TOXICOLOGICAL PROFILE FOR MANGANESE

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Public Health Service
Agency for Toxic Substances and Disease Registry

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DISCLAIMER

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UPDATE STATEMENT

A Toxicological Profile for manganese was released in July 1992. This edition supersedes any previously released draft or final profile.

Toxicological Profiles are revised and republished as necessary, but no less than once every three years. For information regarding the update status of previously released profiles, contact ATSDR at:

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Division of Toxicology/Toxicology Information Branch
1600 Clifton Road NE, E-29
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FOREWORD

This toxicological profile is prepared in accordance with guidelines* developed by the Agency for Toxic Substances and Disease Registry (ATSDR) and the Environmental Protection Agency (EPA). The original guidelines were published in the *Federal Register* on April 17, 1987. Each profile will be revised and republished as necessary.

The ATSDR toxicological profile succinctly characterizes the toxicologic and adverse health effects information for the hazardous substance described therein. Each peer-reviewed profile identifies and reviews the key literature that describes a hazardous substance's toxicologic properties. Other pertinent literature is also presented, but is described in less detail than the key studies. The profile is not intended to be an exhaustive document; however, more comprehensive sources of specialty information are referenced.

The focus of the profiles is on health and toxicologic information; therefore, each toxicological profile begins with a public health statement that describes, in nontechnical language, a substance's relevant toxicological properties. Following the public health statement is information concerning levels of significant human exposure and, where known, significant health effects. The adequacy of information to determine a substance's health effects is described in a health effects summary. Data needs that are of significance to protection of public health are identified by ATSDR and EPA.

Each profile includes the following:

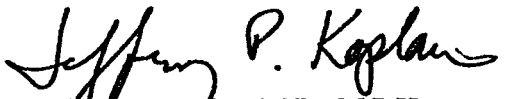
(A) The examination, summary, and interpretation of available toxicologic information and epidemiologic evaluations on a hazardous substance to ascertain the levels of significant human exposure for the substance and the associated acute, subacute, and chronic health effects;

(B) A determination of whether adequate information on the health effects of each substance is available or in the process of development to determine levels of exposure that present a significant risk to human health of acute, subacute, and chronic health effects; and

(C) Where appropriate, identification of toxicologic testing needed to identify the types or levels of exposure that may present significant risk of adverse health effects in humans.

The principal audiences for the toxicological profiles are health professionals at the Federal, State, and local levels; interested private sector organizations and groups; and members of the public.

This profile reflects ATSDR's assessment of all relevant toxicologic testing and information that has been peer-reviewed. Staff of the Centers for Disease Control and Prevention and other Federal scientists have also reviewed the profile. In addition, this profile has been peer-reviewed by a nongovernmental panel and was made available for public review. Final responsibility for the contents and views expressed in this toxicological profile resides with ATSDR.



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*Legislative Background

The toxicological profiles are developed in response to the Superfund Amendments and Reauthorization Act (SARA) of 1986 (Public Law 99-499) which amended the Comprehensive Environmental Response, Compensation, and Liability Act of 1980 (CERCLA or Superfund). This public law directed ATSDR to prepare toxicological profiles for hazardous substances most commonly found at facilities on the CERCLA National Priorities List and that pose the most significant potential threat to human health, as determined by ATSDR and the EPA. The availability of the revised priority list of 275 hazardous substances was announced in the *Federal Register* on October 21, 1999 (64 FR 56792). For prior versions of the list of substances, see *Federal Register* notices dated April 17, 1987 (52 FR 12866); October 20, 1988 (53 FR 41280); October 26, 1989 (54 FR 43619); October 17,1990 (55 FR 42067); October 17, 1991 (56 FR 52166); October 28, 1992 (57 FR 48801); February 28, 1994 (59 FR 9486); April 29, 1996 (61 FR 18744); and November 17, 1997 (62 FR 61332). Section 104(i)(3) of CERCLA, as amended, directs the Administrator of ATSDR to prepare a toxicological profile for each substance on the list.

QUICK REFERENCE FOR HEALTH CARE PROVIDERS

Toxicological Profiles are a unique compilation of toxicological information on a given hazardous substance. Each profile reflects a comprehensive and extensive evaluation, summary, and interpretation of available toxicologic and epidemiologic information on a substance. Health care providers treating patients potentially exposed to hazardous substances will find the following information helpful for fast answers to often-asked questions.

Primary Chapters/Sections of Interest

Chapter 1: Public Health Statement: The Public Health Statement can be a useful tool for educating patients about possible exposure to a hazardous substance. It explains a substance's relevant toxicologic properties in a nontechnical, question-and-answer format, and it includes a review of the general health effects observed following exposure.

Chapter 2: Health Effects: Specific health effects of a given hazardous compound are reported by *route of exposure*, by *type of health effect* (death, systemic, immunologic, reproductive), and by *length of exposure* (acute, intermediate, and chronic). In addition, both human and animal studies are reported in this section.

> ***NOTE:*** Not all health effects reported in this section are necessarily observed in the clinical setting. Please refer to the Public Health Statement to identify general health effects observed following exposure.

Pediatrics: Four new sections have been added to each Toxicological Profile to address child health issues:

Section 1.6 How Can Manganese Affect Children?
Section 1.7 How Can Families Reduce the Risk of Exposure to Manganese?
Section 2.6 Children's Susceptibility
Section 5.6 Exposures of Children

Other Sections of Interest:

Section 2.8 Biomarkers of Exposure and Effect
Section 2.11 Methods for Reducing Toxic Effects

ATSDR Information Center

Phone: 1-800-447-1544 (to be replaced by 1-888-42-ATSDR in 1999)
or 404-639-6357 ***Fax:*** 404-639-6359
E-mail: atsdric@cdc.gov ***Internet: http://atsdr1.atsdr.cdc.gov:8080***

The following additional material can be ordered through the ATSDR Information Center:

Case Studies in Environmental Medicine: Taking an Exposure History—The importance of taking an exposure history and how to conduct one are described, and an example of a thorough exposure history is provided. Other case studies of interest include *Reproductive and Developmental Hazards; Skin Lesions and Environmental Exposures; Cholinesterase-Inhibiting Pesticide Toxicity;* and numerous chemical-specific case studies.

Managing Hazardous Materials Incidents is a three-volume set of recommendations for on-scene (prehospital) and hospital medical management of patients exposed during a hazardous materials incident. Volumes I and II are planning guides to assist first responders and hospital emergency department personnel in planning for incidents that involve hazardous materials. Volume III—*Medical Management Guidelines for Acute Chemical Exposures*—is a guide for health care professionals treating patients exposed to hazardous materials.

Fact Sheets (ToxFAQs) provide answers to frequently asked questions about toxic substances.

Other Agencies and Organizations

The National Center for Environmental Health (NCEH) focuses on preventing or controlling disease, injury, and disability related to the interactions between people and their environment outside the workplace. *Contact:* NCEH, Mailstop F-29, 4770 Buford Highway, NE, Atlanta, GA 30341-3724 • Phone: 770-488-7000 • FAX: 770-488-7015.

The National Institute for Occupational Safety and Health (NIOSH) conducts research on occupational diseases and injuries, responds to requests for assistance by investigating problems of health and safety in the workplace, recommends standards to the Occupational Safety and Health Administration (OSHA) and the Mine Safety and Health Administration (MSHA), and trains professionals in occupational safety and health. *Contact:* NIOSH, 200 Independence Avenue, SW, Washington, DC 20201 • Phone: 800-356-4674 or NIOSH Technical Information Branch, Robert A. Taft Laboratory, Mailstop C-19, 4676 Columbia Parkway, Cincinnati, OH 45226-1998 • Phone: 800-35-NIOSH.

*The National Institute of Environmental Health Sciences (*NIEHS) is the principal federal agency for biomedical research on the effects of chemical, physical, and biologic environmental agents on human health and well-being. *Contact:* NIEHS, PO Box 12233, 104 T.W. Alexander Drive, Research Triangle Park, NC 27709 • Phone: 919-541-3212.

Referrals

The Association of Occupational and Environmental Clinics (AOEC) has developed a network of clinics in the United States to provide expertise in occupational and environmental issues. *Contact:* AOEC, 1010 Vermont Avenue, NW, #513, Washington, DC 20005 • Phone: 202-347-4976 • FAX: 202-347-4950 • e-mail: ***aoec@dgs.dgsys.com*** • AOEC Clinic Director: http://occ-env-med.mc.duke.edu/oem/aoec.htm.

The American College of Occupational and Environmental Medicine (ACOEM) is an association of physicians and other health care providers specializing in the field of occupational and environmental medicine. *Contact:* ACOEM, 55 West Seegers Road, Arlington Heights, IL 60005 • Phone: 847-228-6850 • FAX: 847-228-1856.

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THE PROFILE HAS UNDERGONE THE FOLLOWING ATSDR INTERNAL REVIEWS:

1. Health Effects Review. The Health Effects Review Committee examines the health effects chapter of each profile for consistency and accuracy in interpreting health effects and classifying end points.

2. Minimal Risk Level Review. The Minimal Risk Level Workgroup considers issues relevant to substance-specific minimal risk levels (MRLs), reviews the health effects database of each profile, and makes recommendations for derivation of MRLs.

3. Data Needs Review. The Research Implementation Branch reviews data needs sections to assure consistency across profiles and adherence to instructions in the Guidance.

PEER REVIEW

A peer review panel was assembled for manganese. The panel consisted of the following members:

1. Michael Aschner, Professor, Wake Forest University School of Medicine, Medical Center Building, Winston-Salem, NC 27151
2. Christopher Newland, Professor, Auburn University, Department of Psychology, 110 Thach Hall, Auburn, AL 36849-5212
3. Donna Mergler, Professor, CINBOISE, Universite du Quebec a Montreal, CP 8888, Succ Centreville, Montreal, Quebec, H3C 3P8, Canada
4. Joseph Zayed, Professor, University of Montreal, Faculty of Medicine, Department of Occupational & Environmental Health, TOXHUM (Human Toxicology Research Group), 2375 Cote Ste. Catherine, Montreal, Quebec, H3C 3J7, Canada

These experts collectively have knowledge of manganese's physical and chemical properties, toxicokinetics, key health end points, mechanisms of action, human and animal exposure, and quantification of risk to humans. All reviewers were selected in conformity with the conditions for peer review specified in Section 104(I)(13) of the Comprehensive Environmental Response, Compensation, and Liability Act, as amended.

Scientists from the Agency for Toxic Substances and Disease Registry (ATSDR) have reviewed the peer reviewers' comments and determined which comments will be included in the profile. A listing of the peer reviewers' comments not incorporated in the profile, with a brief explanation of the rationale for their exclusion, exists as part of the administrative record for this compound. A list of databases reviewed and a list of unpublished documents cited are also included in the administrative record.

The citation of the peer review panel should not be understood to imply its approval of the profile's final content. The responsibility for the content of this profile lies with the ATSDR.

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1. PUBLIC HEALTH STATEMENT

This public health statement tells you about manganese and the effects of exposure.

The Environmental Protection Agency (EPA) identifies the most serious hazardous waste sites in the nation. These sites make up the National Priorities List (NPL) and are the sites targeted for long-term federal cleanup activities. Manganese has been found in at least 603 of the 1,517 current or former NPL sites. However, the total number of NPL sites evaluated for this substance is not known. As more sites are evaluated, the sites at which manganese is found may increase. This information is important because exposure to this substance may harm you and because these sites may be sources of exposure.

When a substance is released from a large area, such as an industrial plant, or from a container, such as a drum or bottle, it enters the environment. This release does not always lead to exposure. You are exposed to a substance only when you come in contact with it. You may be exposed by breathing, eating, or drinking the substance or by skin contact.

If you are exposed to manganese, many factors determine whether you'll be harmed. These factors include the dose (how much), the duration (how long), and how you come in contact with it. You must also consider the other chemicals you're exposed to and your age, sex, diet, family traits, lifestyle, and state of health. This chapter discusses adverse (negative) effects from exposure to "high levels" or "too much" manganese. In general, these terms refer to levels of manganese reported in occupational settings, such as battery plants or smelters. Most people are not likely to be exposed to such high levels of manganese in a typical day. However, each person's body handles manganese differently; therefore, it is not possible to predict at what level of manganese a person would begin to show symptoms of health effects from exposure to increased manganese.

1.1 WHAT IS MANGANESE?

Manganese is a naturally occurring substance found in many types of rock. Manganese does not have a special taste or smell. Pure manganese is a silver-colored metal; however, it does not occur in the environment as a pure metal. Rather, it occurs combined with other substances such as oxygen, sulfur, and chlorine. These forms (called compounds) are solids that do not evaporate. However, small dust particles of the solid material can become suspended in air. Also, some manganese compounds can dissolve in water, and low levels of these compounds are normally present in lakes, streams, and the ocean. Manganese can change from one compound to another (either by natural processes or by human activity), but it does not break down or disappear in the environment.

Rocks with high levels of manganese compounds are mined and used to produce manganese metal. This manganese metal is mixed with iron to make various types of steel. Some manganese compounds are used in the production of batteries, in dietary supplements, and as ingredients in some ceramics, pesticides, and fertilizers.

Manganese is an essential trace element and is necessary for good health. The human body typically contains small quantities of manganese, and under normal circumstances, the body controls these amounts so that neither too little nor too much is present.

Different forms of manganese are discussed in this profile. These forms are either inorganic manganese or organic manganese. The inorganic manganese includes those forms of the element such as combustion products from cars or trucks, as well as the dusts that are present in steel or battery factories. Organic forms of manganese that are discussed are a gasoline additive, two pesticides, and a compound used in hospitals to test if a patient has certain types of cancer. The profile discusses what is known about the amount of these compounds that can be toxic to people and how these compounds can affect people's health.

Chapters 3, 4, and 5 have more information on the properties and uses of manganese and how it behaves in the environment.

1.2 WHAT HAPPENS TO MANGANESE WHEN IT ENTERS THE ENVIRONMENT?

Manganese and manganese compounds exist naturally in the environment as solids in the soil and as small particles in water. Manganese may also be present in small dust-like particles in the air. These manganese-containing particles usually settle out of the air within a few days depending on their size, weight, density, and the weather conditions. Manganese exists naturally in rivers and lakes, and is also naturally present in some underground water. Algae and plankton in the water can consume some manganese and concentrate it within themselves.

In addition to occurring naturally in the environment, manganese can be introduced by human activity. Manganese can be released into the air by industry and by the burning of fossil fuels. More specifically, sources of airborne manganese include iron- and steel-producing plants, power plants, coke ovens, and dust from uncontrolled mining operations. Manganese released from burning a gasoline additive may also be a source of manganese in the air. Manganese from these human-made sources can enter surface water, groundwater, and sewage waters. Small manganese particles can also be picked up by water flowing through landfills and soil. The chemical state of manganese and the type of soil determine how fast it moves through the soil and how much is retained in the soil. Maneb and mancozeb, two pesticides that contain manganese, may also add to the amount of manganese in the environment when they are applied to crops or released to the environment from packaging factories. There is information on the amount of maneb and mancozeb released into the environment from facilities that make or use these pesticides. However, the amount of manganese in the environment because of the release and use of these pesticides is not known.

To avoid staining clothes or plumbing fixtures, the EPA recommends that the concentration of manganese in drinking water not be more than 0.05 ppm. FDA has set the same level for bottled water. This concentration is believed to be more than adequate to protect human health. The EPA

has also established rules that set limits on the amount of manganese that factories can dump into water. EPA requires factories that use or produce manganese to report how much they dump in the environment. OSHA has set limits of 5 mg/m^3 for fume and 0.2 mg/m^3 for particulate matter as the average amounts of manganese in workplace air over 8-hour workday (OSHA 1998). Similarly, the ACGIH (American Conference of Governmental Industrial Hygienists) has set a limit of 1 mg/m^3 for manganese fume and 0.2 mg/m^3 for the average amount of manganese, either elemental or as inorganic compounds, that can be present in the air over an 8-hour workday (ACGIH 1998).

For more information on manganese in the environment, see Chapter 5.

1.3 HOW MIGHT I BE EXPOSED TO MANGANESE?

Because manganese is a natural component of the environment, you are always exposed to low levels of it in water, air, soil, and food. In drinking water, levels are usually about 0.004 parts manganese per million parts (ppm) of water. In air, levels are usually about 0.00002 milligrams manganese per cubic meter (mg/m^3) of air. Natural levels in soil usually range from 40 to 900 ppm. Manganese is also a normal part of living things, including both plants and animals, so it is present in foods. For nearly all people, food is the main source of manganese, and usual daily intakes range from about 1 to 10 mg/day. The exact amount you take in depends on your diet.

You are most likely to be exposed to higher-than-usual levels of manganese or manganese-containing chemicals if you work in a factory where manganese metal is produced from manganese ores or where manganese compounds are used to make steel or other products. In these factories you would be exposed mainly by breathing in manganese dust. If you live near such a factory you could also be exposed to higher-than-usual levels of manganese dust in the outside air, although the amounts would be much lower than in the factory. You might be exposed to higher-than-usual levels if you live near a coal- or oil-burning factory because manganese is released into the air when these fossil fuels are burned. Some areas of the country use a

gasoline that has manganese added to it to increase performance. You could also be exposed to higher-than-usual levels of manganese if you live in a major urban area where such gasoline is used, if you have a job in which you make or have contact with that gasoline every day (such as a mechanic), or if you are exposed to a high amount of car exhaust on a daily basis (at bus stops, gas stations, etc.). You can also be exposed to manganese if you use pesticides that contain it. People who deal with such pesticides may be exposed through skin contact, but there have been instances in which workers may have accidentally eaten or inhaled some pesticides. You may also be exposed to manganese by eating foods that contain small, leftover amounts (residues) of these pesticides.

If manganese compounds, either naturally-occurring or from a factory or a hazardous waste site, get into water, you could be exposed to higher-than-usual levels by drinking the water.

See Chapter 5 for more information on how you might be exposed to manganese or its compounds.

1.4 HOW CAN MANGANESE ENTER AND LEAVE MY BODY?

Humans are exposed to manganese in the food and water they eat and drink and in the air they breathe. Infants eat manganese that is present in breast milk, soy-based infant formulas, or cow's milk. The amount of manganese in these sources is generally not a problem, and they provide the manganese that is necessary for normal functioning of the body. If you live near a hazardous waste site, you could possibly eat or drink higher-than usual levels of manganese that are in soil or water or breathe manganese-containing dust particles in the air that come from the waste site. The contribution of these exposure routes to manganese's toxicity is uncertain; in general, adverse effects in people exposed through these routes have only been reported when environmental manganese levels were quite high. If you get manganese-contaminated soil or water on your skin, very little will enter your body, so this is not of concern. If you swallow manganese in water or in soil, most is excreted in the feces. However, about 3–5% is usually taken up and kept in the body. If you breathe air containing manganese dust, many of the smaller

dust particles will be trapped in your lungs. Some of the manganese in these small particles may then dissolve in the lungs and enter the blood. The exact amount that may enter the blood is not known. Larger particles and those that do not dissolve will be coughed up, in a sticky layer of mucus, out of the lungs and into the throat, where they will be swallowed and will enter the stomach.

Manganese is a regular part of the human body; it is a necessary component in order for the body to work properly. The body normally controls the amount of absorbed manganese. For example, if large amounts of manganese are eaten in the diet, the body excretes large amounts in the feces. Therefore, the total amount of manganese in the body tends to stay about the same, even when exposure rates are higher or lower than usual. However, if too much manganese is taken in, the body may not be able to adjust for the added amount.

See Chapter 2 for more information on how manganese enters and leaves the body.

1.5 HOW CAN MANGANESE AFFECT MY HEALTH?

To protect the public from the harmful effects of toxic chemicals and to find ways to treat people who have been harmed, scientists use many tests.

One way to see if a chemical will hurt people is to learn how the chemical is absorbed, used, and released by the body; for some chemicals, animal testing may be necessary. Animal testing may also be used to identify health effects such as cancer or birth defects. Without laboratory animals, scientists would lose a basic method to get information needed to make wise decisions to protect public health. Scientists have the responsibility to treat research animals with care and compassion. Laws today protect the welfare of research animals, and scientists must comply with strict animal care guidelines.

Manganese is an essential nutrient, and eating a small amount of it each day is important to stay healthy. Manganese is present in many foods, including grains and cereals, and is found in high

concentrations in many foods, such as tea. The amount of manganese in typical western diets (about 1–10 mg manganese per day) appears to be enough to meet daily needs. Human diets with too little manganese can lead to slowed blood clotting, skin problems, changes in hair color, lowered cholesterol levels, and other alterations in metabolism. In animals, eating too little manganese can interfere with normal growth, bone formation, and reproduction.

Too much manganese may also cause serious illness. Most manganese compounds seem to cause the same effects, although it is unknown whether exposure to different manganese compounds results in slight differences in adverse effects. Manganese miners or steel workers exposed to high levels of manganese dust in air may have mental and emotional disturbances, and their body movements may become slow and clumsy. This combination of symptoms is a disease called 'manganism.' Workers do not usually develop symptoms of manganism unless they have been exposed to manganese for many months or years. Manganism occurs because too much manganese injures a part of the brain that helps control body movements. Some of the symptoms of manganism may improve upon certain medical treatments, but the improvements are usually temporary, and the brain injury is permanent. Manganism has been reported most often in miners. It has only been reported a few times in other workers exposed to the metal, such as steel workers. The symptoms most commonly observed in occupational workers (other than miners) include difficulty in the following motor skills: holding one's hand steady, performing fast hand movements, and maintaining balance when tested. These symptoms are not as severe as those related to manganism, indicating that the effects caused by manganese over-exposure are related to the level of exposure.

Most people who inhale manganese are involved in jobs where they are exposed to the metal. There is a possibility that people can be exposed to manganese in the air if they live near a plant that uses manganese, or if they live in a high traffic area and the automobiles burn manganese in the gasoline. A recent study showed that people who inhaled manganese from the air and who had high levels of manganese in their blood showed signs of neurological problems that were similar to those reported in occupationally-exposed persons. The neurological problems were most significant in the people aged 50 years and older.

It is not certain whether eating or drinking too much manganese can cause symptoms of manganism. In one report, people who drank water containing high concentrations of manganese developed a number of symptoms that were similar to those seen in manganese miners and steel workers. However, it is not clear whether these effects were caused by the manganese alone; other effects were noted, suggesting that other compounds may have been involved. In another report, people who drank water with above-average levels of manganese seemed to have a slightly higher frequency of symptoms such as weakness, stiff muscles, and trembling hands. However, these symptoms are not specific for manganism and might have been caused by other factors. Another study discovered that people who ate food with high concentrations of manganese, while also eating a diet low in magnesium, suffered nerve disease. Another study in adults over 40 years old who drank water with high manganese levels for at least 10 years reported no changes in behavior and no symptoms, that commonly occur in people exposed to excess levels of manganese. Two studies reported that children who drank water and who ate food with higher-than-usual levels of manganese did more poorly in school and on specific tests that measure coordination than children who had not eaten above-average amounts of manganese. However, these studies included several limitations; it is not clear whether the adverse effects in the children were caused only by eating too much manganese.

Studies in animals have shown that very high levels of manganese in food or water can cause changes in the brain. This information suggests that high levels of manganese in food or water might cause changes in the function of the nervous system. However, people exposed to manganese concentrations typically found in food, water, or air have little cause for concern.

Breathing too much manganese dust over a short or long time can cause irritation of the lungs. Sometimes this makes breathing difficult, and it can also increase the chances of getting a lung infection, such as pneumonia. However, this can happen from breathing in many kinds of dust particles and not just those that contain manganese.

A common effect in men who are exposed to high levels (levels seen in some occupational studies) of manganese dust in the air over a long time is impotence. Studies in animals show that too much

manganese may also injure the testes. Much less is known about the effects of too much manganese on women's ability to reproduce. Studies in animals suggest that too much manganese can negatively affect a female's ability to reproduce.

No studies have been done to determine whether breathing manganese dust causes cancer in humans. Some studies in animals show that eating large amounts of manganese might increase the chances of getting cancer. However, only a few animals in these studies developed cancer, and it was difficult to tell whether the tumors were really caused by the excess manganese. Thus, there is little evidence to suggest that cancer is a major concern for people exposed to manganese in the environment or near hazardous waste sites. The EPA has determined that manganese is not classifiable as a human carcinogen.

One compound that contains manganese, potassium permanganate, damages the skin. Two other compounds that contain manganese, the pesticides maneb and mancozeb, can cause skin reactions in people who have allergies to these pesticides. Skin rashes can occur because of these allergies, but once the exposure to the pesticide is stopped, the rashes and any other effects will usually go away. However, once a person has developed an allergy to a particular manganese-containing pesticide, that person may have similar allergic reactions to different, but related, pesticides.

The negative adverse effects of exposure to excess levels of manganese have been observed in all ages. Several studies in humans and animals indicate that the elderly may be a potentially susceptible population to the adverse effects of manganese exposure. Further, studies show that the young may also be a susceptible population. Effects of exposure to high levels of manganese in children are discussed in section 1.6.

Chapter 2 has more information on the health effects of manganese exposure in humans and animals.

1.6 HOW CAN MANGANESE AFFECT CHILDREN?

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans. Potential effects on children resulting from exposures of the parents are also considered.

Children, like adults, are primarily exposed to manganese through the food they eat. The human diet typically provides the amount of manganese required for the normal functioning of a healthy body. Children, like adults, can also inhale manganese if it is present in the air.

In their daily activities, children contact a very different physical environment than adults do. Therefore, their behavior in their surroundings might allow them to contact manganese in ways in which adults typically would not. Young children sometimes eat dirt on purpose and often eat dirt accidentally by putting their hands into their mouths. If the soil contains manganese, children can be exposed to manganese in this unique way. However, there is little information on how well manganese in soil can be taken up from the stomach into the body if children eat it. Most soils contain a background concentration of the metal (values range from 40–900 ppm, with an average estimated at 330 ppm). However, eating small amounts of soils containing background concentrations of manganese should not cause harm to most healthy children because of the tight control the body has over the amount of manganese it maintains.

No studies have discovered how much manganese children need to stay healthy or how much manganese they absorb from all environmental sources. Therefore, it is not known whether the amount of manganese per kilogram of body weight that children take into their bodies through eating or breathing is different from that amount in adults. Animal studies indicate that infant rats take in and retain more manganese than adult rats; therefore, infants and young children may also take up more manganese than adults.

Children who ate or drank above-average amounts of manganese did more poorly in school and on tests that measure coordination than other children who had not eaten increased amounts of

manganese. Although the amounts of manganese in the water and food were measured, the amounts eaten by the children were not known. However, the studies that reported these results in children had several flaws; it is unclear if eating too much manganese was the cause for the difference in the children.

Adverse health effects have also been observed in children who cannot get rid of extra manganese from their body, such as children whose livers do not function properly. These effects include a lack of control over movements in their arms and legs, a tendency to overbalance when walking, and uncontrollable shaking in their arms and hands. In addition to children with problems removing excess manganese from their bodies, some, but not all, children who must have liquid-form nutrition injected into their veins, called total parenteral nutrition (TPN), have also shown these effects. In the cases involving liquid diets, the children had no control over the foods they ate, and there may have been too much manganese in the liquid food. These same effects have been observed in adults with similar liver conditions or on liquid diets. More serious health effects are typically observed only in people who have inhaled manganese in a work environment for many years. These occupational environments tend to have manganese levels that are much higher than the typical environment (10–70 nanograms/m^3 in urban areas with no significant sources of manganese). The severe and permanent neurological effects and mood swings that might be anticipated from occupational studies of adults have not been reported in children. Workers who have been overexposed to manganese particles in the air have suffered wild mood swings, uncontrollable laughter or crying at inappropriate times, and abnormal facial expressions (stiff with grimacing or blank with no expression). Similar effects have also been seen in monkeys who have been injected with low levels of manganese for only a few days. These serious effects of manganese overexposure might be expected in children who have been exposed to high concentrations of manganese for extended periods, although it is not known for sure. The levels of manganese children would have to breathe or eat before they showed these effects is not known.

Limited information suggests that higher-than-usual amounts of manganese can cause birth defects. One study in humans suggests that high levels of exposure to environmental manganese

(in the soil, water, air, or food) might increase the chances of birth defects. However, it is not possible to reach a conclusion from this study because other factors were present that may have caused the birth defects. Studies involving animals exposed to manganese in air are limited. One study in animals shows that exposure of pregnant females to high levels of manganese in air resulted in decreased body weight in the pups. Other studies investigating birth defects have used different exposure methods. One study that involved exposing pregnant rats and their offspring to manganese in drinking water (over 21,000 times the amount that is typically recommended as safe for people to eat each day) found that the rat pups had a short-lived decrease in body weight and an increase in activity. Higher concentrations (approximately 37,000 times the recommended safe amount for humans) of manganese provided in food to animals were associated with decreased activity, while lower concentrations (approximately 1,100 times the recommended safe amount for humans) given all at once each day to rodents can cause delays in the growth of reproductive organs, decreased pup weight, mistakes in skeletal formation, behavioral differences in animals, and changes in the brain.

Other studies in which pregnant animals have been injected with manganese show that negative effects can be seen in unborn pups. These studies have reported delays in formation of skeletal bones and internal organs, suggesting that the skeletal system is a target for birth defects caused by manganese. However, except when manganese is administered via a liquid form of nutrition injected into their veins, humans are not exposed to manganese through injection.

Because manganese is a normal part of the human body, it is always present in the tissues and bloodstream of the mother; in addition, it can cross the placenta and enter an unborn baby. Manganese has been measured in plasma from the umbilical cord blood of premature and full-term babies, as well as in the blood of their mothers. The concentrations of manganese found in full-term babies were slightly higher than the concentrations found in premature babies, though these levels were not significantly different. Also, manganese levels in the livers of pregnant rats were much higher than those in non-pregnant rats, and the manganese levels in their unborn pups were higher than usual. Although the few available animal studies indicate that excess manganese interferes with normal development of the fetus, the relevance of these studies

to humans is not known. There is no information available on the effects in pregnant women from exposure to excess levels of manganese in air, food, or water.

Manganese is necessary for proper nutrition for a rapidly growing infant. The element is present in breast milk at approximately 4–10 μg/L, an amount that appears to be adequate for a nursing baby. Studies show that infant formulas contain more manganese than breast milk, but that infants absorb the same proportion of manganese from infant formulas, cow's milk, and breast milk. However, because cow milk formulas and soy formulas contain much larger amounts of manganese than breast milk, infants who are fed these formulas ingest much higher amounts of manganese than breast-fed infants. Whether these higher amounts of manganese are unhealthy for the infant is unknown.

Sections 2.6 and 5.6 contain more information on the effects of manganese on children.

1.7 HOW CAN FAMILIES REDUCE THE RISK OF EXPOSURE TO MANGANESE?

If your doctor finds that you have been exposed to significant amounts of manganese, ask whether your children might also be exposed. Your doctor might need to ask your state health department to investigate.

In typical situations, there is no need to reduce exposure to manganese. A healthy body regulates the amount of manganese that it either keeps or eliminates based on the foods eaten and the air breathed. Because manganese is the twelfth most common element in the earth's crust, it is always found in measurable concentrations in topsoil. If young children eat soil, it is unknown whether they are able to absorb the manganese in the soil. No studies were located that would show how much, if any, manganese can be absorbed after eating soil. Despite this lack of information, manganese concentrations in soil are not typically high, and therefore, the amount of manganese that children might take in from eating soil should not be a great concern. However, if soil in your neighborhood contains large amounts of manganese from hazardous waste or other

environmental sources, you should prevent your children from eating it and discourage children from putting their hands in their mouths or performing other hand-to-mouth activity.

Manganese is also present in drinking water. The EPA has set a Secondary Maximum Contaminant Level (MCL) for the metal in drinking water at 0.05 ppm because at higher concentrations it can stain clothes or plumbing fixtures. The Food and Drug Administration (FDA) has also set this level for bottled water, and it is believed to be low enough to protect human health. Individuals with well water that leaves black deposits or dark stains in their sinks and other fixtures may want to have their water tested for high levels of manganese.

The exact amounts of manganese necessary for proper body functioning in an infant or child are not known. However, the effects of getting too little manganese are well known in adults, and recorded cases of manganese deficiency are very rare. Therefore, it appears that humans get adequate amounts of manganese from their diets. Children are not likely to be exposed to toxic amounts of manganese in the diet. However, manganese can be absorbed in higher-than-usual amounts if the diet is low in iron. Therefore, it is very important to provide your child with a well-balanced diet. The Food and Nutrition Board of the National Research Council (NRC) has not established a Recommended Daily Allowance for manganese because too little is known about the dietary requirements of this trace element. However, an Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for manganese has been estimated as 0.3–0.6 mg/day for infants from birth to 6 months, 0.6–1 mg/day for infants aged 6 months to 1 year, 1–1.5 mg/day for children aged 1–3 years, 1–2 mg/day for children aged 4–10 years of age, and 2–5 mg/day for children aged 10 years to adult.

1.8 IS THERE A MEDICAL TEST TO DETERMINE WHETHER I HAVE BEEN EXPOSED TO MANGANESE?

Several tests are available to measure manganese in blood, urine, hair, or feces. Because manganese is a normal part of the body, some is always found in tissues or fluids. Concentrations in blood, urine, hair, or feces are often found to be higher than average in groups of people

exposed to higher-than-usual levels of manganese. Because the levels in different people can vary widely, these methods are not very reliable to determine whether a single person has been exposed to higher-than-usual levels. However, blood or urine levels in groups of people who have been exposed to higher-than-usual amounts are useful indicators of exposure when compared with reference levels from people who have not been exposed. The normal range of manganese levels in blood is 4–14 μg/L, 0.97–1.07 μg/L in urine, and 0.15–2.65 μg/L in serum (the fluid portion of the blood). Because excess manganese is usually removed from the body within a few days, past exposures are difficult to measure with common laboratory tests.

A medical test known as magnetic resonance imaging, or MRI, can detect the presence of increased amounts of manganese in the brain. This test has been very useful in determining whether people have accumulated higher-than-usual amounts of manganese in the body. This tool is often used when a person is showing severe signs of manganese toxicity, as in manganism, or in other diseases that affect the brain, such as Parkinson's disease or Alzheimer's disease. The results must be used along with a complete medical history because other diseases affecting the brain can cause abnormal MRI scans. MRI is not useful, though, in determining the source of increased exposure or in establishing the amount of manganese that you might have been exposed to. Furthermore, MRI analysis will not necessarily detect manganese in the brain after exposure to the metal has ceased. Most people who have increased manganese concentrations in their body do so as a result of increased exposure to the compound (most often by work exposures); others have increased levels because they are unable to clear manganese from their bodies. A medical test would not be able to tell the difference between these two possibilities, and further testing would be needed to find the cause of increased exposure. Also, exposure to high levels of manganese (such as in the case of manganese miners) may cause a permanent effect on the brain, depending on the length and level of manganese exposure.

Chapters 2 and 6 have more information on how manganese can be measured in exposed humans.

1.9 WHAT RECOMMENDATIONS HAS THE FEDERAL GOVERNMENT MADE TO PROTECT HUMAN HEALTH?

The federal government develops regulations and recommendations to protect public health. Regulations can be enforced by law. Federal agencies that develop regulations for toxic substances include the Environmental Protection Agency (EPA), the Occupational Safety and Health Administration (OSHA), and the Food and Drug Administration (FDA). Recommendations provide valuable guidelines to protect public health but cannot be enforced by law. Federal organizations that develop recommendations for toxic substances include the Agency for Toxic Substances and Disease Registry (ATSDR) and the National Institute for Occupational Safety and Health (NIOSH).

Regulations and recommendations can be expressed in not-to-exceed levels in air, water, soil, or food that are usually based on levels that affect animals; then they are adjusted to help protect people. Sometimes these not-to-exceed levels differ among federal organizations because of different exposure times (an 8-hour workday or a 24-hour day), the use of different animal studies, or other factors.

Recommendations and regulations are also periodically updated as more information becomes available. For the most current information, check with the federal agency or organization that provides it. Some regulations and recommendations for manganese include the following:

To avoid staining clothes or plumbing fixtures, the EPA recommends that the concentration of manganese in drinking water be not more than 0.05 ppm. FDA has set the same level for bottled water. This concentration is believed to be more than adequate to protect human health. The EPA has also established rules that set limits on the amount of manganese that factories can dump into water. EPA requires factories that use or produce manganese to report how much they dump in the environment. OSHA has set a limit of 5 mg/m^3 for the average amount of manganese in workplace air over an 8-hour workday (OSHA 1998).

Manganese is an essential element of the diet. Like a number of metals (for example, chromium, copper, iron, and zinc) manganese is important in the normal functioning of the body. Therefore, both too little or too much can be harmful. The Food and Nutrition Board of the National Research Council has set an ESADDI for manganese. The ESADDI for manganese ranges from 0.3 up to 5 mg/day for different age groups (1–10 mg/day is about the amount found in the diet of an adult; Freeland-Graves 1994; Gibson 1994).

Chapter 7 has more information on governmental rules regarding manganese.

