutagenic in *S. typhimurium* strains TA97, TA98, TA100, TA1535 or TA1537, either with or without exogenous metabolic (S9) activation (NTP, 1992).

The manganese ion (Mn^{2+}) has been shown to bind 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). True DNA-strand breaks have also been induced by manganese in Chinese hamster ovary cells and human diploid fibroblasts (Hamilton-Koch et al., 1986; Snyder, 1988). Tests for induction of sister chromatid exchanges and chromosomal aberrations 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, 1992).

Manganese sulfate monohydrate did not induce sex-linked recessive lethal mutations in germ cells of male *Drosophila melanogaster* (NTP, 1992). A study by Joardar and Sharma (1990) demonstrated that both $MnSO_4$ and $KMnO_4$ were clastogenic in mice following oral administration for 3 weeks, with $MnSO_4$ being more potent. The frequencies of chromosomal aberrations in bone marrow cells and micronuclei were

significantly increased by both salts. There was also an enhancement of sperm-head abnormalities which demonstrated a statistically significant dose-response trend.

Reproductive Effects. Gray and Laskey (1980) exposed male mice to 1100 ppm Mn as Mn_3O_4 in a casein diet from gestation day 15 to 90 days of age. 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) found a dose-related decrease in serum testosterone concentration (without a concomitant increase in serum lutinizing hormone concentration) and reduced fertility at the highest dose in rats exposed to 0, 400, 1100 or 3550 ppm Mn (as Mn_3O_4) orally in the diet from day 2 of mother's gestation to 224 days of age. Testes weight as well as litter size, number of ovulations, resorption and preimplantation deaths and fetal weights were not affected.

Laskey et al. (1985) conducted studies to assess the effect of manganese on hypothalmic, pituitary and testicular functions. 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 actual dose of manganese was calculated to be 0, 71 or 214 mg Mn/kg bw/day. Effects attributed to manganese included slight decreases in body and testes weights and a reduction in serum testosterone. There was no indication of hypothalmic or pituitary malfunction, and it was suggested that the decrease in testosterone was due to manganese-induced damage of testicular Leydig cells.

A series of studies by Chandra and colleagues have consistently reported degenerative changes in the seminiferous tubules in the testes after parenteral exposure to manganese (Chandra, 1971; Shukla and Chandra, 1977; Imam and Chandra, 1975; Chandra et al., 1973a, 1975). The U.S. EPA (1984) notes that results from parenteral studies are of limited value in predicting the reproductive hazards of ingested manganese. Table V-9 summarizes studies of the reported reproductive effects of exposure to manganese.

Teratogenicity. In animals, manganese deficiency during pregnancy causes a variety of developmental defects related to impaired mucopolysaccharide formation. Resultant defects include impaired coordination, which was due to defective bone otolith calcification and growth deficiencies, reproductive difficulties and CNS changes (Oberleas and Caldwell, 1981; Hurley, 1981). The effect of manganese excess has been studied by only a few investigators.

The embryotoxic and teratogenic potential of manganese during organogenesis was investigated by Sanchez et al. (1993; abstract only). Pregnant Swiss mice were administered daily subcutaneous injections of 0, 2, 4, 8 or 16 mg/kg of $MnCl_2 4H_2O$ on days 6-15 of gestation, and dams were sacrificed on gestational day 18. Significant reductions in weight gain and food consumption were reported in dams receiving ≥8 mg/kg, and treatment-related deaths were reported at 16 mg/kg. A significant increase in the number of late resorptions was observed at doses ≥4 mg/kg, and reduced fetal body weight and an increased incidence of morphological defects were reported at ≥8

TABLE V-9

Reproductive Effects of Exposure to Manganese

Compound	Species	Route	Dose	Effect	Reference
Mn_3O_4	mice	oral	1100 ppm Mn	Decreased weight of testes, seminal vesicles and preputial glands after 90 days.	Gray and Laskey, 1980
Mn_3O_4	rat	oral	400 ppm Mn 1100 3550	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 214	Decreased body and testes weights. Reduction in serum testosterone.	Laskey et al., 1985
$MnCl_2$	rat	i.p.	8 mg/kg daily	Degenerative changes in ~50% of seminiferous tubules after 150 and 180 days.	Chandra, 1971
$MnCl_2 \cdot 4H_2O$	rat	i.p.	15 mg/kg daily	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
$MnCl_2 \cdot 4H_2O$	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., 1973a

mg/kg. No difference was seen in the incidence of individual or total malformations in treated groups compared with controls.

Excess manganese during pregnancy has been shown to affect behavioral parameters in rodents. Lown et al. (1984) studied behavioral effects in mice of *in utero* and lactational exposure to airborne MnO_2 dust. Preconception exposure was to 49.1$\pm$2.3 mg Mn/m^3 for 12 weeks (7 hours/day, 5 days/week) and to 85.3$\pm$15.6 mg Mn/m^3 for 4 additional weeks. All females were exposed preconceptually and randomly assigned to MnO_2 or control until day 17 of gestation. Pups were fostered equally among exposed and nonexposed mothers. Treatment effects on growth and behavior of offspring were evaluated by multivariate analysis of variance. Prenatal exposure resulted in significantly reduced weight at day 45 and higher mean number of pups. Measures of neonatal gross locomotor activity, maternal retrieval latency and day 45 offspring behavior showed effects on postnatal development. Prenatal exposure resulted in significantly reduced activity scores. Exposures both *in utero* and by suckling depressed adult rearing frequency, exploratory behavior and general activity.

There are other supporting reports of effects of manganese on learning in the adult rat (Murthy et al., 1981), and by a study of the distribution of ^{54}Mn in fetal, young and adult rats (Kaur et al., 1980). Kaur et al. (1980) found that younger neonates and 19-day fetuses were more susceptible to manganese toxicity than the older groups. Manganese was localized to the liver and brain in the younger groups and there was more manganese per unit of weight in the younger animals compared with the older groups (Kaur et al., 1980). No fetal abnormalities were seen when 18-day embryos were

exposed to 16 μmol/200 g maternal weight, but this is a late stage for detecting morphologic defects.

Kontur and Fechter (1985) exposed pregnant Long-Evans rats to 0, 5, 10 or 20 mg/mL of $MnCl_2$ in drinking water throughout the gestational period. Rats in the 10 and 20 mg/mL groups had a reduced water intake and a significant decrease in weight gain. There was also a significant decrease in birth weight in the 20 mg/mL group. At 1 day of age, pups from the 5 and 10 mg/mL group were found to have significantly increased manganese levels in the forebrain, which was no greater in the 10 mg/mL group than in the 5 mg/mL group. The increased manganese levels were not associated with any changes in catecholamine function, nor was there any effect on startle responses in the exposed pups. It was concluded that prenatal exposure to manganese is not toxic to developing rats, probably because of limited placental transfer.

Järvinen and Ahlström (1975) exposed female rats to 4, 24, 54, 154, 504 or 1004 mg Mn/kg (as $MnSO_4 \cdot 7H_2O$) in the diet for 8 weeks after weaning and during pregnancy. No maternal reproductive or fetal teratogenic effects were found. At the higher manganese levels (> 154 mg Mn/kg bw) an increase in whole body content of manganese in fetuses and in the livers of dams was reported. However, no increase in liver manganese was found in nonpregnant females.

Laskey et al. (1982) found a dose-related decrease in serum testosterone concentration (without a concomitant increase in serum LH concentration) in the male offspring of treated dams. Female rats exposed to 0, 400, 1100 or 3550 ppm Mn (as

Mn_3O_4, 50 ppm as $MnSO_4$) orally in the diet from day 2 of gestation to 224 days of age exhibited reduced fertility at the highest dose. Testes weight of the offspring as well as litter size, number of ovulations, resorptions and preimplantation deaths and fetal weights were not affected.

Summary

LD_{50} values for soluble manganese compounds range from an average of 102 mg Mn/kg for parenteral exposure to 683 mg Mn/kg for oral exposure with the highest toxicity occurring in oldest and youngest rats (Kostial et al., 1978). The CNS is the primary system affected by chronic manganese exposure in humans. No accurate dose-response relationship for neurologic effects by inhalation exists at this time. Oral animal exposures are difficult to interpret because laboratory rodents ingesting manganese in food and water do not exhibit the neurobehavioral deficits (muscular rigidity, tremor and paralysis) found in humans, and the one study of ingestion in primates used only one dose. Alterations in neurochemical parameters have been used instead as indicators of CNS effects in animals. Primates appear to be a better model of adverse CNS effects arising from excess manganese exposure.

The intraperitoneal administration of manganese to animals has also been questioned in studies designed to detect the chronic neurotoxic effects of manganese exposure. Scheuhammer (1983) reported that intraperitoneally administered manganese exerts a selectively toxic effect on the pancreas that may render subsequent neurochemical changes difficult to interpret.

Toxic effects of chronic manganese exposure are also seen in the pulmonary, hematopoietic, cardiovascular, reproductive and digestive systems. Pulmonary system effects are limited to inhalation exposure and are reported to be insignificant at low levels (U.S. EPA, 1984). Hematologic and biochemical effects vary depending on age and iron status. Young and irondeficient animals are more likely to exhibit hematologic and biochemical effects (Carter et al., 1980). A single study of cardiovascular effects in animals reported a significant increase in blood serotonin levels and a decrease in blood pressure (Kimura et al., 1978). Although animal studies of the GI effects of manganese exposure are not conclusive, studies of liver function and structure are generally adequate. Large doses of manganese may produce cholestasis in animals, similar to that seen in humans (U.S. EPA, 1984).

The organs with greatest sensitivity to manganese include the brain, lung, liver and endocrine glands. Parenteral, as opposed to oral, exposure to manganese may result in more selective and toxic organ effects, especially those observed in the pancreas (Scheuhammer, 1983).

The U.S. EPA (1984) reports that the data from available studies of the carcinogenic effects of manganese are inadequate for animals and lacking for humans. Thus, the weight-of-evidence for manganese carcinogenicity would currently be rated as Group D (not classified) using the guidelines for carcinogen risk assessment of the U.S. EPA (1986a). This category denotes that more information is needed to reach a definitive conclusion. Testing is underway by the National Toxicology Program to address the carcinogenicity of orally administered manganese sulfate in rats and mice.