1.10 WHERE CAN I GET MORE INFORMATION?

If you have any more questions or concerns, please contact your community or state health or environmental quality department or

Agency for Toxic Substances and Disease Registry
Division of Toxicology
1600 Clifton Road NE, Mailstop E-29
Atlanta, GA 30333

* Information line and technical assistance

Phone: 1-888-422-8737
Fax: (404) 639-6359

ATSDR can also tell you the location of occupational and environmental health clinics. These clinics specialize in recognizing, evaluating, and treating illnesses resulting from exposure to hazardous substances.

* To order toxicological profiles, contact

National Technical Information Service
5285 Port Royal Road
Springfield, VA 22161
Phone: (800) 553-6847 or (703) 605-6000

2. HEALTH EFFECTS

2.1 INTRODUCTION

The primary purpose of this chapter is to provide public health officials, physicians, toxicologists, and other interested individuals and groups with an overall perspective on the toxicology of manganese. It contains descriptions and evaluations of toxicological studies and epidemiological investigations and provides conclusions, where possible, on the relevance of toxicity and toxicokinetic data to public health.

A glossary and list of acronyms, abbreviations, and symbols can be found at the end of this profile.

Manganese is a naturally occurring element found in rock, soil, water, and food. In humans and animals, manganese is an essential nutrient that plays a role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from damaging free radical species, and the formation of glycosaminoglycans (Wedler 1994). Manganese acts as both a constituent of metalloenzymes and as an enzyme activator. Enzymes that contain manganese include arginase, pyruvate carboxylase, and manganese-superoxide dismutase (MnSOD) (Keen and Zidenberg-Cher 1990; NRC 1989; Wedler 1994). Manganese, in its activating capacity, can bind either to a substrate (such as adenosine triphosphate, ATP), or to a protein directly, thereby causing conformational changes (Keen and Zidenberg-Cher 1990). Manganese has been shown to activate numerous enzymes involved with either a catalytic or regulatory function (e.g., transferases, decarboxylases, hydrolases) (Wedler 1994). The nutritional role of manganese is discussed in Section 2.4. Although manganese is an essential nutrient, exposure to high levels via inhalation or ingestion may cause some adverse health effects.

It has been suggested that these adverse health effects, especially neurologic effects, are occurring on a "continuum of ...dysfunction" that is dose-related (Mergler et al. 1999). In other words, mild or unnoticeable effects may be caused by low, but physiologically excessive amounts of manganese, and these effects appear to increase in severity as the exposure level increases. Case reports and occupational studies address this continuum of nervous system dysfunction and help to underscore the apparent dose-response relationship. It is clear that chronic exposure to manganese at very high levels results in permanent neurological damage, as is seen in former manganese miners. Chronic exposure to much lower levels of manganese (as with occupational exposures) has been linked to deficits in the ability to perform rapid hand

movements and some loss of coordination and balance, along with an increase in reporting mild symptoms such as forgetfulness, anxiety, or insomnia. What is still unknown is the lowest level at which adverse neurologic effects can occur after long-term exposures.

Chemical Forms of Concern

Manganese can exist in both inorganic and organic forms. This profile will discuss key manganese compounds in both forms, with inorganic compounds discussed first. The inorganic forms include manganese chloride ($MnCl_2$), manganese sulfate ($MnSO_4$), manganese acetate (MnOAc), manganese phosphate ($MnPO_4$), manganese oxide (MnO_2), and manganese tetroxide (Mn_3O_4).

Emphasis has been placed on the health effects of compounds containing inorganic manganese in the Mn(II), Mn(III), or Mn(IV) oxidation states, since these are the forms most often encountered in the environment and in the workplace. There is evidence in animals and humans that adverse neurological effects can result from exposure to differing manganese compounds. Much of this information is from reports and experiments of acute exposures to very high doses. Though some differences in toxic effects across compounds may be related to differences in toxicokinetics, there is some evidence that MnO_2 is absorbed more slowly and to a lesser extent that $MnCl_2$. However, more information is needed to determine whether this and/or other toxicokinetic differences across manganese compounds contribute to greater or lesser toxicologic risk. Because available information is insufficient to characterize any differences in toxicity induced by different manganese compounds, no distinction is made in the text between various inorganic Mn(II), Mn(III), or Mn(IV) compounds, although the chemical form used in a study is reported when it is known. Manganese in the form of permanganate produces toxic effects primarily through its oxidizing capacity. However, because of its tendency to oxidize organic material, the permanganate ion is not stable in the environment, and the probability of exposure to this species around waste sites is considered very low. For this reason, data on exposures to permanganate are only briefly discussed.

The organic compounds that will be discussed are methylcyclopentadienyl manganese tricarbonyl (MMT), maneb and mancozeb, both fungicides, and mangafodipir (MnDPDP). MMT is a fuel additive developed in the 1950s to increase the octane level of gasoline and thus improve the antiknock properties of the fuel (Davis 1998; Lynam et al. 1999). Additional information concerning MMT is included in Chapter 4.

Exposure to MMT is expected to be primarily through inhalation or oral pathways, although occupational exposure for gasoline attendants or mechanics will be more significant via dermal absorption. MMT is generally extremely unstable in light and degrades very quickly in air; therefore, exposure via gasoline exhausts are likely to be more to the oxidative products of MMT (manganese phosphates, sulfates, oxides and sulfoxides) rather than to the parent compound. However, despite evidence to its photolability, recent information shows that MMT levels in the environment increase with traffic density (Zayed et al. 1999); therefore, inhalation and/or ingestion exposures to the parent compound are possible. Exposure and resultant toxicity from MMT's inorganic combustion products are covered under the inorganic subsections, while toxicity attributable to MMT is covered under the organic subsections.

Maneb and mancozeb are widely used fungicides in the agriculture and forestry industries. Exposure to these compounds can be via ingestion, inhalation, or dermal pathways, especially in sprayers who do not use protective measures. Much of the information about effects in humans from exposure to these pesticides comes from reports of agricultural workers or home use. Often exposure was likely to involve both inhalation and oral routes, or inhalation and dermal routes, and in some cases, all three routes may have contributed to total exposure. Thus, when discussed in this text, levels of exposure to these pesticides is expressed as mg/kg/day to reflect total exposure per estimated body weight.

Mangafodipir is the clinical term for manganese (II) *N,N'*-dipyridoxylethylenediamine-*N,N'*-diacetate 5,5'bis(phosphate). This manganese chelate is a contrast agent for magnetic resonance (MR) imaging. This compound is primarily liver-specific and aids in the diagnosis of hepatic cancers; it has also been found to aid in the identification of kidney and pancreatic tumors. The compound is only used in the diagnosis of organ-specific cancers and is found exclusively in a clinical setting. Mangafodipir is injected intravenously; therefore, inhalation, oral, and dermal pathways of exposure are not a concern. Because exposure to this compound is pathway-specific and the exposure population is inherently limited, toxicity arising from exposure to mangafodipir will be discussed in a separate subsection to Chapter 2, Section 2.2.4, Diagnostic Exposure.

2.2 DISCUSSION OF HEALTH EFFECTS BY ROUTE OF EXPOSURE

To help public health professionals and others address the needs of persons living or working near hazardous waste sites, the information in this section is organized first by route of exposure — inhalation, oral, and dermal; and then by health effect — death, systemic, immunological, neurological, reproductive,

developmental, genotoxic, and carcinogenic effects. These data are discussed in terms of three exposure periods — acute (14 days or less), intermediate (15–364 days), and chronic (365 days or more).

Levels of significant exposure for each route and duration are presented in Tables 2-1 through 2-5 and illustrated in Figures 2-1 through 2-4. The points in the figures showing no-observed-adverse-effect levels (NOAELs) or lowest-observed-adverse-effect levels (LOAELs) reflect the actual doses (levels of exposure) used in the studies. LOAELs have been classified into "less serious" or "serious" effects. "Serious" effects are those that evoke failure in a biological system and can lead to morbidity or mortality (e.g., acute respiratory distress or death). "Less serious" effects are those that are not expected to cause significant dysfunction or death, or those whose significance to the organism is not entirely clear. ATSDR acknowledges that a considerable amount of judgment may be required in establishing whether an end point should be classified as a NOAEL, "less serious" LOAEL, or "serious" LOAEL, and that in some cases, there will be insufficient data to decide whether the effect is indicative of significant dysfunction. However, the Agency has established guidelines and policies that are used to classify these end points. ATSDR believes that there is sufficient merit in this approach to warrant an attempt at distinguishing between "less serious" and "serious" effects. The distinction between "less serious" effects and "serious" effects is considered to be important because it helps the users of the profiles to identify levels of exposure at which major health effects start to appear. LOAELs or NOAELs should also help in determining whether or not the effects vary with dose and/or duration, and place into perspective the possible significance of these effects to human health.

The significance of the exposure levels shown in the Levels of Significant Exposure (LSE) tables and figures may differ depending on the user's perspective. Public health officials and others concerned with appropriate actions to take at hazardous waste sites may want information on levels of exposure associated with more subtle effects in humans or animals or exposure levels below which no adverse effects have been observed. Estimates of levels posing minimal risk to humans (Minimal Risk Levels or MRLs) may be of interest to health professionals and citizens alike.

Estimates of exposure levels posing minimal risk to humans (Minimal Risk Levels or MRLs) have been made for manganese. An MRL is defined as an estimate of daily human exposure to a substance that is likely to be without an appreciable risk of adverse effects (noncarcinogenic) over a specified duration of exposure. MRLs are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration within a given route of exposure. MRLs are

based on noncancerous health effects only and do not consider carcinogenic effects. MRLs can be derived for acute, intermediate, and chronic duration exposures for inhalation and oral routes. Appropriate methodology does not exist to develop MRLs for dermal exposure.

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

Inorganic and organic manganese compounds are discussed separately in this and several subsequent chapters. The organic manganese compounds, MMT, maneb, mancozeb, and mangafodipir, are handled differently as far as exposure dose. For example, for MMT and mangafodipir, exposure dose has been estimated for the amount of manganese in the compound. Therefore, all dose values in the referenced literature have been changed to mg manganese/kg or mg manganese/m^3. Adverse effects resulting from exposure to the manganese pesticides, maneb and mancozeb, have been assumed to arise as a result of the whole compound, not necessarily from exposure to manganese. Therefore, this profile considers exposure doses to be administered doses. However, maneb and mancozeb formulations are typically 80% active ingredient. Doses have been corrected to represent an assumed amount of 80% active ingredient, except where specified by the author(s) of the study. In addition, corrections were made for compound purity when specified. The dose values given in this profile reflect this correction and will be different than those reported in the referenced literature.

One limitation of any human or animal study involving exposure to manganese is the contribution of manganese body burden by the diet. With respect to animal studies, all commercial chow formulations contain manganese, as it is an essential element necessary for good health. However, its concentration in the diet is often not precisely known, is often specified only as a range of values, and will not necessarily be the same from lot to lot. In some cases, the dietary contribution of manganese is larger than the amount in the administered dose. Because dietary manganese may contribute to the observed toxicity in any animal study, this factor causes considerable uncertainty in developing dose-response relationships for observed effects. However, this lack of quantification and reporting of dietary manganese in studies is

widespread and cannot be corrected. Therefore, this profile presents a distillation of the available literature concerning manganese toxicity, and provides, where possible, a dose-response relationship based only on the dose of administered manganese. The uncertainty caused by dietary manganese is acknowledged. Accurate information on dietary manganese contributions in future toxicity studies is necessary for more accurate calculations.

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

2.2.1 Inhalation Exposure

Inorganic manganese compounds are not volatile, but they can exist in the air as aerosols or suspended particulate matter. Table 2-1 and Figure 2-1 summarize the available quantitative information on the health effects that have been observed in humans and animals following inhalation exposure to various inorganic manganese compounds. All exposure levels are expressed as milligrams of manganese per cubic meter (mg manganese/m^3).

Many of the studies, especially those dealing with occupational exposures, make the distinction between respirable and total manganese dust. Respirable dust is usually defined by a particular dust particle size that varies from study to study. It is typically defined as those particles 5 microns or smaller; these smaller dust particles can enter the lower areas of the lungs, including the bronchioles and the alveoli. These particles can be absorbed by the lung, and will enter the bloodstream immediately, thus avoiding clearance by the liver. Total dust represents larger particles that cannot travel as deeply into the lungs as respirable dust, and will largely be coughed up, and swallowed. Although many of the recent occupational studies have provided information on the size of the respirable particles that are associated with the exposure levels documented, some of the occupational studies and historical studies in miners only measure total dust. The profile provides, where possible, the different exposure levels in terms of respirable and total dust, but does not make a further distinction between particle sizes of the respirable dust.

2.2.1.1 Death

Inorganic Manganese

No conclusive studies have been located that show inhalation exposure of humans to manganese resulting in death. Hobbesland et al. (1997a) investigated nonmalignant respiratory diseases as a cause of death in male ferromanganese and silicomanganese workers. The authors found a slight excess in the numbers of deaths caused by pneumonia for manganese furnace workers but could not discount other work-related exposures as potential causes of the pneumonia.

In analyses performed several years ago, MMT in gasoline was found to combust primarily to Mn_3O_4, but in the low levels currently used in gasolines, it is primarily combusted to manganese phosphate and manganese sulfate (Lynam et al. 1999). Therefore, inhalation exposures to exhaust from gasoline containing MMT will be discussed with inorganic manganese exposures. No deaths were observed in male outbred albino rats and male golden hamsters exposed to the exhaust (either irradiated or non-irradiated) from automobiles that were fueled with MMT-containing gasoline (Moore et al. 1975).

No other studies were located regarding death in humans or animals after inhalation exposure to inorganic manganese.

Organic Manganese

MMT. MMT has been used in very few inhalation studies due to the photolability of the compound; its short half-life in air makes it a very difficult compound to administer to laboratory animals in exposure chambers or nose-cones. Hinderer (1979) evaluated the toxicity of various unspecified MMT concentrations administered to 10 male Sprague-Dawley rats per exposure group during 1-hour and 4-hour exposure periods. The inhalation LD_{50} was determined to be 62 mg Mn/m^3 (247 mg MMT/m^3* 55 mg Mn/218.1 mg MMT = 62 mg Mn/m^3) for 1-hour exposure and 19 mg Mn/m^3 for 4-hour exposure. No mention was made in the report of steps taken to prevent MMT photodegradation during the experiment.

Maneb and mancozeb. No studies of death following inhalation exposure to maneb or mancozeb in either humans or animals were located.

2.2.1.2 Systemic Effects

Inorganic Manganese

Table 2-1 and Figure 2-1 show the highest NOAEL and all LOAEL values from each reliable study for these systemic effects in each species and each duration category.

Respiratory Effects.

Inorganic Manganese

In humans, inhalation of particulate manganese compounds such as manganese dioxide (MnO_2) or manganese tetroxide (Mn_3O_4) can lead to an inflammatory response in the lung. This is characterized by an infiltration of macrophages and leukocytes which phagocytize the deposited manganese particles (Lloyd Davies 1946). Damage to lung tissue is usually not extensive but may include local areas of edema (Lloyd Davies 1946). Symptoms and signs of lung irritation and injury may include cough, bronchitis, pneumonitis, and minor reductions in lung function (Abdel Hamid et al. 1990; Akbar-Khanzadeh 1993; Lloyd Davies 1946; Roels et al. 1987a); occasionally, pneumonia may result (Lloyd Davies 1946). These effects have been noted mainly in people exposed to manganese dust under occupational conditions, although there is some evidence that respiratory effects may also occur in residential populations near ferromanganese factories (Kagamimori et al. 1973; Nogawa et al. 1973; WHO 1987). The frequency of effects has been shown to decrease in at least one population when concentrations of total manganese in falling dust declined (Kagamimori et al. 1973). A threshold for respiratory effects has not been established. It is likely that the inflammatory response begins shortly after exposure and continues for the duration of the exposure.

It is important to note that an inflammatory response of this type is not unique to manganese-containing particles but is characteristic of nearly all inhalable particulate matter (EPA 1985d). This suggests that it is not the manganese per se that causes the response but more likely the particulate matter itself.

An increased prevalence of infectious lung disease (especially pneumonia) has also been noted in some studies of workers with chronic occupational exposure to manganese dust (Lloyd Davies 1946) and in residents near a ferromanganese factory (WHO 1987). It seems likely that this increased susceptibility to

TABLE 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
ACUTE EXPOSURE							
Systemic							
1	Rat (Sprague-Dawley)	10 d 6hr/d	Resp			43 (pneumonitis and increased lung weight)	Shiotsuka 1984 MnO2
			Hemato	138			
2	Mouse (CD-1)	2 hr	Resp	2.8 F			Adkins et al. 1980b Mn3O4
3	Gn pig (NS)	1 hr 24 hr/d	Resp	14			Bergstrom 1977 MnO2
Immunological/Lymphoreticular							
4	Mouse (CD-1)	1-4 d 3hr/d			69 (increased susceptibility to pneumonia)		Maigetter et al. 1976 MnO2

TABLE 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
INTERMEDIATE EXPOSURE							
Systemic							
5	Monkey (Rhesus)	10 mo 22hr/d	Resp		0.7 F (mild inflammation)		Suzuki et al. 1978 MnO2
6	Monkey (NS)	9 mo (cont)	Resp	1.1			Ulrich et al. 1979a Mn3O4
7	Rat (NS)	9 mo (cont)	Resp	1.1			Ulrich et al. 1979b Mn3O4
			Hemato	1.1			
			Hepatic	1.1			
8	Rabbit (NS)	4 wk 5d/wk 6hr/d	Resp	3.9 M			Camner et al. 1985 MnCl2
9	Pigeon	5d/wk, 5, 9, or 13 wks	Hemato		0.167 B (decrease in total blood proteins (p<= 0.05) at 13 wks of exposure that persisted 2 wks after exposure ended)		Sierra et al. 1998 Mn3O4

TABLE 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
Neurological							
10	Monkey (NS)	9 mo (cont)		1.1			Ulrich et al. 1979a Mn3O4
11	Rat (NS)	9 mo (cont)		1.1			Ulrich et al. 1979b Mn3O4
12	Mouse (Swiss ICR)	18 wk 5d/wk 7hr/d				61F (decreased maternal pup retrieval latency)	Lown et al. 1984 MnO2
13	Mouse (Swiss ICR)	16-32 wk 5d/wk 7hr/d				72M (increased open-field behavior)	Morganti et al. 1985 MnO2
Reproductive							
14	Mouse (Swiss ICR)	18 wk 5d/wk 7hr/d		61 F			Lown et al. 1984 MnO2

TABLE 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
CHRONIC EXPOSURE							
Systemic							
15	Human	No data (occup)	Resp			3.6 M (pneumonia)	Lloyd Davies 1946 MnO2
16	Human	1-19 yr (occup)	Resp			0.97 M total dust (cough, decreased lung function)	Roels et al. 1987a Mn salts and oxides
			Hemato	0.97 M (median)			
17	Human	5.3 yr (occup)	Resp	0.18 respirable dust (median)			Roels et al. 1992 MnO2
			Endocr	0.18 respirable dust (median)			
18	Monkey (Rhesus)	66 wk	Hemato	0.1			Coulston and Griffin 1977 Mn3O4

TABLE 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)		LOAEL Serious (mg/m3)		Reference
Neurological									
19	Human	1.1-15.7 yr			1.59 total dust	(postural sway with eyes closed)			Chia et al. 1995 MnO2
20	Human	NS (occup)					22M	(bradykinesia, mask-like face)	Cook et al. 1974 NS
21	Human	12.7 yr (mean)		0.051 respirable dust (median)					Gibbs et al. 1999 NS
22	Human	1-35 yr (2.6 median) (occup)			0.14 total dust (median)	(decreased reaction time, finger tapping)			Iregren 1990 MnO2
23	Human	1-28yr			0.027-0.27 (range total dust;CEI= 0.199mg/m^3*yr, g.mean, med.exp.	(decreased neurobehavioral performance finger tapping, symbol digit, digit span, additions)			Lucchini et al. 1995 (primarily MnO2) (MnOx - Mn oxides)

TABLE 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
24	Human	11.5 yr (mean)			0.0967 (CEI/yrs. exp., med. exp. grp.) (decreased performance on neurobehavioral exams)		Lucchini et al. 1999 MnO2, Mn3O4
25	Human	16.7 yr (mean) (occup)			0.032 respirable dust (median) (decreased motor function)		Mergler et al. 1994 NS
26	Human	1-19 yr (occup)			0.97 total dust (median) (altered reaction time, short-term memory, decreased hand steadiness)		Roels et al. 1987a Mn salts and oxides
27	Human	5.3 yr (occup)			0.179[c] respirable dust (median) (impaired visual time, eye-hand coordination, and hand steadiness)		Roels et al. 1992 [UPDATE2] MnO2
28	Human	NS (occup)				2.6M (tremor, decreased reflexes)	Saric et al. 1977 NS
29	Human	1-9 yr (occup)				6M (psychomotor disturbances, weakness, pain)	Schuler et al. 1957 MnO2

TABLE 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL Less serious (mg/m3)	LOAEL Serious (mg/m3)	Reference
30	Human	NS (occup)				5M (weakness, ataxia, pain)	Tanaka and Lieben 1969 NS
31	Human	1 yr (occup)				3.5M (weakness, anorexia, ataxia)	Whitlock et al. 1966 NS
32	Monkey (Rhesus)	2 yr 5d/wk 6hr/d			30 F (altered DOPA levels)		Bird et al. 1984 MnO2
33	Monkey (Rhesus)	66 wk		0.1			Coulston and Griffin 1977 Mn3O4
	Reproductive						
34	Human	1-19 yr (occup)				0.97M (decreased fertility in males as assessed by number of observed vs expected children)	Lauwerys et al. 1985 Mn salts and oxides
35	Human	at least 1yr				6.5-82.3M (abnormal sperm)	Wu et al. 1996 (Mn fumes)

TABLE 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (continued)

Key to figure[a]	Species (strain)	Exposure/ duration/ frequency	System	NOAEL (mg/m3)	LOAEL		Reference
					Less serious (mg/m3)	Serious (mg/m3)	
36	Human	at least 1yr				0.14-5.5M (abnormal sperm)	Wu et al. 1996 (MnO2)

[a]The numbers correspond to entries in Figure 2-1.
[b]All doses expressed as mg manganese/m^3.
[c]The chronic-duration inhalation minimal risk level (MRL) of 0.00004 mg Mn/m^3 was derived by using a benchmark dose analysis NOAEL of 74µg Mn/m^3. This value was adjusted using the following uncertainty and modifying factors (10 for human variability, 5/7 for intermittent exposure (5 days/wk), 8/24 for intermittent exposure (8 hr/day), and 10 for potential differences in toxicity due to the different forms of Mn and other limitations in the database, and 5 for potentially increased susceptibility in children based on differential pharmacokinetics in the young.

cont = continuous; d = day(s); DOPA = dihydroxyphenylalanine; Endo = Endocrine; F = female; Gn Pig = guinea pig; Hemato = hematological; hr = hour(s); Immuno = immunological; LOAEL = lowest-observed-adverse-effect level; M = male; Mn = manganese; $MnCl_2$ = manganous chloride; mo = month(s); Mn_3O_4 = manganese tetraoxide; MnO_2 = manganese dioxide; NOAEL = no-observed-adverse-effect level; occup = occupational; Resp = respiratory; TWA = time weighted average; wk = week(s) ; yr = year(s)

Figure 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation

Acute (≤14 days)

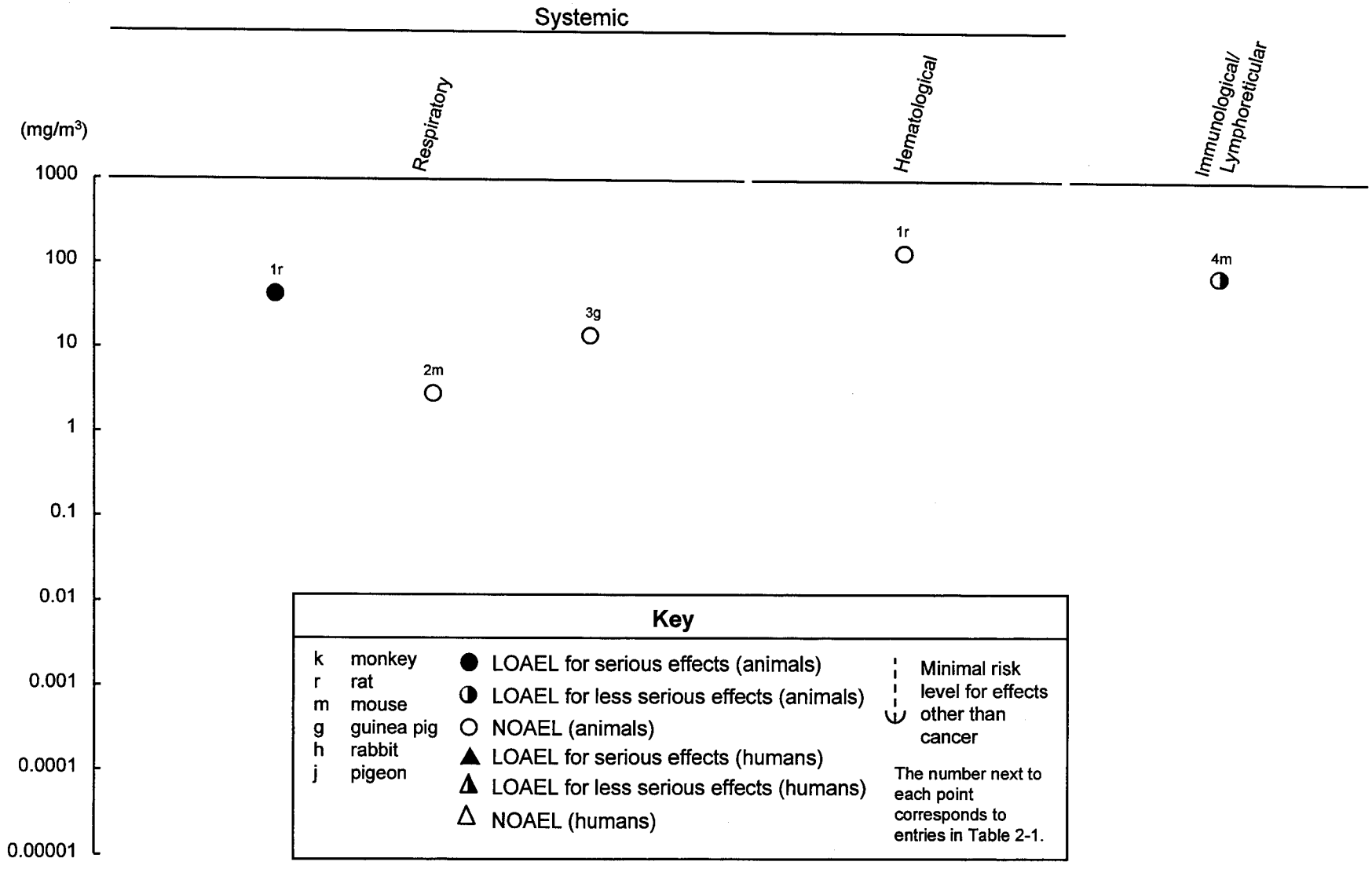


Figure 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (cont.)

Intermediate (15-364 days)

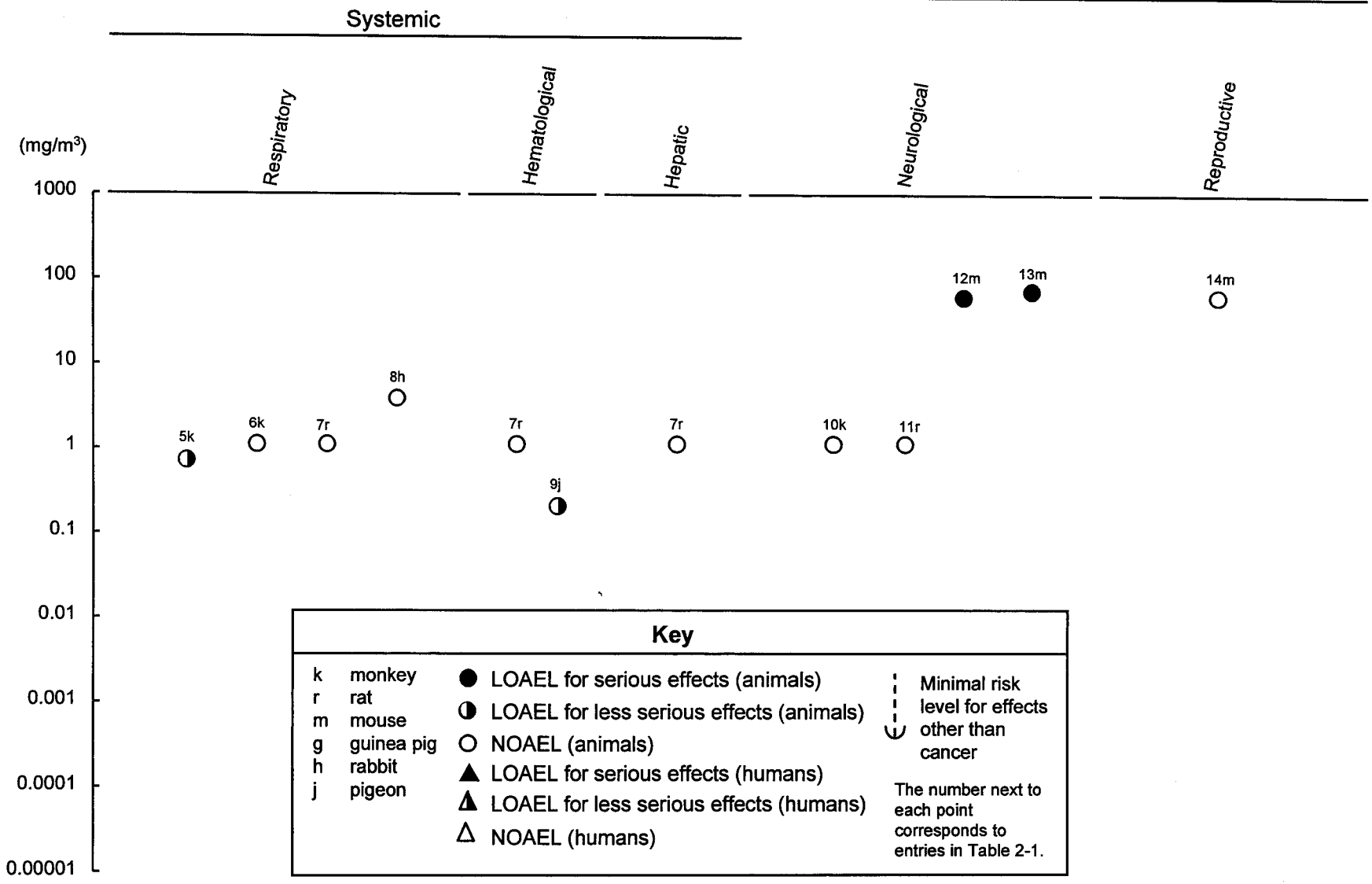
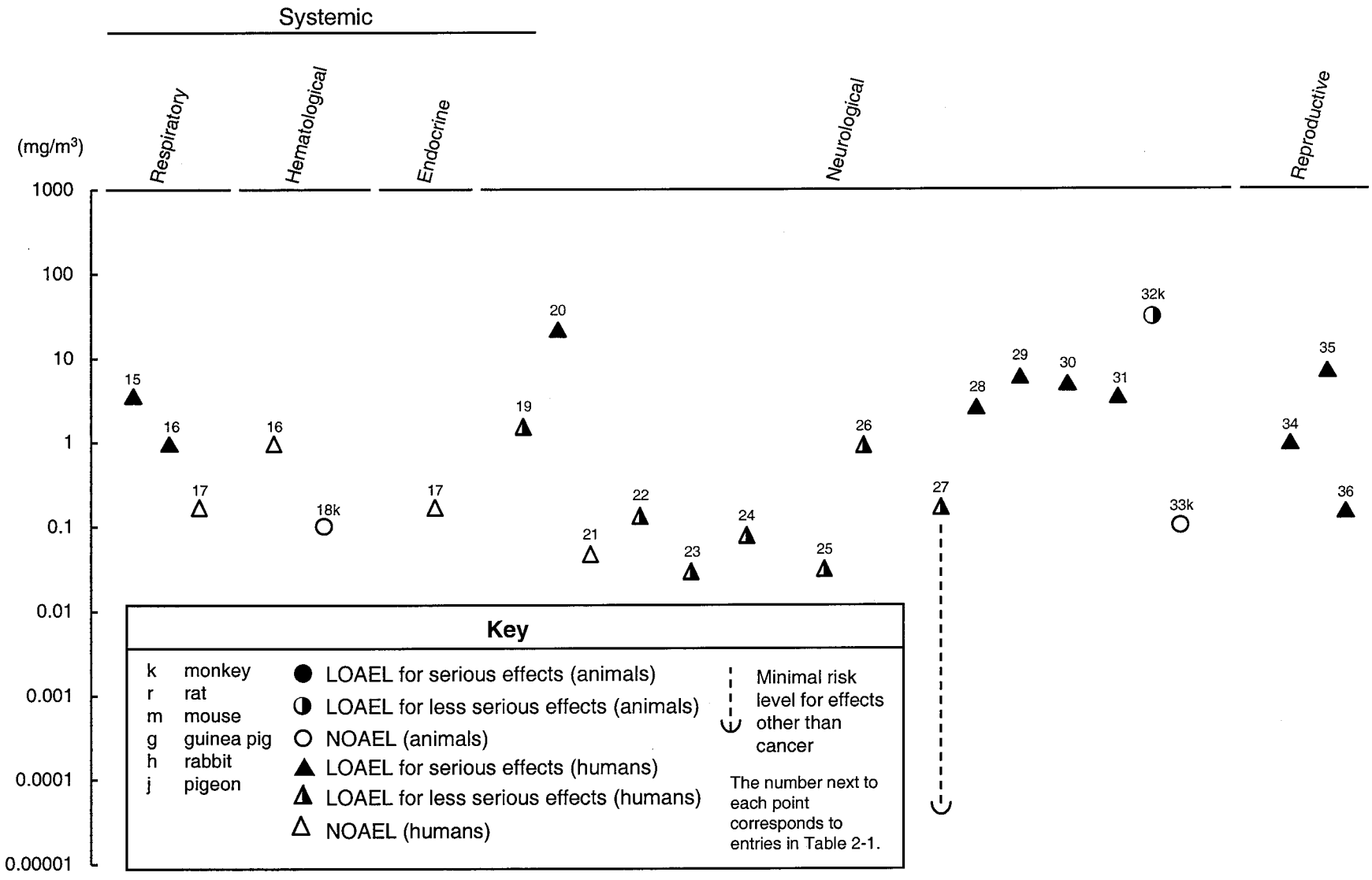


Figure 2-1. Levels of Significant Exposure to Inorganic Manganese - Inhalation (cont.)

Chronic (≥365 days)



respiratory infection is mainly secondary to the lung irritation and inflammation caused by inhaled particulate matter, as discussed above.

Inhalation of particulate manganese compounds such as MnO_2 or Mn_3O_4 also leads to an inflammatory response in the lungs of animals, although inhalation of $MnCl_2$ did not cause lung inflammation in rabbits (Camner et al. 1985). Several acute- and intermediate-duration studies in animals report various signs of lung inflammation following periods ranging from 1 day to 10 months at manganese concentrations ranging from 0.7 to 69 mg/m^3 (Bergstrom 1977; Camner et al. 1988; Shiotsuka 1984; Suzuki et al. 1978; Ulrich et al. 1979a, 1979b). Bergstrom (1977) and Ulrich et al. (1979a, 1979b) determined NOAELs, which are reported in Table 2-1. Increased susceptibility to lung infection by bacterial pathogens following inhalation of manganese dusts has been noted in acute animal studies (Maigetter et al. 1976). Conversely, Lloyd Davies (1946) reported no increase in the susceptibility of manganese-treated mice to pneumococci or streptococci.

Moore et al. (1975) exposed male golden hamsters and outbred albino rats to automobile exhaust from a car that burned MMT-containing fuel. The animals were exposed to non-irradiated exhaust or irradiated exhaust; the irradiation served to convert hydrocarbon gases and vapors to particulate form. Controls for each species were exposed to clean air. The animals were exposed for 8 hours/day, for 56 consecutive days. While the hamsters were fed a diet containing an adequate amount of manganese for normal development, the rats were divided into 2 groups, 1 group was fed a manganese-sufficient diet (42.2 µg Mn/g diet) and 1 was fed a manganese-deficient diet (5 µg Mn/g diet). After the exposure, the authors observed a thickening of the cuboidal epithelium at the level of the terminal bronchiole in the golden hamsters. The lesion was not classified as severe and only affected one to two sites per lung section. Further, the lesions did not increase with length of exposure to the exhaust products (from 1 week to 9 weeks). The incidence of lesions in the lung was 21% after exposure to irradiated exhaust, 14% after exposure to non-irradiated exhaust, and 6% after exposure to clean air.

Organic Manganese

MMT. No studies were located concerning respiratory effects in humans following inhalation exposure to MMT.

Male rats exposed to high concentrations of MMT (exposure doses not reported) via inhalation exhibited labored breathing during and after 1-hour and 4-hour exposures (Hinderer 1979). Gross necropsy or histopathological analyses on these animals were not performed.

Maneb and mancozeb. No studies were located concerning respiratory effects in humans or animals following inhalation exposure to maneb or mancozeb.

Cardiovascular Effects.

Inorganic Manganese

Three studies reported adverse cardiovascular effects after occupational exposure to manganese. Saric and Hrustic (1975) observed a lower mean systolic blood pressure in male workers at a ferromanganese plant. Manganese concentrations in the plant ranged from 0.4 to 20 mg/m^3, but specific data on exposure levels were lacking. More recently, Jiang et al. (1996a) studied the potential cardiotoxicity of MnO_2 exposure in 656 workers (547 males, 109 females) involved in manganese milling, smeltering, and sintering. The authors took 181 samples of airborne manganese (not specified if respirable or total dust), with a geometric mean of 0.13 mg/m^3. The workers, whose work tenure ranged from 0 to 35 years, had a greater incidence of low diastolic blood pressure. The incidence of this effect was highest in young workers with the lowest tenure in the plant. There was no increase of abnormal electrocardiograms between workers and their matched controls. The authors surmised that manganese's ability to lower the diastolic blood pressure weakens with age as the elasticity of the blood vessels deteriorates.

Hobbesland et al. (1997b) reported a significantly increased incidence in sudden death mortality for workers in ferromanganese/silicomanganese plants during their employment period (Standardized Mortality Ratio, SMR, = 2.47). The sudden deaths included cardiac deaths and other natural causes. More specifically, among furnace workers, who are more likely to be exposed to manganese fumes and dusts than non-furnace workers, the mortality during active-person time was statistically significantly elevated (38.7%) compared to non-furnace workers (23.3%; $p<0.001$). However, the authors caution that the association of increased death and manganese exposure is speculative, and the increase in sudden death could also be caused by common furnace work conditions (heat, stress, noise, carbon monoxide, etc.)

Organic Manganese

MMT. No studies on cardiovascular effects from inhalation exposure to MMT in humans or animals were located.

Maneb and mancozeb. Three case reports exist concerning possible cardiac effects following acute exposure to maneb. The first report involved a farm worker who sprayed 100 g of maneb on a potato field without taking protective measures. This is estimated to be equivalent to a dose of 1.1 mg/kg/day (100 g* 0.8/70kg; the 0.8 conversion accounts for the 80% content of manganese-containing fungicide in most formulations of maneb, and the 70 kg is the assumed weight of the man). No adverse cardiac effects (as measured by heart auscultation, pulse, blood pressure, and blood laboratory studies) following exposure were observed (de Carvalho et al. 1989). A second case report of a 42-year-old man who sprayed a cumulative dose of 2,750 g of manzidan (combined dithiocarbamate of maneb and zineb [zinc ethylene BIS dithiocarbamate]) in 2 sprayings over 6 days (estimated as equivalent to 15,700 mg/kg/day) indicated no cardiotoxicity as measured by heart rate and electrocardiogram (ECG) (Israeli et al. 1983a). A third case report involved a 62-year-old man who spread 40 g of maneb (estimated as equivalent to 229 mg/kg/day) on his garden over 2 consecutive days without taking protective measures; ECG analysis of heart function suggested myocardial ischemia (Koizumi et al. 1979). The man underwent hemodialysis after developing renal failure from exposure to the fungicide. ECG analysis 2 months following the exposure revealed no abnormalities.

One case report of intermediate exposure to maneb involves a 47-year-old man who was exposed to maneb for an average of 4 hours/day, 4 days/week, 4 months/year, for 2 years, with a daily dose of approximately 16,000 mg/kg/day (Meco et al. 1994). The man worked in a closed environment with a window and ventilation system, but worked without gloves or a mask. ECG analysis 2 years post-exposure indicated no adverse cardiovascular effects.

No studies of cardiovascular effects following inhalation exposure to maneb in animals or mancozeb in humans or animals were located.

Gastrointestinal Effects.

Inorganic Manganese

There are no reports of gastrointestinal effects following inhalation exposure to inorganic manganese in humans or animals.

Organic Manganese

MMT. There are no reports concerning the gastrointestinal effects following inhalation exposure to MMT in humans or animals.

Maneb and mancozeb. Case reports indicate that acute exposure to maneb at 229 mg/kg/day for 2 days resulted in diarrhea (Koizumi et al. 1979) and exposure to 1.1 mg/kg/day of maneb for 1 day resulted in nausea, vomiting, and diarrhea (de Carvalho et al. 1989) in male farmers.

There are no reports of gastrointestinal effects in animals following inhalation exposure to maneb or in humans or animals following inhalation exposure to mancozeb.

Hematological Effects.

Inorganic Manganese

Examination of blood from persons chronically exposed to high levels of manganese in the workplace has typically not revealed any significant hematological effects (Mena et al. 1967; Roels et al. 1987a; Smyth et al. 1973; Whitlock et al. 1966). Yiin et al. (1996) observed increased erythrocyte superoxide dismutase (SOD) and plasma malondialdehyde in male employees who worked at manganese smelters. Malondialdehyde is a product of lipid peroxidation; lipid peroxidation is believed to be a mechanism for cell toxicity (see Section 2.3). The authors observed that plasma malondialdehyde and manganese levels were strongly correlated in exposed workers and interpreted this response to be an indicator of manganese toxicity via lipid peroxidation.

Organic Manganese

MMT. No studies on hematological effects from inhalation exposure to MMT in humans or animals were located.

Maneb and mancozeb. No adverse hematological effects were observed after clinical examination of a 54-year-old farm worker who was exposed to a dose of 1.1 mg/kg/day of maneb without taking protective measures (de Carvalho et al. 1989). A 47-year-old man exposed to 16,000 mg/kg/day of maneb during a 2 year period in which he was treating barley seeds with the fungicide (Meco et al. 1994) had normal blood analyses 2 years after the exposure ended. No analyses were taken during or shortly after the exposure.

Hepatic Effects.

Inorganic Manganese

Even though the liver actively transports manganese from blood to bile (see Section 2.3.4), there is no information to indicate that the liver is adversely affected by manganese; however, there are few specific studies on this subject. Workers chronically exposed to manganese dust in the workplace exhibited no abnormalities in serum levels of alkaline phosphatase. Of 13 patients who were hospitalized with chronic manganese poisoning, 1 had a 20% sulfobromophthalein (SBP) retention and 1 had a 12% SBP retention, although histological examination of a liver biopsy from the latter revealed no abnormal tissue (Mena et al. 1967). No significance was ascribed to the elevated SBP retention.

Rats exposed to Mn_3O_4 dusts for 9 months exhibited no adverse effects or histopathological lesions; however, slight increases in liver weights were noted (Ulrich et al. 1979b). These data, although limited, indicate that the liver is not significantly injured by manganese.

Organic Manganese

MMT. No studies on hepatic effects from inhalation exposure to MMT in humans or animals were located.

Maneb and mancozeb. No studies on hepatic effects from inhalation exposure to maneb or mancozeb in humans or animals were located.

Musculoskeletal Effects.

Inorganic Manganese

No studies were located concerning musculoskeletal effects from inhalation exposure to inorganic manganese.

Organic Manganese

MMT. No studies were located concerning musculoskeletal effects from inhalation exposure to MMT in humans or animals.

Maneb and mancozeb. A gardener exposed to 229 mg/kg/day of maneb while mixing the fungicide without taking personal protective measures developed muscular weakness after the exposure (Koizumi et al. 1979). Tonic and clonic convulsions and signs of right hemiparesis were observed in an unconscious man exposed to manzidan for 2 days at a dose of 15,700 mg/kg/day (Israeli et al. 1983a). These effects are likely secondary to acute central nervous system changes and are discussed further in Section 2.2.1.4. A worker in a malt-producing mill who had developed a method for treating barley seeds with maneb developed resting tremors in all 4 limbs and the lips after 2 years of exposure to 16,000 mg/kg/day of maneb in the absence of personal protective equipment (Meco et al. 1994). The tremor was secondary to neurological effects; this case report is discussed in more detail in Section 2.2.1.4

No studies have been located concerning musculoskeletal effects following inhalation exposure of animals to maneb or of humans or animals to mancozeb.

Renal Effects.

Inorganic Manganese

The kidney is not generally considered to be a target for manganese, but specific studies are rare. No abnormalities in urine chemistry were detected in workers chronically exposed to manganese dusts in the workplace (Mena et al. 1967), but other more sensitive tests of renal function were not performed.

No studies were located regarding renal effects in animals after inhalation exposure to inorganic manganese.

Organic Manganese

MMT. No studies on renal effects from inhalation exposure to MMT in humans or animals were located.

Maneb and mancozeb. One case study (Koizumi et al. 1979) reports acute renal failure in a man exposed to 229 mg/kg/day for 2 days without taking any protective measures (e.g., no gloves, mask, or respirator). Clinical biochemistry studies revealed levels of blood urea nitrogen (BUN), creatinine, uric acid, and potassium that were indicative of acute renal failure (144.3 mg/dL, 14 mg/dL, 21.5 mg/dL, and 5.8 meq/L, respectively). The man recovered after hemodialysis.

In another case study (de Carvalho et al. 1989), a 54-year-old male farm worker sprayed a maneb solution equivalent to a daily dose of 1.1mg/kg/day. The man took no protective measures during preparation or spraying of the solution. On the following day, the man developed nausea, vomiting, and diarrhea. He was taken to a hospital after 3 consecutive days of worsening symptoms. A physical exam revealed peripheral edema, most evident on the face and ankles. Serum levels of nitrogen, creatinine, and uric acid were 93.5, 2.3, and 8 mg/dL, respectively. While in the hospital, the man's renal function decreased progressively, and he was given a low salt diet with a balanced water intake. A closed renal biopsy revealed severe tubular lesions, mainly within the proximal convoluted tubules. Cell swelling, vacuolization, disappearance of the brush border, reduced tubular lumina, and necrosis were observed. Hyaline casts were reported in the distal tubules and leukocyte accumulations were observed in the medullary vasa recta. The man began steroid therapy with oral prednisolone and a low-salt albumin infusion was administered for 12 days. Daily urine volume increased and the edema lessened. One month later, serum values had returned to normal. A second renal biopsy revealed a reconstituted tubular structure without remarkable signs of toxicity. The proximal tubules had completely recovered but glomerular lesions persisted with fusion of the epithelial foot processes and greater mesangial reaction. No studies have been located concerning renal effects following inhalation exposure of animals to maneb or of humans and animals to mancozeb.

Endocrine Effects.

Inorganic Manganese

Few studies have measured endocrine effects in humans exposed to inorganic manganese. Two studies measured hormonal levels after exposure to manganese. The first study (Alessio et al. 1989) involved chronic exposure of foundry workers to manganese for approximately 10 years. The exposure levels were reported as 0.04–1.1 mg manganese/m^3 (particulate matter) and 0.05–0.9 mg/m^3 as fumes. These levels overlap the current American Congress of Governmental Industrial Hygiene (ACGIH) threshold limit value-time weighted average (TLV-TWA) of 0.2 mg/m^3 for particulate, but are less than the limit of 1 mg/m^3 for manganese fumes. The study reported both elevated prolactin levels and elevated cortisol levels; however, no changes in the levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) were noted.

Smargiassi and Mutti (1999) reported effects in a group of workers from a ferroalloy plant who were exposed occupationally to elevated levels of airborne manganese. Serum prolactin levels in these workers were evaluated in a 1992 study and again in a 1997 study. Serum prolactin levels, which were significantly elevated in the earlier analysis, had also increased significantly over the earlier measurement ($p<0.001$). This difference was especially noticeable in those with abnormally high prolactin levels. During the five year period between studies, exposure levels were consistent and were not reduced; therefore, it is unclear whether prolactin levels reflect current or cumulative exposure.

Other elements of endocrine function (reproductive function, etc.) are discussed elsewhere in the text.

No studies were located regarding endocrine effects in animals after inhalation exposure to inorganic manganese.

Organic Manganese

MMT. No studies on endocrine effects from inhalation exposure to MMT in humans or animals were located.

Maneb and mancozeb. In the case of a man acutely exposed to 229 mg/kg/day of maneb for 2 days, thyroid function tests (including assay of thyroid-stimulating hormone, TSH) were not performed shortly after exposure, but normal results were reported when tests were performed 6 months later (Koizumi et al. 1979).

Dermal Effects.

No studies have been located concerning dermal effects in humans or animals following inhalation exposure to inorganic or organic manganese.

Ocular Effects.

Inorganic Manganese

No studies have been located concerning ocular effects in humans or animals following inhalation exposure to inorganic manganese.

Organic Manganese

MMT. There are no studies reporting ocular effects following inhalation exposure of humans to MMT. One-hour and 4-hour exposures to doses of MMT used in lethality studies resulted in conjunctivitis in rats (Hinderer 1979).

Maneb and mancozeb. Conjunctivitis has not been observed in case reports involving acute inhalation exposures to high concentrations of maneb (de Carvalho et al. 1989; Koizumi et al. 1979).

Body Weight Effects.

Inorganic Manganese

No studies were located regarding body weight effects in humans following exposures to inorganic manganese.

Organic Manganese

MMT. No studies were located regarding body weight effects in humans following inhalation exposure to MMT. Hinderer (1979) observed a decrease in weight gain in Sprague-Dawley rats during the first 7 days following a 1-hour or 4-hour exposure to unspecified MMT concentrations in an acute toxicity test. The rats resumed their normal weight gain by 14 days post-exposure.

Maneb and mancozeb. A man who was exposed via inhalation to 1.1 mg maneb/kg during a single application of the fungicide to a field was taken to the hospital with peripheral edema (de Carvalho et al. 1989). The man's weight had increased 11% over his typical weight due to the edema, which was precipitated by renal failure. The man's weight returned to normal following a special diet and steroid therapy. No studies were located concerning body weight effects from inhalation exposure to maneb or in humans or animals following inhalation exposure to mancozeb.

Metabolic Effects.

Inorganic Manganese

No studies were located concerning metabolic effects from inhalation of inorganic manganese in humans or animals.

Organic Manganese

MMT. No studies were located concerning metabolic effects following inhalation exposure to MMT in humans or animals.

Maneb and mancozeb. Acute exposure to maneb at doses of 1.1 mg/kg/day (de Carvalho et al. 1989) and 229 mg/kg/day (Koizumi et al. 1979) were associated with metabolic acidosis in male fungicide sprayers.

2.2.1.3 Immunological and Lymphoreticular Effects

Inorganic Manganese

One study on immunological effects in humans following inhalation to inorganic manganese was located. Male welders exposed to manganese (0.29–0.64 mg/m^3 for an unspecified duration), vibration, and noise exhibited suppression of the T and B lymphocytes characterized by reductions in serum immunoglobin G (IgG) and total E-rosette-forming cells (Boshnakova et al. 1989). However, the welders in this study were exposed to numerous other compounds, including cobalt, carbon dioxide, and nitric oxide. Therefore, it is impossible to determine whether exposure to manganese caused the effects. It is not known whether any of these changes are associated with significant impairment of immune system function. No studies were located on lymphoreticular effects in humans exposed to manganese by the inhalation route.

No studies were located on immunological or lymphoreticular effects in animals exposed to inorganic manganese by the inhalation route.

As noted above, inhalation exposure to particulate manganese compounds usually leads to an inflammatory response in the lung (i.e., pneumonitis), and this is accompanied by increased numbers of macrophages and leukocytes in the lung (Bergstrom 1977; Lloyd Davies 1946; Shiotsuka 1984; Suzuki et al. 1978). However,
this is an expected adaptive response of the immune system to inhaled particulates, and these data do not indicate that the immune system is injured. Conflicting data are reported concerning increased susceptibility to bacterial infection after exposure to airborne manganese. Lloyd Davies (1946) indicated that manganese exposure did not increase the susceptibility of mice to bacterial infection, whereas Maigetter et al. (1976) reported that exposure to aerosolized MnO_2 altered the resistance of mice to bacterial and viral pneumonias.

Organic Manganese

MMT. No studies on immunological or lymphoreticular effects from inhalation exposure to MMT in humans or animals were located.

Maneb and Mancozeb. No studies on immunological or lymphoreticular effects from inhalation exposure to maneb or mancozeb in humans or animals were located.

2.2.1.4 Neurological Effects

Inorganic Manganese

There is conclusive evidence from studies in humans that inhalation exposure to high levels of manganese compounds [usually MnO_2, but also compounds with Mn (II) and Mn (III)] can lead to a disabling syndrome of neurological effects referred to as 'manganism.' Manganism is a progressive condition that usually begins with relatively mild symptoms but evolves to include dull affect, altered gait, fine tremor, and sometimes psychiatric disturbances. Some of these symptoms also occur with Parkinson's disease, which has resulted in the use of terms such as "Parkinsonism-like disease" and "manganese-induced Parkinsonism" to describe those symptoms observed with manganese poisoning. Despite the similarities, significant differences between Parkinsonism and manganism do exist (Barbeau 1984; Calne et al. 1994; Chu et al. 1995). Barbeau (1984) reported that the hypokinesia and tremor present in patients suffering from manganism differed from those seen in Parkinson's disease. Calne et al. (1994) noted other characteristics that distinguish manganism from Parkinson's disease: psychiatric disturbances early in the disease (in some cases), a "cock-walk," a propensity to fall backward when displaced, less frequent resting tremor, more frequent dystonia, and failure to respond to dopaminomimetics (at least in the late stages of the disease).

Manganism and Parkinson's disease also differ pathologically. In humans and animals with chronic manganese poisoning, lesions are more diffuse, found mainly in the pallidum, caudate nucleus, the putamen, and even the cortex. In people with Parkinson's disease, lesions are found in the substantia nigra and other pigmented areas of the brain (Barbeau 1984). Moreover, Lewy bodies are usually not found in substantia nigra in manganism cases, but are almost always found in cases of Parkinson's disease (Calne et al. 1994). Manganese appears to affect pathways that are post-synaptic to the nigrostriatal system, most likely the globus pallidus (Chu et al. 1995). Magnetic resonance imaging (MRI) of the brain reveals accumulation of manganese in cases of manganism but few or no changes in people with Parkinson's disease; fluorodopa positron emission tomography (PET) scans are normal in cases of manganism but abnormal in people with Parkinson's disease (Calne et al. 1994). It is likely that the terms Parkinson-like-disease and manganese-induced-Parkinsonism will continue to be used by those less knowledgeable

about the significant differences between the two. Nonetheless, comparison with Parkinson's disease and the use of these terms may help health providers and health surveillance workers recognize the effects of manganese poisoning when encountering it for the first time.

Typically, the clinical effects of high-level inhalation exposure to manganese do not become apparent until exposure has occurred for several years, but some individuals may begin to show signs after as few as 1–3 months of exposure (Rodier 1955). The first signs of the disorder are usually subjective, often involving generalized feelings of weakness, heaviness or stiffness of the legs, anorexia, muscle pain, nervousness, irritability, and headache (Mena et al. 1967; Nelson et al. 1993; Rodier 1955; Tanaka and Lieben 1969; Whitlock et al. 1966). These signs are frequently accompanied by apathy and dullness along with impotence and loss of libido (Abdel-Hamid et al. 1990; Emara et al. 1971; Mena et al. 1967; Nelson et al. 1993; Rodier 1955; Schuler et al. 1957). Early clinical symptoms of the disease include a slow or halting speech without tone or inflection, a dull and emotionless facial expression, and slow and clumsy movement of the limbs (Mena et al. 1967; Nelson et al. 1993; Rodier 1955; Schuler et al. 1957; Shuqin et al. 1992; Smyth et al. 1973; Tanaka and Lieben 1969). In a study by Wolters et al. (1989), 6-fluorodopa (6-FD) and ^{18}F-2-fluoro-2-deoxyglucose (FDG) positron emission tomography (PET) were used to investigate the neurochemistry of four patients with "early manganism." FDG PET demonstrated decreased cortical glucose metabolism. No anomalies were noted in the 6-FD scans. This led the authors to suggest that, in early manganism, damage may occur in pathways that are postsynaptic to the nigrostriatal system, and most likely involve striatal or pallidal neurons.

As the disease progresses, walking becomes difficult and a characteristic staggering gait develops. Muscles become hypertonic, and voluntary movements are accompanied by tremor (Mena et al. 1967; Rodier 1955; Saric et al. 1977a; Schuler et al. 1957; Smyth et al. 1973). Few data are available regarding the reversibility of these effects. They are thought to be largely irreversible, but some evidence indicates that recovery may occur when exposure ceases (Smyth et al. 1973). Manganism has been documented in welders and in workers exposed to high levels of manganese dust or fumes in mines or foundries. Extreme examples of psychomotor excitement have been observed in manganese miners and, to a lesser extent, in industrial workers (Chu et al. 1995; Mena et al. 1967; Nelson et al. 1993). The behavior, known as "manganese madness" (Mena 1979) includes nervousness, irritability, aggression and destructiveness, with bizarre compulsive acts such as uncontrollable spasmodic laughter or crying, impulses to sing or dance, or aimless running (Emara et al. 1971; Mena et al. 1967; Rodier 1955; Schuler et al. 1957). Patients are aware of their irregular actions, but appear incapable of controlling the behavior.

The reports of frank manganism (Rodier 1955; Schuler et al. 1957; Smyth et al. 1973) observed in manganese miners clearly indicate that the onset of manganism results from chronic exposure to high concentrations of the metal. Documented cases indicate that the most important pathway of exposure is inhalation of manganese dusts or fumes, while other pathways such as ingestion of the metal from mucociliary transport of larger particles and hand-to-mouth activity, may contribute a smaller amount. Based on the data provided by Rodier (1955) and Schuler et al. (1957) it appears that the frequency of manganism cases increased with prolonged exposure, suggesting that the seriousness of the symptoms presented increases with cumulative exposure. For example, Rodier (1955) reports that the highest percentage of manganism cases (28, or 24.4%) occurred in miners with 1-2 years experience. Only 6 cases of manganism (5.2%) were reported in males with 1–3 months exposure, and 68% of the cases reported occurred after exposures greater than 1-2 years in length. Rodier did not present statistics on the number of men in the mine who were employed for comparable durations who did not suffer from manganism. Schuler et al. (1957) studied fewer manganism cases, but showed that the number of men with manganism increased with time spent mining, with the average time delay before onset of the disease being 8 years, 2 months. In fact, the minimum duration of exposure to the metal was 9 months before signs of manganism became recognizable, and the maximum exposure was 16 years. However, Schuler et al. (1957) point out that their study was not intended to "suggest incidence rates" and of the 83 miners selected for examination of potential manganism, only 9 were chosen as actually suffering from manganese poisoning. As with the Rodier (1955) study, the Schuler (1957) study did not discuss the exposure duration or symptomatology of those men not displaying "frank manganism;" therefore, these collective data, although suggestive of a cumulative effect of manganism neurotoxicity, must be interpreted with caution.

Recently, Huang et al. (1998) documented the progression of clinical symptoms of manganism in five surviving workers (from an original six) chronically exposed to manganese in a ferroalloy plant. These men were exposed from 3 to 13 years and were examined 9 to 10 years after manganese exposure had ceased. Neurologic examination revealed a continuing deterioration of health exhibited in gait disturbance, speed of foot tapping, rigidity, and writing. The men had high concentrations of manganese in blood, urine, scalp, and pubic hair at the time of the original neurologic evaluation. Follow-up analyses revealed a drastic drop in manganese concentrations in these fluids and tissues (e.g., 101.9 µg/g manganese in blood from patient 1 in 1987; 8.6 µg/g manganese in blood in 1995). Further, T1-weighted MRI analysis did not reveal any high-signal intensity areas. These data support the progression and permanence

of clinical effects from excess manganese exposure, even when tissue levels of the metal had returned to normal. Further, it shows that this neurotoxicity can continue in the absence of continuing manganese exposure.

More recent studies to estimate the impact of occupational exposure to manganese on neurological health have employed a number of sensitive tests designed to detect early signs of neuropsychological and neuromotor deficit in the absence of overt symptoms (Iregren 1999). These analyses allow the comparison of discrete performance values that are associated with either biological levels of manganese or approximations of exposure levels. Thus, they allow for the comparison of one exposure group to another without the subjective description of neurological symptoms that were prevalent in the studies with miners and others with frank manganism.

There are some quantitative data on the exposure levels leading to manganism. However, the available values are only estimates of actual exposure levels. Often, time-weighted averages of workplace exposures are reported, and dose-response relationships cannot be determined. As shown in Table 2-1 and Figure 2-1, manganese levels reported to lead to early signs of nervous system toxicity after inhalation exposure range from 0.027 to 1 mg Mn/m^3 (Chia et al. 1993, 1995; Iregren 1990; Lucchini et al. 1995; Mergler et al. 1994; Roels et al. 1987, 1992; Wennberg et al. 1991). Overt manganism has been observed at exposure levels ranging from 2 to 22 mg Mn/m^3 (Cook et al. 1974; Rodier 1955; Saric et al. 1977; Schuler et al. 1957; Tanaka and Lieben 1969; Whitlock et al. 1966). None of the recent occupational studies report a dose-response curve or determine the existence of a threshold for the effects observed. They do, however, clearly indicate the strong potential for significant, measurable neurological effects that are believed to be precursors to the clinical signs associated with frank manganism seen in the older studies in miners (Rodier 1955; Schuler et al. 1957). Given the consistency of these early neurological effects, a dose-response curve has been developed using raw data from two of these studies (Iregren 1990; Roels et al. 1992) and a modeling technique (Benchmark Dose Analysis) as discussed in Section 2.5.

Roels et al. (1987a) detected early preclinical neurological effects (alterations in simple reaction time, audioverbal short-term memory capacity, and hand tremor) in workers exposed to 0.97 mg manganese (median concentration in total dust)/m^3 as MnO_2, Mn_3O_4, $MnSO_4$, $MnCO_3$, $MnNO_2$ for a group average of 7.1 years. Similarly, Iregren (1990) used neurobehavioral tests (simple reaction time, digit span, finger tapping, verbal ability, hand dexterity, and finger dexterity tests from the Swedish Performance Evaluation System, SPES) to study adverse effects in 30 male workers from 2 different manganese foundries exposed to an estimated median concentration of 0.14 mg manganese (in total dust)/m^3 as MnO_2 for 1–35 years

(mean, 9.9). The exposed workers had below-average scores on a number of the tests, such as reaction time and finger tapping, when compared to matched controls with no occupational manganese exposure. A more recent study by Roels et al. (1992) provided results similar to these earlier reports. Workers in a dry alkaline battery factory exhibited impaired visual reaction time, hand-eye coordination, and hand steadiness when exposed to concentrations of MnO_2 in total dust ranging from 0.046 to 10.840 mg manganese/m^3 and in respirable dust from 0.021 to 1.32 mg manganese/m^3 (exposure ranged from 0.2 to 17.7 years). A lifetime integrated exposure (LIE) for both total manganese dust and respirable manganese was estimated for each of the exposed workers [$LIE = 3((C_{job\ 1} \times T_1) + (C_{job\ 2} \times T_2) + ... (C_{job\ n} \times T_n))$, where C is concentration, T is years of exposure, and LIE is expressed as mg manganese/m^3 times year]. Based on the analysis of data by a logistic regression model, it was suggested that there was an increased risk (OR=6.43, 95% CI=0.97–42.7) of decreased hand steadiness at a lifetime integrated exposure level of 3.575 mg/m^3*year for total dust or 0.730 mg/m^3*year for respirable dust. It should be noted that the LIE at which an increased risk of abnormal neurological function occurs is based on exposures in an occupational setting. Therefore, periods of exposures would be followed by periods that would be relatively free of manganese contamination. Presumably, during these "rest" periods the homeostatic mechanism would metabolize and excrete excess manganese to maintain the concentration within physiologic limits. Further, the LIE for deleterious neural effects may be biased in favor of a higher concentration due to the "healthy worker effect" (i.e., the most susceptible individuals are not incorporated into the study).

A study by Mergler et al. (1994) also supports the work of Iregren and Roels. This epidemiologic study included 115 (95% of the total) male workers from a ferromanganese and silicomanganese alloy factory who were matched to other workers from the region with no history of exposure. The groups were matched on the following variables: age, sex, educational level, smoking, and number of children. These workers were exposed to both manganese oxide dusts and manganese fumes. Environmental levels of manganese in total dust were measured at 0.014–11.48 mg/m^3 (median, 0.151 mg/m^3; arithmetic mean, 1.186 mg/m^3), while manganese levels in respirable dust were 0.001–1.273 mg/m^3 (median, 0.032 mg/m^3; arithmetic mean, 0.122 mg/m^3), and mean duration of exposure was 16.7 years. The exposed workers had significantly greater blood manganese levels, but urinary manganese did not differ between groups. Manganese workers showed decreased performance on tests of motor function (including those from the SPES) as compared to matched control workers with no manganese exposure. Using test results obtained from performance of the groups on the Luria-Nebraska Neuropsychological Battery and other tests, the authors reported that manganese-exposed workers performed more poorly than controls on tests of motor function, particularly on tests that required alternating and/or rapid hand movements and hand steadiness.

The exposed workers also differed significantly from the controls in cognitive flexibility and emotional state. They also exhibited significantly greater levels of tension, anger, fatigue, and confusion. Further, these workers had a significantly lower olfactory threshold than controls; this is the first study to report this effect following inhalation exposure to manganese.

Similar effects to those observed in the Mergler et al. (1994) study were observed by Chia et al. (1993). Workers in a manganese ore milling plant exposed to 1.59 mg manganese (mean concentration in total dust)/m^3 exhibited decreased scores in several neurobehavioral function tests including finger tapping, digit symbol, and pursuit aiming. Further, the workers exhibited an increased tendency for postural sway when walking with their eyes closed (Chia et al. 1995).

A more recent epidemiologic study (Lucchini et al. 1995) also supports findings of these earlier studies concerning the preclinical neurological effects of manganese exposure. This study, which evaluated performance on neuromotor tests (seven tests from the SPES, including simple reaction time, finger tapping, digit span, additions, symbol digit, shapes comparison, and vocabulary) involved 58 male workers from a ferroalloy plant. The workers had been exposed for 1–28 years (mean, 13; standard deviation, 7) to geometric mean airborne concentrations of manganese, as MnO_2, in total dust as high as 0.070–1.59 mg/m^3. These concentrations had decreased in the last 10 years to a range of 0.027–0.270 mg manganese (in total dust)/m^3. At the time of the study, the exposed workers were undergoing a forced cessation from work of 1–48 days. Blood and urine manganese levels were analyzed. A cumulative exposure index (CEI) was calculated for each subject by multiplying the average annual airborne manganese concentration in respirable dust characteristic of each job by the number of years for which this activity was performed. Significant correlations were found between the log value of blood manganese concentrations in exposed workers and the tests of additions, digit span, finger tapping, and symbol digit (log values for the last two tests); between the log value of urinary manganese levels and the performance on the additions test; and between the log value of the CEI and the log value of the symbol digit score. Further, a significant correlation on an individual basis was found between external exposure, represented by CEI, and blood and urine manganese levels. These results are unique in that they are the first to suggest that blood and urine manganese concentrations are indicative of exposure on an individual basis. As suggested by Lucchini et al. (1995), the correlations may be observable in this study, when they have not existed in past studies (Roels et al. 1987a; 1992), because the workers were assessed at a time when they were not currently being exposed to manganese. In support of this possibility, the correlation coefficients

between the urine and manganese levels and the CEI increased with time elapsed since the last exposure to airborne manganese (Lucchini et al. 1995).

Crump and Rousseau (1999) performed a follow-up study of 213 men occupationally exposed to manganese, 114 of whom were subjects in the Roels et al. (1987a, 1987b) studies. Exposure data were unavailable during the 11 years of study (1985–1996) during which blood and urine samples were taken and neurological tests (short-term memory, eye-hand coordination, and hand steadiness) were administered as in the Roels studies. Yearly blood and urine manganese levels remained fairly consistent throughout the study period, and were comparable to the levels reported in the previous studies. The authors suggest that the consistency of these data on manganese levels indicates that the airborne manganese concentrations to which the subjects were exposed during the study period were likely comparable to those at the time of the Roels studies. The average age and exposure duration of the subjects increased from 36 and 7 years, respectively, in 1985, to 41 and 14 years, respectively, in 1996. Variations in year-to-year test results were observed that were not attributable to age of the subject or exposure to manganese. The authors observed decreases in errors in the short-term memory test (number of repeated words and number of errors). During 1987, 1988, and 1989, the average number of words remembered on the memory test was lower than in any other year. However, there was a progressive improvement in percent precision and percent imprecision on the eye-hand coordination test during 1985–1988 (after 1991 the design of the test was modified and percent imprecision was lower in that year and all subsequent testing years). The authors suggest several reasons for the inter-year variability in test results (Crump and Rousseau 1999), including variations in test conditions, different groups of workers being tested in different years, the mood of the workers following a plant restructuring, and increased caution on the part of the subjects when answering test questions. When data analysis was controlled for year of testing, older workers performed significantly worse than younger workers on total words recalled in the memory test, and on percent precision and percent sureness in the eye-hand coordination test. Further, blood and urine manganese levels were not significantly associated with performance on memory or eye-hand coordination tests, but blood manganese was associated with performance on the hand steadiness test ($p<0.05$). Age was not a factor in hand steadiness when the year of test was controlled for in the analysis. Crump and Rousseau investigated whether individual test scores worsened with time by studying the group of 114 men from the original Roels et al. (1987a, 1987b) studies and a subset of 44 long-term employees who had been given both memory and hand steadiness tests on 2 occasions, 8 years apart. These analyses revealed decreases in performance over time for a particular hole in the hand steadiness test and improvements in repetitions and errors on the memory test, both of which were statistically significant. The authors suggest that the

improvements in the memory test were likely the result of increased caution on the part of the subject. The changes in performance over time could not be attributed solely to manganese exposure because it was impossible to control for age and year of testing in all of the analyses. They note the lack of an age-matched control group with which to compare test results and the absence of data caused by workers ending their terms of employment. Some have questioned whether inter-year variability in test results, potentially caused by different test administrators over time, would affect interpretation of the findings. While this may contribute to the changes in performance over time seen in the Crump and Rousseau (1999) study, this factor will potentially impact any study of this type.

Roels et al. (1999) performed an 8-year prospective study with 92 subjects exposed to MnO_2 at a dry-alkaline battery plant (Roels et al. 1992) to determine if poor performance on tests measuring visual reaction time, eye-hand coordination, and hand steadiness could be improved if occupational manganese exposure were decreased. The workers were divided into "low" (n=23), "medium" (n=55), and "high" (n=14) exposure groups depending on location within the plant and job responsibility. At the end of the 1987 study, technical and hygienic improvements had been implemented within the plant to decrease atmospheric manganese concentrations. Yearly geometric mean values for airborne MnT (total manganese dust) in the "low," "medium," and "high" exposure areas decreased in the following manner, respectively: ~0.310 to ~0.160 mg/m^3; ~0.900 to ~0.250 mg/m^3; ~3 to ~1.2 mg/m^3. The cohort decreased from 92 subjects in 1987 to 34 subjects in 1995 due to turnover, retirement, or dismissal, but no worker left due to neurological signs or symptoms. A separate group of workers was selected who had prior manganese exposure (ranging from 1.3 to 15.2 years). These subjects had left the manganese processing area of the plant prior to the end of 1992, and therefore, their exposure to manganese had ceased at that time; these workers were still employed in other areas of the plant. The control group consisted of 37 workers employed at the same polymer factory that had provided the control population in the previous study (Roels et al. 1992). This group, with an average age of 38.5 (range was 32–51 years) allowed for the analysis of age as a confounder. Exposure data (respirable manganese and total manganese dust, MnT) were taken with personal air samplers. Time-trend analysis of air sampler data revealed a significant decrease in total manganese from 1987 to 1995, with a more pronounced decline from 1992 forward. From 1987 to 1990, the authors observed that the precision of the hand-forearm movement (PN1) in the eye-hand coordination test for the whole cohort worsened, but then got progressively better. Hand steadiness and visual simple reaction time variables were inconsistent over time and time-trends were not observed. When the cohort was divided into exposure groups, and analyzed for performance on the eye-hand coordination test, it was revealed that in general, the performance on the PN1 aspect of the test

improved from 1987 to 1995, especially after 1991. The performance of the "low-dose" group was comparable to that of the control group in 1987 (Roels et al. 1992) and to that of the control group in 1997. The performance of the "medium-dose" group was intermediate between the "low-dose" and "high-dose" group. The only significant differences in performance were in the "high-dose" group as compared to the "low-dose" group during the years 1988–1990 (test scores of 49–51 for the high-dose group and 63–65 for the "low-dose" group). However, it was noted that performance on the eye-hand coordination test for the "medium" and "high-dose" groups was considerably poorer than the controls.