Reproductive studies present histologic and biochemical evidence of toxicity to reproductive organs (Chandra et al., 1973a, 1975; Gray and Laskey, 1980; Laskey et al., 1982). The U.S. EPA (1984) has questioned the value of using parenteral studies in predicting the reproductive hazards of ingested manganese.

The teratogenic effects of excess manganese exposure during pregnancy may include altered behavioral parameters in offspring, but the evidence at this time is insufficient to define manganese as teratogenic (Lown et al., 1984).

VI. HEALTH EFFECTS IN HUMANS

Introduction

Most of the information on the toxicity of manganese in humans is derived from the inhalation of large amounts of manganese oxides by occupationally exposed groups. Although the pulmonary effects of manganese inhalation are not relevant to the ingestion of manganese, other systemic effects are. Table VI-1 summarizes some of the studies of manganese health effects in humans and exposure-response relationships. The psychologic and neurologic effects of manganese exposure upon the CNS, collectively referred to as manganism, have been the primary focus of these studies. The syndrome is described in the next section, Clinical Case Studies. For years manganism has been considered a model of Parkinson's disease, but Barbeau (1984) suggested that it is better characterized as a mixture of bradykinesia and dystonia.

The U.S. EPA (1984) reported that >550 cases of manganism have been recorded in the literature since the first report by Couper (1837). Case reports have consistently reported that human manganese exposure produces signs and symptoms of neurotoxicity, which include both psychologic disturbances and neurologic disorders; the latter especially seem irreversible (U.S. EPA, 1984). Although the neurotoxic effects of manganese exposure can erupt after only a few months, the latency typically is 2-3 years or longer.

Clinical Case Studies

Most of the studies, particularly the older ones, concentrate on clinical descriptions (case reports or clinical studies) rather than rates of response for a given

TABLE VI-1

Studies of Manganism in Humans and Exposure-Response Relationship[a]

Source of Inhalation Exposure	Chemical (particle size)	Exposure Level (mg Mn/m³)	Duration of Exposure (range)	Number Affected/ Number Studied	Signs and Symptoms[b]	Reference
Ore crushing mill/dust	oxides, mostly MnO, (NR)	10-30 30-180	3.3 year average	0/9 11/25	None 44% manganism	Flinn et al., 1941
Manganese mine	NR	62.5-250	178 days	12/72	manganism	Ansola et al., 1944a,b
Manganese mine; dusts	NR (90% <5 μ)	25-450	~1 month to 10 years	NR	150 cases manganism	Rodier, 1955
Manganese mine	oxides (NR)	1.5-16[c]	8.2 year average	15/83 (9 months to 16 years)	manganism	Schuler et al., 1957
Industrial plants	NR	<5 5-30	NR	0/38 7/117	none 6% manganism	Tanaka and Lieben, 1969
Dry-cell battery industry; dusts	65% MnO, (NR)	6.8-42.2[c]	7.5 year average (1-16 years, cases)	8/36	22.2% manganism psychosis	Emara et al., 1971
Ferromanganese production and processing	ferromanganese, small amounts of MnO, Mn_3O_4 (95% <5 μ) and/or 100% Mn oxides, mainly Mn_2O_3 (<2 μ)	2.1-12.9 and/or 0.12-13.3	8-26 years in five cases	5/71	7% manganism	Smyth et al., 1973
Ferromanganese industry	NR (0.5-6 μ; mostly 4.5)	0.06-4.9	12 years (12 hours/day)	26/160	30% subjective symptoms; 2% "health disorders due to manganism"; symptoms increased with number of years of employment	Suzuki et al., 1973a
Ferromanganese industry; electric furnace workers	NR (<1.5 μ)	3.2-8.6	8.5±6.8 years	40/100	40% subjective symptoms; 8-10% single neurologic signs, e.g., tremor of fingers	Suzuki et al., 1973a
Ferromanganese plant, dust and fumes	NR	0.3-20.44	27% <4 years 9.8% >20 years	62/369	16.8% slight neurologic signs, e.g., tremor at rest, pathologic reflexes	Saric et al., 1977

TABLE VI-1 (cont.)

Source of Inhalation Exposure	Chemical (particle size)	Exposure Level (mg Mn/m³)	Duration of Exposure (range)	Number Affected/ Number Studied	Signs and Symptoms[b]	Reference
Control I elctrode plant		0.002-0.03 (emissions from ferromanganese plant)	NR	11/190	5.8% neurologic findings	Saric et al., 1977
Control II aluminum rolling mill (ambient levels)		≤0.07 µg/m³	NR	0/204	none	Saric et al., 1977
Welding fumes	NR	0.44-0.99[d] 0.5-0.8[e] 0.88-2.6[e]	20.2 (mean year) (10-31) 21.9 (mean year) (2-32) 14.1 (mean year) (6-27)	5/20 10/20 9/20	25% slight neurologic signs (brisk deep reflexes) 50% 45%	Chandra et al., 1981b
Manganese salts and oxides plants	Mn dust	0.07-8.61[c] (median 0.97) control	7.1 mean (1-19) 0	NR/141 NR/140	Exposed performed "less well" in psychomotor tests	Roels et al., 1987a

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

[b]Percentage is given if sample has been selected such that the rate can be considered an estimate of prevalence.

[c]Range of averages for different areas or workstations sampled

[d]In worker's breathing zone

[e]Personal samplers

NR = Not reported

exposure (epidemiologic studies). Kilburn (1987) has published a report on the possible role of manganese in the neurologic disorders found to occur in the isolated Aboriginal population of Groote Eylandt, a large island off the northern coast of Australia. This area is rich in manganese deposits. Although it is difficult to determine actual levels of manganese exposure, elevated levels found universally in the hair of the Aborigines is testament to increased exposure. Elevated whole blood manganese has also been reported in a few individuals. The small population of Groote Eylandt and problems in defining an appropriate control group have made a statistical analysis of clinical problems impossible. However, high levels of stillbirths and congenital malformations have been revealed, and an association with manganese is implicated. A study of the neurologic status of the Aborigines has found two general syndromes: one characterized by amyotrophy and weakness and the other by ataxia and oculomotor disturbances. While this study is still in progress, the role of manganese in these neurologic deficits cannot be clearly defined but must be considered as a possible cause.

A case report by Yamada et al. (1986) published the findings of an autopsy performed on a patient who had worked for 12 years in a manganese ore crushing plant. Two years after he began work, he developed difficulty in walking and diminished libido. Years later, neuropsychiatric symptoms developed, including euphoria, emotional incontinence, masked face, monotonous speech, "cock walk," weakness of extremities, tremor of the eyelids, and exaggeration of knee jerks. Autopsy revealed that the major neuropathologic change was degeneration of the basal ganglia with severe affliction of

Cook et al. (1974) described symptoms and signs of chronic manganese intoxication in six American workers in a manganese ore crushing plant. Symptoms included somnolence, gait imbalance, slurred speech and impaired fine movements, consistent with other descriptions in the literature. However, none of these individuals demonstrated "manganese psychosis" before onset of these symptoms as had miners in other studies. Signs included bradykinesia, postural instability, impaired arising ability, masked faces and speech disorder. One patient did not exhibit major symptoms until 3 years after cessation of exposure.

Kawamura et al. (1941) reported on health effects resulting from the ingestion of manganese-contaminated well water by 25 individuals. The well water had been contaminated with manganese dissolved from dry cell batteries buried near the well. The length of exposure to manganese was estimated to be 2-3 months. The concentration of manganese in the well water was analyzed 7 weeks after the first case appeared and was determined at that time to be ~14 mg Mn/L (as Mn_3O_4). However, when re-analyzed 1 month later, the levels were decreased by about half. Therefore, the actual exposure was probably to drinking water containing 28 mg Mn/L or higher. Assuming a daily water intake of 2 L, 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 10-20 times the level considered to be safe and adequate by the Food and Nutrition Board of the National Research Council (NRC, 1989). Health effects included lethargy, increased muscle tonus, tremor and mental disturbances. The elderly were more frequently affected; children were affected less. Three deaths occurred, one from suicide. Upon autopsy, the concentration of manganese in the brain of one case was

found to be 2-3 times higher than in two controls. In the brain, extreme macroscopic and microscopic changes were seen, especially in the globus pallidus. The authors also reported excess zinc in the well water, but concluded that the zinc appeared to have no relation to symptoms produced and pathologic changes found in the tissues. This conclusion was based upon the fact that, upon autopsy, morphologic changes were observed in the corpus striatum, which is characteristic of manganese poisoning, but not of zinc poisoning. While manganese appears to be the cause of toxicity in these individuals, several aspects of this outbreak are inconsistent with traits of manganism in humans resulting from inhalation exposure. First, the symptoms appeared to come on very quickly; for example, two adults who came to tend the members of one family developed symptoms within 2-3 weeks. Also, the course of the disease was very rapid, progressing in one case from initial symptoms to death in 3 days. Those who did survive recovered from the symptoms, even before the manganese content of the well had decreased significantly after removal of the batteries. This is in contrast to the longer latency period and irreversible damage caused by inhalation exposure to manganese. These differences may represent differences in the pharmacokinetics of ingested vs. inhaled manganese, but there is little information to support this. Therefore, while there is no question that these individuals were exposed to high levels of manganese, it is not clear that the observed effects were due to manganese alone.

Individual case reports have focused on acute exposure to manganese that, although rare, has been found to occur following accidental or intentional ingestion of large amounts of manganese as potassium permanganate. Oral ingestion of 300 mg of potassium permanganate was reported to result in extensive damage to the distal

stomach and pyloric stenosis in a case described by Dagli et al. (1973). Two cases of methemoglobinemia were reported following ingestion of an unspecified amount of potassium permanganate, which had been prescribed by African witch doctors (Mahomedy et al., 1975). The lowest dose of potassium permanganate found to produce toxic effects in a human was 2400 μg/kg bw/day orally ingested by a woman. This information, as reported in a 1933 French study cited in NIOSH (1984), was not available for review.

Additional case studies have also pointed to the potential for manganese poisoning, but are difficult to assess quantitatively. One involved a 59-year-old male who was admitted to the hospital with symptoms of classical 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," perhaps a result of his consumption of "large doses of vitamins and minerals for 4 to 5 years." Unfortunately, no quantitative data were reported.