Significant differences were noted in variables in the hand steadiness test between the exposure groups during 1987–1992 (data not reported), when manganese concentrations were at their highest. However, no readily identified temporal changes in performance among the groups on this test was found, nor with the visual reaction time test. When the authors performed separate time-trend analysis on MnT levels and PN1 (eye-hand coordination test) values, a significant time effect was present for each variable. An analysis of covariance was performed for each exposure group (low, medium and high)in which log MnT was considered as covariate in order to adjust for estimation of PN1 variations as log MnT changed over time. The resultant data suggested that a reduction in log MnT was associated with an improvement in PN1 for each group. The authors also found that when time was also considered with log MnT as an interaction term, it did not influence PN1 variations over the years and the effect of time on PN1 values disappeared when log MnT was maintained as an ordinary covariate. The authors interpreted this to mean that performance on the eye-hand coordination tests were only related, and inversely so, to the exposure to manganese. In other words, when manganese exposure was increased, test performance decreased and vice versa (Roels et al. 1999). However, in the high-exposure group, the performance increased from 71 to 83% of that of the control group, and leveled off at this point, despite decreased manganese exposure occurring from 1991/1992 with most dramatic improvements occurring in 1994. The authors suggest that this leveling off of performance by the high-exposure group may be indicative of a permanent effect of manganese on eye-hand coordination. The authors tested PN1 values in exposed subjects 3 years following a cessation of exposure. They found that in 20/24, the PN1 values were below the mean PN1 values of the control group, but 16 of these individuals showed an improvement in 1996 (% improvement unspecified). The remaining four subjects (three "low-exposure" and one "medium-exposure" subjects) had PN1 values that exceeded the mean value of the control group. However, these data indicate that although there was improvement in performance on the coordination test, the vast majority of the exposed group still could not perform to the level of an unexposed worker 3 years after manganese exposure ceased. In addition, the exposed workers who did perform as well or better than the control subjects were

among the least exposed workers while at the plant. As discussed previously, performance of the "low-exposure" group on eye-hand coordination tests during 1992–1995 was comparable to that of the control groups from 1987 and 1997, indicating that manganese exposure of these individuals during that time did not severely impact their ability to perform this neurobehavioral test. Comparable performance on the tests by the same control group in 1987 and 10 years later, in 1997, indicates that age was not a confounder in this study. None of the variables except visual reaction time was significantly correlated with age, and the existing correlation in the visual reaction time test only represented a 3% difference (Roels et al. 1999).

Lucchini et al. (1999) also investigated differences in neurobehavioral test performance over time as exposure to manganese (MnO_2 and Mn_3O_4) decreased. The study group consisted of 61 men who worked in different areas of a ferroalloy plant. The plant was divided into 3 exposure areas with total manganese dust (geometric mean) values decreasing from 1981 to 1995: "high-exposure" values decreased from 1.6 to 0.165 mg/m^3; "medium-exposure" values decreased from 0.151 to 0.067 mg/m^3; and "low-exposure" values decreased from 0.57 to 0.012 mg/m^3. Respirable dust constituted 40–60% of the total dust value. Control subjects consisted of 87 maintenance and auxiliary workers from a nearby hospital who had not been exposed to neurotoxins. The study and control groups were well matched except for years of education and the percentage of subjects working night shifts.

The study groups answered a questionnaire concerning neuropsychological and Parkinsonian symptoms and underwent testing to determine the effect of manganese on neuromotor performance. Four tests were from the Swedish Performance Evaluations System SPES (addition, digit span, finger tapping, symbol digit) and five timed tasks were from the Luria Nebraska Neuropsychological Battery (open-closed dominant hand--Luria 1, open-closed non-dominant hand--Luria 2, alternative open-closed hands--Luria 3, thumb-fingers touch dominant hand--Luria 4, and thumb-fingers touch non-dominant hand--Luria 5). Individual scores were taken from these subtests, and the sum of the Luria tests was taken (Luria sum). Postural tremor was also measured, as was visual reaction time and coordination ability via the hand pronation/supination test. Manganese levels in blood and urine, as well as blood lead levels were analyzed prior to each neurobehavioral test. Manganese levels in both blood and urine were significantly elevated in exposed workers compared to controls ($p<0.0001$). Blood lead levels were also significantly higher in the ferroalloy workers ($p=0.0002$). The authors noted that the study groups did not report as many complaints as those reported in the Mergler et al. (1994) study.

After correcting for age, education, alcohol, smoke, coffee, shift work, and blood lead levels, an analysis of test results indicated that performance of the exposed workers was significantly different than that of controls on all tests except for Luria 5 and Luria sum. A comparison of SPES test results from workers tested in 1990 or 1991 and those from this study did not indicate any difference in paired t-test values; this indicates that performance did not improve over time or with decreasing exposure to manganese. Cumulative Exposure Index (CEI) values were calculated (in the same manner as in Lucchini et al. (1995)) for each exposure group and performance on the neurobehavioral tests was analyzed for correlation to these values and to manganese levels in body fluids. Significant differences were found between those with low CEI values of <0.5 mg/m^3*yrs, mid CEI values of 0.5-1.8 mg/m^3*yrs, and high CEI values of >1.8 mg/m^3*yrs and performance on the following tests: symbol digit, finger tapping, dominant and non-dominant hand, and digit span. A positive correlation was observed between the log CEI value and these tests, indicating that performance decreased as exposure increased. No correlations were found between CEI values and manganese levels in blood and urine; these results differ from the correlation between CEI and manganese levels in fluids from the previous study (Lucchini et al. 1995). Lucchini estimated a manganese dose (total dust) that would represent the annual airborne manganese concentration indicative of neurobehavioral deficit in this study by dividing the geometric mean CEI of the mid-exposure subgroup, 1.1 mg/m^3*yrs, by the geometric mean value of years of exposure for this same subgroup, 11.51, yielding a value of 0.096 mg/m^3. A comparable respirable dust value would be 0.038 mg/m^3 (0.096*0.40).

Gibbs et al. (1999) studied a population of workers in a U.S. plant that produces electrolytic manganese metal. These 75 workers and a well-matched group of control workers with no manganese exposure were administered a computerized questionnaire concerning neurological health issues (including mood, memory, fatigue, and other issues) and were analyzed for performance on several neurobehavioral tests including hand steadiness (Movemap steady, Movemap square, and tremor meter), eye-hand coordination (orthokinisimeter), rapidity of motion (4 choice reaction time and finger tapping). The Movemap test is a relatively recent test that has not undergone widespread use, and it has not been validated by other researchers. Further, although technically sophisticated, the test has not been observed to discriminate between exposure groups any better than simpler current methods (Iregren 1999).

Airborne levels of total and respirable manganese were obtained using personal samplers and were not available for years prior to 1997. Using the arithmetic mean of samples collected in 12 different job categories, exposure was estimated for the years prior to 1997. Cumulative exposure values for each worker were estimated for the 30-day and 12-month exposure periods just prior to neurobehavioral testing.

Multiple regressions of the test scores were performed using age and each of the following manganese exposure variables individually as explanatory variables: duration of exposure; 30-day cumulative exposure; 1-year cumulative exposure; and cumulative occupational exposure to either respirable or total manganese. Shift work was also used as a variable in conjunction with age and cumulative 30 day exposure to respirable or total manganese. The authors threw out outlying data points if they were greater than three times the standard deviation of the residual after a model fit. Exposures to respirable and total dust were highly correlated (r^2, 0.62–0.75), as were cumulative exposures over the previous 30 days and the previous year (r^2, 0.72–0.82); however, lifetime integrated exposure was not correlated with either 30-day or 12-month exposure values. The average exposure value for manganese-exposed workers was estimated at 0.066±0.059 mg/m3 (median, 0.051 mg/m3) for respirable dust, and 0.18±0.21 mg/m^3 for total dust (median, 0.086 mg/m^3).

Responses to the questionnaire and performance on the neurobehavioral tests did not differ significantly between exposed and control groups. When the outliers were included in the analyses, the cumulative years of exposure did have an effect on tapping speed—speed increased with increased exposure. The authors also reported an inverse correlation between age and performance on tests measuring eye-hand coordination but positively correlated between age and complex reaction time. The study by Gibbs et al. (1999)is the first to report a lack of poorer performance on neurobehavioral tests by workers chronically exposed to manganese. Interestingly, the median exposure estimates for respirable dust in this population (0.051 mg/m^3) is slightly higher than the lowest level of respirable dust at which preclinical neurological effects have been seen (0.032 mg/m^3) as reported by Mergler et al. (1994).

Gorell et al. (1999) noted a high Odds Ratio (OR) of 10.51 for the development of Parkinson's disease in individuals older than 50 who were occupationally exposed to manganese for greater than 20 years, but not for those exposed for fewer than 20 years. However, the numbers of individuals with a >20 year exposure was rather small (4), and occupational exposures to other metals (copper, and lead-iron, lead-copper, and iron-copper combinations) for greater than 20 years were also associated with increased risk for the disease.

Mergler et al. (1999) studied environmental exposure to manganese and its possible effect on mood (Bowler et al. 1999), neuromotor function (Beuter et al. 1999), and levels of the metal in biological fluids (Baldwin et al. 1999). The study group was a community in southwest Quebec, Canada, near which a former manganese alloy production plant served as a point source for environmental manganese pollution.

Due to the presence of MMT in gasoline in Canada, inhaled manganese from car exhaust is a potential contributor to manganese exposures experienced in the population studied. A total of 273 persons comprised the test population. These individuals were selected using a stratified random sampling strategy from the Quebec Health Plan Register which includes all residents. This strategy helps to ensure that no selection bias was introduced. These individuals were administered a test battery including a computerized neuromotor test, blood sampling, visual function tests from the Neurobehavioral Evaluation System-2, an extensive neuropsychological test battery, and diverse tests covering such areas as olfactory threshold, finger tapping, digit span, and postural sway.

Blood sampling data for the study subjects (Baldwin et al. 1999) indicated that manganese levels in women (geometric mean = 7.5 µg/L) were significantly higher than in men (6.75 µg/L). No relationship was found between the overall level of manganese in blood and those of lead or iron in serum. However, blood manganese levels were negatively correlated with serum iron in women and had a tendency to decrease with increasing age. Serum iron levels in men were higher than in women. The authors analyzed manganese in drinking water from the study subjects' residences and analyzed air samples from four different locations for total manganese particulates and PM_{10} values. The geometric mean value for manganese in drinking water was 4.11 µg/L; there was no correlation between individual values in drinking water manganese and manganese blood levels. Intersite differences in manganese values in total particulate were not observed in the air samples, but intersite differences did exist for manganese in PM_{10} values. Two geographical areas were identified where manganese in air contributed to blood manganese levels; serum iron was negatively related to blood manganese levels in this analysis (Baldwin et al. 1999).

The Profile of Moods State and Brief Symptom Inventory self-report scales were used to assess condition of mood in the study population (Bowler et al. 1999). The results from these analyses indicated that men who are older (>50 years) and have higher blood manganese levels ($7.5 µg/L) showed significant disturbances in several mood symptoms with significantly increased values for anxiety, nervousness, and irritability; emotional disturbance; and aggression and hostility when compared to those with lower levels of blood manganese. Neuromotor, neurological, and neurobehavioral analyses revealed that subjects with higher blood manganese levels ($ 7.5 µg/L) performed significantly worse on test for coordinated upper limb movements, with poorest performance in older men (Mergler et al. 1999). Also in men, proximal events on the qualified neurological examination, involving arm movements were significantly slower for those with higher blood manganese, and hand movements (distal events) tended to be in the same direction. No correlation was observed in women. Other measures of motor performance (e.g., hand-arm

tremor and tapping movements) were not related to blood manganese levels, although a significant decrease in tremor frequency dispersion was observed with log MnB (manganese blood level). For both men and women, performance on the learning and memory tests was inversely correlated with MnB values, although performance on individual portions of the overall test varied significantly with gender. For men, higher levels of MnB were associated with poorer performance on list acquisition, delayed auditory recall, and visual recognition following a distracter. Females, in contrast, tended to recall fewer geometric shapes, made more errors on the visual reproduction test, but remembered more numbers on the digit span forward test. This study is unique in that it is the first to study both males and females in an exposed population, and it shows an association between elevated manganese blood levels linked to elevated environmental manganese and poor performance on neurobehavioral and neuropsychiatric tests. This study also reported that neurological effects associated with higher levels of blood manganese were more likely to be observed in persons >50 years of age. In contrast, Roels et al. (1999) reported that age was a significant factor only in performance of the visual reaction time test, but not for the eye-hand coordination test or the measure of hand steadiness used in their longitudinal studies. However, Crump and Rousseau (1999) reported that older age was a significant factor in poor performance in tests of short-term memory and eye-hand coordination. Although there were no statistically significant neurological effects associated with manganese exposure among workers of a metal-producing plant evaluated by Gibbs et al. (1999), these investigators also noted that test performance in eye-hand coordination and reaction time decreased with increasing age.

In several animal studies, intermediate or chronic inhalation exposure of monkeys and rats to manganese dusts has not produced neurological signs similar to those seen in humans (Bird et al. 1984; EPA 1983c; Ulrich et al. 1979a, 1979b). However, in a chronic study with rhesus monkeys, decreased levels of dopamine were found in several regions of the brain (caudate and globus pallidus) (Bird et al. 1984). Behavioral tests (especially those that involve measurements of physical activity) have detected signs of neurological effects in mice, although these are only seen at relatively high exposure levels (60–70 mg manganese/m^3) (Lown et al. 1984; Morganti et al. 1985). The available evidence suggests that rodents may be less susceptible than humans to neurological damage from manganese. The available evidence also suggests that adverse neurological effects in rodent models are only measurable at extremely high doses (as high as 61–72 mg/m^3 in total dust via inhalation) compared to humans (as low as 0.14 mg/m^3 in total dust via inhalation). This difference may indicate that rodents are less susceptible than humans to manganese neurotoxicity or that there are differences in bioavailability or biokinetics between these species.

Organic Manganese

MMT. No studies on neurological effects from inhalation exposure to MMT in humans or animals were located.

Maneb and mancozeb. A 62-year-old man who had prepared and applied 229 mg maneb/kg/day over 2 days developed exaggeration of both knee jerk and ankle jerk in the left lower extremity. No other neurological effects were noted during examination (Koizumi et al. 1979). A 42-year-old man mixed 250 g of manzidan and, using personal protective equipment (protective clothing, mask and gloves), sprayed the solution on a plantation (Israeli et al.1983a). The next day he walked through the plantation without protective equipment. Later that same day, the man refused to talk to his family, he was nervous, complained of tiredness, dizziness, and weakness. The same symptoms were present the following day, and the man did not return to work. Two days later, the man felt better. Six days after the first pesticide application, the man made a manzidan solution 10 times stronger than the first (2,500 g, resulting in an average dose of 15,700 mg/kg/day) and sprayed it on the plantation, again with preventative measures. The man took a second walk through the plantation without any protection. He complained of weakness, headache, nausea, and fatigue. The man had difficulty removing his clothing and then began to redress, but did so incorrectly. His speech was unclear, he lost consciousness, and began to show tonic and clonic convulsions. Upon admittance to a hospital, the man again presented with the convulsions and showed right hypertonic hemiparesis. Reflexes were augmented on the right side, and Kernig sign was positive. An electroencephalogram (EEG) showed a diffuse slow rhythm without evidence of focal irritation or epileptiform events. A cerebrospinal fluid-pandy-protein test was negative. The man recovered normal neurological function after 2 days of clinical treatment with Dexacort (dexamethasone, a synthetic adrenocorticosteroid), Tagamet (an H_2-histamine receptor antagonist), and ritalin (a mild CNS stimulant).

A 47-year-old man was exposed to 16,000 mg/kg/day of maneb over the course of 2 years while developing a method to treat barley seeds with the fungicide (Meco et al. 1994). The man was in a closed environment with a ventilator, but did not use a mask or gloves during the maneb application. Two years after the exposure, the man developed a mild tremor associated with paresthesias (numbness with tingling) in his right leg, which later spread to the ipsilateral arm. The man was prescribed amantadine by a neurologist who diagnosed extrapyramidal syndrome; the drug had a beneficial effect on the symptoms. However, during the subsequent 3 years, the symptoms worsened, with the tremor spreading to the contralateral limbs. The symptoms remained stable for approximately 7 years, after which time another neurological

exam (Meco et al. 1994) was performed. At this time, the man exhibited a mild, generalized bradykinesia and rigidity, postural tremor in the right limbs, mild tremor of the lips, mild slowness of gait with reduced swinging of the arms, seborrhea, mild hypomimia, and slurred speech. A computerized tomography (CT) scan of the skull was normal, with a mild enlargement of the right lateral ventricle the only notable exception. Acute administration of apomorphine or L-dopa had no effect. The man was maintained on the previously-prescribed medication. Approximately 9 months following this exam, the man returned for observation with a worsening of symptoms. Examination revealed a resting tremor in all four limbs and the lips. MRI (T2-weighted scans) showed small hyperintense areas in the bilateral frontal and left parietal white matter, which were interpreted as nonspecific gliotic foci. The basal ganglia were normal. A neuropsychological exam revealed no relevant abnormalities, except for some perseverative errors and difficulties on attentive tests and in shifting between semantic and alphabetic categories.

One chronic study in farm workers exposed to maneb was located (Ferraz et al. 1988). This study involved 50 male workers who had either prepared solutions of maneb or had fumigated with it. The workers were exposed for at least 6 months and had an average age of 37 years (range, 17–58 years). The workers, and a control group of 19 men with no exposure to maneb, were administered a questionnaire concerning the duration and degree of exposure, and the existence of symptoms found in manganese poisoning. Neurological exams were also performed. Analyses of the results indicated that there was a statistically significant increase in the complaints of headache, nervousness, memory complaints, and sleepiness ($p<0.05$) in the exposed group as compared to the controls. The only significant neurological finding was the increased incidence of rigidity (with cogwheeling) ($p<0.01$). Only one worker showed a mild bradykinetic facies and gait associated with rigidity.

Ruitjen et al. (1994) studied 131 flower-bulb farmers (average age, 43 years) for neurological effects subsequent to 20 years (mean exposure, SD = 7) of exposure to a mixed pesticide including maneb and zineb for an average length of 20 years. The authors observed exposure-related decreases in conduction velocities in motor fibers of the median and peroneal nerves, and in the sensory fibers of the median and sural nerves. The authors noted that they cannot ascribe the effects to a particular pesticide, since rarely are pesticides used singly. The study population was also exposed to captan and formaldehyde during the exposure period.

2.2.1.5 Reproductive Effects

Inorganic Manganese

As discussed earlier (see Section 2.2.1.4), impotence and loss of libido are common symptoms in male workers afflicted with clinically identifiable signs of manganism attributed to occupational exposure to manganese for 1–21 years (Emara et al. 1971; Mena et al. 1967; Rodier 1955; Schuler et al. 1957). Obviously, these symptoms could lead to reduced reproductive success in men. Impaired fertility (measured as a decreased number of children/married couple) has been observed in male workers exposed for 1–19 years to manganese dust (0.97 mg/m^3) at levels that did not produce frank manganism (Lauwerys et al. 1985). This suggests that impaired sexual function in men may be one of the earliest clinical manifestations of manganism, but no dose-response information was presented so it is not possible to define a threshold for this effect. Jiang et al. (1996b) performed a reproductive epidemiological study on 314 men in a manganese plant. The men, from 6 different factories, performed milling, smeltering, and sintering duties for up to 35 years. The geometric mean airborne manganese concentration (assumed to be total dust) was 0.145 mg/m^3 as MnO_2. The researchers found no significant differences in reproductive outcomes between exposed and control workers (controls were matched for several factors, including age, smoking, personal hygiene, living habits, and cultural background). The incidences of sexual dysfunction were evaluated through researchers' questions and judged by the occurrence of two positive responses to three potential conditions: impotence, abnormal ejaculation (early ejaculation or nonejaculation), and lack of sexual desire. Impotence and lack of sexual desire were higher in the exposed group than in the controls (Jiang et al. 1996b). Wu et al. (1996) reported increased semen liquefaction time and decreased sperm count and viability in 3 groups of men occupationally exposed to manganese: 63 miners or ore processors, 38 electric welders in mechanical fields, and 110 electric welders in shipbuilding. Matched controls consisted of 99 men who were employed in the same occupation and from the same area, but were not exposed to manganese or other reproductive toxins. The men had been exposed to manganese for 1 or more years. Geometric means of total manganese dust (as MnO_2) ranged from 0.14 mg/m^3 for mining operations to 5.5 mg/m^3 for manganese powder processing. Manganese fume concentrations varied; the mechanical welders were exposed to a concentration of 0.25 mg/m^3 (geometric mean), while the shipbuilding area concentrations ranged from geometric means of 6.5 to 82.3 mg/m^3, depending on the location within the ship. The miners had a significant percentage (14.3%; $p<0.01$) of samples with increased liquefaction time, decreased sperm count (34.9%; $p<0.01$), and decreased percentage of total viable sperm (33.3% had abnormal counts; $p<0.01$) compared to controls. Welders in shipbuilding had

decreased sperm viability levels that were significantly different from controls ($p<0.01$). Manganese concentrations in semen were significantly increased compared to controls in the mechanical welders; copper, nickel, chromium, and iron concentrations were also elevated in semen in welders in both mechanical and shipbuilding careers. Further, stepwise regression analysis of the impact of these other metals on the measured reproductive parameters indicated that the higher the nickel concentration, the lesser the semen volume and the greater the number of deformed sperm. Copper in the seminal fluid was also positively linked with the viable sperm percentage, sperm viability and number of sperm. Although this study indicates that manganese exposure can cause sperm toxicity, the presence of other metals prevents any conclusive statements concerning its importance. Gennart et al. (1992) performed a reproductive study on 70 male workers exposed to manganese dioxide at a median concentration of 0.71 mg manganese/m^3 in total dust for an average of 6.2 years in a dry alkaline battery plant. Results from a questionnaire answered by the workers and controls in the study and from analysis of birthrates of exposed and control workers revealed no difference in birthrates between the groups.

These results in human studies reveal conflicting evidence for whether occupational exposure to manganese causes adverse reproductive effects. Effects reported may occur as a secondary result of neurotoxicity but do not provide information on any direct effect manganese may have on the reproductive organs. No information was found regarding reproductive effects in women.

Intratracheal instillation studies in rabbits indicate that single high doses of manganese (158 mg/kg, as MnO_2) can cause severe degenerative changes in the seminiferous tubules and lead to sterility (Chandra et al. 1973; Seth et al. 1973). This effect did not occur immediately but developed slowly over the course of 4–8 months following the exposure. Direct damage to the testes has not been reported in humans occupationally exposed for longer periods, suggesting that this effect may not be of concern under these exposure circumstances. However, it is unclear if specific studies to investigate possible testicular damage have been performed.

None of the studies located reported adverse effects in female animals following inhalation exposure to manganese. In a study with female mice (Lown et al. 1984), the average number of pups born to exposed females was increased when dams were exposed to MnO_2 before conception through gestation.

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 2-1 and plotted in Figure 2-1.

Organic Manganese

No studies were located concerning reproductive effects following inhalation exposure to organic manganese compounds in humans or animals. Although studies exist suggesting that exposure to pesticides may result in adverse reproductive effects, these studies do not involve exposure to maneb or mancozeb alone; therefore, no conclusions can be made concerning the potential adverse reproductive effects from exposure to these fungicides.

2.2.1.6 Developmental Effects

Inorganic Manganese

Very little information is available on the developmental effects of inorganic manganese from inhalation exposure. The incidences of neurological disorders, birth defects, and stillbirths were elevated in a small population of people living on an island where there were rich manganese deposits (Kilburn 1987). However, no conclusions could be reached on the causes of either the neurological effects or the increased incidence of birth defects and stillbirths because there were insufficient exposure data. Control data were not provided, and the study population was too small for meaningful statistical analysis. Although inhalation exposure was not ruled out, the route of exposure was assumed to be primarily oral.

Lown et al. (1984) evaluated the developmental effects of inhaled manganese in mice. The study involved exposing dams and non-pregnant female mice to either filtered air or manganese at an average concentration of 61 mg/m^3 (as MnO_2) 7 hours/day, 5 days/week, for 16 weeks prior to conception. The authors then exposed the mice to either air or manganese post-conception, irrespective of preconception exposure. Once delivered, six pups (three of each sex) were distributed to foster mothers and then nursed in the absence of exposure to manganese. The pups were then evaluated on postpartum day 7 for weight gain and gross locomotor activity and on day 45 for different behavioral parameters and learning performance. The authors observed that pups raised by foster mothers that had been exposed to manganese preconception and filtered air postconception had reduced weights compared to pups raised by foster mothers exposed only to filtered air. The activity data indicated that there were no observable differences in activity between pups who had been exposed to manganese *in utero* and those who had not. Therefore, the data did not provide evidence that manganese exposure resulted in adverse neurological developmental effects.

Organic Manganese

No studies were located concerning developmental effects in humans or animals following inhalation exposure to organic manganese.

2.2.1.7 Genotoxic Effects

Inorganic Manganese

One study was located regarding genotoxic effects in humans following inhalation exposure to manganese. In this study, the incidences of chromosomal aberrations in 3 groups of welders with occupational exposures (10–24 years) to metals including manganese, nickel, and chromium were examined (Elias et al. 1989). An increase in chromosomal aberrations was found in the group working with the metal active gas welding process; however, since their exposures included nickel as well as manganese, the authors could not attribute the results to any one metal exposure (nickel is known to cause chromosomal aberrations by the inhalation route). The median manganese concentrations during the survey were 0.18 mg/m^3 for respirable dust and 0.71 mg/m^3 for total dust. No information was available regarding the genotoxicity of manganese alone.

No studies were located regarding genotoxic effects in animals after inhalation exposure to inorganic manganese.

Organic Manganese

MMT. No studies were located concerning genotoxic effects in humans or animals following inhalation exposure to MMT.

Maneb and mancozeb. The genotoxic effects of occupational exposure to mancozeb were analyzed in a cohort of 14 women and 30 men, all of reproductive age (mean age for women, 31 years; mean age for men, 28 years) (Jablonická et al. 1989). A control group of 30 workers from the same plant, but who were not involved in mancozeb production was used; the controls were matched for age, social positions, and habits. Occupational exposure was for a period of up to 2 years in the production of mancozeb; dust concentrations in the air of the packing area of the final product ranged from 2.6–6.0 mg/m^3 active

ingredient of mancozeb (assuming the final product was 80% active ingredient and 20% dispersion ingredients). Peripheral blood cells were incubated in the presence of growth stimulator to test for cytogenetic effects, and in the presence of 5-bromodeoxyuridine to test for sister-chromatid exchange (SCE). The incidence of chromatid breaks was statistically significantly increased in exposed women compared to control women ($p<0.001$) and the combined exposed cohort ($p<0.01$) compared to the combined control group; isochromatid breaks were significantly increased in exposed men and the combined group as compared to their respective controls ($p<0.001$); gaps were significantly increased in all exposed groups ($p<0.001$). Chromatid exchanges were not significantly increased in any group. SCEs were not different between exposed and control groups when the influence of smoking was taken into account. Mancozeb-exposed smokers had a significantly increased incidence of SCEs as compared to exposed non-smokers ($p<0.05$). There was no difference between exposed non-smokers and control non-smokers in the incidence of SCEs.

Other genotoxicity studies are discussed in Section 2.5.

2.2.1.8 Cancer

No studies were located regarding carcinogenic effects in humans or animals after inhalation exposure to inorganic or organic manganese.

2.2.2 Oral Exposure

Although humans are often exposed to significant quantities of inorganic manganese compounds in food and water (see Sections 5.4 and 5.5), reports of adverse effects in humans from ingestion of excess manganese are limited. Most information on the effects of oral exposure to inorganic manganese is derived from studies in animals. These studies are summarized in Table 2-2 and Figure 2-2, and the findings are discussed below. All doses are expressed as mg manganese/kg/day.

Health effects following oral exposure to the organic manganese compounds MMT, maneb, and mancozeb were observed in animals. Studies involving oral exposure of animals to MMT are summarized in Table 2-3 and Figure 2-3. Studies involving oral exposure of animals to maneb and mancozeb are considered together in Table 2-4 and Figure 2-4. As discussed previously, because inhalation, oral, and dermal pathways are not a concern regarding exposure to mangafodipir, this

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)		LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
	ACUTE EXPOSURE							
	Death							
1	Rat (Sprague-Dawley)	Once GW					412 M (LD50 - $MnCl_2$)	Holbrook et al. 1975 MnCl2
2	Rat (albino)	Once GW					804 M (LD50)	Kostial et al. 1978 MnCl2
3	Rat (Wistar)	Once GW					275 (LD50 - pups)	Kostial et al. 1989 MnCl2
							342 M (LD50)	
							331 F (LD50)	
4	Rat (Wistar albino)	Once G					642 M (LD50 - MnCl2)	Singh and Junnarkar 1991 MnSO4, MnCl2
							782 M (LD50 - MnSO4)	
5	Rat (NS)	Once GW					1082 (LD50)	Smyth et al. 1969 MnAc
	Systemic							
6	Rat (F344/N)	14 d (F)	Resp	1300				NTP 1993 MnSO4
			Cardio	1300				
			Hemato	650	M			
			Hemato	1300	F	1300 M	(decreased leukocyte and neutrophil counts)	
			Hepatic	650	M			
			Hepatic	1300	F	1300 M	(reduced liver weight)	
			Renal	1300		1300	(decreased body weight - 57% in males, 20% in females)	
			Endocr	1300				
			Bd Wt	650				
7	Mouse (B6C3F1)	14 d (F)						NTP 1993 [UPDATE2] MnSO4

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
Neurological							
8	Rat (Wistar)	6d (GW)			22 M (increase in dihydroxyphenylacetic acid and uric acid in striatum)		Desole et al. 1994 MnCl2
9	Rat (F344/N)	14 d (F)					NTP 1993 MnSO4
Reproductive							
10	Rat (Sprague-Dawley)	Gd6-17 (GW)		22 F			Grant et al. 1997 MnCl2
11	Rat (F344/N)	14 d F					NTP 1993 [UPDATE2] MnSO4
Developmental							
12	Rat (Sprague-Dawley)	Gd6-17 (GW)		22 B			Grant et al. 1997 MnCl2

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
INTERMEDIATE EXPOSURE								
Death								
13	Rat (Long- Evans	21 d GW				225	(LD50 - 21 days)	Rehnberg et al. 1980 Mn3O4
Systemic								
14	Rat (Long- Evans	224 d F	Hemato	180 M				Carter et al. 1980 Mn3O4
15	Rat (Wistar)	1x/d;20d (F)				6 M	(rats gained only 44% of what control rats gained with normal food consumption)	Exon and Koller 1975 Mn3O4
16	Rat (F344/N)	13 wk F			40 F (reduced lung weight) 33 M (increased neutrophil count) 155 F (decreased leukocyte count) 33 M (decreased liver weight) 618 F (decreased liver weight) 155 F (11% decreased body weight)			NTP 1993 MnSO4
17	Rat (Sprague-Dawley)	Gd 0-21 (GW)			11 F (increased cytochrome P450)			Szakmary et al. 1995 (MnCl2)
18	Rat (white)	10 wk W						Wassermann and Wassermann 1977 MnCl2

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)		Serious (mg/kg/day)	
19	Mouse (CD-1)	90 d F						Gray and Laskey 1980 Mn3O4
20	Mouse (ddY)	100 d F			284	M	(decreased red blood cell count, white blood cell count, and hematocrit)	Komura and Sakamoto 1991 MnCl2, MnAc, MnCO3, MnO2
					284	M	(10% decrease in body weight gain)	
21	Mouse (B6C3F1)	13 wk F						NTP 1993 MnSO4
					1950	M	(mild hyperplasia and hyperkeratosis of the forestomach)	
					1950		(decreased hematocrit, hemoglobin, and erythrocyte count)	
					1950	M	(reduced liver weight)	
					1950	M	(13% lower body weight compared to controls)	
22	Gn Pig	30d; 1 d (G)			4.4	M	(patchy necrosis, decreased ATPase, G6Pase in stomach and small intestine)	Chandra and Imam 1973 MnCl2
	Immunological/Lymphoreticular							
23	Rat (F344/N)	13 wk F		77 F	33	M	(increased neutrophil count)	NTP 1993 MnSO4
					155	F	(decreased leukocyte count)	
	Neurological							
24	Rat (Sprague-Dawley)	2 mo W			594	M	(increased gamma-aminobutyric acid levels)	Bonilla 1978b MnCl2

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
25	Rat (Sprague-Dawley)	8mo (W)			380 M (increased L-tyrosine hydroxylase activity in neostriatum, midbrain, hippocampus, and hypothalamus)		Bonilla 1980 MnCl2
26	Rat (Sprague-Dawley)	8 mo W			14 M (decreased norepinephrine levels)		Bonilla and Prasad 1984 MnCl2
27	Rat (CD)	PND 1-49 (GW)			22 Increased spontaneous motor activity		Brenneman et al. 1999 MnCl2
28	Rat (ITRC)	30 d W			140 M (increased activity and aggression, turnover of striatal dopamine, tyrosine and homovanillic acid, altered neurotransmitter levels)		Chandra 1983 MnCl2
29	Rat (Neonatal)	60 d GW				1 M (neuronal degeneration, altered brain enzymes)	Chandra and Shukla 1978 MnCl2
30	Rat (ITRC albino)	360d (W)			40 M (increase of dopamine, norepinephrine, and homovanillic acid in striatum, followed by a decrease of all three compounds)		Chandra and Shukla 1981 MnCl2*4H20
31	Rat (CD Neonatal)	24 d GW			10 M (decreased dopamine levels in the hypothalamus, altered enzyme levels)		Deskin et al. 1980 MnCl2
32	Rat (CD)	ppd 0-24 (GW)			20 M (increased serotonin in hypothalamus, decreased acetylcholinesterase in striatum)		Deskin et al. 1981 MnCl2*4H20

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference
33	Rat	21 d 1 x/d (GW)			11 significant increase in pulse elicited startle reflex at PND21		Dorman et al. 2000 MnCl2
34	Rat	100-265d W			390 M (increased dopamine and dopamine metabolite levels)		Eriksson et al. 1987a MnCl2
35	Rat (Long- Evans	14-21 d GO					Kontur and Fechter 1988 MnCl2
36	Rat	44 d GW				150 (ataxia)	Kristensson et al. 1986 MnCl2
37	Rat (F344/N)	13wk F					NTP 1993 MnSO4
38	Rat (albino)	90 d W			11.8 M (altered brain regional dopamine and serotonin levels and monoamine oxidase activity)		Subhash and Padmashree 1991 MnCl2
39	Mouse (CD-1)	6 mo F			2270 M (decreased dopamine levels)		Gianutsos and Murray 1982 MnCl2
40	Mouse (CD-1)	90 d F			1050 M (decreased locomotor activity)		Gray and Laskey 1980 Mn3O4
41	Mouse (ddY)	100 d F			284 M (decreased motor activity)		Komura and Sakamoto 1991 MnCl2, MnAc, MnCO3, MnO2
42	Mouse (B6C3F1)	13 wk F					NTP 1993 MnSO4

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
Reproductive							
43	Rat (Long- Evans	20 d Gd 0-20 W		620 F	1240 F (decreased litter weight)		Kontur and Fechter 1985 MnCl2
44	Rat (Long- Evans	100-224d F			350 M (reduced testosterone levels)		Laskey et al. 1982 Mn3O4
45	Rat (Sprague-Dawley)	Gd1-pgd30 (W)					Pappas et al. 1997 MnCl2
46	Rat (Sprague-Dawley)	Gd 0-21 (GW)			22 F (increase in relative weight of liver, thymus, and brain)	33 F (post implantation loss)	Szakmary et al. 1995 (MnCl2)
47	Mouse (CD-1)	90 d F			1050 M (delayed growth of testes)		Gray and Laskey 1980 Mn3O4
48	Mouse (B6C3F1)	13 wk F					NTP 1993 MnSO4
49	Rabbit (New Zealand)	Gd 6-20 (GW)					Szakmary et al. 1995 (MnCl2)
Developmental							
50	Rat (CD)	PND 1-49 (W)			22 ~20% decrease in bw at PND 49		Brenneman et al. 1999 MnCl2
51	Rat (CD)	PND 1-49 (GW)			22 Increased spontaneous motor activity		Brenneman et al. 1999 MnCl2
52	Rat	21 d 1 x/d (GW)			22 40% decrease in wt. gain compared to controls		Dorman et al. 2000 MnCl2

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference
53	Rat	21 d 1 x/d (GW)			11 significant increase in pulse elicited startle reflex at PND21		Dorman et al. 2000 MnCl2
54	Rat	44 d GW				150 (ataxia)	Kristensson et al. 1986 MnCl2
55	Rat (Sprague-Dawley)	Gd1-pgd30 (W)			620 M (transient decrease in body weight on pgd 9-24) 620 M (increased activity at pgd 17)		Pappas et al. 1997 MnCl2
56	Rat (Sprague-Dawley)	Gd 0-21 (GW)			33 M F (increased retardation in skeletal/organ development)		Szakmary et al. 1995 (MnCl2)
57	Rabbit (New Zealand)	Gd 6-20 (GW)					Szakmary et al. 1995 (MnCl2)

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)		LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
CHRONIC EXPOSURE								
Death								
58	Rat (F344/N)	2 yr F					232 M (14% survival compared to 49% in controls)	NTP 1993 MnSO4
Systemic								
59	Rat (F344/N)	2 yr F	Resp	200	M			NTP 1993 MnSO4
				232	F			
			Cardio	200	M			
				232	F	200 M (increased severity of chronic progressive nephropathy)		
			Gastro	200	M			
				232	F			
			Hemato	200	M	200 M (body weight 10% less than controls)		
				232	F			
60	Mouse (B6C3F1)	2 yr F	Musc/skel	200	M			NTP 1993 MnSO4
				232	F			
			Hepatic	200	M			
				232	F	585 M (hyperplasia, erosion)		
			Renal	65	M			
				232	F		732 F (ulceration and inflammation of the forestomach)	
			Endocr	200	M	585 M (increased hematocrit, hemoglobin, and erythrocyte counts)		
				232	F			
			Dermal	200	M	585 M (thyroid follicular hyperplasia and dilatation)		
				232	F			
			Bd Wt	64	M	64 F (thyroid follicular hyperplasia)		
			Bd Wt	232	F	732 F (13% lower body weight than controls)		
Immunological/Lymphoreticular								
61	Rat (F344/N)	2yr F		200	M			NTP 1993 MnSO4
				232	F			
62	Mouse (B6C3F1)	2yr F						NTP 1993 [UPDATE2] MnSO4

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
Neurological							
63	Human	50 yr W		0.0048	0.059 (mild neurological signs)		Kondakis et al. 1989 NS
64	Human	10 yr or more (W)					Vieregge et al. 1995 NS
65	Monkey (Rhesus)	18mo (GW)				25 M (weakness, rigidity, neuronal loss and depigmentation of the substantia niagra)	Gupta et al. 1980 MnCl2
66	Rat (Wistar)	2 yr W			40 (altered neurotransmitter uptake)		Lai et al. 1984 MnCl2
67	Rat (Sprague-Dawley)	65wk W			40 M (increased activity)		Nachtman et al. 1986 MnCl2
68	Mouse (ddY)	3 gen W				10.6 (altered gait)	Ishizuka et al. 1991
69	Mouse (ddY)	12mo F			275 M (decreased brain dopamine, norepinephrine, and epinephrine levels; increased brain homovanilic acid levels; decreased locomotor activity)		Komura and Sakamoto 1992 [UPDATE2] MnCl2, MnAc, MnCO3 , MnO2
70	Mouse (B6C3F1)	2 yr F					NTP 1993 MnSO4
Reproductive							
71	Rat (F344/N)	2 yr F		201 M 232 F			NTP 1993 MnSO4

TABLE 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL		Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)	
72	Mouse (B6C3F1)	2yr F					NTP 1993 MnSO4

[a]The numbers correspond to entries in Figure 2-4.