Another case study of manganese intoxication involved a 62-year-old male who had been receiving total parenteral nutrition that provided 2.2 mg of manganese (form not stated) daily for 23 months (Ejima et al., 1992). The patient's whole blood manganese was found to be elevated, and he was diagnosed as having parkinsonism, with dysarthria, mild rigidity, hypokinesia with masked face, a halting gait, and severely impaired postural reflexes. Assuming an average absorption of roughly 5% of an oral

dose, the i.v. dose of 2.2 mg Mn/day would be approximately equivalent to an oral intake of 40 mg Mn/day.

Epidemiologic Studies

There was one epidemiologic study of manganese in drinking water performed by Kondakis et al. (1989). Three areas in northwest Greece were chosen for this study, with manganese concentrations of 3.6-14.6 µg/L in area A, 81.6-252.6 µg/L in area B, and 1800-2300 µg/L in area C. The total population in the three areas being studied ranged from 3200 to 4350 people. The study included only individuals over the age of 50 drawn from a random sample of 10% of all households (n=62, 49 and 77 for areas A, B and C). The authors reported that "all areas were similar with respect to social and dietary characteristics," but few details were reported. The investigator subsequently estimated a dietary intake of 5-6 mg Mn/day (Kondakis, 1993), but data have not been supplied to substantiate this estimate. Because of the uncertainty in the amount of manganese in the diet, it is difficult to estimate a total oral intake. The lack of dietary data is recognized as a source of significant uncertainty in this assessment.

The individuals in this study were submitted to a neurologic examination, the score of which represents a composite of the presence and severity of 33 symptoms (e.g., weakness/fatigue, gait disturbances, tremors, dystonia). Whole blood and hair manganese concentrations were also determined. 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 ($p<0.001$ for area C vs. A). The concentration of manganese in whole blood did not differ between the three areas, but this is not considered to be a reliable indicator

of manganese exposure. The mean (x) and range (r) of neurologic scores were as follows:

Area A (males: x=2.4, r=0-21; females: x=3.0, r=0-18; both: x=2.7, r=0-21).

Area B (males: x=1.6, r=0-6; females: x=5.7, r=0-43; both: x=3.9, r=0-43).

Area C (males: x=4.9, r=0-29; females: x=5.5, r=0-21; both: x = 5.2, r = 0-29).

A higher neurological score indicates an increased frequency and/or severity of the 33 symptoms that were evaluated. The authors indicate that the difference in mean scores for area C vs. A was significantly increased (Mann-Whitney z=3.16, p=0.002 for both sexes combined), indicating possible neurologic impairment in people living in Area C. In a subsequent analysis, logistic regression indicated that there is a significant difference between areas A and C even when both age and sex are taken into account (Kondakis, 1990). Therefore, the LOAEL for this study is defined by Area C (mean=1950 µg/L) and the NOAEL by Area B (mean=167 µg/L).

Additional concern for possible health effects resulting from an excessive intake of manganese has come from studies with infants. Collipp et al. (1983) found that hair manganese levels in newborn infants was found to increase significantly from birth (0.19 µg/g) to 6 weeks of age (0.865 µg/g) and 4 months of age (0.685 µg/g) when the infants were given formula, but that the increase was not significant in babies who were breast-fed (0.330 µg/g at 4 months). While human breast milk is relatively low in manganese (7-15 µg/L), levels in infant formulas are 3-100 times higher. It was further reported in this study that the level of manganese in the hair of learning disabled children (0.434

μg/g) was significantly increased in comparison with that of normal children (0.268 μg/g). Other investigators have also 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 determined for learning disabilities and manganese intake, further research in this area is warranted. High levels of manganese in infant formulas may be of concern because of the increased absorption and retention of manganese that has been reported in neonatal animals (Lönnerdal et al., 1987). Also, manganese has 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). It was suggested by Dieter et al. (1992) 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 for infants as a group at risk..."

Although conclusive evidence is lacking, some investigators have also linked increased intakes of manganese with violent behavior. Gottschalk et al. (1991) found statistically significantly elevated levels 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 authors suggest 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." Caution should be exercised to prevent reading too much into these data, but support for this hypothesis is provided by studies of a population of Aborigines in Groote Eylandt. Several clinical symptoms consistent with manganese intoxication are present in about 1% of the inhabitants of this Australian island and it may not be coincidental that the proportion of arrests in this native

population is the highest in Australia (Cawte and Florence, 1989; Kilburn, 1987). The soil in this region is very high in manganese (40,000-50,000 ppm), and the fruits and vegetables grown in the region are also reported to be high in manganese. Quantitative data on oral intakes have not been reported, but elevated concentrations of manganese have been determined in the blood and hair of the Aborigines (Stauber et al., 1987). In addition to the high levels of environmental manganese, other factors common to this population may further increase the propensity for manganism: high alcohol intake, anemia, and a diet deficient in zinc and several vitamins (Florence and Stauber, 1989).

Most of the studies of the health effects of manganese exposure in humans involve inhalation exposures. They tend to be collections of clinical studies, simply listing observations rather than analytical epidemiologic studies, which test statistical associations between exposure and effects. In addition, most of the studies have been cross-sectional in approach rather than the preferred prospective or retrospective design. Limitations to these studies include the inability to obtain incidence rates or to examine the effects of exposure duration as well as selection biases and lack of appropriate controls. The levels of exposure in the following reports are time weighted averages.

Flinn et al. (1941) described neurotoxic effects in 34 workers exposed to manganese in ore crushing mills. The U.S. EPA (1984) reported that 11/34 workers had neurologic symptoms indicative of manganese poisoning; those most affected had an average length of exposure to manganese of 5.3 years and those least affected, 2.4 years. All 11 cases of manganism occurred in workers exposed to 30-180 mg Mn/m^3. Nine workers exposed to <30 mg Mn/m^3 had no signs of manganese poisoning. In

addition to neurotoxic effects, Flinn et al. (1941) also found evidence of hematologic effects in humans. Those most affected neurologically also had a low white cell count, which became more pronounced with the progress of manganism.

Kesic and Hausler (1954) found hematologic effects in 52 exposed miners without symptoms of manganism. The miners had higher mean levels of erythrocytes, hemoglobin, and monocytes compared with levels in 60 sawmill workers of similar age and social conditions. The level and duration of exposure were not specified.

Ansola et al. (1944a,b) found neurotoxic effects in 12/72 miners exposed to 62.5-250 mg Mn/m^3 for 178 days. The classic study by Rodier (1955) described clinical details of cases of manganese poisoning in miners exposed to 250-450 mg Mn/m^3. The length of exposure varied from 1 month to 10 years. Schuler et al. (1957) studied 83 miners exposed to 1.5-16 mg Mn/m^3 for 9 months to 16 years and found neurotoxic effects among 15 workers.

Sabnis et al. (1966) found no manganism among workers (number unspecified) in a ferromanganese alloy factory exposed to <2.3 mg Mn/m^3, but did find cases (number unspecified) of manganism among those who were exposed to 8.4 mg Mn/m^3. The duration of exposure was not specified.

Tanaka and Lieben (1969) studied 117 workers in industrial plants exposed to 5-30 mg Mn/m^3, and 38 workers exposed to <5 mg Mn/m^3, which is the Threshold Limit Value (TLV) established for occupational exposures by ACGIH (1986). They reported

seven cases of manganism among those exposed above the TLV. The length of exposure was not reported. A subsequent clinical report by Cook et al. (1974) included workers from these plants.

Emara et al. (1971) found manganism in 8/36 workers exposed to 6.2-42.2 mg Mn/m^3 as manganese dioxide dust in a factory manufacturing dry cell batteries. Exposure ranged from 1-16 years among the affected cases.

Smyth et al. (1973) reported five cases of manganism among 71 workers in a ferromanganese production and processing plant. Manganese exposure concentrations ranged from 0.12-13.3 mg Mn/m^3 for fumes and 2.1-12.9 mg Mn/m^3 for dust. Length of exposure ranged from 8-26 years among cases.

Suzuki et al. (1973a) studied workers in a ferromanganese plant who were exposed to 0.06-4.9 mg Mn/m^3 12 hours/day for 12 years. They reported 26 cases showing signs and symptoms of manganism among 160 workers, which increased with the number of years of employment. Suzuki et al. (1973b) also found 40/100 workers affected by exposure to 3.2-8.6 mg Mn/m^3 for 1.7-15.3 years.

The biochemical effects of manganese exposure have been studied by Jonderko et al. (1971, 1973, 1974). In the 1971 study, workers exposed to manganese who did not exhibit symptoms or signs of manganism were compared with nonexposed controls. Lower levels of magnesium, hemoglobin, and reduced glutathione in addition to higher levels of calcium and cholesterol were found among exposed workers. Interpretation is

difficult because the duration and level of exposure were not specified. In the 1973 study, 110 workers exposed to manganese in a steel mill at levels 1.3-50 times above the maximum allowable concentration for an average of 9 years were compared with 90 unexposed controls. Statistically significant ($p<0.01$) increases in mean cholesterol, β-lipoproteins and total lipoproteins, as well as increased incidences of hypertension and atherosclerosis were found in the exposed group. The U.S. EPA (1984) noted that confounding variables such as smoking and obesity were not considered. In the 1974 study, 34 iron-manganese plant workers were examined during employment and 2-4 years after cessation of occupational exposure. Changes in levels of lactate dehydrogenase, alanine and aspartate aminotransferase, cholesterol, and glutathione were found to have normalized after exposure ceased when compared with controls. Hemoglobin levels increased after cessation of exposure as well.

Chandra et al. (1974) studied clinical and biochemical parameters in 12 cases of suspected manganism and found a statistically significant ($p<0.01$) increase in serum calcium and adenosine deaminase levels, which was greatest in the most severe cases. The author suggested using serum calcium levels to detect early manganism. Also reported were lower erythrocyte counts and lower hemoglobin concentrations in the manganism cases as compared with controls. White cell counts were normal and did not differ between the two groups.

Saric and Hrustic (1975), studying cardiovascular system effects of manganese exposure, compared the diastolic and systolic blood pressure of 367 workers in a ferromanganese plant where there were exposures to 0.39-20.44 mg Mn/m^3 with that of

189 workers in electrode production within the same plant where exposures were 0.002-0.30 mg Mn/m^3. The study also included 203 workers unexposed to manganese. The length of exposure for 75% of the workers was >4 years. The workers with the highest exposures were found to have the lowest mean systolic blood pressure followed by the lowest exposed and nonexposed workers. This was true regardless of age. The lowest mean diastolic blood pressure was found in the unexposed workers followed by the highest and lowest exposed workers. This was also true for all age groups. Saric (1978) suggests that an action of manganese ions on the myocardium may be responsible for cardiovascular system effects. The U.S. EPA (1984) notes, however, that other potentially confounding risk factors were insufficiently controlled in the study.

Saric et al. (1977) published a report that compared 369 workers exposed to 0.3-20.44 mg Mn/m^3 at a ferroalloy plant with 190 workers at an electrode plant exposed to 0.002-0.03 mg Mn/m^3 and 204 workers at an aluminum rolling mill exposed to ambient levels <0.0001 mg Mn/m^3. Signs of manganism were found in 17% of workers in the ferroalloy plant, 6% in the electrode plant, and 0% in the aluminum plant. The ferroalloy workers were subsequently categorized into three groups by mean manganese concentrations at working places: <5 mg/m^3, 9-11 mg/m^3 and 16-20 mg/m^3. Table VI-2 presents the findings of effects at different levels of exposures and suggests that slight neurologic disturbances may occur at exposures <5 mg/m^3 and appear to be more prevalent at higher exposures.

Chandra et al. (1981b) studied neurotoxic effects in welders from a heavy engineering shop, a railway workshop and a ship repair shop. Welders in the heavy

TABLE VI-2

Ferroalloy Workers with Neurologic Signs by Level of Exposure to Manganese*

Signs	Mean Manganese Concentrations at Working Places (mg/m^3)				
	~0 (electrode plant) (n=190)	~0 (aluminum rolling mill) (n=204)	<5 (n=369)	9-11 (n=17)	16-20 (n=18)
Cogwheel phenomenon	0	0	1 (0.3%)	0	0
Difficulty in initiating voluntary movements	0	0	2 (0.5%)	0	0
Pathologic reflexes	1 (0.3%)	0	6 (1.6%)	1 (5.7%)	1 (5.6%)
Tremor at rest	10 (5.3%)	0	42 (11.4%)	2 (11.8%)	2 (11.1%)
Pathologic reflexes and tremor at rest	0	0	3 (0.8%)	0	0
Cogwheel phenomenon and tremor at rest	0	0	0	0	1 (5.6%)
Cogwheel phenomenon and pathologic reflexes	0	0	0	0	1 (5.6%)
Total	11 (5.8%)	0	54 (14.6%)	3 (17.6%)	5 (27.8%)

*Source: Adapted from Saric et al., 1977

engineering shop were exposed to manganese from welding fumes at breathing zone concentrations of 0.44-0.99 mg/m^3 and an airborne mean of 0.31 mg/m^3 for 10-31 years. In the railway workshop, welders' breathing zone concentrations ranged from 0.5-0.8 mg Mn/m^3 with an airborne mean of 0.57 mg Mn/m^3 for 2-32 years. Ship repair shop welders had the highest breathing zone concentration of 0.88-2.6 mg Mn/m^3 and airborne mean of 1.75 mg Mn/m^3 for 6-27 years. Neurologic signs in the form of brisk deep reflexes of limbs and tremors were reported for 5/20 engineering shop welders, 10/20 railway workshop welders, and 9/20 ship repair shop welders. Twenty controls showed no effects but no statistical analysis nor analysis by person-years was presented.

A questionnaire was used by Lauwerys et al. (1985) to assess the effect of manganese dust on male fertility. The manganese-exposed group consisted of 85 male workers from a factory producing manganese salts. The airborne concentration of manganese dust ranged from 0.07-8.61 mg/m^3 with an average value of ~1 mg/m^3. The control group consisted of 81 male factory workers who were never exposed to manganese. The exposed and control groups were matched for age, age of wife, age of wife at marriage, duration of employment in the factory, smoking habits, alcohol consumption, education, professional activity of wife, and desire to have children. While manganese blood levels were not reported, it was stated that the level was, on average, 2.3 times higher in the exposed group than in the controls. Manganese levels in the urine were said to fluctuate, but median values of 1.17 and 0.16 μg Mn/g creatinine were reported for the exposed and control groups, respectively. During their period of exposure to manganese there was a statistically significant ($p<0.05$) decrease in the

number of children born to exposed workers. There was no indication that any other factors may have accounted for the difference in fertility between the exposed and control groups.

Roels et al. (1987a) conducted an epidemiologic study on 141 male workers exposed to inorganic manganese in a manganese oxide and salt producing plant (mean age = 34.3 years, mean duration of exposure = 7.1 years, range = 1-19 years). They were matched with a control group of 104 workers from a nearby chemical plant. The manganese exposed group was found to have a significantly increased incidence of several respiratory tract symptoms (coughing, dyspnea during exercise, bronchitis). Psychomotor tests proved to be the most useful indicator of adverse effects of manganese on the CNS. The manganese exposed workers exhibited significant adverse changes in simple reaction time, audioverbal short-term memory capacity, and hand tremor. Hematologic parameters were all normal except for a significant increase in neutrophil count. There was also a significant increase in several serum parameters (ceruloplasmin, copper, ferritin and calcium). There were no monitoring data available, but during the survey the time-weighted average concentration of total airborne manganese ranged from 0.07-8.61 mg/m^3 with an overall average of ~1 mg/m^3.

When the above CNS and biologic effects were examined as a function of blood-manganese and of duration of manganese exposure, no statistically significant dose-response relationship was found. Blood-manganese levels were related to serum calcium, hand steadiness and eye-hand coordination. This last parameter was the basis for the suggestion that the threshold level for blood-manganese is ~1 µg/100 mL blood.

Levels of manganese in the blood and urine (Mn-B and Mn-U, respectively) of workers in the above study were reported in a separate publication (Roels et al., 1987b). Mn-B ranged from 0.1-3.59 µg/100 mL (arithmetic mean = 1.36) in exposed workers while levels in the control group ranged from 0.04-1.31 µg/100 mL (mean = 0.57). Mn-U levels ranged from 0.06-140.6 µg/g creatinine (geometric mean = 1.56) in exposed workers while control levels ranged from 0.01-5.04 (mean = 0.15) µg/g creatinine. No relationship was found between Mn-B and Mn-U and neither concentration correlated on an individual basis with the current level of Mn-air or the duration of manganese exposure. This is expected as blood and urine levels of manganese are not considered to be good indicators of manganese exposure.

Carcinogenicity. There are no epidemiologic studies relating manganese exposure to cancer occurrence in humans. The available evidence for manganese carcinogenicity in humans would be rated Group 3 (not classifiable) using the International Agency for Research on Cancer (IARC) Criteria.

Marjanen (1969) correlated the amount of soluble manganese in cultivated mineral soil with 5-year cancer incidence rates in Finland and found that cancer incidence rates decreased with increasing content of manganese. The data were not age-adjusted and other confounding variables were not considered.

Mutagenicity and Teratogenicity. No studies were found for humans relating manganese exposure to mutagenic or teratogenic effects.

Summary

Although no clear dose-response relationship is evident, the studies cited in this report support the association of neurotoxic effects with exposure to manganese in humans. Other effects, including hematologic, biochemical and cardiovascular have been reported, but, in most cases, are based on a single study or on studies whose primary purpose was the investigation of neurotoxic effects. There are no epidemiologic studies relating manganese exposure to carcinogenic, mutagenic or teratogenic effects in humans.

The lowest reported exposure levels associated with neurotoxic effects in humans range from $\geq$0.3 mg/m^3 for inhaled manganese (Saric et al., 1977; Chandra et al., 1981b; Roels et al., 1987a). However, the findings reported at 0.3 mg/m^3 could not be definitely attributed to manganese exposure (U.S. EPA, 1984). Levels >5 mg/m^3 have been more consistently associated with neurotoxic effects.

One study of health effects resulting from the ingestion of manganese-contaminated drinking water found neurotoxic signs and symptoms occurring at drinking water concentrations >28 mg Mn/L (Kawamura et al., 1941). Another epidemiologic study suggests increased manganese retention and possible adverse neurologic effects from chronic ingestion of drinking water containing ~2 mg Mn/L (Kondakis et al., 1989).

VII. MECHANISMS OF TOXICITY

Mechanisms of Neurotoxicity

Manganese is an essential metal in mammals and is required for the activity of many degradative enzymes such as pyruvate carboxylase, arginase, phosphatases, as well as the biosynthetic enzymes of lipids and mucopolysaccharides of cartilages (Venugopal and Lucky, 1978). Exposure to excess amounts of manganese may result in adverse health effects, primarily of the CNS.

The mechanism by which manganese crosses the blood-brain barrier (BBB) to gain access to neuronal tissue has not been fully elucidated, but may be a function of binding to transferrin (Aschner and Aschner, 1990). In the portal circulation, manganese binds to alpha-2-macroglobulin, which is removed by the liver (Tanaka, 1982). This complex, however, cannot cross the BBB. Transferrin, which has a strong affinity for iron, has also been shown to bind manganese (+3 oxidation state) and may be responsible for its transport into the brain. This argument is substantiated by the fact that those regions of the brain that accumulate manganese (e.g., ventral pallidum, globus pallidus and substantia nigra) receive neuronal input from the nucleus accumbens and the caudate-putamen, both being areas rich in transferrin receptors. More direct evidence 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 because both metals are transported by transferrin and may be competing for binding sites.

Several neurotransmitter systems in the brain appear to be affected by manganese, primarily monoamines such as dopamine, noradrenaline and serotonin (Neff et al., 1969; Mustafa and Chandra, 1971), but also gamma amino butyric acid (GABA) (Gianutsos and Murray, 1982). Manganese neurotoxicity is generally associated with a selective depletion of dopamine in the striatum (Neff et al., 1969; Bernheimer et al., 1973). It has been demonstrated that the striatum preferentially accumulates manganese (Scheuhammer and Cherian, 1981), particularly within the mitochondria (Maynard and Cotzias, 1955).