Bd Wt = body weight; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; G = gavage;Gd = gestation day; GO = gavage oil; GW = gavage water;1x = once; LD50 = Lethal Dose (50% kill); LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); PND = post natal day(s)

Figure 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral

Acute (≤14 days)

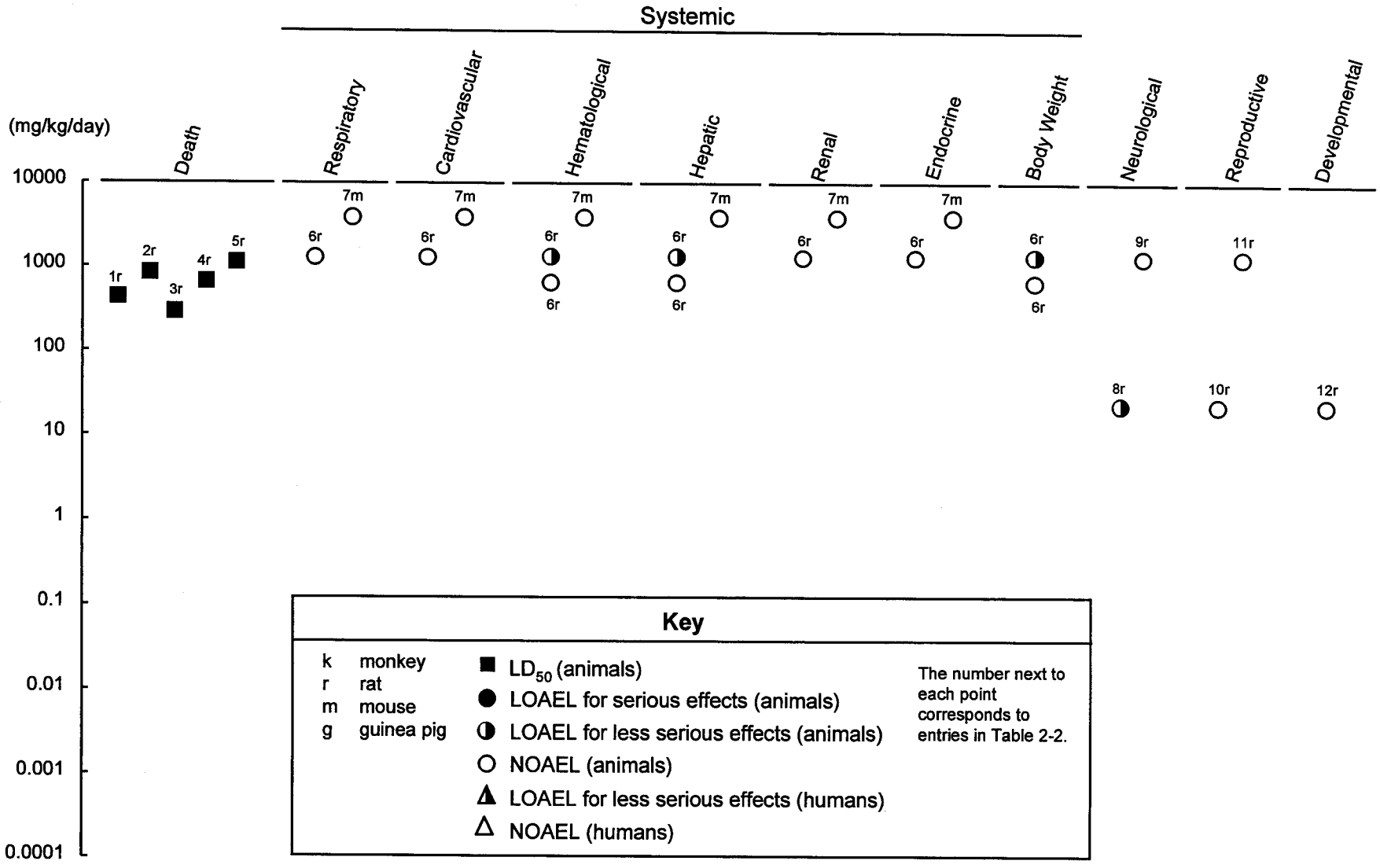


Figure 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (cont.)

Intermediate (15-364 days)

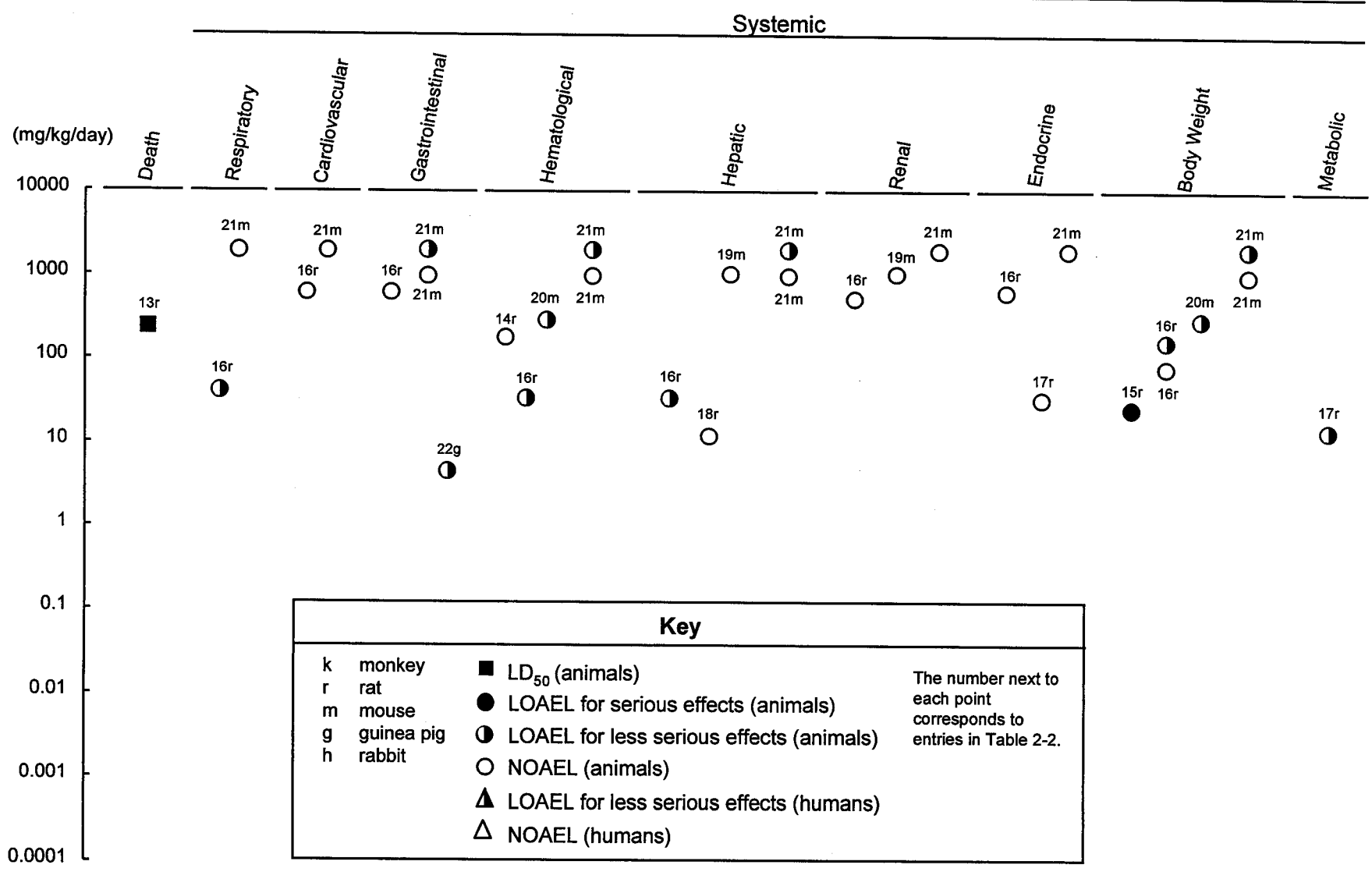


Figure 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (cont.)

Intermediate (15-364 days)

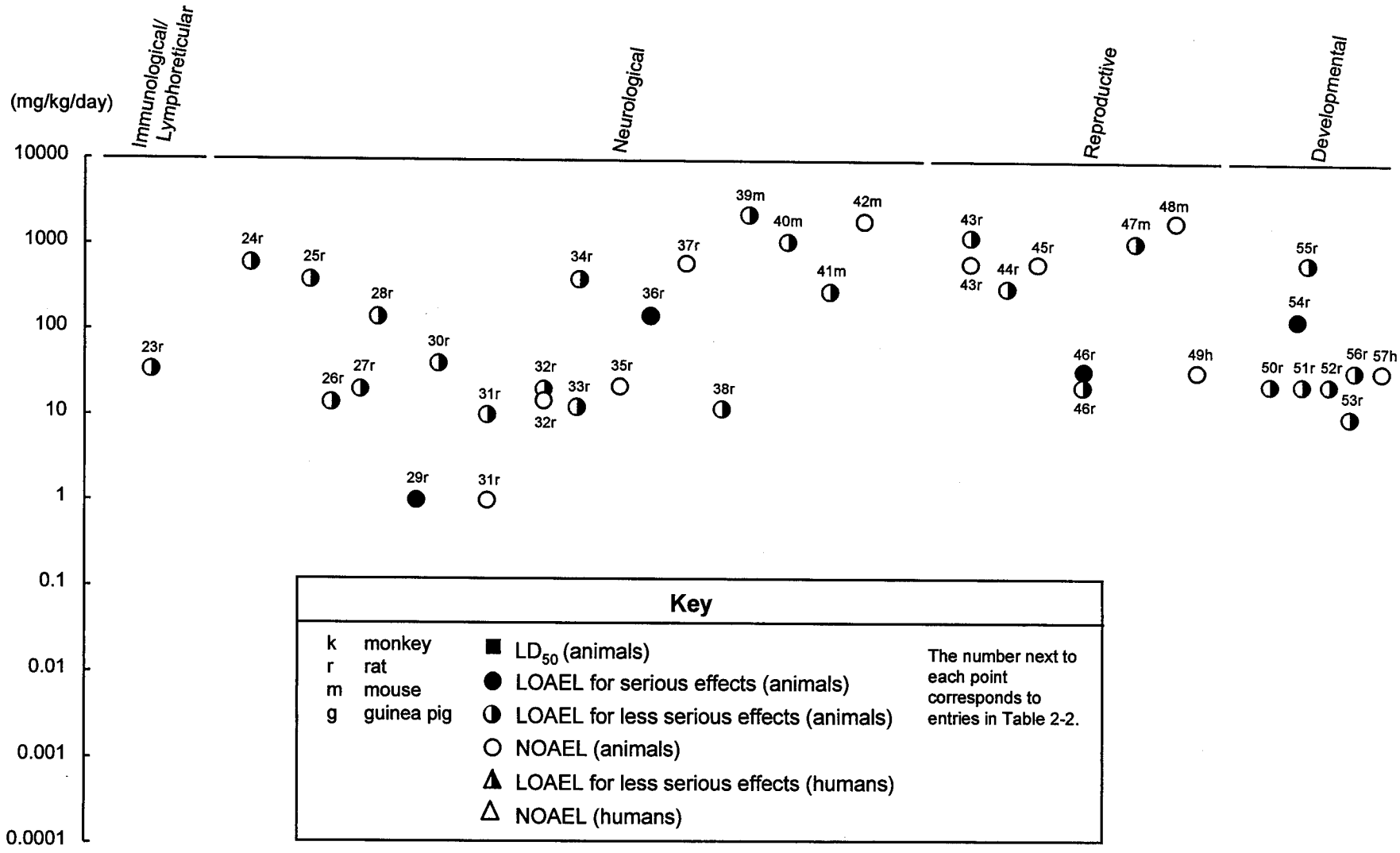


Figure 2-2. Levels of Significant Exposure to Inorganic Manganese - Oral (cont.)

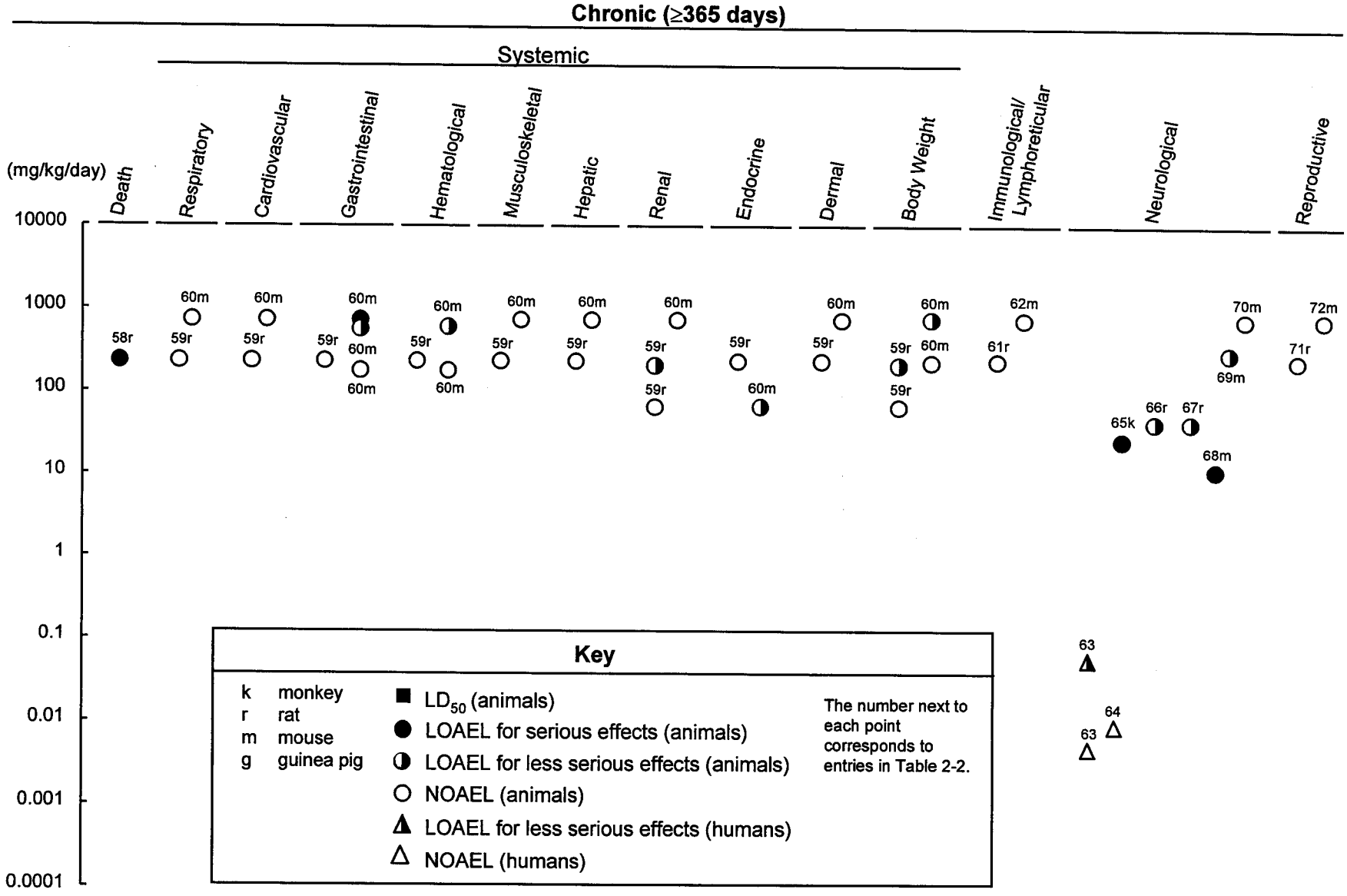


TABLE 2-3. Levels of Significant Exposure to MMT - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg)	LOAEL		Reference
					Less Serious (mg/kg)	Serious (mg/kg)	
	ACUTE EXPOSURE						
	Death						
1	Rat (Sprague-Dawley)	1x (GO)				13 M	Hanzlik et al. 1980a
2	Rat (Sprague-Dawley)	1x (GO)				15 B	Hinderer et al. 1979
	Systemic						
3	Rat (Sprague-Dawley)	1x (GO)				30 M	Hanzlik et al. 1980a

TABLE 2-3. Levels of Significant Exposure to MMT - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg)	LOAEL		Reference
					Less Serious (mg/kg)	Serious (mg/kg)	
	CHRONIC EXPOSURE		Bd Wt		(>10% decrease in body weight in exposed group)		
	Systemic						
4	Mouse (ddY)	1x/d; 12mo (F)			11 M		Komura and Sakamoto 1992b
	Neurological						
5	Mouse (ddY)	1x/d; 12mo (F)			11 M		Komura and Sakamoto 1992b
6	Mouse (ddY)	12 mo (F)			11 M		Komura and Sakamoto 1994

[a]The numbers correspond to entries in Figure 2-4.

Bd Wt = body weight; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; G = gavage;Gd = gestation day; GO = gavage oil; GW = gavage water;1x = once; LD50 = Lethal Dose (50% kill); LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); PND = post natal day(s)

Figure 2-3. Levels of Significant Exposure to Organic Manganese - MMT - Oral

Acute (≤14 days)

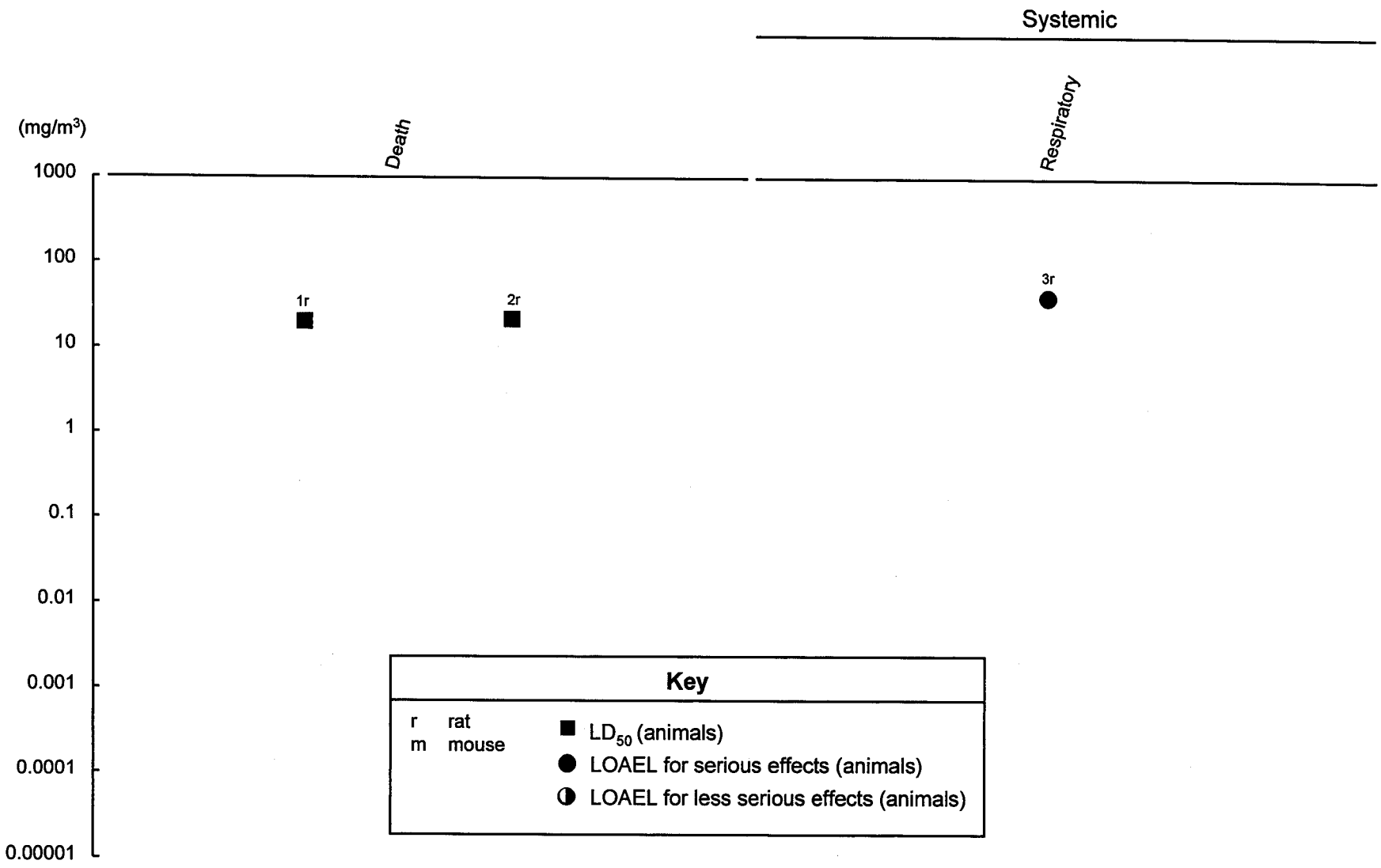


Figure 2-3. Levels of Significant Exposure to Organic Manganese - MMT - Oral (cont.)

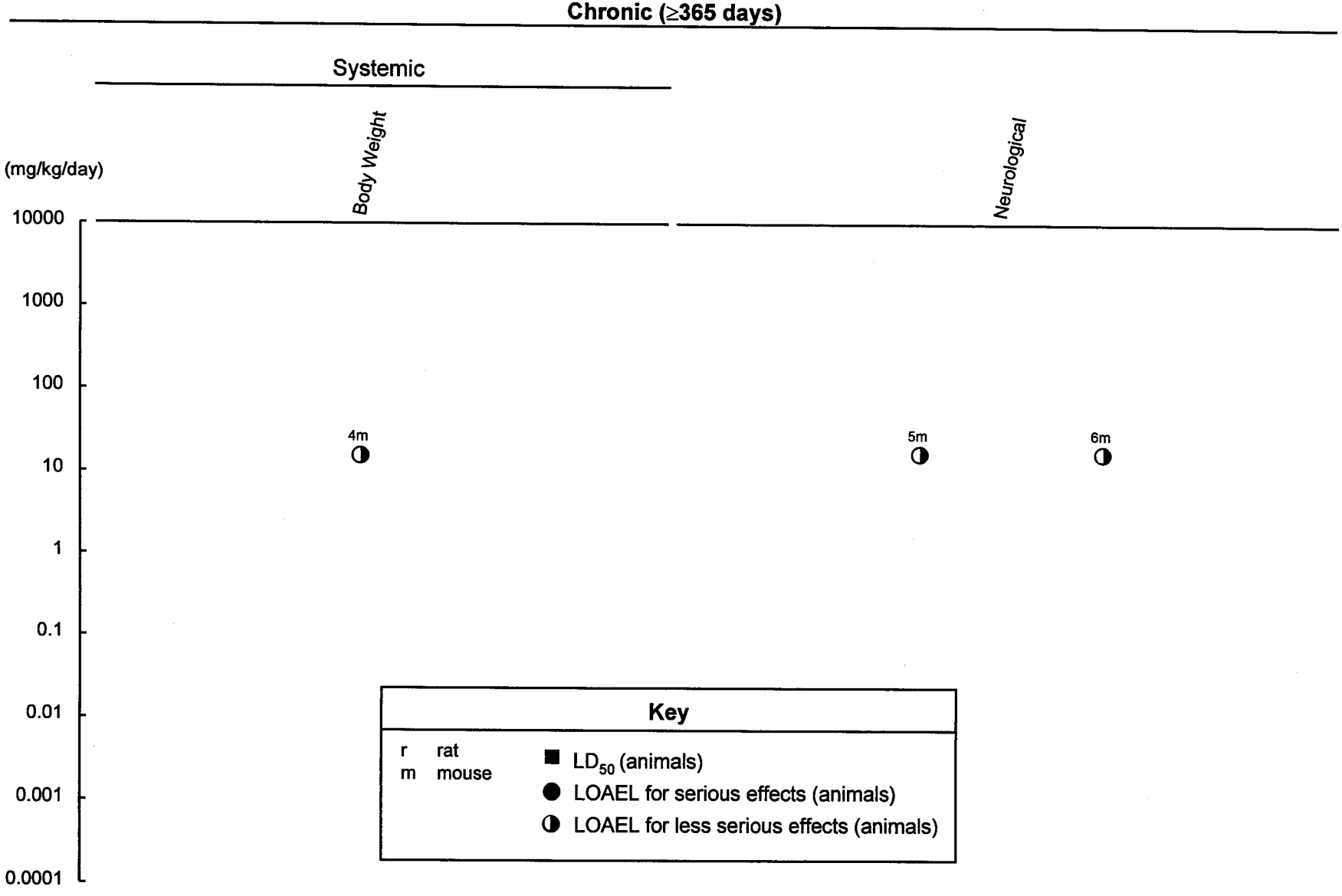


TABLE 2-4. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Oral

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)		Serious (mg/kg/day)	
ACUTE EXPOSURE								
Death								
1	Rat (Wistar)	once (GO)					11250M (LD_{50})	Kackar et al. 1997a Mancozeb
Systemic								
2	Rat (Wistar)	7d (GW)	Metab		5.7 M	(significant decrease in liver p-nitroanisole O-dealkylase and aniline hydroxylase)		Siddiqui et al. 1993 Mancozeb
Reproductive								
3	Rat (Sprague-Dawley)	10 d Gd7-16 (GW)					100 F (33% decrease in maternal body weight gain compared to controls)	Chernoff et al. 1976 Maneb
4	Rat (Sprague-Dawley)	once Gd11 (GW)		1060 F				Larsson et al. 1976 Mancozeb
5	Rat (Sprague-Dawley)	once Gd11 (GW)		655; no F ZnOAc				Larsson et al. 1976 Maneb
6	Rat (Sprague-Dawley)	once Gd11 (GW)					655 F (55% resorption)	Larsson et al. 1976 Maneb
7	Rat (NS)	once Gd11 or Gd13 (GW)					3200 F (significant increase in number of resorptions)	Petrova-Vergieva and Ivanova-Tchemishanska 1973 Maneb
8	Mouse (CD-1)	10 d 1 x/d Gd6-15 (G)					960 F (increased maternal death)	Beck 1990 Maneb
9	Mouse (CD-1)	10 d Gd7-16 (GW)			300 F			Chernoff et al. 1979 Maneb

TABLE 2-4. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)		Reference
10	Mouse (NMRI)	once Gd 9 or 13 (GW)		1060 F				Larsson et al. 1976 Mancozeb
11	Mouse (NMRI)	once Gd9 or 13 (GW)		1200 F				Larsson et al. 1976 Maneb
12	Chicken Sexsal laying hens	once (G-DMSO)			40 F			Serio et al. 1984 Maneb
13	Chicken (Shaver laying hens)	7 d (F)		180 F				Weppelman et al. 1980 Maneb
	Developmental							
14	Rat (Sprague-Dawley)	10 d Gd7-16 (GW)				384	(9% decrease in fetal body weight w/ decrease in caudal ossification)	Chernoff et al. 1979 Maneb
15	Rat (Sprague-Dawley)	9 d Gd7-15 (GW)			380 M			Chernoff et al. 1979 Maneb
16	Rat (Sprague-Dawley)	once Gd11 (GW)				1060 F	(22/87 surviving fetuses were malformed with effects including neromelia, adactyly, short tail, malformed vertebral column and spinal cord)	Larsson et al. 1976 Mancozeb
17	Rat (Sprague-Dawley)	once Gd11 (GW)				340 F	(skeletal malformations, short tail, open eye, umbilical hernia)	Larsson et al. 1976 Maneb
18	Rat (NS)	once Gd11 or Gd13 (GW)				400	(decrease in the number of live fetuses)	Petrova-Vergieva and Ivanova-Tchemish anska 1973 Maneb
19	Rat (Wistar)	7 d (GW)			5.7 M			Siddiqui et al. 1993 Mancozeb

TABLE 2-4. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
20	Mouse (CD-1)	10 d 1 x/d Gd6-15 (G)				960 F	(decrease in ossification of skeleton, bent tails, and increase in supernumerary ribs, increase in stillborn litters)	Beck 1990 Maneb
21	Mouse (CD-1)	10 d Gd7-16 (GW)			300 F			Chernoff et al. 1979 Maneb
22	Mouse (NMRI)	once Gd9 or 13 (GW)		1060 F				Larsson et al. 1976 Mancozeb

TABLE 2-4. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
INTERMEDIATE EXPOSURE							
Death							
23	Rat (albino)	30-360 d total 6d/wk (GO)				375M (lethality)	Kackar et al. 1997a Mancozeb
24	Rat (Wistar)	148 d Gd10-PND1 68 (F)				11 (decreased survival 7% when given with saline (compared to controls), when administered with NMU, survival decreased by27%)	Monis and Valentich 1993 Mancozeb
25	Rat (ITRC albino)	15-90 d 1 x/d (GO)				375M (death)	Trivedi et al. 199 Maneb
26	Rat (ITRC albino)	15-90 d 1 x/d (GO)				500M (death)	Triveldi et al. 1993 Maneb
Systemic							
27	Rat (albino)	30-360 d total 6 d/wk (GO)	Bd Wt		375 M (13% decrease in body weight over 180 days of study)		Kackar et al. 1997a Mancozeb
28	Rat (Wistar)	360 d 1 x/d (GO)				375M (thyroid hyperplasia, increase in thyroid:bw ratio, decreased ^{125}I uptake at 180 days, decrease in T_4 at 90 days) 375M (24% decrease in body weight gain)	Kackar et al. 1997b Mancozeb
29	Rat (Osborne-Mendel)	28 d PND1-28 (F)		20 M			Sobotka et al. 1972 Maneb

TABLE 2-4. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL Less Serious (mg/kg/day)	LOAEL Serious (mg/kg/day)	Reference
30	Rat (Osborne-Mendel)	180 d PND1-180 (F)		106 M			Sobotka et al. 1972 Maneb
31	Rat (Osborne-Mendel)	150 d PND29-180 (F)			4.8 M		Sobotka et al. 1972 Maneb
32	Rat (albino)	30 d 21x 5d/wk (GW)			188 M		Subramoniam et al. 1991 Mancozeb
33	Rat (ITRC albino)	15-90 d 1 x/d (GO)			375 M		Trivedi et al. 1993 Maneb
					375 M		
					375 M		
					375 M		
	Neurological						
34	Rat (Osborne-Mendel)	28 d PND1-28 (F)			1 M (significant decreases in exploratory activity and changes in regional neurochemical levels)		Sobotka et al. 1972 Maneb
35	Rat (albino)	30d 21x 5d/wk (GW)			188 M		Subramoniam et al. 1991 Mancozeb
36	Rat (ITRC albino)	15-90d 1 x/d (GO)				375M (hind limb paralysis)	Trivedi et al. 1993 Maneb
37	Rat (ITRC albino)	15-90 d (GO)				500M (hind limb paralysis)	Triveldi et al. 1993 Maneb
	Reproductive						
38	Rat (albino)	30-360 d total 6d/wk (GO)			375 M (significant changes in enzyme activities in testes and epididymis at 180d and beyond, significant decrease in concentration of sialic acid in testes and protein in epididymis at 360d)		Kackar et al. 1997a Mancozeb

TABLE 2-4. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Oral (continued)

Key to figure[a]	Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg/day)	LOAEL			Reference
					Less Serious (mg/kg/day)	Serious (mg/kg/day)		
39	Rat (NS)	19 d 1 x/d Gd2-20 (GW)		400 F				Petrova-Vergieva and Ivanova-Tchemishanska 1973 Maneb
40	Mouse (Swiss albino)	35 d 1 x/d (GW)				750M	(80% decrease in sperm count and approximately 9% increase in incidence of sperm with abnormal morphology)	Khan and Sinha 1996 Mancozeb
	Developmental							
41	Rat (NS)	19 d 1 x/d Gd2-20 (GW)		400 F				Petrova-Vergieva and Ivanova-Tchemishanska 1973 Maneb
42	Rat (Osborne-Mendel)	28-150 d PND 1-28; PND 30-180 (F)			1 M			Sobotka et al. 1972 Maneb
	Cancer							
43	Rat (Wistar)	148 d Gd10-PND168 (F)				11	(significant increase in incidence of dysplastic foci in pancreas and carcinoma in situ when administered with NMU)	Monis and Valentich 1993 Mancozeb

[a]The numbers correspond to entries in Figure 2-4.