Mapping studies have shown that most of the neuronal degeneration attributed to manganese exposure lies close to monoamine cell bodies and pathways. However, histopathology in primates shows rather widespread damage, including the subthalamic nuclei and the globus pallidus. The globus pallidus was also found to be most severely affected in an autopsy performed on a worker with 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 mechanisms remain obscure. There is a close resemblance between the symptoms of manganism and Parkinsonian syndrome that has been further substantiated by the demonstration that 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). One theory involves the effect of manganese on brain cytochrome P-450 activity. Liccione and Maines (1989) demonstrated a high degree of sensitivity of rat striatal mitochondria to manganese-induced increases in cytochrome P-450 activity. The authors hypothesized that this increase in mixed function oxidase activity may result in a concomitant increase in the formation of active oxygen species (e.g., superoxide anions) that may result in toxic effects to the dopamine pathways.

Manganese (Mn^{+3}) has also been shown to oxidize dopamine to its cyclized o-quinone (cDAoQ) (Archibald and Tyree, 1987); this is an irreversible process ultimately resulting in decreased dopamine levels. The formation of cDAoQ may subsequently initiate the generation of reactive oxygen species that may lead to oxidative stress and cell death (Segura-Aguilar and Lind, 1989).

It is noteworthy that, while alterations in neurotransmitters have been observed in rodents administered high levels of manganese, the psychologic disturbances seen in primates are not observed. Primate brain tissue contains more pigmented areas (e.g., the substantia nigra) that are known to sequester manganese. Marsden and Jenner (1987) hypothesized that the ability of certain drugs to induce parkinsonism in primates but not in rodents is because of the relative lack of neuromelanin in rodents.

The effects of manganese on the levels of monoamines also appear to be age-dependent. It has been shown that neonatal rats and mice exposed to manganese from birth up to 15-30 days of age actually have an increased level of dopamine and

norepinephrine in the brain (Chandra et al., 1979; Cotzias et al., 1976; Shukla et al., 1980).

Parenti et al. (1986) indicated that the alterations of postsynaptic dopaminergic receptors seen in manganese poisoning may be different from that seen in Parkinson's disease and that current therapy for Parkinson's disease (administration of L-DOPA) may be contra-indicated in manganese poisoning. Despite similarities in symptoms, a comparative study of a worker exposed to manganese in an ore crushing plant and a 52-year-old patient with Parkinson's disease did not reveal any similarity in neuropathology (Yamada et al., 1986). Since the issue is unresolved, extensive discussion is beyond the scope of this document and the reader is referred to the cited literature and to more detailed reviews (Shukla and Singhal, 1984; U.S. EPA, 1984; Seth and Chandra, 1988).

Studies based on altered neurotransmitter metabolism have examined the following:

- the synthesis of dopamine and the susceptibility of the rate-limiting synthesizing enzyme, tyrosine hydroxylase (TOH) to manganese; the changes in TOH activities closely parallel dopamine levels (Bonilla, 1980; Chandra and Shukla, 1981)
- alterations in TOH activity (as well as other monoxygenases) may also be related to manganese-induced alterations in brain heme metabolism (Qato and Maines, 1985).

VIII. QUANTIFICATION OF TOXICOLOGIC EFFECTS

Introduction

The quantification of toxicologic effects of a chemical consists of separate assessments of noncarcinogenic and carcinogenic health effects. Chemicals that do not produce carcinogenic effects are believed to have a threshold dose below which no adverse, noncarcinogenic health effects occur, while carcinogens are assumed to act without a threshold.

In the quantification of noncarcinogenic effects, a Reference Dose (RfD), [formerly termed the Acceptable Daily Intake (ADI)] is calculated. 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 health effects during a lifetime. The RfD is derived from a no-observed-adverse-effect level (NOAEL), or lowest-observed-adverse-effect level (LOAEL), identified from a subchronic or chronic study, and divided by an uncertainty factor(s) times a modifying factor. The RfD is calculated as follows:

$$RfD = \frac{(NOAEL \text{ or } LOAEL)}{[Uncertainty\ Factor(s) \times Modifying\ Factor]} = \_\_ mg/kg\ bw/day$$

Selection of the uncertainty factor to be employed in the calculation of the RfD is based upon professional judgment, while considering the entire data base of toxicologic effects for the chemical. In order to ensure that uncertainty factors are selected and

applied in a consistent manner, the U.S. EPA (1993) employs a modification to the guidelines proposed by the National Academy of Sciences (NAS, 1977, 1980) as follows:

Standard Uncertainty Factors (UFs)

- Use a 10-fold factor when extrapolating from valid experimental results from studies using prolonged exposure to average healthy humans. This factor is intended to account for the variation in sensitivity among the members of the human population. [10H]

- Use an additional 10-fold factor when extrapolating from valid results of long-term studies on experimental animals when results of studies of human exposure are not available or are inadequate. This factor is intended to account for the uncertainty in extrapolating animal data to the case of humans. [10A]

- Use an additional 10-fold factor when extrapolating from less than chronic results on experimental animals when there is no useful long-term human data. This factor is intended to account for the uncertainty in extrapolating from less than chronic NOAELs to chronic NOAELs. [10S]

- Use an additional 10-fold factor when deriving an RfD from a LOAEL instead of a NOAEL. This factor is intended to account for the uncertainty in extrapolating from LOAELs to NOAELs. [10L]

Modifying Factor (MF)

- Use professional judgment to determine another uncertainty factor (MF) that is greater than zero and less than or equal to 10. The magnitude of the MF depends upon the professional assessment of scientific uncertainties of the study and data base not explicitly treated above, e.g., the completeness of the overall data base and the number of species tested. The default value for the MF is 1.

The uncertainty factor used for a specific risk assessment is based principally upon scientific judgment rather than scientific fact and accounts for possible intra- and interspecies differences. Additional considerations not incorporated in the NAS/ODW guidelines for selection of an uncertainty factor include the use of a less than lifetime

study for deriving an RfD, the significance of the adverse health effects and the counterbalancing of beneficial effects.

From the RfD, a Drinking Water Equivalent Level (DWEL) can be calculated. The DWEL represents a medium specific (i.e., drinking water) lifetime exposure at which adverse, noncarcinogenic health effects are not anticipated to occur. The DWEL provides the noncarcinogenic health effects basis for establishing a drinking water standard. For ingestion data, the DWEL is derived as follows:

$$DWEL = \frac{RfD \times (Body\ weight\ in\ kg)}{Drinking\ Water\ Volume\ in\ L/day} = \_\_ mg/L$$

where:

Body weight = assumed to be 70 kg for an adult
Drinking water volume = assumed to be 2 L/day for an adult

The DWEL for manganese, as described in detail later in this chapter, is calculated from a drinking water-specific RfD (U.S. EPA, 1993). It is assumed that a separate dietary contribution will be made to the total oral intake.

In addition to the RfD and the DWEL, Health Advisories (HAs) for exposures of shorter duration (1-day, 10-day and longer-term) are determined. The HA values are used as informal guidance to municipalities and other organizations when emergency spills or contamination situations occur. The HAs are calculated using an equation

similar to the RfD and DWEL; however, the NOAELs or LOAELs are identified from acute or subchronic studies. The HAs are derived as follows:

$$HA = \frac{NOAEL \text{ or } LOAEL \times (bw)}{UF \times (\_\_ L/day)} = \_\_ mg/L$$

Using the above equation, the following drinking water HAs are developed for noncarcinogenic effects:

1. 1-day HA for a 10 kg child ingesting 1 L water per day.
2. 10-day HA for a 10 kg child ingesting 1 L water per day.
3. Longer-term HA for a 10 kg child ingesting 1 L water per day.
4. Longer-term HA for a 70 kg adult ingesting 2 L water per day.

The 1-day HA calculated for a 10 kg child assumes a single acute exposure to the chemical and is generally derived from a study of <7 days duration. The 10-day HA assumes a limited exposure period of 1-2 weeks and is generally derived from a study of <30 days duration. The longer-term HA is derived for both the 10 kg child and a 70 kg adult and assumes an exposure period of ~7 years (or 10% of an individual's lifetime). The longer-term HA is generally derived from a study of subchronic duration (exposure for 10% of animal's lifetime).

The U.S. EPA categorizes the carcinogenic potential of a chemical, based on the overall weight-of-evidence, according to the following scheme:

> Group A: Human Carcinogen. Sufficient evidence exists from epidemiology studies to support a causal association between exposure to the chemical and human cancer.

Group B: Probable Human Carcinogen. Sufficient evidence of carcinogenicity in animals with limited (Group B1) or inadequate (Group B2) evidence in humans.

Group C: Possible Human Carcinogen. Limited evidence of carcinogenicity in animals in the absence of human data.

Group D: Not Classifiable as to Human Carcinogenicity. Inadequate human and animal evidence of carcinogenicity or for which no data are available.

Group E: Evidence of Noncarcinogenicity for Humans. No evidence of carcinogenicity in at least two adequate animal tests in different species or in both adequate epidemiologic and animal studies.

If toxicologic evidence leads to the classification of the contaminant as a known, probable or possible human carcinogen, mathematical models are used to calculate the estimated excess cancer risk associated with the ingestion of the contaminant in drinking water. The data used in these estimates usually come from lifetime exposure studies using animals. In order to predict the risk for humans from animal data, animal doses must be converted to equivalent human doses. This conversion includes correction for noncontinuous exposure, less than lifetime studies and for differences in size. The factor that compensates for the size difference is the cube root of the ratio of the animal and human body weights. It is assumed that the average adult human body weight is 70 kg and that the average water consumption of an adult human is 2 L of water per day.

For contaminants with a carcinogenic potential, chemical levels are correlated with a carcinogenic risk estimate by employing a cancer potency (unit risk) value together with the assumption for lifetime exposure from ingestion of water. The cancer unit risk is usually derived from a linearized multistage model with a 95% upper confidence limit

providing a low dose estimate; that is, the true risk to humans, while not identifiable, is not likely to exceed the upper limit estimate and, in fact, may be lower. Excess cancer risk estimates may also be calculated using other models such as the one-hit, Weibull, logit and probit. There is little basis in the current understanding of the biologic mechanisms involved in cancer to suggest that any one of these models is able to predict risk more accurately than any other. Because each model is based upon differing assumptions, the estimates derived for each model can differ by several orders of magnitude.

The scientific data base used to calculate and support the setting of cancer risk rate levels has an inherent uncertainty that is due to the systematic and random errors in scientific measurement. In most cases, only studies using experimental animals have been performed. Thus, there is uncertainty when the data are extrapolated to humans. When developing cancer risk rate levels, several other areas of uncertainty exist, such as the incomplete knowledge concerning the health effects of contaminants in drinking water, the impact of the experimental animal's age, sex and species, the nature of the target organ system(s) examined and the actual rate of exposure of the internal targets in experimental animals or humans. Dose-response data usually are available only for high levels of exposure and not for the lower levels of exposure closer to where a standard may be set. When there is exposure to more than one contaminant, additional uncertainty results from a lack of information about possible synergistic or antagonistic effects.