Bd Wt = body weight; d = day(s); Endocr = endocrine; F = female; Gastro = gastrointestinal; G = gavage;Gd = gestation day; GO = gavage oil; GW = gavage water;1x = once; LD50 = Lethal Dose (50% kill); LOAEL = lowest-observed-adverse-effect level; M = male; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s); PND = post natal day(s)

Figure 2-4. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Oral

Acute (≤14 days)

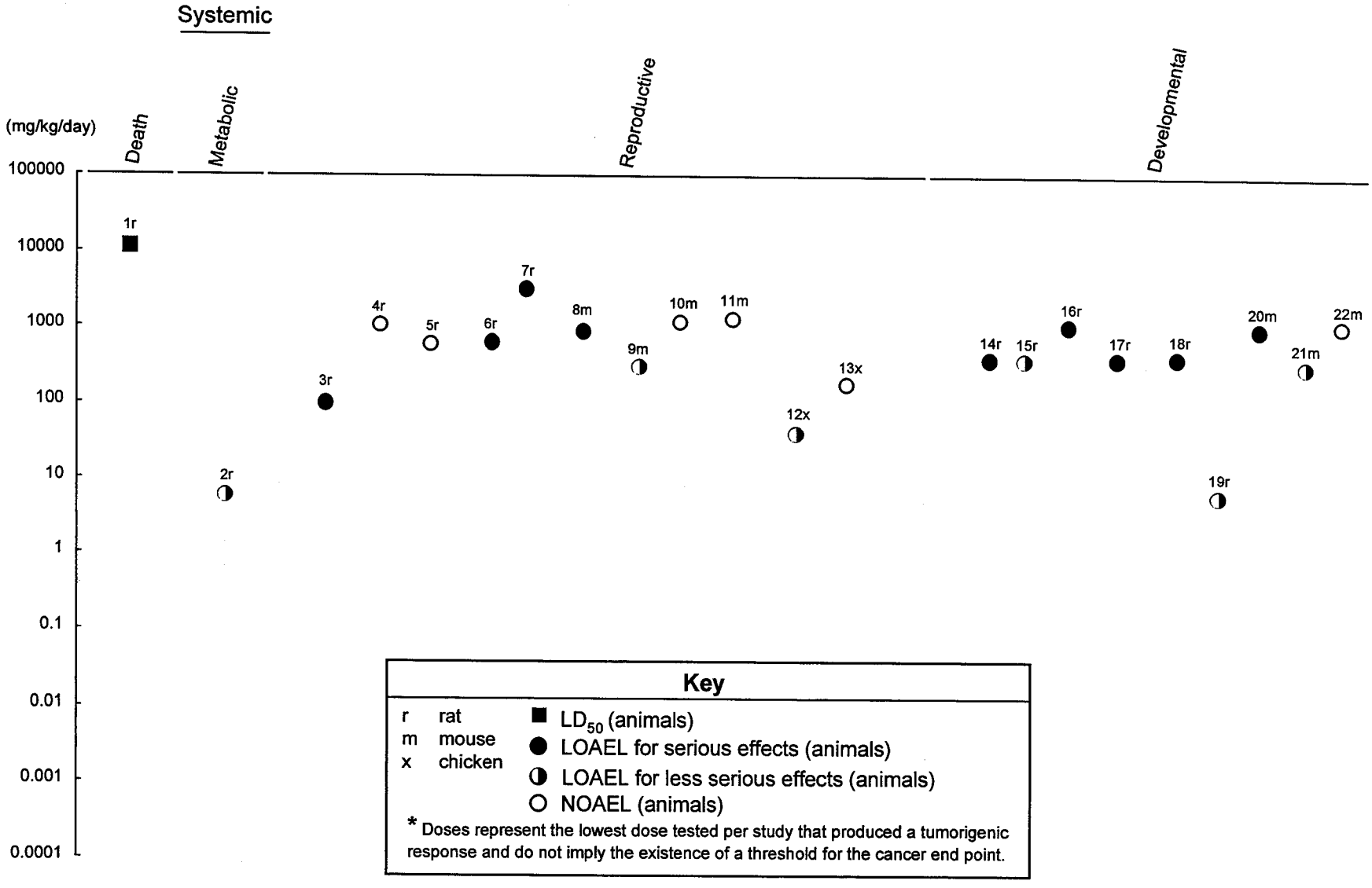
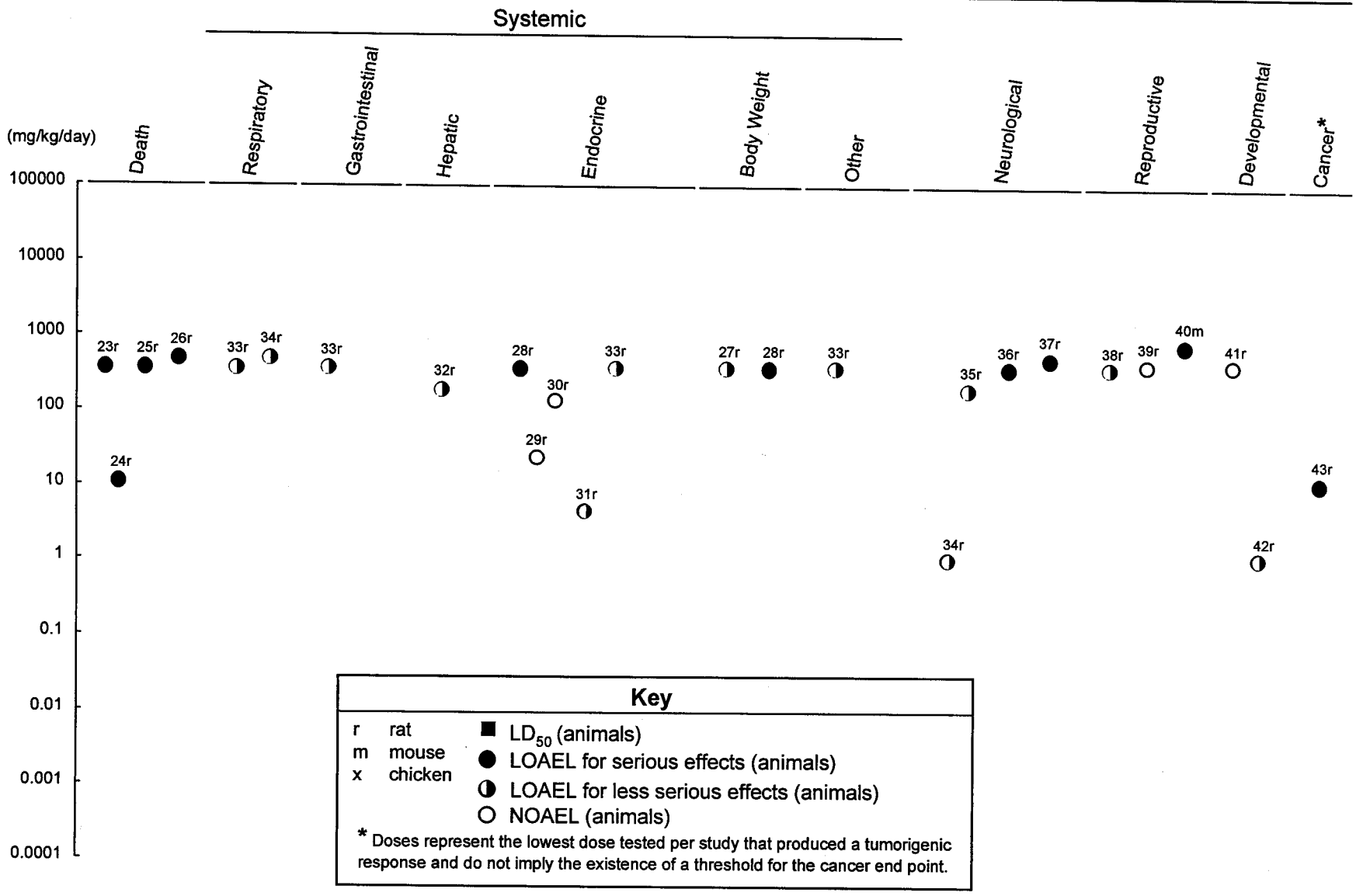


Figure 2-4. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Oral cont.

Intermediate (15-364 days)



compound's studies are not presented in a Levels of Significant Exposure table or figure; instead, it is discussed in Section 2.2.4.

2.2.2.1 Death

Inorganic Manganese

Only one study was located in which death in humans may have been associated with ingestion of manganese (Kawamura et al. 1941). In this report, death from "emaciation" occurred in two adults who ingested drinking water contaminated with high levels of manganese. However, as discussed in detail in Section 2.2.2.4, several aspects of this incident suggest that manganese may not have been responsible for the deaths.

In animals, most studies indicate that manganese compounds have low acute oral toxicity. In rats, daily doses of 1,300 mg manganese/kg/day (as $MnSO_4$ in the feed) for 14 days did not affect survival (NTP 1993). Survival was decreased in male rats fed 331 mg manganese/kg/day for 2 years (Hejtmancik et al. 1987a) and also in a study in which males received 200 manganese/kg/day as $MnSO_4$ for a similar duration (NTP 1993). In the latter study, the cause of death was attributed to increased severity of nephropathy and renal failure.

However, females fed 271 mg manganese/kg/day for 2 years were not affected in this manner (Hejtmancik et al. 1987a). The survival of male and female mice that received 722 mg manganese/kg/day as $MnSO_4$ or 905 mg manganese/kg/day as $MnSO_4$, respectively, for 2 years was not affected (Hejtmancik et al. 1987b). Similarly, doses as high as 2,270 mg manganese/kg/day ($MnCl_2$) in the diet were tolerated by mice for 6 months without lethality (Gianutsos and Murray 1982). The survival of mice was also unaffected by feeding as much as 731 mg manganese/kg/day as $MnSO_4$ for 2 years (NTP 1993).

In contrast to these studies, when exposure is by gavage (usually as highly concentrated solutions of $MnCl_2$ in water), measured LD_{50} values for 1–21 days of exposure range from 225 to 1082 mg manganese/kg/day in mice and rats (Holbrook et al. 1975; Kostial et al. 1978, 1989; Rehnberg et al. 1980; Singh and Junnarkar 1991; Smyth et al. 1969). This result suggests that gavage dosing with a bolus of a concentrated soluble manganese compound in water is not a good model for determining the toxic effects of manganese

ingested by humans from environmental sources because death in animals resulted from bolus dosing at concentrations near those tolerated in food given for longer durations. These results suggest that bolus dosing may circumvent the homeostatic control of manganese absorption. In addition, the concentrations used in the bolus dosing studies are much higher than even excess levels to which certain humans might be exposed. In addition to route of exposure, strain and species differences may account for some of the observed variations in toxicity.

Organic Manganese

MMT. No studies were located concerning death in humans following ingestion of MMT.

MMT, dissolved in oil and administered by gavage, was found to have LD_{50} values of 15 mg Mn/kg (LD_{50} of 58 mg/kg MMT * 55 mg Mn/218.1 mg MMT = 15 mg Mn/kg) in the male and female Sprague-Dawley rat and 58 mg Mn/kg in the adult female CD-1 mouse (Hinderer 1979).

Hysell et al. (1974) administered via gavage increasing amounts of MMT (dissolved in oil) to adult COBS rats, 10 animals/group. No lethality was observed at the lowest 2 doses of 3.8 and 7.5 mg Mn/kg, but 5/10 rats died within 2–6 days postdosing at a dose of 11.3 mg Mn/kg. Increasing numbers of rats died at higher doses, with decreasing times of death post-dosing; complete mortality occurred at the highest dose of 37.5 mg Mn/kg. The survivors appeared normal by 14 days. The LD_{50} (14-day) was estimated at 14.5 mg Mn/kg.

Hanzlik et al. (1980) determined the 14-day LD_{50} for purified MMT administered in corn oil via gavage to adult male Sprague-Dawley rats to be 12.5 mg Mn/kg (95% confidence interval, 9.5–16.8 mg Mn/kg). The animals survived similar times post-dosing as those in the Hysell et al. (1974) study.

Maneb and mancozeb. No studies were located regarding death in humans following oral exposure to maneb or mancozeb.

Mitchell et al. (1989) observed no mortalities in adult male Swiss mice in 96 hours post-dosing with 1 gavage dose of up to 32 mg/kg maneb.

Kackar et al. (1997a) determined the acute oral LD_{50} for mancozeb (75% active ingredient) dissolved in peanut oil and administered via gavage to male albino rats to be 11,250 mg Mn/kg.

In a subsequent chronic study, Kackar et al. (1997b) administered the same fungicide in the same manner to male albino rats for a period of 360 days; mortality was recorded at intervals. Rats died in a dose-dependent manner at each interval. The respective number of rats dying at each interval at the doses of 375, 750, and 1,125 mg Mn/kg are the following: 2, 4, and 5 rats dying in the first 30 days; 3, 5, and 10 rats died during days 30–90; 2, 3, and 3 rats died during days 90–180; and 3, 2, and 3 rats died during days 180–360. Only four rats total from the control group died during the last three exposure intervals. Monis and Valentich (1993) observed a significant mortality rate in offspring of Wistar rat dams in an intermediate mancozeb toxicity study. The dams were fed 11 mg mancozeb/kg/day in chow from gestation day 10 through birth of the pups. The pups were allowed to nurse while the dams were maintained on the mancozeb diet, then were weaned at 30 days of age and were placed on the mancozeb diet. When the pups were 3 days old they were injected with saline or nitrosomethylurea (NMU). The mancozeb-saline group had an overall mortality rate of 24%, whereas the mancozeb-NMU combined treatment caused 44% mortality by the end of the 24-week study.

All LD_{50} values from each reliable study for death in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

2.2.2.2 Systemic Effects

In general, there is a lack of data concerning systemic toxic effects in humans who have ingested manganese. This is likely due to the strong homeostatic control the body exerts on the amount of manganese absorbed following oral exposure; this control protects the body from the toxic effects of excess manganese. Studies in humans and animals provide limited data regarding the effects of manganese ingestion on systemic target tissues. This information is discussed below. Table 2-2 and Figure 2-2 present the highest NOAEL and all LOAEL values from each reliable study for these effects for each species and each duration category.

Respiratory Effects.

Inorganic Manganese

No studies were located regarding respiratory effects in humans after oral exposure to inorganic manganese.

No respiratory effects were reported in mice fed up to 3,900 mg manganese/kg/day as $MnSO_4$ or rats fed 1,300 mg manganese/kg/day as $MnSO_4$ for 14 days (NTP 1993). Male rats fed $MnSO_4$ for 13 weeks showed no respiratory effects at 520 mg manganese/kg/day; however, females exhibited decreased lung weight at 40–618 mg manganese/kg/day (NTP 1993). No respiratory effects were noted in mice of either sex fed 122–1,950 mg manganese/kg/day as $MnSO_4$ for 13 weeks (NTP 1993). Neither were respiratory effects observed in rats fed up to 232 mg manganese/kg/day $MnSO_4$ or mice fed up to 731 mg manganese/kg/day as $MnSO_4$ for 2 years (NTP 1993). No histological effects on the lung or any clinical signs of impaired respiratory functions were observed in mice or rats exposed for 2 years to average oral doses, respectively, of 905 or 331 mg manganese/kg/day as $MnSO_4$ (Hejtmancik et al. 1987a, 1987b).

Organic Manganese

MMT. The lungs of adult male Sprague-Dawley rats administered 1 dose of MMT via gavage in corn oil (31.25 mg Mn/kg) showed signs of hemorrhage, alveolar, and perivascular edema, with an accumulation of proteinaceous material in the alveoli. As early as 12 hours following gavage administration of this same dose, the lung/body weight ratio increased to 2.5 times the control value (Hanzlik et al. 1980). Hinderer (1979) observed dark red lungs in Sprague-Dawley rats and CD-1 mice administered sublethal doses (values unspecified) of MMT in an acute toxicity study. Gross necropsy of the lungs of COBS rats administered 1 dose of MMT in Wesson oil (dose range, 20–37.5 mg Mn/kg) revealed severe congestion and the release of a serosanguinous fluid upon sectioning; histopathology of lungs from rats dying within 24 hours post-exposure showed severe congestion, perivascular and alveolar edema, and alveolar hemorrhage (Hysell et al. 1974). Sections of lungs from rats surviving until 14 days post-exposure revealed extensive areas of consolidation, thickened alveolar septa, and focal areas of alveolar macrophage activity.

Maneb or mancozeb. No studies were located regarding respiratory effects in humans or animals following oral exposure to maneb or mancozeb.

Cardiovascular Effects.

Inorganic Manganese

No studies were located regarding cardiovascular effects in humans after oral exposure to inorganic manganese.

None of the located studies in animals reported adverse cardiovascular effects. In a 1993 NTP study, no cardiovascular effects (pathological lesions) were observed in mice or rats fed 3,900 or 1,300 mg manganese/kg/day, respectively, for 14 days. No cardiovascular effects were observed in rats or mice exposed for 13 weeks to doses as high as 1,950 mg manganese/kg/day as $MnSO_4$ or for 2 years to doses as high as 731 mg manganese/kg/day as $MnSO_4$ (NTP 1993). No histological effects on heart or blood vessels were observed in rats or mice exposed for 2 years to average oral doses, respectively, of 331 or 905 mg manganese/kg/day (as $MnSO_4$) (Hejtmancik et al. 1987a, 1987b).

Organic Manganese

No studies were located regarding the cardiotoxic effects of organic manganese in either humans or animals following oral exposure.

Gastrointestinal Effects.

Inorganic Manganese

No studies were located regarding gastrointestinal effects in humans after oral exposure to manganese, except for one case report of a child who accidentally ingested some potassium permanganate (Southwood et al. 1987). This led to severe local corrosion of the mouth, esophagus, and stomach due to the caustic effects of potassium permanganate ($KMnO_4$) on the tissue, but there was no evidence of systemic toxicity.

Adverse gastrointestinal effects have been reported in guinea pigs and mice but not in rats. Guinea pigs administered 4.4 mg manganese/kg/day (as $MnCl_2$ by gavage) did not suffer any gross abnormalities in either the stomach or small or large intestines as a result of treatment but did have patchy necrosis and decreased adenosine triphosphatase and glucose 6-phosphatase levels in both the stomach and small intestine (Chandra and Imam 1973). This study differs from the others in its delivery of manganese (by gavage); the gavage treatment may have partially or completely contributed to the adverse effects seen in the stomach and small intestine of the guinea pigs. No gastrointestinal effects were observed in female mice fed 1,950 mg manganese/kg/day as $MnSO_4$ (in food) or rats fed up to 618 mg manganese/kg/day as $MnSO_4$ (in food) for 13 weeks, but male mice exhibited mild hyperplasia and hyperkeratosis of the forestomach at 1,950 mg manganese/kg/day, also in food (NTP 1993).

In a 1993 NTP study, rats fed as much as 232 mg manganese/kg/day as $MnSO_4$ for 2 years showed no gastrointestinal effects; however, mice treated with $MnSO_4$ for 2 years exhibited hyperplasia, erosion, and inflammation of the forestomach at 585 mg manganese/kg/day for males and 731 mg manganese/kg/day for females. No histological effects on the gastrointestinal system were observed in rats given oral doses of 331 mg manganese/kg/day (as $MnSO_4$ in food) for 2 years (Hejtmancik et al. 1987a), although mild acanthosis (epithelial hypertrophy) was noted in the forestomach of mice receiving 905 mg manganese/kg/day (Hejtmancik et al. 1987b). The acanthosis was judged by the authors to be a result of direct irritation of the gastrointestinal epithelium and to be of minor consequence.

Organic Manganese

MMT. No studies were located concerning gastrointestinal effects following oral exposure to MMT in humans. Hinderer (1979) observed discolored intestinal tracts in Sprague-Dawley rats and fluid-filled intestines and spotting of the intestine in CD-1 mice dosed by gavage with high concentrations (values not provided) of MMT in a 14-day toxicity study. Hysell et al. (1974) observed that single lethal doses of 20–37.5 mg Mn/kg (as MMT, given by gavage) produced small intestines that were distended with clear watery contents and thin, friable walls.

Maneb and mancozeb. One case report involved an adult male who ingested small amounts of young vegetables in his garden without washing the vegetables (Koizumi et al. 1979). During the previous 2 days, the man had applied 229 mg/kg/day of maneb in his garden. The man developed diarrhea during the night after ingesting the unwashed vegetables. In a second study, a man who sprayed 1.1 mg/kg/day of

maneb on a field without using protective measures developed nausea, vomiting, and diarrhea (de Carvalho et al. 1989). The man had not washed his hands or body after pesticide application, and he had used his lips to clean the holes of the sprayer.

No studies were located regarding gastrointestinal effects in animals following oral exposure to mancozeb.

Hematological Effects.

Inorganic Manganese

In a dietary study with female subjects (Davis and Greger 1992), no changes in hematocrit, serum transferrin, or serum ferritin were reported following supplementation with 0.25 mg manganese/kg/day for 119 days. Vieregge et al. (1995) found no effects on hemoglobin, ceruloplasmin, copper and iron levels in serum for a population of 40-year-olds who had ingested at least 0.3 mg manganese/L in drinking water for a minimum of 10 years. These data indicate that exposure to increased manganese in water did not result in observable hematological toxicity.

Alterations in hematological parameters have been reported in rats and mice although they were found to vary depending on species, duration, and the form of manganese administered. No conclusive evidence regarding a significant functional deficit has been reported. In mice fed 284 mg manganese/kg/day for 100 days, red blood cell count was decreased by manganese acetate and $MnCl_2$; white blood cell count was decreased by manganese acetate, $MnCl_2$, and MnO_2; and hematocrit was decreased by $MnCO_3$ (Komura and Sakamoto 1991). However, $MnCO_3$ had no effect on red blood cells or white blood cells, MnO_2 had no effect on red blood cells or total hematocrit, and manganese acetate and $MnCl_2$ had no effect on total hematocrit. It has been suggested that the manganese-related effects on red blood cells may be related to the displacement of iron by manganese. The significance of the other hematological effects was not noted. In a study in rats and mice dosed with $MnSO_4$ for 14 days, 13 weeks, or 2 years, minor changes in hematology parameters were reported; these changes varied depending on species, dose, and duration, and the study authors did not consider them to be clearly related to compound administration (NTP 1993). No significant hematological effects were observed in mice exposed to 180 mg manganese/kg/day (as Mn_3O_4) for 224 days (Carter et al. 1980) or in rats or mice exposed for 2 years to average oral doses of 331 or 905 mg manganese/kg/day (as $MnSO_4$), respectively (Hejtmancik et al. 1987a, 1987b).

Organic Manganese

MMT. No studies were located concerning hematological effects following oral exposure to MMT in humans or animals.

Maneb and mancozeb. Hematological assays were normal in an adult male who may have ingested maneb while preparing and spraying up to 1.1 mg/kg/day in a single application on a field (de Carvalho et al. 1989). Studies concerning hematological effects in animals following oral exposure to maneb or in humans or animals following oral exposure to mancozeb were not available.

Musculoskeletal Effects.

Inorganic Manganese

No studies were located regarding musculoskeletal effects in humans after oral exposure to inorganic manganese.

In young rats, high concentrations of $MnCl_2$ in the diet (218–437 mg manganese/kg/day) led to rickets (Svensson et al. 1985, 1987); however, this was found to be due to a phosphate deficiency stemming from precipitation of manganese phosphate salt ($MnHPO_4$) in the intestine rather than to a direct biological effect of manganese on bone formation. No significant musculoskeletal effects were observed in mice or rats exposed to average oral doses of 905 or 331 mg manganese/kg/day (as $MnSO_4$), respectively, for 2 years (Hejtmancik et al. 1987a, 1987b) or in rats or mice fed up to 731 mg manganese/kg/day for 2 years (NTP 1993).

Organic Manganese

MMT. No studies were located concerning musculoskeletal effects following oral exposure to MMT in humans or animals.

Maneb and mancozeb. A man developed muscular weakness after nibbling unwashed young vegetables in his garden that had been sprayed with the equivalent of 229 mg/kg/day maneb (Koizumi et al. 1979).

There are no studies of musculoskeletal effects following oral exposure to maneb in animals or to mancozeb in humans. Hind limb paralysis has been observed in adult male rats administered a dose as low as 375 mg/kg/day mancozeb for 90–360 days (Kackar et al. 1997a, 1997b; Trivedi et al. 1993). Because there is little other evidence for an adverse effect of manganese on the musculoskeletal system, this is likely related to a neurologic effect with effects on the musculature being secondary.

Hepatic Effects.

Inorganic Manganese

A single study of human oral exposure of manganese investigated potential hepatotoxicity by analyzing liver enzymes in serum. Vieregge et al. (1995) reported no effects on bilirubin, alkaline phosphatase, glutamic pyruvic transaminase, glutamic oxalacetic transaminase, and gamma glutamyl transferase in humans, aged 40 and above, who had ingested well water containing $0.30 mg/L for at least 10 years. These limited data indicate that chronic exposure to elevated levels of manganese did not result in observable liver toxicity in this population.

In animals, a variety of histological changes in subcellular organelles (e.g., rough and smooth endoplasmic reticulum, Golgi apparatus) were observed in the livers of rats exposed to 12 mg manganese/kg/day for 10 weeks (as $MnCl_2$) (Wassermann and Wassermann 1977). However, these changes were not considered to be adverse but to be adaptive, possibly in response to the increased requirement for manganese excretion in the bile (see Section 2.3.4). Reductions in liver weight have also been reported in male Fischer 344 rats fed 1,300 mg manganese/kg/day as $MnSO_4$ for 14 days. However, these effects were not seen in $B6C3F_1$ mice fed dosages up to 3,900 mg manganese/kg/day as $MnSO_4$ for 14 days (NTP 1993). In rats fed up to 618 mg manganese/kg/day as $MnSO_4$ for 13 weeks, decreased liver weights were reported in males at $33 mg manganese/kg/day and females at 618 mg manganese/kg/day (NTP 1993). When mice were fed 122–1,950 mg manganese/kg/day as $MnSO_4$ for 13 weeks, the females showed no hepatic effects; however, the males exhibited both relative and absolute reduced liver weights at 1,950 mg manganese/kg/day (NTP 1993). In CD-1 mice, no hepatic changes were seen in males fed 137 mg manganese/kg/day as Mn_3O_4 (Gray and Laskey 1980). No significant hepatic histological changes were observed in either mice or rats exposed for 2 years to average oral doses of 905 or 331 mg manganese/kg/day (as $MnSO_4$), respectively (Hejtmancik et al. 1987a, 1987b). Neither were there hepatic changes reported in a 2-year

NTP (1993) study in which rats were fed up to 232 mg manganese/kg/day as $MnSO_4$, and mice were fed up to 731 mg manganese/kg/day as $MnSO_4$.

Organic Manganese

MMT. There are no studies concerning hepatic effects following oral exposure to MMT in humans.

Hinderer (1979) observed mottling of the liver in CD-1 mice administered high doses (unspecified) of MMT via gavage in a 14-day acute toxicity study. Histological evaluation of livers of adult male Sprague-Dawley rats administered 31.3 mg Mn/kg/day (as MMT) revealed scattered hepatocytes throughout the lobule that contained cytoplasmic vacuoles (Hanzlik et al. 1980). Twelve hours after administration of the same dose, no changes in plasma glutamic pyruvic transaminase (GPT) or liver glucose 6-phosphatase (G6P) activities were observed. After the death of 8/14 animals at this dose level (24 hours post-dosing), there were still no changes in plasma GPT, liver G6P, or hepatic triglycerides (Hanzlik et al. 1980). Hysell et al. (1974) observed that COBS rats that were gavage-dosed with 20–37.5 mg Mn/kg (as MMT) once and died within 24 hours post-dosing had livers with acute centrolobular passive congestion. This damage progressed to hepatic parenchymal necrosis and leukocytic infiltration in those rats surviving 48–72 hours (15–37.5 mg Mn/kg/day), and extensive cytoplasmic vacuolar change in rats surviving to 14 days.

Maneb and mancozeb. No studies of hepatic effects following oral exposure to maneb or mancozeb in humans or animals were located.

Renal Effects.

Inorganic Manganese.

No studies were located regarding renal effects in humans after oral exposure to inorganic manganese.

In animal studies, no significant renal histopathological changes were observed in any of the following: in mice and rats fed up to 3,900 or 1,300 mg manganese/kg/day ($MnSO_4$) for 14 days (NTP 1993); in mice exposed to 140 mg manganese/kg/day (as Mn_3O_4) in their diet for 90 days (Gray and Laskey 1980); in mice or rats fed up to 1,950 mg manganese/kg/day for 13 weeks (NTP 1993); in rats or mice exposed for 2 years to average oral doses of 331 or 905 mg manganese/kg/day (as $MnSO_4$), respectively, (Hejtmancik

et al. 1987a, 1987b); or in mice fed up to 731 mg manganese/kg/day for 2 years and female rats fed 232 mg manganese/kg/day as $MnSO_4$ (NTP 1993). Contrary to these findings, increased severity of chronic progressive nephropathy was noted in male rats fed 200 mg manganese/kg/day as $MnSO_4$ for 2 years (NTP 1993).

Organic Manganese

MMT. No studies were located concerning renal effects in humans following oral exposure to MMT.

Hanzlik et al. (1980) observed occasional vacuolar degeneration of proximal convoluted tubules of the kidney in Sprague-Dawley rats administered a single gavage dose of 31.3 mg Mn/kg (as MMT). Histopathologic renal effects observed within 24 hours of a gavage dose of 20–37.5 mg Mn/kg (Hysell et al. 1974) included hyaline droplet change, cytoplasmic vacuolation of the proximal convoluted tubules, and distention of the glomerular space and tubule lumens with a finely granular material that stained lightly basophilic. Within 48 hours post-dosing there was severe tubular degeneration in the form of nuclear pyknosis and cell lysis. Animals surviving the administration of 3.75–25 mg Mn/kg did not have any adverse renal effects.

No studies were located regarding renal effects in humans or animals after oral exposure to maneb or mancozeb.

Endocrine Effects.

Inorganic Manganese

No studies were located regarding endocrine effects in humans after oral exposure to inorganic manganese; however other elements of endocrine function (e.g., reproductive effects) following oral exposure to inorganic manganese are discussed elsewhere.

In mice fed up to 3,900 mg manganese/kg/day as $MnSO_4$ and rats fed 1,300 mg manganese/kg/day as $MnSO_4$ for 14 days, no endocrine effects (pathological lesions) were observed (NTP 1993). The adrenal gland was assessed for atypical cells and hyperplasia. In the pituitary gland, the pars distalis was assessed for cyst, hyperplasia, and hypertrophy. The pars intermedia was checked for cysts. C-cells and hyperplasia

were examined in the thyroid gland. No endocrine effects were observed in mice or rats fed up to 1,950 mg manganese/kg/day as $MnSO_4$ for 13 weeks. A 2-year study in rats fed up to 232 mg manganese/kg/day as $MnSO_4$ reported no endocrine effects (NTP 1993). However, in a 2-year mouse study, thyroid follicular hyperplasia and dilatation were observed in males fed 584 mg manganese/kg/day, and thyroid follicular hyperplasia was observed in females fed 64 mg manganese/kg/day (NTP 1993).

Organic Manganese

MMT. No studies were located regarding endocrine effects in humans or animals following oral exposure to MMT.

Maneb and mancozeb. Thyroid function tests in an adult male farmer who ingested unwashed vegetables in his garden after spraying 2 applications of maneb at a dose of 229 mg/kg/day were normal when taken 6 months after the exposure and subsequent to hemodialysis. No thyroid function tests were taken at the time of the maneb exposure (Koizumi et al. 1979).

Trivedi et al. (1993) investigated the effect of intermediate oral dosing of mancozeb (75% active ingredient) on thyroid function in the male albino rat. The fungicide was dissolved in peanut oil and given via gavage at doses of 375, 750, or 1,125 mg/kg/day for 90 days. Thyroid:body weight ratios increased in all groups, but at 30 days only the highest dose produced a statistically significant increase ($p<0.001$), whereas at 60 and 90 days, all doses resulted in a significant increase over controls gavaged only with peanut oil. Thyroid peroxidase activity was significantly decreased only in the highest dose group at 30, 60, and 90 days, with no difference seen at 15 days. Circulating thyroxine (T_4) levels were noticeably decreased starting at 30 days, with all doses causing a significant decrease compared to untreated controls ($p<0.01$ or lower). Interestingly, the control group had a significant decrease in T_4 levels at 90 days ($p<0.01$), as compared to the other time intervals, though the change was still not as great as seen in treated animals. However, several factors are known to affect thyroid hormone levels in rat plasma including environmental temperature; the strain, age, and sex of the rat; and the time of day (Thomas and Thomas 1994). Microscopic examination of rats treated for 90 days showed hypertrophy and hyperplasia of follicular cells with loss of colloid. The authors explain the dose-dependent hyperplasia and loss of colloid as likely results of increased thyroid stimulating hormone (TSH), but the activity of this hormone was not measured in this report. However, if TSH were increased in the rats, one might expect that plasma T4 levels would be increased, not decreased, because TSH stimulates the secretion and release of this

hormone (Thomas and Thomas 1994). A decrease in thyroid peroxidase activity might affect thyroxine production, even in the presence of increased TSH. Significant decreases in peroxidase were reported only at 1,125 mg/kg/day at 30, 60, and 90 days, whereas thyroxine levels were decreased at all dose levels at 30 days of exposure and longer (Trivedi et al. 1993).

In a follow up study to the Trivedi et al. (1993) investigation, Kackar et al. (1997b) dosed adult male albino rats via gavage with 0, 375, 750, and 1,125 mg/kg/day of mancozeb dissolved in peanut oil for up to 360 days. All 3 doses resulted in a significant increase in the thyroid:body weight ratio ($p<0.001$) at 180 and 360 days (>46% increase seen at 180 days with 375 mg Mn/kg/day); a slightly less significant response was seen at 90 days (23% at the lowest dose; $p<0.05–0.001$), and no increase in relative thyroid weight was seen at 30 days post-exposure. Histopathology revealed hyperplasia and hypertrophy of follicular cells with appreciable loss of colloid mass at 180 and 360 days that were dose-dependent in severity. A significant inhibition ($p<0.01$-0.001) in thyroid radioiodine (^{125}I) uptake was observed at the 2 highest dose levels at 90 days, and with all doses at 180 or 360 days. The highest dose administered for 90 days significantly reduced the serum levels of protein bound ^{125}I ($p<0.01$), but all dose levels reduced protein bound ^{125}I at 180 and 360 days. Circulating T_4 levels were reduced in all treatment groups except in rats exposed to 375 mg Mn/kg/day for 30 days. The highest dose caused 18, 30, 27, and 55% decreases in T_4, respectively, after 30, 60, 90 and 180 days of exposure. In general, the latter study confirms the results in the Trivedi et al. (1993) study, except that it did not report a decrease in circulating T_4 levels at the lowest dose or in the controls, and the severe histopathological effects seen previously were not observed at 90 days. This latter study did not determine the mechanism by which manganese achieved the observed decreases in thyroid function.

No studies were located regarding endocrine function in animals from exposure to maneb or in humans exposed to mancozeb.

Dermal Effects.

Inorganic Manganese

No studies were located regarding dermal effects in humans after oral exposure to inorganic manganese.

In animals, no significant dermal histopathological changes were observed in mice or rats exposed for 2 years to doses up to 731 or 232 mg manganese/kg/day, respectively, (NTP 1993) or to average oral doses of 905 or 331 mg manganese/kg/day (as $MnSO_4$), respectively (Hejtmancik et al. 1987a, 1987b).

Organic Manganese

No studies were located regarding dermal effects following oral exposure to organic manganese. Reports of contact dermatitis in people exposed to maneb or mancozeb are discussed in Section 2.2.3.2 because the route of exposure is assumed to be dermal. None of these studies indicate that ingestion of either pesticide occurred or would contribute to the allergic skin reactions.

Ocular Effects.

Inorganic Manganese

No studies were located regarding ocular effects in humans after oral exposure to inorganic manganese.

In animals, no significant ocular histopathological changes were observed in mice or rats exposed for 2 years to average oral doses of 905 or 331 mg manganese/kg/day (as $MnSO_4$), respectively (Hejtmancik et al. 1987a, 1987b).

Organic Manganese

No studies were located regarding ocular effects in humans or animals after oral exposure to organic manganese.

Body Weight Effects.

Inorganic Manganese

No studies were located regarding body weight effects in humans after oral exposure to inorganic manganese.

In some animal studies, lower body weights were observed in rats and mice in manganese-dosed groups. For example, an NTP study (1993) reported decreases in body weight gain of 57% in male rats and 20% in female rats fed 1,300 mg manganese/kg/day as $MnSO_4$ (in food) for 14 days. Exon and Koller (1975) reported that rats fed daily doses of Mn_3O_4 as low as 6 mg manganese/kg/day (mean ingestion value over the duration of the experiment) for 28 days gained only 44% as much weight over the course of the study as control rats. No changes in eating habits in this lowest dose group were observed, although rats in the highest dose group at 4,820 mg manganese/kg/day did exhibit decreased weight gain due to starvation and the effects of the manganese. No histopathological changes were reported in the exposed animals. The authors suggested that the decrease in weight gain might have been due to manganese interference in metabolism of calcium, phosphorous, and iron.

In chronic studies, a similar sex-related difference in the response to this effect was reported. By the end of a 2-year exposure to the maximum daily dose of 200 mg manganese/kg/day (as $MnSO_4$ in food), male rats had a final mean body weight that was 10% lower than that of controls; however, females' mean body weights were not significantly different from those of controls throughout the study at all dose levels (232 mg manganese/kg/day was the maximum dose for female rats) (NTP 1993). Food intake (as mg/kg/day) was similar for exposed groups and control groups and for males and females (NTP 1993).

Laskey et al. (1982) investigated body weight changes in a study of adverse reproductive toxicity in male and female Long-Evans rats exposed to manganese. Pregnant dams were fed 0, 350, 1050, and 3500 mg manganese/kg/day (in conjunction with a low-iron diet [20 mg iron/kg/day] or a diet adequate in iron [200 mg iron/kg/day]); the pups were continued on their respective diets from day 14–15 postpartum to the end of the study (224 days). Manganese treatment did not have any effect on body weight, in either sex fed adequate iron. In iron-deficient male rats, however, body weights were significantly decreased from controls at 24 days postpartum in the 1,050 mg manganese/kg/day diet and at all doses at 40 and 60 day time points. Interestingly, body weight was not significantly different in iron-deficient male rats fed manganese at 350 mg/kg/day at 100 days and at 224 days (no dose group had weight values significantly different from control at day 224). Female body weights were only significantly different in the highest dose at day 24 and in the remaining 2 manganese doses at day 60. Body weights were not significantly different from controls for the remainder of the study. Significant mortality in both sexes from the highest manganese group fed an iron-deficient diet limited the available data.

Organic Manganese

MMT. No studies were located concerning body weight effects following oral exposure to MMT in humans. Hanzlik et al. (1980) observed no significant differences in acutely exposed rats at a dose of 31.3 mg Mn/kg as MMT. Hinderer (1979) also observed normal weight gain in surviving Sprague-Dawley rats and CD-1 mice administered doses of MMT ranging from 7–159 mg Mn/kg in a 1-dose 14-day lethality study.