Noncarcinogenic Effects of Manganese in the Diet

The health effects associated with the ingestion of manganese are highly dependent on its bioavailability. This may be affected by several factors including species, age, form of manganese, medium (e.g., drinking water vs. food), nutritional status and other dietary constituents. These factors, addressed in Chapters III, V and VI, will also be discussed below, particularly as they impact the quantitative risk assessment of manganese.

It is well recognized that there are significant differences in species' requirements for manganese intakes and in the health effects observed in different species resulting from excessive manganese exposure. Primates are acknowledged to be a better experimental animal than rodents for studying the neurobehavioral manifestations of manganese intoxication (U.S. EPA, 1984). In the brain, several neurotransmitter systems appear to be affected by excess manganese exposure such as dopamine, noradrenaline and serotonin (Neff et al., 1969). Mapping studies have shown that most of the neuronal degenerative alterations in the CNS syndrome of toxicity (manganism) occur where pathways of the monoamines are anatomically located (Pentschew et al., 1963). It has been proposed that the accumulation of manganese in the brain occurs more readily in pigmented tissue, which is distributed differently in primates than in rodents. Also, the human neurobehavioral deficits (e.g., tremor, gait disorders) stemming from manganese toxicity can be reproduced in primates, but not in rodents. For these reasons, rodent species may be less appropriate for studying manganese neurotoxicity.

Although no recommended dietary allowance (RDA) has been established for manganese, it is recognized as an essential element for the activity of many enzymes in humans. Several studies have been performed to determine the average daily intake of manganese from a "typical" American diet. The Total Diet Study conducted in the United States between 1982 and 1986 reported the mean dietary intake to be 2.2 mg Mn/day for women and 2.7 mg Mn/day for men (Pennington et al., 1989). The NAS Food and Nutrition Board has estimated that, based upon manganese intake and balance studies, a 2-5 mg daily intake of manganese (for food and beverages, which includes drinking water) is adequate and safe for adults (NRC, 1989). A World Health Organization report (WHO, 1973) on trace elements in human nutrition suggests that dietary manganese intakes of 8-9 mg/day are safe, since balance studies on normal men and women consuming these levels revealed no evidence of manganese toxicity. The criteria for determining safety were not presented, but it may be assumed that no toxic effects were observed at these levels. Schroeder et al. (1966) reported that patients (number not specified) given 30 mg manganese citrate (equivalent to 9 mg manganese) daily for many months did not show any signs of toxicity. Assuming the patients consumed another 2.5 mg manganese in their diet, the total intake would be ~11.5 Mn/day. Schroeder et al. (1966) has estimated that a 2300 calorie vegetarian diet of whole grains, fresh vegetables, fruits, nuts and tea (all rich sources of manganese) would provide an intake as high as 13-20 mg Mn/day. These levels are also considered to be safe. However, the bioavailability of manganese from various food sources may vary substantially. For example, several constituents of vegetarian diets (e.g., fiber, lectins, phytates) may result in decreased bioavailability of manganese. High or low

levels of other dietary minerals such as iron, calcium and phosphorus may also affect manganese uptake.

Kawamura et al. (1941) reported on health effects resulting from the ingestion of manganese-contaminated well water by 25 individuals. The well water had been contaminated with manganese dissolved from dry cells batteries buried near the well. The length of exposure to manganese was estimated to be 2-3 months. The concentration of manganese in the well water was analyzed 7 weeks after the first case appeared and was determined at that time to be ~14 mg Mn/L (as Mn_3O_4). However, when reanalyzed 1 month later, the levels were decreased by about half. Therefore, the actual exposure was probably to drinking water containing 28 mg Mn/L or higher. Assuming a daily water intake of 2 L, this represents a dose of at least 56 mg Mn/day, plus that which was in the diet. This represents a dose about 10-20 times the dietary intake considered to be safe and adequate by the Food and Nutrition Board of the National Research Council (NRC, 1989). Health effects included lethargy, increased muscle tonus, termor and mental disturbances. The elderly were more frequently and more severely affected; children were affected less. Three deaths occurred, one from suicide. Upon autopsy, the concentration of manganese in the brain of one case was found to be 2-3 times higher than in two controls. In the brain, extreme macroscopic and microscopic changes were seen, especially in the globus pallidus.

Kawamura et al. (1941) also reported excess zinc in the well water, but concluded that the zinc appeared to have no relation to the reported symptoms and pathologic changes found in the tissues. This conclusion was based upon the fact that, upon

autopsy, morphologic changes were observed in the corpus striatum, which is characteristic of manganese poisoning, but not of zinc poisoning. While manganese appears to be the cause of toxicity in these individuals, several aspects of this outbreak are inconsistent with traits of manganism in humans resulting from inhalation exposure. First, the symptoms appeared to come on very quickly; for example, two adults who came to tend the members of one family developed symptoms within 2-3 weeks. Also, the course of the disease was very rapid, progressing in one case from initial symptoms to death in 3 days. Those who did survive recovered from the symptoms, even before the manganese content of the well had decreased significantly after removal of the batteries. This is in contrast to the longer latency period and irreversible damage caused by inhalation exposure to manganese. These differences may represent differences in the pharmacokinetics of ingested vs. inhaled manganese, but there is little information to support this. Therefore, while there is no question that these individuals were exposed to high levels of manganese, it is not clear that the observed effects were due to manganese alone.

There was one epidemiologic study of manganese in drinking water performed by Kondakis et al. (1989). Three areas in northwest Greece were chosen for this study, with manganese concentrations of 3.6-14.6 μg/L in area A, 81.6-252.6 μg/L in area B, and 1800-2300 μg/L in area C. The total population in the three areas being studied ranged from 3200 to 4350 people. The study included only individuals over the age of 50 drawn from a random sample of 10% of all households (n=62, 49 and 77 for areas A, B and C). The authors reported that "all areas were similar with respect to social and dietary characteristics," but few details were reported. The individuals chosen were

submitted to a neurologic examination, the score of which represents a composite of the presence and severity of 33 symptoms (e.g., weakness/fatigue, gait, disturbances, tremors, dystonia). Whole blood and hair manganese concentrations were also determined. 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 ($p < 0.001$ for area C vs. A). The concentration of manganese in whole blood did not differ between the three areas, but this is not considered to be a reliable indicator of manganese exposure. The mean (x) and range (r) of neurologic scores were as follows:

Area A (males: x=2.4, r=0-21; females: x=3.0, r=0-18;
both: x=2.7, r=0-21).

Area B (males: x=1.6, r=0-6; females: x=5.7, r=0-43;
both: x=3.9, r=0-43).

Area C (males: x=4.9, r=0-29; females: x=5.5, r=0-21;
both: x=5.2, r=0-29).

The authors indicate that the difference in mean scores for area C vs. A was significantly increased (Mann-Whitney $z=3.16$, $p=0.002$ for both sexes combined). In a subsequent analysis, logistic regression indicated that there is a significant difference between areas A and C even when both age and sex are taken into account (Kondakis, 1990). Therefore, the LOAEL for this study is defined by Area C (mean = 1950 μg/L) and the NOAEL by Area B (mean = 167 μg/L).

The report by Kondakis et al. (1989) is the only epidemiologic study of the effects associated with low-level ingestion of manganese in drinking water. Most of the studies of the health effects of manganese exposure in humans involve inhalation exposures.

Animal studies of manganese toxicity arising from oral exposure generally do not provide evidence of a dose-response relationship for neurologic effects similar to those observed in humans. Reported effects in rodents exposed to manganese in drinking water include alterations in neurotransmitter systems but not neurobehavioral effects. It is uncertain whether the effects on neurotransmitters should be defined as adverse, since they could represent compensatory responses. Also, these effects are highly dependent on many variables, such as the exposure regimen and the age of the animal.

Several studies with monkeys exposed to large doses of manganese by parenteral routes have consistently reported extrapyramidal symptoms and histologic lesions that resemble those described in advanced human manganism (U.S. EPA, 1984). However, only one limited study of oral administration has been published (Gupta et al., 1980). Four rhesus monkeys (*M. mulatta*) administered an oral dose of 6.9 mg Mn/kg/day (25 mg/kg $MnCl_2 \cdot 4H_2O$) for 18 months developed neurologic signs and showed histologic evidence of damage to the substantia nigra. No biochemical data were reported.

Chandra et al. (1979a) reported CNS effects in growing male mice exposed to 1 mg Mn/kg bw/day (3 µg Mn/mL drinking water x 10 mL water/day ÷ 0.03 kg bw) for the first 6 months of life. These effects included a significant increase in motor activity at 60 and 90 days associated with a significant elevation in levels of dopamine and norepinephrine. However, the reported exposure levels are below the NAS recommendation for rodents for the average daily intake of 3-6 mg/kg/day deemed necessary for development (NAS, 1980) making their validity questionable, particularly

given the recent studies by Lown et al. (1984) and Gianutsos and Murray (1982). The studies by Chandra and coworkers have also been questioned because of the strain of animals used (I.T.R.C. rats and mice). There are no historic data on this strain and it is possible that metabolic or other differences between strains account for discrepancies between these and other studies.

Singh et al. (1979) reported significant alterations of brain enzymes in mature rats exposed to 4.4 mg Mn/kg in drinking water for 30 days. The study found no brain morphologic changes but noted that biochemical changes occur before morphologic damage is visible under light microscope.

The next lowest reported dose producing CNS effects has been reported by Lai, Leung and colleagues (Lai et al., 1981a, 1982a, 1984; Leung et al., 1981, 1982; Nachtman et al., 1986). In developing and aging rats orally exposed to 38.9 mg Mn/kg/day (1 mg $MnC1_2 \cdot 4H_2O$/mL drinking water = 0.278 mg Mn/mL x 0.49 mL/day ÷ 0.35 kg = 38.9 mg Mn/kg) different neurotoxic effects were reported depending on the age of the rat and the duration of exposure. Lai et al. (1982a) concluded that although the rat may not appear to serve as an ideal model for studying the neurotoxic effects of manganese, some neurochemical effects may be discernible when selected analyses are made at the appropriate period.

Chandra and Shukla (1981) exposed young male rats to 38.9 mg Mn/kg/day (0.278 mg Mn/mL x 0.49 mL/day ÷ 0.350 kg = 38.9 mg Mn/kg) as 1 mg $MnCl_2 \cdot 4H_2O$/mL drinking water for as long as 360 days. Dopamine, norepinephrine and homovanillic acid

levels were found to increase initially, then to return to normal and finally to decrease significantly after 300 days of exposure. The authors suggested that the early biochemical elevations might explain the psychiatric signs often associated with the early phases of manganese toxicity, while later biochemical declines may produce the neurologic manifestations.

Newborn rats exposed to 150 mg Mn/kg/day by gavage for 41 days displayed a rigid and unsteady gait from 15-22 days of age (Kristensson et al., 1986). The gait was normal by 44 days of age. Transient effects were also observed in some neurotransmitter levels.

Bonilla and Diez-Ewald (1974) found decreased dopamine levels in female adult rats exposed in drinking water for 7 months to 255 mg Mn/kg/day (2.18 mg Mn/mL x 35 mL + 0.300 kg = 255 mg Mn/kg). Behavioral and histologic parameters were not examined.

The studies reviewed above show marked inconsistencies, even conflicts in the dose-effect function for neurologic effects. Liver effects have also been reported after oral manganese exposure, but these data are also not consistent. Wasserman and Wasserman (1977) reported ultrastructural changes of the liver cell in young male rats exposed to 12.2 mg Mn/kg/day in drinking water for 10 weeks. Shukla et al. (1978) found biochemical changes in the livers of adult male rats exposed to 4.4 mg Mn/kg/day for 30 days. Kimura et al. (1978), however, reported no liver effects in male rats exposed to 56.4 mg Mn/kg/day for 3 weeks. Leung et al. (1982) found higher liver MAO

plateau values in female rats exposed to 38.9, 389 or 778 mg Mn/kg/day for 80 days; however, the effects were not dose related. Hietanen et al. (1981) administered 700 mg Mn/kg/day in drinking water to rats and found changes in several hepatic enzyme activities at 1 week but not at 6 weeks.

Oral administration of manganese to experimental animals has also produced some reproductive effects. Laskey et al. (1982) reported a dose-related decrease in serum testosterone concentrations in young male rats exposed for 100 days to 20, 55 or 177.5 mg Mn/kg/day in the diet. In addition, reduced fertility was found after 224 days in female mice exposed to 177.5 mg Mn/kg/day. Gray and Laskey (1980) reported decreased weight of testes, seminal vesicles and preputial glands in male mice exposed in the diet to 143 mg Mn/kg/day for 90 days.

There are several issues to be considered in performing a risk assessment for ingested manganese. One factor is that of selecting the most appropriate species. The most sensitive and subtle expressions of manganese toxicity reflect action upon the CNS. The neurobehavioral effects observed in humans, however, have not been reproduced in rodents by oral, inhalation or parenteral routes. These exposure routes have produced the characteristic neurobehavioral effects in monkeys. Thus, from the standpoint of modeling the neurotoxic effects observed in humans, studies involving rodents are of limited use.

Another issue to be considered is that of the route of administration of manganese. While the toxicity of ingested manganese is low in laboratory animals,

adverse effects on the central nervous system are apparent at much lower doses following exposure by inhalation. Therefore, in deriving an RfD, an oral study is required because of the large amount of uncertainty involved in route-to-route extrapolation. Unfortunately, only one primate study involving oral administration of manganese is available (Gupta et al., 1980), and it is confined to a single dose level, 6.9 mg/kg/day, that is associated with significant toxicity.

Finally one of the more significant factors that appears to impact the toxicity of manganese is the medium in which it is ingested, particularly food vs. drinking water. Accordingly, the following discussion is divided into two sections, the first describing the development of a dietary RfD for manganese and the second describing the development of a drinking water RfD.

Development of the Dietary RfD for Manganese

Schroeder et al. (1966) reported that patients (number not specified) given 9 mg Mn/day (as manganese citrate) for many months did not show any signs of toxicity. Assuming the patients consumed another 2.5 mg manganese in their normal diet, the total intake would be ~11.5 mg Mn/day. Schroeder et al. (1966) has estimated that a vegetarian diet may provide a manganese intake as high as 13-20 mg/day. These levels are also considered to be safe, but it should be kept in mind that the manganese present in a vegetarian diet may be less bioavailable.

The NAS Food and Nutrition Board has estimated a daily intake of 2-5 mg Mn for adults as being "safe and adequate" (NRC, 1989) and the WHO (1973) concluded that

there was no evidence of manganese toxicity in individuals consuming 8-9 mg Mn/day in food. Little information is available to indicate at what levels manganese in the diet presents a health threat. It is clear however, that the dietary RfD should be establishied above the "safe and adequate levels" of 2-5 mg/day established by NRC.

Based on information from the NAS Food and Nutrition Board (NRC, 1989), Schroeder et al. (1966), and WHO (1973), a dietary manganese intake of 10 mg/day has been chosen to represent a chronic oral human NOAEL. Furthermore, because of the efficient homeostatic control of manganese and its essentiality, this level is thought to be safe for all humans. For a 70 kg adult, this dose converts to 0.14 mg Mn/kg bw/day.

$$RfD\ (food) = \frac{0.14\ mg\ Mn/kg/day}{1} = 0.14\ mg\ Mn/kg/day$$

where:

0.14 mg Mn/kg/day	=	a chronic human NOAEL
1	=	uncertainty factor to be used in conjunction with chronic human data identifying a NOAEL that is safe for all subpopulations.

The oral RfD of 0.14 mg Mn/kg/day for a dietary intake was verified by the RfD Work Group in September 1992 (U.S. EPA, 1993). It is emphasized that this oral RfD is based on total dietary intakes; a separate RfD was derived for manganese in drinking water.

It is important to recognize, however, that while the RfD process involves the determination of a single point estimate of an oral intake, a range of intakes more appropriately fits the science. This is consistent with the definition of the RfD, which is associated "with uncertainty spanning perhaps an order of magnitude." Numerous factors, both environmental (e.g., the presence or absence of many dietary constituents) and biological or host-related (e.g., age, nutritional status, alcohol consumption), can significantly influence an individual's uptake of manganese from the diet. As discussed in Chapter III, there is significant variability in the absorption of manganese by humans. The determination of a single intake of manganese in the diet must be recognized as a process that is limited in its ability to reflect the variable nature of manganese toxicity. It may both over- and underestimate the risk depending on the specific combination of environmental and individual circumstances.

Development of the Drinking Water RfD for Manganese

In contrast to manganese in the diet, two studies using humans have associated high levels of manganese in drinking water with neurologic effects. The first, a case study by Kawamura et al. (1941) reported frank effects in humans who drank well water contaminated with manganese at levels of about 28 mg/L for a few months (see Chapter VI for full discussion). A Greek epidemiologic study by Kondakis et al. (1989; also described in Chapter VI) examined individuals over 50 years of age who consumed water containing manganese at concentrations of 3.6-14.6 µg/L (Area A, mean = 9.1 µg/L); 81.6-252.6 µg/L (Area B, mean = 167 µg/L); or 1600-2300 µg/L (Area C, mean = 1950 µg/L). No effects were observed in individuals from Area B, but some degree of

neurologic impairment was reported in residents of Area C. This study is used to support the calculation of a drinking water RfD for manganese.

$$RfD\ (water) = \frac{0.167\ mg\ Mn/L}{1} \times \frac{2\ L/day}{70\ kg} = 0.0048\ mg/kg/day$$

(rounded to 0.005 mg/kg/day)

where:

0.167 mg Mn/L	=	drinking water concentration of manganese consumed for a lifetime without adverse health effects (Kondakis et al., 1989)
2 L/day	=	assumed water consumption by an adult
70 kg	=	assumed body weight of an adult
1	=	uncertainty factor to be used in conjunction with chronic human data identifying a NOAEL that is safe for all subpopulations

Quantification of Noncarcinogenic Effects for Manganese in Drinking Water

Derivation of 1-Day and 10-Day HAs. There are two human studies involving exposure through drinking water. Kondakis et al. (1989) reported increased manganese content in the hair and possible neurologic impairment of individuals drinking water containing ~2 mg Mn/L. Kawamura et al. (1941) reported that 3 of 25 individuals died following a few months of exposure to at least 28 mg Mn/L of contaminated well water. Several others exhibited neurologic impairment, but children were not affected to the degree that adults were. The study by Kondakis et al. (1989) was used to establish a water-specific RfD of 0.005 mg/kg/day for manganese. Assuming a body weight of 70

kg and a drinking water consumption of 2 L/day, this RfD is equivalent to about 0.2 mg Mn/L drinking water. However, this RfD is for chronic exposure to manganese. Acute exposures do not warrant the same concern. Also, children appear to be less sensitive to the effects of ingested manganese than are adults, particularly the elderly. This is substantiated by the greater requirement of manganese for growth and health maintenance in children (NRC, 1989) and also by the Japanese poisoning (Kawamura et al., 1941) that reported frank effects (including neurologic impairment and deaths) in elderly humans but no effects in children up to 10 years of age.

Unfortunately, there are relatively few data that are appropriate to use in setting short-term health advisories. The NRC has estimated that for infants 6 months to 1 year of age, an intake of 0.6-1 mg Mn/day is safe and adequate. Taking the upper end of this range (1 mg Mn/day) and assuming that the infant's nutrition comes from a maximum of about 1 L of formula per day, this would correspond to a manganese concentration of 1 mg/L. This concentration is higher than the NOAEL of 0.2 mg/L, but lower than the LOAEL of 2 mg/L, identified by Kondakis et al. (1989).

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

where:

1 mg/day	=	intake of manganese considered to be "safe and adequate" for infants (NRC, 1989)
1 L/day	=	assumed water consumption by a child

1	=	uncertainty factor to be used with intake known to be safe for short-term ingestion by humans

Derivation of Longer-term HA. As with the 1- and 10-day HAs, there are no studies appropriate for the derivation specifically of a longer-term health advisory. The basis for the water-specific RfD is considered to provide the best basis upon which to base a longer-term HA.