In a chronic study, Komura and Sakamoto (1992) administered 11 mg Mn/kg/day (as MMT) in chow to male ddY mice for 12 months. A 12% decrease in weight gain was observed at 9 months between exposed mice and mice fed unmodified chow, increasing to a 17% difference at 12 months. All differences in these time points were statistically significant. There was no observed difference in food intake between the exposed and control groups.

Maneb and mancozeb. A man who was exposed to 1.1 mg maneb/kg/day during a 1-time application of the fungicide to a field had a weight increase of 11% due to peripheral edema from renal failure (de Carvalho et al. 1989). Kackar et al. (1997a, 1997b) observed a reduced increase in weight gain with increasing doses of mancozeb administered to male rats. However, food intake was not measured in these studies, therefore the decreased weight gain might be due to a decreased food intake.

No studies were located regarding body weight effects following oral exposure to maneb in animals or mancozeb in humans.

Metabolic Effects

Inorganic Manganese

No studies were located regarding metabolic effects following oral exposure to inorganic manganese in humans or animals.

Organic Manganese

MMT. No studies were located regarding metabolic effects following oral exposure to MMT in humans or animals.

Maneb and mancozeb. Two men who potentially ingested maneb at concentrations equivalent to or greater than 1.1 mg/kg/day showed signs of metabolic acidosis (de Carvalho et al. 1989; Koizumi et al. 1979). No studies were located regarding metabolic effects in animals after oral exposure.

2.2.2.3 Immunological and Lymphoreticular Effects

Inorganic Manganese

No studies were located regarding immunological or lymphoreticular effects in humans after oral exposure to inorganic manganese.

Alterations in white blood cell counts have been reported in rats and mice following oral exposure to manganese. One NTP study reported immunological effects in rodents treated for 13 weeks but not in those treated for 2 years (NTP 1993). Mice were fed 122–1,950 mg manganese/kg/day as $MnSO_4$ for 13 weeks. Males exhibited decreased leukocyte counts at $975 mg manganese/kg/day; however, these effects may not have been treatment related; females were unaffected. For 13 weeks, rats were fed 33–520 mg manganese/kg/day (males) and 40–618 mg manganese/kg/day (females); neutrophil counts were increased in males at $33 mg manganese/kg/day, lymphocytes were decreased in males at $130 mg manganese/kg/day, and total leukocytes were decreased in females at $155 mg manganese/kg/day (NTP 1993). Rats fed up to 232 mg manganese/kg/day as $MnSO_4$ and mice fed up to 731 mg manganese/kg/day as $MnSO_4$ for 2 years exhibited no gross or histopathological changes or organ weight changes in the lymph nodes, pancreas, thymus, or spleen (NTP 1993). Komura and Sakamoto (1991) reported decreased white blood cell counts in mice following dosing at 284 mg manganese/kg/day with manganese acetate, $MnCl_2$, or MnO_2 for 100 days. It is not known if any of these changes are associated with significant impairment of immune system function.

Organic Manganese

MMT. No studies were located regarding immunological or lymphoreticular effects following oral exposure to MMT in humans or animals.

Maneb or mancozeb. A cursory immunological assay of blood taken from a man exposed 1 time to 1.1 mg maneb/kg revealed no abnormalities (de Carvalho et al. 1989). No studies regarding immunological or lymphoreticular effects were located following oral exposure to maneb or mancozeb in animals.

2.2.2.4 Neurological Effects

Inorganic Manganese

Although inhalation exposure to high levels of manganese is known to result in a syndrome of profound neurological effects in humans (see Section 2.2.1.4, above), there is only limited evidence that oral exposure leads to neurological effects in humans. An outbreak of a disease with manganism-like symptoms was reported in a group of 6 Japanese families (about 25 people) exposed to high levels of manganese in their drinking water (Kawamura et al. 1941). Noted symptoms included a masklike face, muscle rigidity and tremors, and mental disturbance. Five people were severely affected (2 died), 2 were moderately affected, 8 were mildly affected, and 10 were not affected. These effects were postulated to be due to the contamination of well water with manganese (14 mg/L) that leached from batteries buried near the well. Although many of the symptoms reported were characteristic of manganese toxicity, several aspects of this outbreak suggest that factors in addition to manganese may have contributed to the course of the disease. First, symptoms appeared to have developed very quickly. For example, two adults who came to tend the members of one family developed symptoms within 2–3 weeks. Second, the course of the disease was very rapid, in one case progressing from initial symptoms to death in 3 days. Third, all survivors recovered from the symptoms even before the manganese content of the well had decreased significantly after removal of the batteries. Thus, while there is no doubt these people were exposed to manganese, there is considerable doubt that all of the features of this outbreak (particularly the deaths) were due to manganese alone.

A manganism-like neurological syndrome has been noted in an aboriginal population living on an island near Australia where environmental levels of manganese are high (Kilburn 1987). Symptoms include

weakness, abnormal gait, ataxia, muscular hypotonicity, and a fixed emotionless face. Although it seems likely that excess manganese exposure is an etiologic factor in this disease (based on occupational exposure data from a study where exposure was assumed to be primarily by inhalation although oral exposure was not ruled out), absence of data on dose-response correlations and absence of data from a suitable control group preclude a firm conclusion on the precise role of manganese (Cawte et al. 1987). It is possible that other factors besides manganese exposure may have contributed to the neurological effects, including genetic factors, dietary deficiencies in antioxidants and calcium, and excess alcohol consumption (Cawte et al. 1989). Also, it should be noted that if manganese intake is a causal factor for neurological damage, exposure of the population evaluated in this study could occur not only through the oral route (e.g., food, water, soil) but also by inhaling manganese-containing dusts in environmental or workplace air (Cawte et al. 1987).

In another study, Kondakis et al. (1989) reported that chronic intake of drinking water containing elevated levels of manganese (1.8–2.3 mg/L) led to an increased prevalence of neurological signs in the elderly residents (average age, 67 years) of 2 small towns in Greece. Effects in these residents were compared with effects in similarly aged residents in a town where manganese levels were 0.004–0.015 mg/L and 0.082–0.25 mg/L. These levels are within and slightly above levels found in U.S. drinking water, respectively (see Section 5.4.2). Over 30 different neurological signs and symptoms were evaluated, each being weighted according to its diagnostic value for Parkinsonism. Based on this system, the average neurological scores for the residents of the control town (0.004–0.015 mg Mn/L), the town with mid-range levels (0.08–0.25 mg Mn/L), and the town with elevated manganese (1.8–2.3 mg Mn/L) were 2.7, 3.9, and 5.2, respectively. Results from this study suggest that higher-than-usual oral exposure to manganese might contribute to an increased prevalence of neurological effects in the aged population.

However, there are a number of limitations to this study which make this conclusion uncertain. First, no details were reported regarding which neurological signs or symptoms were increased, so it is difficult to judge if the differences were due to effects characteristic of manganism or to nonspecific parameters. Second, the weighting factors assigned to each neurological symptom were based on the symptom's diagnostic value for Parkinsonism; however, there are clinically significant differences between manganism and Parkinsonism. Therefore, the weighting scheme should have placed more weight on those symptoms (e.g., sleep disorders, emotional lability, weakness, fatigue, and irritability) reported in humans with manganism, such as manganese-exposed miners. The report does not indicate whether efforts were made to avoid bias in the examiner or in the study populations. Nonetheless, the use of the weighting

scheme does strengthen the authors' assertion of an association between elevated manganese concentration in the water source and increased susceptibility to neurological symptoms in older populations. Although the subjective parameters included in this scoring are indicative of alterations in mood or emotional state, and affective disorders often accompany other more objective nervous system effects, the authors did not state whether individuals who experienced neurological signs did in fact ingest higher levels of manganese than unaffected individuals. The authors reported that the populations in the towns were very similar to each other, but they provided few data to substantiate this. In this regard, even small differences in age, occupational exposures, or general health status could account for the small differences observed. Thus, this study suggests, but does not prove, that chronic oral intake of high levels of manganese can lead to neurological changes in humans.

A more recent study by Vieregge et al. (1995) reported no difference in performance on neurological function studies by people who had ingested well water with high concentrations of manganese. These individuals (high-exposure group), aged 40 and above, were exposed to manganese at a minimum concentration of 0.3 mg manganese/L in water for at least 10 years. The controls consisted of a matched group of people who ingested well water with a manganese concentration no higher than 0.05 mg/L. Mean blood manganese concentrations in the high-concentration group were 8.5+/-2.3 µg/L compared to the control value of 7.7+/-2.0 µg/L. Performance on motor coordination tests in the 'high-exposure' group was no different than the performance of the control group. The authors noted that they could not control for the ingestion of water from sources other than the wells described. Ingestion of manganese in food is also a major contributor, but the authors did not report an estimate of manganese levels ingested from foodstuffs. However, these possible confounders were considered negligible because no differences between groups were revealed in a risk factor analysis for nutritional factors performed by the authors and because manganese concentrations in the blood were not statistically different between the two groups. Manganese drinking water levels for the 'control group' in this study were within the range of levels reported in U.S. drinking water (see Section 5.4.2). As with the report by Kondakis et al. (1989), a limitation of this study is the use of a neurological assessment scale for 'Parkinsonian signs' rather than an evaluation of symptoms associated with manganism, though the authors observed no 'detectable' neurological impairment.

Goldsmith et al. (1990) investigated a cluster of Parkinson's disease in the southern region of Israel. They reported an increased prevalence of Parkinsonism particularly among those 50–59 years old, which suggested early onset of the disease. The authors believed that a potential environmental cause was the

water source common to residents in the region where the cluster of Parkinson's disease was observed. It was noted that maneb, a manganese containing pesticide, and paraquat were among the agricultural chemicals used in the region. Although the authors reported that the water samples examined showed a "substantial excess of aluminum and a smaller excess of iron and manganese," the concentrations were not reported. Soil samples were reported to contain excess concentrations of manganese as well as beryllium, chromium, europium, and ytterbium, though no quantitative values were provided. The residents were connected to a national water system, so it could not be determined when the water supply may have become contaminated with excess levels of manganese and other metals. Moreover, there was no clear evidence that persons living in the region were actually exposed to a contaminated water supply. Although identified as a cluster of Parkinson's disease rather than manganism, the authors suggested that the disease cluster might be related to an environmental source. However, the limitations in this study make it difficult to make any clear association between chronic oral intake of excess levels of manganese and the prevalence of neurological disease.

Iwami et al. (1994) studied the metal concentrations in rice, drinking water, and soils in Hohara, a small town on the Kii peninsula of Japan. This town had a high incidence of motor neuron disease (MND). The researchers observed that a significantly-increased manganese content in local rice and a decreased concentration of magnesium in drinking water were positively correlated with the incidence of MND in Hohara ($r^2 = 0.99$).

Two recent studies (He et al. 1994; Zhang et al. 1995) have reported adverse neurological effects in children (aged 11–13) who were exposed to excess manganese in well water and in foods fertilized with sewage water. However, these two studies have several flaws that preclude their use as substantial support for the link between ingestion of excess manganese and the incidence of preclinical neurological effects in children. These studies utilized a group of 92 children pair-matched to 92 controls who lived in a nearby region. The pairs were matched for age, sex, grade, family income level, and parental education level; in addition, all children lived on farms. Although the groups were well-matched, the duration and amount of manganese uptake from the flour (from wheat fertilized with sewage) and drinking water containing excess levels was not well characterized. Moreover, the studies did not indicate if nutritional status, such as low iron or calcium intake which could greatly enhance manganese uptake, were evaluated as potential confounding factors.

The exposed population drank water with manganese levels of 0.241 mg/L on average. The control group drank water containing 0.04 mg manganese/L. These values were measured over 3 years, although it was not stated if the children were exposed during the entire 3 years, nor what the children's daily manganese intakes were. The exposed children performed significantly more poorly ($p<0.01$) in school and on neurobehavioral exams than control students. School performance was measured as mastery of the native language and other subjects; neurobehavioral performance was measured using the WHO core test battery. However, the report did not state what measures, if any, were taken to ensure that the individuals administering the tests were blind to the exposure status of the subject. Such safeguards would be necessary to prevent the introduction of bias in measurement and analysis of the performance data of the subjects. The exposed children's hair, blood, and urine manganese levels were significantly increased relative to controls. A simple correlation analysis indicated the performance of exposed children on 5/6 of the neurobehavioral tests administered (digit span, Santa Ana manual dexterity, digit symbol, Benton visual retention test, and pursuit aiming test) was inversely correlated with hair manganese levels. Although the authors reported that iron, copper, and zinc were measured in blood and hair, no other metals were measured in these tissues. Because the exposed group presumably ingested food from sources irrigated with sewage, the children may have been exposed to increased levels of other metals, such as lead or mercury. The authors indicate that the children were exposed to increased manganese in their diet from excess levels in foodstuffs and drinking water. Of the foodstuffs evaluated (cabbage, spinach, potatoes, eggplant, sorghum, and flour) only wheat flour contained excess manganese compared to that from the control area. Although the total amount of manganese ingested from the wheat flour and drinking water was not estimated, the authors suggest that the elevated manganese level in drinking water was the key factor contributing to the observed effects. Based on the highest concentration of manganese reported in drinking water (0.346 mg/L) and an intake of 2 liters drinking water per day (estimate for adult intake), manganese intake from drinking water alone (0.7 mg) may have been well below the ESADDI of 2–5 mg/day for children in this age group. Although it is possible, these studies do not provide clear evidence that the daily intake of manganese in the exposed group exceeded this ESADDI. The authors report that children ingesting food and water containing elevated manganese showed poor performance in neurobehavioral tests and poorer school performance when compared to children from a control area. Because exposure levels and duration were not well defined, these studies as reported are not rigorous enough to establish causality between ingestion of excess manganese and preclinical neurological effects in children. Nonetheless, these studies are strongly suggestive that early neurobehavioral effects often seen in industrial workers exposed to excess manganese via inhalation are observed in children.

Though limited, these studies also provide further evidence for a link between ingestion of elevated levels of manganese and learning problems. Other studies have found that manganese levels in hair are higher in learning-disabled children than in normal functioning children (Collipp et al. 1983; Pihl and Parkes 1977). The route of excess exposure is not known but is presumed to be mainly oral. These observations are consistent with the possibility that excess manganese ingestion could lead to learning or behavioral impairment in children as suggested by the results of He et al. (1994) and Zhang et al. (1995). However, an association of this sort is not sufficient to establish a cause-effect relationship because a number of other agents, including lead, might also be involved (Pihl and Parkes 1977). Moreover, other potentially confounding factors, e.g., health and nutritional status, must be taken into consideration in interpreting such studies.

Other evidence of neurological effects following oral exposure was noted in a case report of a man who accidentally ingested low doses of potassium permanganate (about 1.8 mg manganese/kg/day) for 4 weeks (Holzgraefe et al. 1986). After several weeks the man began to notice weakness and impaired mental capacity. Although exposure was stopped after 4 weeks, the authors reported that a syndrome similar to Parkinson's disease developed after about 9 months. Though suggested by the appearance of a syndrome resembling Parkinsonism, it is difficult to prove that these neurological effects were only caused by exposure to the manganese compound. The authors speculated that the ingested MnO_4^- was reduced to Mn(II) or Mn(III); however, while this would be expected, it was not measured. Since MnO_4^- is a corrosive agent, it seems likely that it may have caused significant injury to the gastrointestinal tract (the patient did experience marked stomach pain), perhaps leading to a larger-than-normal gastrointestinal absorption of manganese.

Further evidence of manganese-induced neurotoxicity potentially caused by oral exposure is provided in a study by Banta and Markesbery (1977). This case report involved a 59-year-old man with no occupational or environmental exposure to manganese. The man exhibited dementia and neuromuscular deficiencies including bradykinesia, shuffling gait, retropulsion, and rigidity in the upper extremities. Masked facies with infrequent blinking and stooped posture were also observed. Manganese concentrations were significantly elevated in serum, urine, hair, feces, and cerebrum. Although the authors posit that the man may have had Alzheimer’s disease as well as manganese toxicity, they question how the individual could build up significant body stores of manganese in the absence of occupational exposure or any other known source of excess manganese. The authors suggest that the manganese overload may have been caused by abuse of vitamins and minerals.

Several recent studies report the link between hepatic encephalopathy and an increased manganese body burden following chronic liver disease in adults (Hauser et al. 1994; Pomier-Layrargues et al. 1998; Spahr et al. 1996) and children (Devenyi et al. 1994) and in individuals with surgically-induced portacaval shunts (PCS) (Hauser et al. 1994). The manganese exposure in these studies was assumed to originate from a normal diet. Hepatic encephalopathy comprises a spectrum of neurological symptoms commonly occurring in individuals with chronic liver disease; these symptoms include varying degrees of mental dysfunction, although extrapyramidal symptoms may also be identified during a clinical examination (Spahr et al. 1996). In the Hauser et al. (1994) study, 2 men aged 49 and 65, both with chronic liver disease, and one 56-year-old man with cirrhosis of the liver and a portacaval shunt, showed a variety of neurological symptoms including bradykinesia, postural tremor of the upper extremities, and gait disturbances, as well as a decrease in cognitive function. These men all had significant elevations ($p<0.05$) in blood manganese as compared to healthy male and female controls, and had hyperintense signals in the basal ganglia bilaterally as measured by T1-weighted MRI. Similar elevations of blood manganese were reported in a population of 57 cirrhotic patients with an absence of clinical encephalopathy (Spahr et al. 1996). Blood manganese was elevated in 67% of the patients and was significantly higher in those patients with previous portacaval anastomoses or transjugular intrahepatic portosystemic shunt. MRI signal hyper intensity was observed in the globus pallidus; the elevated blood manganese levels were significantly correlated with the intensity of the signal in affected patients. Neurological evaluation of extrapyramidal symptoms using the Columbia rating scale indicated a significant incidence of tremor, rigidity, or akinesia in ~89% of the patients, although there was no significant correlation between blood manganese level and these symptoms.

Similar results were observed in a young girl with Alagille's syndrome (involving neonatal cholestasis and intrahepatic bile duct paucity) with end-stage cholestatic liver disease who exhibited several neurological dysfunctions including dystonia, dysmetria, propulsion, retropulsion, and poor check response bilaterally (Devenyi et al. 1994). The girl had elevated blood manganese (27 µg/L compared to normal value of ~9.03 µg/L) and exhibited hyperintense signal in the basal ganglia. After a liver transplant, the MRI signal abated and the blood manganese level returned to normal (8.6 µg/L). This study and those in adults indicate that the increased manganese body burden (as evidenced by increased manganese blood and brain levels) may be the cause of the resultant neurological symptoms and encephalopathy in individuals with cirrhosis or chronic liver disease.

Most recently, Rose et al. (1999) evaluated brain manganese levels in 12 autopsied cirrhotic individuals who died from hepatic coma and 12 control subjects with no history of hepatic, neurological, or psychiatric disorders at time of death. Neutron activation analysis of the brain tissue revealed an increase in manganese content in the cirrhotic individuals, particularly in the globus pallidus, which had 186% more manganese than that of controls (significant at a level of $p<0.001$). Significant, although less extreme, increases in manganese were also found in the putamen and caudate nucleus from cirrhotic patients. However, the increased brain manganese did not correlate with patient age, the etiology of the cirrhosis, or the history of recurrent hepatic encephalopathy (reported in 6 patients).

An association has been suggested between violent behavior and excess manganese exposure; this was investigated by measuring the correlation between the manganese content in hair and violent behavior in prison subjects and controls (Gottschalk et al. 1991). The prisoners did have a significantly higher hair manganese content than controls, but further research was indicated to determine whether manganese was a causative factor in violent behavior. The highest concentrations of manganese demonstrated in the hair samples (1.8–2.5 ppm) were, however, within the control ranges reported by Kondakis et al. (1989) (0–13 ppm) and Huang et al. (1989) (0.1–2.2 ppm for scalp and 0.3–9.8 ppm for pubic hair). Another factor to be considered in the interpretation of these results is the hair color composition within the samples evaluated. At least one study (Cotzias et al. 1964) has reported that manganese content was greater in dark hair when compared to that found in lighter colored hair. Another study showed that manganese accumulated in melanin-containing tissues including the melanin from human hair (Lydén et al. 1984). In their study of inhabitants living in Angurugu on Groote Eylandt, Australia, Stauber et al. (1987) found the same manganese content in samples of grey and black hair from one elderly Aborigine participant and in the black and white hairs of a local dog. Based on this evidence, these investigators stated that there was no evidence to support previous reports that dark colored hair concentrated more manganese than light hair. Interestingly, the average manganese content in scalp hair among male and female Aborigine residents was 3.5- to 5-fold greater than the average scalp hair manganese in male and female Caucasian residents, respectively. The authors cautioned that interpretation of data on manganese content in scalp hair should take into consideration endogenous as well as potential exogenous sources. Moreover, long-term manganese exposure that may be associated with adverse effects may not be represented by manganese content in hair growth from only a few months (Stauber et al. 1987). Thus, further investigations are needed to determine whether manganese content can vary significantly due to hair color pigment alone.

Manganese has also been associated with amyotrophic lateral sclerosis (ALS). In a human study, spinal cord samples from ALS patients were found to have higher manganese concentrations in the lateral fasciculus and anterior horn than in the posterior horn (Kihira et al. 1990). Also, ALS patients exhibited a positive correlation between manganese and calcium spinal cord content, whereas controls exhibited a negative correlation. It was suggested that an imbalance between manganese and calcium in ALS patients plays a role in functional disability and neuronal death. There was also some indication from previous studies that an excess intake of manganese in drinking water may have caused this imbalance, although data to support this were not presented. While this is suggestive of an association between manganese and ALS, it is equally plausible that ALS leads to an imbalance in manganese-calcium metabolism.

There are significantly more studies on the neurological effects of manganese ingestion in animals as compared to humans. A few of these report observed effects that were comparable to clinical signs seen in people. Gupta et al. (1980) reported that monkeys given 25 mg manganese/kg/day (as $MnCl_2$) for 18 months developed weakness and muscular rigidity (however, no data were provided to support these observations). In another study, rats dosed with 150 mg manganese/kg/day (as $MnCl_2$) developed a rigid and unsteady gait after 2–3 weeks, but this was a transient condition that was not apparent by 7 weeks (Kristensson et al. 1986). In addition, in 2 separate studies, the authors reported a decrease in spontaneous activity, alertness, muscle tone, and respiration in mice dosed once with 58 mg manganese/kg/day by oral gavage (Singh and Junnarkar 1991) and staggered gait and histochemical changes in 2 third-generation mice treated with 10.6 mg manganese/kg/day as $MnCl_2$ in drinking water (Ishizuka 1991). Ali et al. (1983a) investigated the role of dietary protein on the neurological effects of excess manganese in drinking water using rats. Manganese exposure originated 90 days prior to mating and continued throughout gestation and nursing. The offspring of rats who drank the equivalent of 240 mg manganese (as $MnCl_2$)/kg/day, irrespective of diet, had pups with delayed air righting reflexes. No treatment-related effects were observed in body weight or brain weight in pups from dams fed adequate protein. Significant delays in age of eye opening and development of auditory startle were observed only in the pups of dams fed protein-deficient diets. Most other studies in animals have reported changes in brain neurotransmitter levels or alterations in motor activity with both hypo– and hyperactivity reported. As shown in Table 2-2 and Figure 2-2, changes of this sort have been reported at oral exposure levels that ranged from 1 up to 2,270 mg manganese/kg/day (as $MnCl_2$, manganese acetate, or Mn_3O_4) (e.g., Bonilla 1978b; Bonilla and Prasad 1984; Chandra 1983; Erikson et al. 1987a; Gianutsos and Murray 1982; Gray and Laskey 1980; Komura and Sakamoto 1991, 1992; Lai et al. 1984; Nachtman et al. 1986; Subhash and Padmashree 1991). Thus, the database on neurological effects in animals ingesting high levels of manganese does not

provide a clear picture of manganese-induced effects and the significance of these results is difficult to interpret.

Recently, Rose et al. (1999) reported the effects on manganese body burden (exclusively from the diet) in rats with either induced cirrhosis of the liver, acute liver failure (induced by portacaval anastomosis followed by hepatic artery ligation), or a surgically-administered portacaval shunt (PCS). Brain manganese levels in these three groups of rats were compared to control rats and sham-operated rats. PCS and sham-operated rats were evaluated 4 weeks following surgery, while cirrhotic rats were studied 6 weeks following surgery. Rats with acute liver failure were studied 15–18 hours following devascularization at coma stage of encephalopathy. Manganese levels were statistically significantly increased as compared to non-treated controls and sham-operated controls in both cirrhotic and PCS rats in the frontal cortex, globus pallidus, and caudate/putamen; manganese levels were highest in the globus pallidus. For example, in the globus pallidus, brain manganese was increased 57% in the PCS rats as compared to the control rats ($p<.0001$). However, the level of manganese in the globus pallidus in the PCS rats was significantly elevated as compared to cirrhotic rats, indicating that shunting is a strong determinant of manganese deposition in the brain.

A study by Kontur and Fechter (1988) reported no difference in levels of monoamines and related metabolites in neonatal rats at 22 mg manganese/kg/day as $MnCl_2$ (14–21 days), although Dorman et al. (2000) reported elevated striatal dopamine (DA) and 3,4-dihydroxyphenylacetic acid (DOPAC, an oxidation product of DA)in 21-day old rats administered the same high daily dose used by Kontur and Fechter (1988) from postnatal day 1-21. Effect of manganese treatment on neurobehavior was also evaluated in this study. There was a significant decrease in body weight gain in pups at the highest manganese exposure dose. Although there were no statistically significant effects on motor activity or performance in the passive avoidance task in the neonates, manganese treatment induced a significant increase in amplitude of the acoustic startle reflex at PND 21. However, in adult rats, there was a significantly decreased amplitude of acoustic startle reflex at the lowest dose tested. An NTP (1993) study reported no gross or histopathological lesions in the nervous system of rats fed 1,300 mg manganese/kg/day as $MnSO_4$ for 14 days or in mice given 1,950 mg manganese/kg/day as $MnSO_4$ for 13 weeks. However, other studies in neonatal animals have detected neurostructural and neurochemical changes at doses similar to or slightly above dietary levels (1–10 mg manganese/kg/day) (Chandra and Shukla 1978; Deskin et al. 1980), suggesting

that young animals might be more susceptible to manganese than adults. A more thorough discussion of the significance of these studies is presented in Sections 2.5 and 2.6.

Organic Manganese

MMT. No studies regarding neurological effects following oral exposure to MMT by humans were identified.

Komura and Sakamoto (1992b) administered 11 mg Mn/kg/day as MMT to ddY mice in food for 12 months. To measure differences in behavior between exposed and control mice that were fed normal chow, Spontaneous motor activity was measured at regular intervals during exposure to determine differences in behavior between exposed and control mice fed normal chow. The authors observed a significant increase in spontaneous activity at day 80; no other significant differences were noted. In a separate study (Komura and Sakamoto 1994), the authors analyzed brain levels of different neurotransmitters and metabolites after identical MMT treatment. MMT resulted in a 66% decrease in dopamine (DA; $p<0.05$) and a 95% decrease in normetanephrine (NMN, ; $p<0.01$) in the hypothalamus; in the hippocampus, DA was unchanged, while the level of DOPAC was reduced 41% ($p<0.05$), and the 3-methoxytyramine (3MT) level increased 3.5-fold ($p<0.01$). In the midbrain, the only significant changes noted were an almost 6-fold increase in 3MT ($p<0.01$), and a 1.75-fold increase of homovanillic acid (HVA, metabolite of DOPAC via conjugation by catechol-o-methyl transferase; $p<0.05$). In the cerebral cortex, HVA was decreased by 61%, norepinephrine (NE) by 64%, and epinephrine by 43% (all were $p<0.05$) due to MMT administration. In the cerebellum, DOPAC was decreased 51% ($p<0.05$), while NMN was increased 7.7-fold ($p<0.01$). Finally, in the medulla oblongata, DOPAC was decreased by 45% ($p<0.05$), HVA was decreased by 55% ($p<0.01$), and serotonin (5HT) was decreased 81% ($p<0.01$); metanephrine was increased approximately 2.75-fold in the medulla ($p<0.05$).

Through analysis of the distribution of manganese in the different brain regions of the mice, the authors observed relationships between manganese content and neurotransmitter levels. For example, a weak relationship was found between the manganese level in the corpus striatum and the level of NE. There was no relationship between the increase in HVA and the manganese levels in this same region. The relationship between the increase in 3MT and manganese levels in the midbrain was weak, as was the relationship between DOPAC and manganese levels in the cerebellum. There were no relationships between amines and manganese levels in the hippocampus, cerebral cortex, or medulla oblongata, although some changes

were found. A significant correlation was found between the level of NMN and manganese in the cerebellum. As discussed more fully in Section 2.3.2, the cerebellum contained the most manganese of any brain region following MMT administration (Komura and Sakamoto 1994).

Maneb and mancozeb. A man who ate unwashed vegetables from his garden after applying 229 mg maneb/kg/day for 2 days developed exaggerated knee and ankle jerks in his lower left leg, but no other notable neurological effects (Koizumi et al. 1979). A man who had potentially ingested maneb during the preparation and spraying of 1.1 mg maneb/kg/day during a single application had an unremarkable neurological examination (de Carvalho et al. 1989).

Maneb administered once by gavage in dimethylsulfoxide to laying hens at doses of 16 or 32 mg/kg resulted in a 25 and 45% decrease (statistically significant at $p<0.05$), respectively, in the metabolism of injected radiolabeled dopa (dopamine precursor), as measured by incorporation of label into norepinephrine (Serio et al. 1984). The hens had been injected prior to sacrifice with 50 mg/kg carbidopa, which is a dopa decarboxylase inhibitor that is not transported through the blood-brain barrier. Carbidopa is believed to increase the amount of dopa taken up by the brain by reducing peripheral decarboxylation. Neither dose had a statistically significant effect on the concentration of dopa or dopamine in the brain.

In a taste aversion paradigm, male Swiss mice were given either water, xylene (positive control), or maneb (0.32, 3.2, or 32 mg/kg). The percent saccharin intake was significantly reduced in the highest dose group (Mitchell et al. 1989).

Chernoff et al. (1979) reported that gestational doses of 0, 190, or 380 mg maneb/kg given on days 7–15 to pregnant rats via gavage (in water) had no effect on the behavior of male offspring at postnatal week 6. Behavioral tests included latency to leave the center circle in an open field, defecations, urinations, rearings, and activity. Male offspring from exposed dams also did not have any differences in development of startle reflex and air righting, as measured during the first 6 postnatal weeks, but did have a significant ($p<0.05$) retardation in eye opening. The authors did not specify if both doses caused the effect.

Sobotka et al. (1972) investigated potential neurological effects in neonatal/postnatal Osborne-Mendel rats from a diet containing maneb. Upon delivery, the dams were placed on maneb-containing diets at the following concentrations: 0, 0.4 ppm , 0.8 ppm, and 8.0 ppm (5–6 litters/dietary level). Study groups were divided into three exposure groups. In group 1, the dams were fed the maneb diet only while the pups

were nursing; upon weaning at day 28, the pups were maintained on a control diet (pre-weaning group). In group 2, the pups were exposed to maneb through mother's milk, and maintained on a maneb diet until sacrifice at 6 months of age (pre- and post-weaning group). In group 3, the pups were only exposed to maneb post-weaning (post-weaning group). Behavioral testing included measurements of emotional reactivity of 30 day old rats and of learning ability in 6-month-old offspring. Emotional reactivity was measured using two procedures: exploratory activity in an open-field situation and fear-induced suppression of motor activity in a passive avoidance situation. Due to the complexity of the dietary regimen (pre-weaning exposure group is exposed to the maneb or metabolites that enter mother's milk, while the other groups are exposed directly to maneb through their chow), the doses are presented as cumulative doses for each group. For example, the pre-weaning exposure group was exposed to 0.4 mg maneb/kg diet * 0.035 kg diet eaten/0.35 kg body weight of dam * 28 days exposure = 1 mg/kg cumulative dose. The other groups had cumulative doses that took into account the ingestion rate and weight of the young rat during the 6-month exposure period. Pre-weaning exposure to 0.4 and 8.0 ppm maneb (cumulative doses of 1 and 20 mg/kg) resulted in a statistically significant decrease in pre-shock latency to enter a new environment (compared to control rats), but only the high-dose group showed a significant decrease in exploratory activity in the open-field situation. There were inadequate numbers of rats in the intermediate-dose group to complete these behavioral tests. These data indicate that cumulative pre-weaning exposure to 1 and 20 mg/kg maneb negatively affected behavior in the weanling rat.

Learning ability was measured using a lever-press avoidance/escape operant-conditioning procedure set up in Skinner boxes. The design of this conditioning procedure is such that increased avoidance is indicative of learning. These learning tests were performed when the mice were 6 months old. Pre-weaning group rats exposed to the intermediate and high doses (2 and 20 mg/kg cumulative doses, respectively, given over 28 days) and pre- and post-weaning group rats exposed to the high dose (135 mg/kg cumulative dose given over 208 days) exhibited increased avoidance at a statistically significant level compared to controls in the exercise. These data show that maneb exposure had a positive effect on learning in the adult mouse; this latent effect was most dramatic in the pre-weaning mice exposed to the intermediate dose (2 mg/kg cumulative). Post-weaning rats did not exhibit any differences in the procedures at any dose (5.75, 11.5, or 115 mg/kg cumulative doses over 180 days).

At sacrifice, rat brain regional cholinesterase activity and plasma corticosterone levels were determined; changes in the levels of these compounds were found, although they were unrelated to the behavioral changes reported (Sobotka et al. 1971). Plasma corticosterone levels were roughly doubled at the lowest

and highest doses in the post-weaning exposure group only; the intermediate dose group could not be analyzed due to insufficient numbers. Maneb treatment in the pre-weaning and post-weaning groups resulted in a decrease in cholinesterase activity in the telencephalon, midbrain-diencephalon, and the pons-medulla. All of the decreases were significant in the pre-weaning group, except for the 0.8 mg/kg/day dose in the midbrain (3.8% decrease) and the 0.4 and 0.8 mg/kg/day doses in the pons-medulla (1.3 and 4.6% decreases, respectively). In the post-weaning group, all decreases were significant except for the 0.4 mg/kg/day doses in the midbrain (5.5%) and pons-medulla (5.4%); again, the 0.8 mg/kg/day exposure group was not analyzed due to insufficient numbers. The pre- and post-weaning exposure group showed decreases in cholinesterase activity at the two highest doses in the telencephalon, and in the highest dose group in the midbrain. All other doses resulted in an increase in cholinesterase activity, with the 0.4 and 0.8 mg/kg/day doses resulting in significant increases in the enzyme in the midbrain.

No studies were located regarding neurological effects in humans or animals following oral exposure to mancozeb.

2.2.2.5 Reproductive Effects

Inorganic Manganese

No studies were located regarding reproductive effects in humans after oral exposure to inorganic manganese.

In a 14-day study in rats, no changes in testicular weight were reported at 1,300 mg manganese/kg/day (NTP 1993). However, several intermediate-duration studies in rats and mice indicate that manganese ingestion can lead to delayed maturation of the reproductive function in males. One study investigated the effect of 1050 mg manganese (as Mn_3O_4)/kg/day, provided to weanling mice and their dams starting when the pups were 15 days old (Gray and Laskey 1980). On day 30, the mice were weaned and maintained on the high-manganese diet until sacrificed for analysis at 58, 73, and 90 days old. The growth and general appearance of the weanling rats appeared normal. At sacrifice, preputial gland, seminal vesicle, testes, and body weights were measured. The high-manganese diet resulted in a significant decrease in growth of these reproductive tissues but no growth retardation of the body and no change in liver or kidney weights.

A later study by Laskey et al. (1982) evaluated the reproductive functioning of male and female Long-Evans rats who had been exposed to 0, 350, 1050, and 3500 mg manganese/kg/day (in conjunction with a low-iron diet [20 mg iron/kg/day] or a diet adequate in iron [200 mg iron/kg/day]) while *in utero* (dams were fed the described diets during gestation) and from day 14–15 postpartum. The rats were maintained on the diet throughout the remainder of the study (224 days). The rats were mated at 100 days postpartum and the reproductive success of these matings was evaluated.

In males, manganese treatment resulted in decreased testes weights observed at 40 days (at the 1,050 and 3,500 mg manganese/kg/day dose levels) and 100 days (at the 1,050 mg manganese/kg/day dose level) of age, only when administered with the low-iron diet. Hormone levels in male rats were also evaluated. No treatment-related effect was seen in 40-day-old males. At 60 and 100 days of age, however, dose-related decreases in serum testosterone were observed, while serum LH (luteinizing hormone) levels remained relatively unchanged. Luteinizing hormone is secreted by the pituitary to stimulate testosterone production in the Leydig cells. Testosterone levels control LH production through a negative feedback loop. An increase in testosterone would normally be associated with a subsequent decrease in LH. The decrease in testosterone simultaneous with a stable LH levels suggests that manganese is targeting the Leydig cells. Manganese treatment in both iron regimens prevented the normal decrease in serum follicle-stimulating hormone (FSH) from 60 to 100 days. In addition, manganese only negatively affected epididymal sperm counts at 100 days in the iron-deficient group. Interestingly, when serum concentrations of LH, FSH, and testosterone and epididymal sperm counts from the 60- and 100-day old rats were used to predict the reproductive age of the males, the 60-day old animals were predicted correctly. Of the 100-day old animals, 2/12 controls, 7/12 at 350 mg manganese/kg, and 12/12 at 1,050 mg manganese/kg were classified as 60-days old. These data indicate that manganese induced a significant maturational delay in the reproductive organs of the male rat (Laskey et al. 1982).