It is recommended that this level, 0.2 mg Mn/L, be adopted for the longer-term health advisory for manganese. Calculation of separate concentrations for children and adults is not warranted.

Assessment of Lifetime Exposure and Derivation of a DWEL. In the study by Kawamura et al. (1941), three people died and several others were neurologically impaired following exposure for several months to drinking water containing at least 28 mg Mn/L. The study by Kondakis et al. (1989) suggests that a lifetime exposure to drinking water containing ~2 mg Mn/L results in an increased retention of manganese (as demonstrated by an increased concentration of manganese in hair) and possible neurologic impairment. It is noted, however, that the confidence in this assessment is compromised by the lack of data on dietary manganese in the three populations under study. Also, many of the endpoints scored in the neurological examination are not specific for manganese poisoning, and are, in fact, associated with the normal process of aging.

Based on the study by Kondakis et al. (1989), the RfD/RfC Work Group verified a water-specific RfD for manganese of 0.005 mg/kg/day (U.S. EPA, 1993). This is used as the basis for the DWEL:

$$DWEL = \frac{0.005\ mg/kg/day \times 70\ kg}{2\ L/day} = 0.175\ mg/L\ (rounded\ to\ 0.2\ mg/L)$$

where:

0.005 mg/kg/day	=	RfD (drinking water-specific)
70 kg	=	assumed body weight of an adult
2 L/day	=	assumed water consumption of an adult

Because the DWEL is based on a water-specific RfD that assumes a normal dietary intake of manganese, it is not necessary to factor in a relative source contribution when establishing drinking water standards. This assumption is made primarily because the differences in the bioavailability of manganese in food as compared with that of manganese in water may be such that it is inappropriate to add these intakes together. Unfortunately, while it is agreed that the bioavailability of manganese may vary substantially, relatively few data are available to quantitate these differences, and the number of variables that may affect the uptake of manganese are such that to determine a single value for the absorption of manganese from any medium is not appropriate. These issues have been discussed in Chapter III of this document.

While the RfD process involves the determination of a single point estimate of an oral intake, it must be recognized that a range of intakes more appropriately fits the science. This is consistent with the definition of the RfD, which is associated "with

uncertainty spanning perhaps an order of magnitude." Numerous factors, both environmental (e.g., the presence of high or low levels of other inorganics in drinking water) and biological or host-related (e.g., age, nutritional status, alcohol consumption), can significantly influence the uptake of manganese by an individual. The determination of a single concentration of manganese in drinking water, then, must be recognized as a process that is limited in its ability to reflect the variable nature of manganese toxicity.

Finally, while a concentration of 0.2 mg Mn/L is recommended for health based reasons, it is noted that a concentration of <0.05 mg Mn/L should be maintained to prevent undesirable taste and discoloration (U.S. EPA, 1984).

Weight-of-Evidence for Carcinogenic Effects

No epidemiologic information relating manganese exposure to cancer occurrence in humans is available. Although there is some evidence of carcinogenic activity in laboratory animals exposed to manganese, problems exist with regard to the relevance of these studies to human carcinogenesis.

In a 2-year bioassay, groups of F344 rats (70/sex) were administered 0, 1500, 5000 or 15,000 ppm manganese sulfate monohydrate (NTP, 1992). These dietary concentrations were reported to be equivalent to an intake ranging from 91 mg/kg/day (30 mg Mn/kg/day) for low-dose males to 1019 mg/kg/day (331 mg Mn/kg/day) for high-dose males. For females, the range of intakes was from 81 mg/kg/day (26 mg Mn/kg/day) for the low-dose group to 833 mg/kg/day (270 mg Mn/kg/day) for the high-

dose males. For females, the range of intakes was from 81 mg/kg/day (26 mg Mn/kg/day) for the low-dose group to 833 mg/kg/day (270 mg Mn/kg/day) for the high-dose group. No increases in any tumor type reported were attributed to manganese exposure in rats.

In the same study, groups of B6C3F1 mice (70/sex) were administered 0, 1500, 5000 or 15,000 ppm manganese sulfate monohydrate (NTP, 1992). These dietary concentrations were reported to be equivalent to an intake ranging from 194 mg/kg/day (63 mg Mn/kg/day) for low-dose males to 2222 mg/kg/day (722 mg Mn/kg/day) for high-dose males. For females, the range of intakes was from 238 mg/kg/day (77 mg Mn/kg/day) for the low-dose group to 2785 mg/kg/day (905 mg Mn/kg/day) for the high-dose group. Incidences of thyroid follicular cell hyperplasia were significantly greater in high-dose males and females than in controls. The incidence of follicular cell adenomas was 0/50, 0/49, 0/51 and 3/50 (6%) for control, low-, mid- and high-dose males, respectively. The historical control range for males was reported to be 0-4%. For females, the incidence of follicular cell adenomas was 2/50, 1/50, 0/49 and 5/51 (10%) for control, low-, mid- and high-dose groups, respectively. The historical control range for females was reported to be 0-9%. None of the reported incidences were statistically significantly increased over historical controls, nor were they clearly dose-related. Also, the follicular cell tumors were seen only at the termination of the study (729 days) and only slightly increased relative to the historical control range in the highest dose groups. NTP (1992) reported that the manganese intakes in the high-dose mice was 107 times higher than the recommended dietary allowance. While NTP concluded that the data provide "equivocal evidence" of carcinogenic activity of manganese in mice, the

relevance of these findings to human carcinogenesis is questionable, particularly because of the very large intakes of manganese required to elicit a response seen only at the end of the study, and at frequencies not statistically significantly different from historical controls.

In a study by DiPaolo (1964), a larger percentage of DBA/1 mice exposed subcutaneously and intraperitoneally to manganese chloride developed lymphosarcomas compared with controls. A thorough evaluation of these results was not possible because they were published in abstract form and lacked sufficient detail (U.S. EPA, 1984). Stoner et al. (1976) found a higher frequency of lung tumors in strain A/Strong mice exposed intraperitoneally to manganese sulfate compared with controls. The study results, although suggestive of carcinogenic activity, do not conclusively meet the criteria for establishment of a positive response, namely, an increase in the mean number of tumors per mouse and an evident dose-response relationship (Shimkin and Stoner, 1975). Furst (1978) found an increased incidence of fibrosarcomas at the injection site in F344 rats exposed intramuscularly to manganese acetylacetonate, but not other tumors.

In a series of genetic toxicology assays performed by NTP (1992), manganese sulfate monohydrate was not found to be mutagenic in *Salmonella typhimurium* strains TA97, TA98, TA100, TA1535 or TA1537, either with or without metabolic (S9) activation. Likewise, mutations were not induced in the sex-linked recessive lethal assay in *Drosophila melanogaster*. However, sister chromatid exchanges and chromosomal

aberrations were induced in Chinese hamster ovary cells in the absence of S9; only the sister chromatid exchange test was positive with S9 (NTP, 1992).

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 (05/25/88) by the CRAVE Work Group of the U.S. EPA. This classification will be re-evaluated when the NTP bioassay (NTP, 1992) is available in final form.

Existing Guidelines, Recommendations and Standards

A maximum concentration of 0.05 mg/L has been recommended for manganese in freshwater to prevent undesirable taste and discoloration (WHO, 1970; U.S. PHS, 1962; U.S. EPA, 1976). No criteria or standards based upon toxicity have been proposed. For the protection of consumers of marine mollusks, a criterion for manganese of 0.1 mg/L for marine waters has been recommended (U.S. EPA, 1976). The rationale for this criterion has not been specified, but is partially based on the observation that manganese can bioaccumulate in marine mollusks (U.S. EPA, 1984).

In quantifying acceptable intakes for manganese, it is important to consider the essentiality of this metal. The Food and Nutrition Board of the National Research Council (NRC, 1989) has determined the Estimated Safe and Adequate Daily Dietary Intake (ESADDI) range for manganese to be 0.7-1.5 mg/day for infants and children and 2-5 mg/day for teenagers and adults.

exposure (Mena et al., 1969, 1974). Mena et al. (1974) reported that the early neonatal period may be critical for manganese accumulation because very young rats have increased intestinal absorption and retention of manganese. Other heavy metals show similarly increased absorption in the young; this does not necessarily mean increased potential for toxicity because it may reflect a higher nutritional requirement.

The developing fetus may also be at risk. Manganese penetrates the placental barrier (Schroeder et al., 1966) and accumulates in the fetus such that its concentration is 7-9% higher than in adult tissues (Widdowson et al., 1972). Manganese also penetrates the blood brain barrier with the rate being 4 times higher in the newborn rat compared with adults (Mena, 1974). Again, the increased uptake of manganese by the fetus and neonate may reflect a higher nutritional need and may not necessarily indicate an increased risk of toxicity.

The aged may be at increased risk for manganese toxicity because of a decrease in adaptive responsiveness (Rothand and Adleman, 1975). Silbergeld (1982) also points out that in manganese toxicity, neurotoxicity involves the basal ganglia and monoaminergic pathways that are themselves commonly affected by aging.

In a study sponsored by the Food and Drug Administration (Pennington et al., 1986), the daily intake of 11 essential minerals was estimated based on the consumption of 234 foods by eight different age-sex groups. The daily intake of manganese was 1.1-1.5 mg/day for infants and children and 1.8-2.7 mg/day for teenagers and adults.

The forementioned studies are all based on a total dietary intake of manganese. This information was used by the RfD Work Group as the basis for the oral RfD (food), which was calculated to be 0.14 mg/kg/day (verified in September, 1992). A separate water RfD of 0.005 mg/kg/day was also verified based on the Greek epidemiologic study by Kondakis et al. (1989). Additional data on the bioavailability of manganese from water and various foods are needed to increase the level of confidence that can be placed on these estimates.

Special Groups at Risk

Although several researchers have noted marked differences in individual susceptibility to inhaled manganese (Rodier, 1955; Penalver, 1955; Cotzias, 1958), suggesting that an impaired ability to clear inhaled manganese or to excrete absorbed manganese results in an increased risk of adverse effects, no studies exist to confirm these hypotheses. Individuals suffering from alcoholism, syphilis and lesions of the excretory system have been inferred to be at greater risk (U.S. EPA, 1984), but there is no supporting epidemiologic evidence.

Individuals with iron deficiency show increased rates of manganese absorption. They are, therefore, assumed to be at greatest risk of adverse effects from manganese

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