To further assess the mechanism of toxicity of manganese in the pre-weanling rat, Laskey et al. (1985) dosed rats from birth to 21 days of age with particulate Mn_3O_4 in 50% sucrose solution by gavage at doses of 0, 71, or 214 mg manganese/kg/day. They then assessed the hypothalamic, pituitary, and testicular functions in the rat by measuring the endogenous or stimulated serum concentrations of FSH, LH, and testosterone at 21 or 28 days of age. LH-releasing hormone (LH-RH) was used to stimulate the pituitary-testicular axis to secrete FSH, LH, and subsequently testosterone; human chorionic gonadotropin (hCG) was used to stimulate acutely (2 hour time period) the testicular secretion of testosterone and repeatedly (7 day time period) to assess the ability of the Leydig cells to maintain maximal testosterone synthesis and

secretion. Some rats from both controls and manganese-dosed groups were castrated to determine the effect this would have on the study endpoints. Manganese treatment had only a slight effect on body and testes weights, while no effects were observed on unstimulated or stimulated FSH or LH serum levels. In addition, manganese did not affect endogenous or acute hCG-stimulated serum testosterone concentrations, but did decrease serum testosterone level following repeated hCG stimulation. Liver manganese at the 71 mg/kg/day manganese dose was significantly elevated over controls in both castrated (8.42+/-7.23 mg/kg for treated vs. 1.96+/-0.22 mg/kg for controls) and noncastrated (3.36+/-0.91 mg/kg for treated vs. 1.81+/-0.11 mg/kg for controls) rats. In addition, hypothalamic manganese concentrations were significantly increased at the 71 mg/kg/day dose in both castrated (6.10+/-3.0 mg/kg in treated vs. 0.59+/-0.11 mg/kg in controls) and noncastrated (3.73+/-1.18 mg/kg in treated vs. 0.65+/-0.057 mg/kg in controls) rats. The authors speculate that since their earlier results had shown changes in male reproductive development in postpubertal animals with minimal manganese concentrations in tissues (Gray and Laskey 1980; Laskey et al. 1982), it seemed likely that the changes in this later study (Laskey et al. 1985) would result from high manganese concentrations in the hypothalamus, pituitary, or testes, with the tissue with the highest manganese concentration being the site of the toxic reproductive effect. However, the results from this latest study reveal that manganese had no effect on the hypothalamus or pituitary to produce LH or FSH in pre-weanling rats, despite the increased manganese concentrations. Rather, the data indicate that it is delayed production of testosterone, shown by the inability of the Leydig cells to maintain maximum serum concentrations of the hormone, that results in the delayed sexual maturation. This delay in testosterone was not significant enough, however, to impair rodent fertility at manganese doses as high as 1,050 mg/kg/day (Laskey et al. 1982).

A slight decrease in pregnancy rate was observed in rats exposed to 3,500 mg manganese/kg/day as Mn_3O_4 in the diet for 90–100 days prior to breeding (Laskey et al. 1982). Since both sexes were exposed, it is not possible to conclude whether the effect was in males, females, or both. However, this exposure regimen did not have significant effects on female reproductive parameters such as ovary weight, litter size, ovulations, or resorptions (Laskey et al. 1982).

Manganese was found to affect sperm formation in another intermediate study (Joardar and Sharma 1990). The metal was administered to mice, as $KMnO_4$ or $MnSO_4$, at 23–198 mg/kg/day by gavage for 21 days. The treatment resulted in sperm head abnormalities, and the percentage of abnormal sperm was significantly elevated in all exposed mice as compared to controls.

In another intermediate feeding study, Jarvinen and Ahlstrom (1975) administered varying doses of $MnSO_4$, from nutritionally deficient levels to excess amounts, to Sprague-Dawley female rats for 8 weeks prior to mating. The rats were continued on manganese diet (0.75, 4.5, 10, 29, 94, 187 mg manganese/kg/day) until gestational day 21. The authors found no effect of manganese on maternal weight gain, implantation number, resorptions, or percentage of dead fetuses. The authors did observe that manganese doses of 94 mg manganese/kg/day and higher resulted in significant increases in liver manganese concentrations, whereas non-pregnant females had consistent liver manganese concentrations, irrespective of dose. These data suggest that pregnancy allows the female to develop significant liver manganese stores, and it is possible these stores may be mobilized during gestation or at a future time. The authors also noted that pregnant rats had consistent liver iron concentrations, whereas nonpregnant rats suffered a dose-dependent decrease in liver iron concentrations. Further, the highest dose in dams caused a significant increase in fetal manganese content.

Szakmáry et al. (1995) studied the reproductive effects of $MnCl_2$, administered by gavage to pregnant rabbits and rats at concentrations of 0, 11, 22, and 33 mg manganese/kg/day on gestational days 6–20 in the rabbit and throughout gestation in the rat. Manganese did not result in any reproductive effect in the rabbit, but the highest manganese dose did cause an increase in postimplantation loss in the rat. In 13-week dietary studies, no gross or histopathological lesions or organ weight changes were observed in reproductive organs of rats fed up to 618 mg manganese/kg/day or mice fed 1,950 mg manganese/kg/day, but the reproductive function was not evaluated (NTP 1993).

Two more recent oral studies indicate that ingested manganese does not result in female reproductive toxicity. The first study involved a dose of 22 mg manganese/kg/day administered as $MnCl_2$ by gavage to female rats on days 6–17 of gestation (Grant et al. 1997). No treatment-related mortality, clinical signs, changes in food or water intake, or body weights were observed in the dams. In the second study (Pappas et al. 1997), $MnCl_2$ was provided to pregnant rats in drinking water at doses up to 620 mg manganese /kg/day throughout gestation. The manganese did not adversely affect the health of the dams, litter size, or sex ratios of the pups. More extensive analyses of female reproductive organs were not performed. Similarly, Kontur and Fechter (1985) found no significant effect on litter size in female rats exposed to $MnCl_2$ in drinking water except at concentrations so high (1,240 mg manganese/kg/day) that water intake by the dams was severely reduced.

In a 2-year NTP study, no adverse reproductive effects from $MnSO_4$ exposure were reported for rats at up to 232 mg manganese/kg/day or mice at up to 731 mg manganese/kg/day (NTP 1993).

The highest NOAEL values and all LOAEL values from each reliable study for reproductive effects in each species and duration category are recorded in Table 2-2 and plotted in Figure 2-2.

Organic Manganese

MMT. No studies were located regarding reproductive effects in humans or animals following oral exposure to MMT.

Maneb or mancozeb. Maneb fed in mash to laying hens at a dose of 180 mg/kg/day for 7 days had no effect on egg production on any of the 7 days (Weppelman et al. 1980).

Petrova-Vergieva and Ivanova-Tchemishanska (1973) analyzed the effect of day of administration of maneb in reproductive toxicity in female white rats. In an acute experiment, the pregnant females were administered a single gavage dose of 0, 400, 800, 1,600, or 3,200 mg maneb/kg in water on either gestation day 11 or 13. The rats underwent Caesarian section on gestation day 21 and reproductive toxicity was assayed by comparing implantation sites between control and exposed dams. The only statistically significant reproductive effect was an increase in the number of resorptions in the 3,200 mg/kg dose group (17 occurred) as compared to controls (1 occurred; $p<0.001$) when the maneb was administered on gestation day 11. There were no adverse reproductive effects when the pesticide was administered on day 13.

Larsson et al. (1976) conducted an acute reproductive toxicity study in pregnant Sprague-Dawley rats and NMRI mice using maneb and mancozeb. The fungicides were administered in two different experiments (preliminary and final) by gavage, dissolved in water. The preliminary experiment involved doses of maneb at 0, 340, 650, 1,200 mg/kg/day, or mancozeb at 0, 300, 580, or 1060 mg/kg/day on gestation day 9 or 13 with groups of 7–8 mice and the same doses on day 11 in 1–7 rats. Neither maneb nor mancozeb had any effect on reproductive success in mice. In rats, maneb caused a significant increase in resorption, with resorption rates as high as 60% at 1,200 mg maneb/kg/day. Mancozeb did not cause an appreciable increase in resorption, even at the highest dose (5.4 vs. 2.9% in controls administered distilled water). In a second experiment with rats, maneb at doses of 300, 640, 655, or 1,180 mg/kg was administered on

gestation day 11, either alone or in the presence of 15, 30, 60, or 108 mg zinc acetate/kg. The administration of zinc acetate exerted a protective effect on reproduction, decreasing the resorption incidence, although the protective effect was most significant at concentrations of 60 and 108 mg/kg zinc acetate. The authors surmised that the maneb was chelating the available zinc in the pregnant dam, thereby causing reproductive toxicity since zinc is necessary for development. The effects were counteracted when zinc acetate was co-administered with the maneb. The lower reproductive toxicity of mancozeb in the preliminary study supports the theory; mancozeb contains zinc, thereby providing an additional supply of the essential mineral (Larsson et al. 1976).

Chernoff et al. (1979) administered maneb in distilled water via gavage to pregnant Sprague-Dawley rats and CD-1 mice on gestation days 7–16 at doses of 0, 100, 190, or 380 mg/kg in the rat, or 0, 300, 600, or 1200 mg/kg/day in the mouse. Mice were sacrificed on gestation day 18, rats on day 21. Uteri were removed and examined for live, dead, and resorbed fetuses. Maternal weights and liver weights were also recorded. Maneb caused a significant dose-dependent decrease in maternal weight gain in the rat, but not the mouse ($p<0.01$). In both species, maneb treatment at all doses caused a significant increase in liver:body weight ratio as compared to controls ($p<0.05$). No other reproductive effects were noted. Pregnant rats were used in a second study with maneb at doses of 0, 190, and 380 mg/kg/day. In this study, the pups were allowed to be born the dams were allowed to deliver the pups. The maneb treatment did not affect parturition or litter size (Chernoff et al. 1979).

Oral doses of mancozeb in male albino rats (0, 375, 750, or 1125 mg/kg/day) were given by gavage in peanut oil for up to 360 days to determine the fungicide's reproductive toxicity in the male rodent. A dose of 375 mg/kg/day, given for 180 days, significantly reduced the gonadal activities of acid phosphatase and succinate dehydrogenase (both at $p<0.05$), while increasing the levels of alkaline phosphatase and lactate dehydrogenase (both $p<0.05$) in the same time frame (Kackar et al. 1997a). The absolute weight of the testes was unaffected at any dose, but the relative weight of this organ was significantly increased at the 2 higher doses after 180 days exposure. After 180 days, the relative weight of the epididymis was decreased at the 1,125 mg/kg/day dose ($p<0.02$), whereas the decrease was observed at the 750 mg/kg/day dose at the 360 days time point ($p<0.02$). Decrease in the absolute weight of the epididymis was statistically significant at both doses at the 360 days time point.

Beck (1990) administered 960 mg maneb/kg/day in carboxymethylcellulose by gavage to pregnant CD-1 mice on gestation days 6–15. The treatment resulted in a significant ($p<0.001$) decrease in mating

efficiency as evidenced by an increase in maternal mortality (7 pregnant females died in the treated group vs. 0 in the untreated group and 0 in the vehicle control), occurrence of stillborn litters (6 in treated animals, 1 in vehicle control, and 0 in untreated control), and nonpregnant females. Other parameters of reproductive success (e.g., corpora lutea or resorptions) were not measured.

Petrova-Vergieva and Ivanova-Tchemishanska (1973) performed a study of intermediate duration to evaluate effect of administration of varying doses of maneb given by gavage in water to pregnant albino rats. This study involved the administration of 0, 100, 200, and 400 mg maneb/kg/day on gestation days 2–20. No statistically significant adverse effects were seen, even at the highest dose of 400 mg maneb/kg.

2.2.2.6 Developmental Effects

Inorganic Manganese

Very little information is available on the developmental effects of manganese in humans. The incidences of neurological disorders and the incidences of birth defects and stillbirths were elevated in a small population of people living on an island where there were rich manganese deposits (Kilburn 1987); however, the lack of exposure data, the small sample sizes, and the absence of a suitable control group preclude ascribing these effects to manganese. The route of exposure was assumed to be primarily oral, but inhalation exposure was not ruled out.

Two recent studies evaluated adverse neurological results in children exposed to increased manganese concentrations in both water and food. The first study (He et al. 1994), evaluated 92 children, aged 11–13, who drank water containing manganese at average levels of at least 0.241±0.051 mg/L for 3 years or more and who also ate foodstuffs (wheat flour) with excess manganese (due to the high concentration of the metal in sewage water used to irrigate/fertilize the fields). These children were compared to 92 children from a nearby village whose manganese concentration in water did not exceed 0.040±0.012 mg/L (controls). The children who consumed higher manganese concentrations performed more poorly on the WHO neurobehavioral core tests (the emotional status test was omitted) than the control children. Further, the blood and hair manganese concentrations of exposed children were significantly higher than those of the control population. The negative results on the tests were correlated with hair manganese concentration. The children with increased manganese exposure also performed more poorly in school (as measured by mastery of their native language, mathematics, and overall grade average), and their serum levels of

serotonin, norepinephrine, dopamine, and acetylcholinesterase were significantly decreased compared to controls (Zhang et al. 1995).

Several studies of the effects of manganese on reproductive development show developmental effects. One study involved pre-weanling mice (Gray and Laskey 1980) who were fed 1,050 mg manganese/kg/day as Mn_3O_4 beginning on postnatal day 15. On days 58, 73, and 90, mice were sacrificed and reproductive organ (preputial gland, seminal vesicle, and testes) weights and body weights were measured. The manganese decreased the growth of these reproductive organs but had no effect on body growth or liver or kidney weights.

In another study, Laskey et al. (1982) evaluated the effect of dietary manganese exposure on rats during gestation and continued during nursing and after weaning at doses of 0, 350, 1,050, and 3,500 mg/kg/day. The manganese was given in combination with either 20 or 200 mg iron/kg/day (the former is deficient in iron, the latter is adequate). Manganese treatment was lethal at the highest dose in the iron-deficient diet but had no effect on male or female body weight at any age in animals receiving an iron-sufficient diet. In the iron-poor diet, body weights of males were significantly depressed ($P<0.05$) through day 100 of the study, whereas the females' body weights were depressed only through day 60. Select females and males were mated at day 90–100 of the study and the reproductive outcomes were analyzed. The manganese treatment did not have any significant adverse effects at any dose except to significantly decrease the number of pregnancies at the highest dose ($P<0.05$). Litter size, ovulations, resorptions, preimplantation deaths and fetal weights were unaffected by the metal. Testes weights in males were significantly decreased from controls only when administered manganese in conjunction with an iron-poor diet: at day 40 at 1,050 and 3,500 mg manganese/kg/day and at day 100 at 1,050 mg/kg/day. Hormone levels in male rats were also evaluated. No effect was seen from manganese treatment in 40-day-old male rats. At 60–100 days of age, however, dose-related decreases in serum testosterone were observed, when age-related increases were expected and no increase in serum LH was observed. Manganese given in both iron regimens prevented the normal decrease in serum follicle-stimulating hormone (FSH) from 60 to 100 days. Manganese decreased epididymal sperm count only when given with the iron-poor diet as measured at 100 days.

A third study involved gavage administration of 0, 71, or 214 mg manganese as Mn_3O_4/kg/day to pre-weanling rats from birth to 21 days of age (Laskey et al. 1985). Functioning of the hypothalamus, pituitary, and testicular tissues were measured by assaying endogenous or stimulated serum concentrations

of FSH, LH, and testosterone at days 21 or 28. No manganese-related effects were observed on unstimulated or stimulated FSH or LH serum levels. In addition, manganese did not affect endogenous or acute hCG-stimulated serum testosterone concentrations but did decrease serum testosterone level following chronic hCG stimulation. Liver and hypothalamic manganese concentrations were significantly increased in treated rats given the 71 mg/kg/day dose over controls. The authors hypothesized that the manganese had an unknown affect on the testicular Leydig cell that resulted in the delayed production of testosterone. This delayed production was presumably causing the delayed reproductive maturation seen in the earlier study (Gray and Laskey 1980) but was not enough to affect fertility outcomes at doses as high as 1,050 mg/kg/day (Laskey et al. 1982).

Kristensson et al. (1986) investigated the developmental effects of $MnCl_2$ on 3-day old male rat pups. The authors dosed the pups with 150 mg manganese/kg/day by gavage in water for 41 days. The pups developed a transient ataxia on days 15–22, which was resolved by the end of the dosing period. The exposed pups also had increased levels of manganese in the blood and the brain (7–40 fold increase in 15- and 20-day-old rats, with cortex and striatum concentrations being relatively equal). In 43-day-old rats, the brain manganese levels had decreased to approximately 3 times the control levels, but the striatal levels were higher than in the cortex. Manganese treatment decreased the concentration of homovanillic acid (metabolite of dopamine) in the striatum and the hypothalamus, but not in other brain regions. No other monoamines and metabolites were affected. In a similar study, neonatal rats given bolus doses of $MnCl_2$ in water of 1 mg manganese/kg/day for 60 days suffered neuronal degeneration and increased monoamine oxidase on days 15 and 30 of the study, but did not show any clinical or behavioral signs of neurotoxicity (Chandra and Shukla 1978).

Deskin et al. (1980, 1981) also found changes in brain chemistry in rat pups dosed with manganese. In the first study, male rat pups were administered 0, 1, 10, or 20 mg manganese/kg/day, as $MnCl_2$, via gavage in 5% sucrose solution for 24 days postnatal. The authors observed that the two highest doses resulted in decreased dopamine levels in the hypothalamus, while the highest dose resulted in a significant decrease in tyrosine hydroxylase activity and a significant increase in monoamine oxidase activity in the hypothalamus. Hypothalamic norepinephrine was unaffected by any manganese dose, and no significant changes in neurochemistry were noted in the corpus striatum. The authors suggested that the observed effects were probably due to decreased activity of tyrosine hydroxylase and increased levels of monoamine oxidase.

The second study (Deskin et al. 1981) involved dosing male rat pups with 0, 10, 15, and 20 mg manganese/kg/day, as $MnCl_2$, via gavage in 5% sucrose solution, for 24 days starting at birth. The authors performed neurochemical analyses of hypothalamus and corpus striatum as before and observed that serotonin was increased in the hypothalamus at the highest dose, but was not elevated significantly in the striatum. Acetylcholinesterase levels were significantly decreased in the striatum at the highest dose but were unchanged in the hypothalamus. The authors believed the decrease in acetylcholinesterase to be of minor functional significance given that other mechanisms can also regulate acetylcholine metabolism.

Lai et al. (1984) studied the effect of chronic dosing of 40 mg manganese/kg/day (as Mn_3O_4 given in drinking water) to neonatal rats who were exposed from conception, throughout gestation, and up to 2 years of age. The authors found that manganese treatment led to small decreases in choline acetyltransferase activities in cerebellum and midbrain of 2-month-old rats. The regional distribution of glutamic acid decarboxylase or acetylcholinesterase was unchanged.

Kontur and Fechter (1988) observed that maternal exposure to $MnCl_2$ in drinking water during gestation days 0–20 at daily doses as high as 1,240 mg manganese/kg/day resulted in increased manganese levels in rat pups. However, treatment did not affect dopamine or norepinephrine turnover, nor the development of startle response in the pups.

Szakmáry et al. (1995) studied the developmental toxicity of manganese in the rabbit and rat. The metal, as $MnCl_2$, was administered by gavage during the whole period of gestation in the rat, and during organogenesis (day 6–20) in the rabbit at concentrations of 0, 11, 22, and 33 mg/kg/day. In the rabbit, manganese treatments did not result in decreases in fetal weights, skeletal retardation, extra ribs, or in an increase in fetuses afflicted with major anomalies. In the rat, the highest dose resulted in retardation of development of the skeleton and internal organs. In addition, manganese at the highest dose caused a significant increase in external malformations, such as clubfoot. However, when pups from dams treated at the same dose were allowed to grow for 100 days after birth, no external malformations were observed, indicating that these effects were self-corrected. No significant differences were found in any of the groups concerning the development of the ears, teeth, eyes, forward motion, clinging ability, body posture correction reflex, or negative geotaxis reflex.

Acute administration of $MnCl_2$ by gavage to pregnant rats at a dose of 22 mg manganese/kg/day on gestational days 6–17 resulted in no adverse fetal developmental effects, measured as weight gain, gross

malformations, or skeletal malformations (Grant et al. 1997). An intermediate drinking water study in pregnant rats (Pappas et al. 1997) investigated the toxicity of $MnCl_2$ doses of either 120 or 620 mg manganese/kg/day given on gestation days 1–21. Following birth, the dams were continued on manganese until weaning at postnatal day 22. When the dams were removed, the pups were continued on the same manganese doses until postnatal day 30. Male pups were observed on several days subsequent to exposure in a number of behavioral tests that measured spontaneous motor activity, memory, and cognitive ability. The manganese-treated rats' performance was not significantly different from control rats. Pups from the highest-dose group exhibited a significantly decreased weight gain on several days post-dosing, as well as an increased activity level on postnatal day 17 that was no longer evident by postnatal day 30. The high-dose rats were not overactive on other days, and the decreased weight gain was resolved by postnatal day 90. Neurochemical analyses of the brains from treated pups indicated that brain manganese concentrations were significantly elevated in the high-dose group, as compared to controls. Brain enzyme and dopamine concentrations were not significantly different between groups, but cortical manganese concentrations were significantly elevated in the high-dose group. Cortical thickness was significantly different in several areas of the brains of pups in the high-dose group but was only found to be significantly different in one area of the low-dose group. The significance of the cortical thinning is not clear.

In a longer intermediate study, Jarvinen and Ahlstrom (1975) fed female rats up to 187 mg manganese/kg/day (as $MnSO_4$) for 8 weeks prior to conception. The rats were continued on manganese treatment until the 21st day of gestation. The unborn pups from dams administered 94 mg manganese/kg/day had significantly decreased weights as compared to the other groups. No gross malformations were observed in the fetuses, and alizarin-stained bone preparations revealed no abnormalities in any dose group. However, fetuses from dams fed the highest manganese dose had significantly higher concentrations of manganese in their bodies than fetuses from the other groups. These data indicate that a level of 187 mg manganese/kg/day overwhelmed the rat's homeostatic control of manganese and the metal accumulated in the fetus. The highest manganese dose also resulted in a significant decrease in the iron content of the fetuses.

Ali et al. (1983a) conducted a gestational study investigating the neurological effects of excess manganese in drinking water on rats maintained on either a normal or low-protein diet. Manganese exposure originated 90 days prior to mating and continued throughout gestation and nursing. The offspring of rats who drank the equivalent of 240 mg manganese as $MnCl_2$/kg/day had pups with delayed air righting reflexes. No treatment-related effects were observed in body weight or brain weight in pups from dams fed

the normal amount of protein. Significant delays in age of eye opening and development of auditory startle were observed only in the pups of dams fed protein-deficient diets. In a recent study, Dorman et al. (2000) evaluated the effects of oral manganese treatment in neonatal CD rats. Pups were administered manganese chloride in water at 11 or 22 mg manganese/kg for 21 days by mouth with a micropipette and were dosed starting after birth, postnatal day 1(PND1) until weaning, PND21. At PND 21, the effect of manganese treatment on motor activity, learning and memory (passive avoidance task), evoked sensory response (acoustic startle reflex), brain neurochemistry, and brain pathology was evaluated. Manganese treatment at the highest dose was associated with decreased body weight gain in pups, although the authors indicated absolute brain weight was not significantly altered. There were no statistically significant effects on motor activity or performance in the passive avoidance task. However, manganese treatment induced a significant increase in amplitude of the acoustic startle reflex. Significant increases in striatal DA and DOPAC concentrations were also observed in the high-dose treated neonates. No pathological lesions were observed in the treated pups. The authors indicated that these results suggest that neonatal rats are at greater risk than adults for manganese-induced neurotoxicity when compared under similar exposure conditions.

Organic Manganese

MMT. No studies of developmental effects following oral exposure to MMT in humans or animals were located.

Maneb or mancozeb. A single dose of 0, 400, 800, 1,600, or 3,200 mg maneb/kg was administered to pregnant albino rats on gestation day 11 or 13 (Petrova-Vergieva and Ivanova-Tchemishanska 1973). When administered on gestation day 11, maneb at 1,600 and 3,200 mg/kg/day resulted in a statistically significant increase in the total number of late fetal deaths; there were 18 deaths at the highest dose, 21 at the intermediate dose, and 2 each at the 2 lowest doses ($p<0.001$). When given on day 13, maneb did not cause an increase in late fetal deaths. The 3 highest doses resulted in a significant increase ($p<0.001$) in the incidence of grossly malformed fetuses; on gestation day 11, the 800, 1,600, and 3,200 mg/kg doses resulted in 8, 26, and 25 malformed fetuses, respectively, and on day 13, the same doses caused 33, 27, and 63 malformed fetuses. No malformed fetuses were observed in either the control groups or the lowest dose administered on either day. The malformations included exencephaly, encephalocele, hydrocephalus, cleft lip and palate, micrognathia, retarded/clubbed forelimbs and hind limbs, forelimb and hindlimb ectrodactyly/ oligodactyly, and short kinked tail.

Larsson et al. (1976) administered maneb doses of 0, 340, 655, or 1,200 mg/kg or mancozeb doses of 0, 300, 580, or 1,060 mg/kg by gavage in water to pregnant Sprague-Dawley rats on gestation day 11. Surviving fetuses (those that were not resorbed) were studied for hemorrhages and gross malformations. None of the surviving fetuses in the lowest maneb and mancozeb doses had either hemorrhages or malformations. All of the maneb-dosed fetuses in the two highest-dose groups had malformations, but none had hemorrhages. One percent of the control fetuses suffered malformations. The 2 highest mancozeb doses, 580 and 1,060 mg/kg, resulted in 14.8 and 20.7% incidences of hemorrhage in the surviving fetuses, respectively (compared to an incidence of 4.9% in the vehicle control). Maneb-induced gross malformations included meromelia, adactyly/oligodactyly, short tail, umbilical hernia, edema, constriction of neck region, cleft palate, low external ears, open eye, exophthalmia, anophthalmia or microphthalmia, and hydrocephaly. Skeletal and internal malformations were also investigated and included fused ribs, malformed vertebral column, malformed spinal cord, and malformed eyes. When zinc acetate was coadministered with maneb, it had a protective effect against the incidence of malformations, but only at concentrations of 30 and 60 mg/kg. For example, when 640 mg maneb/kg was given with 30 mg/kg zinc acetate, 63.9% of the surviving fetuses had malformations; when 60 mg/kg zinc acetate was given with the same maneb dose, only 11.2% of the fetuses had malformations. However, when 108 mg/kg zinc acetate was administered with 1,100 mg maneb/kg, 100% of the surviving fetuses had malformations, indicating that this dose of zinc acetate was insufficient to protect against teratogenesis caused by the highest maneb dose.

Beck (1990) studied the induction of skeletal malformations in maneb-exposed CD-1 mice. Administration of 960 mg/kg/day maneb in 1% carboxymethylcellulose by gavage to pregnant dams on gestation days 6–15 resulted in a highly significant ($p<0.001$) increase in fetal mortality (51 stillborn fetuses in treated group compared to 10 in the vehicle control and 0 in the untreated control). There were also increases ($p<0.02$ in minor abnormalities, such as head and nasal shape and spinal curvature, as well as increases in the incidence of bent tails. Maneb treatment had no effect on fetal body weight, on the incidence of malformed hind limbs, or on the mean litter size. Although maneb treatment did result in 14 fetuses with growth retardation compared to only 5 in the control group, this difference was not statistically significant. There was a significant ($p<0.001$) increase in both the number of fetuses with reduced ossification of the centra of the cervical vertebrae, and in bilaterally- or symmetrically-expressed instance of 14-ribs.

Chernoff et al. (1979) administered maneb via gavage in water to pregnant Sprague-Dawley rats and CD-1 mice at doses of 0, 100, 190, and 380 mg/kg/day on gestation days 7–16. The rats were sacrificed on day

21, the mice on day 18; the fetuses were removed from the uteri, weighed as a litter, and examined for gross abnormalities. Half were necropsied, the remaining fetuses were fixed and stained for skeletal examination. The highest dose of maneb caused a 9% decrease in rat fetal weight, that was statistically significant compared to controls. This dose also resulted in a significant decrease (24%; $p<0.05$) in the number of caudal ossification centers in rat fetuses. There were also 18 rat fetuses in 4 litters with hydrocephalus in the highest dose group, compared to none in the control and lower dose groups. The mice were slightly more susceptible to maneb treatment in that all doses resulted in a significant decrease in caudal ossification centers (at least 21% decrease; $p<0.05$). There were no effects on fetal body weight and no incidences of hydrocephalus. There was 1 mouse fetus with cleft palate in the 300 mg/kg group.

In a second experiment on postnatal effects of gestational exposure to maneb in the rat, Chernoff et al. (1979) treated pregnant rats with 0, 190, or 380 mg maneb/kg on gestation days 7–15, also by gavage. The rats were allowed to deliver their litters, which were normalized to four individuals of each sex, weighed weekly, and examined for the development of eye opening, startle reflex, and air righting. The litters were weaned at 22 days of age and the females were discarded. Females exhibited a significant ($p<0.01$) increase in weight immediately after birth which did not persist until the next weekly weighing. The males continued to be weighed weekly, and at postnatal week 6, two males from each litter were tested in a circular open field. Behavioral tests included latency to leave the center circle in which they were placed, defecations, urinations, rearings, and activity. The only notable effect observed in male offspring was the significant ($p<0.05$) retardation in eye opening (assumed to be at both doses since the authors did not specify a difference). No significant differences were noted between exposed and control males in the behavioral tests.

Maneb administered to pregnant white rats by gavage in water at doses of 0, 100, 200, and 400 mg/kg/day on gestation days 2–21 had no significant effect on the number of late deaths or malformed fetuses (Petrova-Vergieva and Ivanova-Tchemishanska 1973).

Three-day-old Wistar rats received a single i.p. 50 mg/kg injection of the carcinogen nitrosomethylurea (NMU), and then were subsequently nursed by dams fed either a control diet, or one containing 100 mg mancozeb/kg diet (Monis and Valentich 1993). The pups were weaned at 30 days of age, then were maintained on the same diet for 24 weeks. The rats eating the mancozeb-supplemented diet suffered a slightly higher incidence of focal acinar cell hyperplasia (FACH; 14/14 rats had the hyperplasia) of the pancreas as compared to rats eating a control chow (13/17 rats) following NMU injection. Whereas none

of the rats receiving the NMU and eating control diet developed dysplastic foci of the pancreas, 9/14 of the mancozeb-treated rats developed the abnormalities. Further, 5/14 of mancozeb-treated rats developed pancreatic carcinoma *in situ* (CIS). None of the rats on the control diet developed CIS.

2.2.2.7 Genotoxic Effects

Inorganic Manganese

No studies were located regarding genotoxic effects in humans after oral exposure to inorganic manganese.

In male Swiss albino mice, $MnSO_4$ and $KMnO_4$ have both been found to be clastogenic, and their effects were found to be dependent primarily on the concentration (not duration) of exposure (Joardar and Sharma 1990). In this *in vivo* study, oral doses were administered at varying levels over a 3-week period. The $MnSO_4$ doses were 10.25, 20.25, and 61.00 mg/100 g body weight, and the $KMnO_4$ doses were 6.5, 13, and 38 mg/100 g body weight. Sperm head abnormalities and the frequency of chromosomal aberrations in bone marrow cells and micronuclei were significantly increased. In male rats, repeated oral doses of 0.014 mg manganese/kg/day (as $MnCl_2$) for 180 days did not produce any significant chromosomal damage in either bone marrow or spermatogonial cells (Dikshith and Chandra 1978).

Organic Manganese

MMT. No studies regarding genotoxic effects following oral exposure to MMT in humans or animals were located.

Maneb or mancozeb. No studies were located regarding genotoxic effects in humans following oral exposure to maneb or mancozeb.

Mancozeb did not result in any chromosomal aberrations in spermatogonia or bone marrow cells taken from Swiss albino mice that had been gavage dosed for 5 days with the pesticide in distilled water at doses of 0, 1,350, 2,700, or 4,050 mg/kg (Vasudev and Krishnamurthy 1994). A second genotoxicity study involved mancozeb dissolved in olive oil administered via gavage for 5, 10, or 13 days at a dose of 20 mg/kg/day in male mice (Sobti et al. 1987). This study reported that mancozeb resulted in a significant increase in chromosomal aberrations in somatic cells when given for both 5 and 10 days. Mancozeb

administered for 13 days also resulted in a significant increase ($p<0.01$) in the incidence of abnormal sperm head shape, even after a delay of 5 weeks following the last day of treatment.

Other genotoxicity studies are discussed in Section 2.5.

2.2.2.8 Cancer

Inorganic Manganese

No studies were located regarding carcinogenic effects in humans after oral exposure to inorganic manganese.

Chronic (2-year) feeding studies in rats and mice have yielded equivocal evidence for the carcinogenic potential of manganese. For example, rats exposed to up to 232 mg manganese/kg/day as $MnSO_4$ for 2 years showed no increases in tumor incidence (NTP 1993). However, in another study male rats exposed to up to 331 mg manganese/kg/day (as $MnSO_4$) for 2 years had an increased incidence of pancreatic cell adenomas and carcinomas (Hejtmancik et al. 1987a). Although the tumor incidence in the dosed groups was low and was not dose responsive (4 out of 50 in all 3 dose groups), the authors concluded that the tumors were compound related because the incidence of these tumors in the controls was zero. This tumor type was noted in only 1 female (in the mid-dose group) and no increases in tumor frequency were detected in any other tissues. Though incidences of hyperplasia and adenomas in the pancreatic islets were slightly higher in male rats, the incidences were well within those of historical controls and were not believed to be treatment related. Mice fed up to 731 mg manganese/kg/day as $MnSO_4$ for 2 years had a marginally increased incidence of thyroid gland follicular cell adenomas (high-dose animals) and a significantly increased incidence of follicular cell hyperplasia (NTP 1993); this was considered by NTP to be "equivocal evidence of carcinogenic activity of manganese (II) sulfate monohydrate in male and female $B6C3F_1$ mice" (there was "no evidence of carcinogenic activity" in rats in this study). In mice, a small increase in the incidence of pituitary adenomas was noted in females at 905 mg manganese/kg/day (as $MnSO_4$) but not in males at 722 mg manganese/kg/day (as $MnSO_4$) (Hejtmancik et al. 1987b). However, the increase was within historical control rates, and the significance was considered to be equivocal. No increases in tumor frequency were detected in other tissues or at lower doses (78 or 275 mg/kg).

Currently, it is not known whether inorganic manganese is carcinogenic. There is only limited evidence for the carcinogenic potential of manganese, and the results are equivocal.

Organic Manganese

MMT. No studies were located regarding carcinogenic effects in humans or animals following oral exposure to MMT.

Maneb or mancozeb. No studies were located regarding carcinogenic effects in humans following oral exposure to maneb or mancozeb and in animals exposed to maneb.

Wistar rats administered a single i.p. 50 mg/kg injection of the carcinogen nitrosomethylurea (NMU) at 3 days of age, who were nursed by dams fed a diet containing 100 mg mancozeb/kg diet, then maintained on the diet for 20 more weeks following weaning, suffered a slightly higher incidence of focal acinar cell hyperplasia (FACH; 14/14 rats had the hyperplasia) as compared to rats eating a control chow (13/17 rats) following NMU injection (Monis and Valentich 1993). Whereas none of the rats receiving the NMU and eating control diet developed dysplastic foci of the pancreas, 9/14 of the mancozeb-treated rats developed the abnormalities. Further, 5/14 of mancozeb-treated rats developed pancreatic carcinoma *in situ* (CIS), characterized by anaplastic elements or foci of malignant cells arranged in acinar and ductular structures containing cuboidal cells with scant cytoplasm. None of the rats on the control diet developed CIS.

2.2.3 Dermal Exposure

For inorganic manganese compounds, dermal exposure is not a typical pathway of exposure because manganese does not penetrate the skin readily. For organic manganese, dermal exposure is a possibility with all compounds discussed in this profile. This exposure pathway is most likely, however, with MMT, maneb and mancozeb, where occupational workers (mechanics, workers in the gasoline industry, pesticide manufacturers and sprayers) are likely to handle large quantities of these compounds.

Studies involving health effects from dermal exposure to maneb and mancozeb are presented in Table 2-5.

TABLE 2-5. Levels of Significant Exposure to Organic Manganese - Maneb &-Mancozeb Dermal

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (ppm)	LOAEL Less Serious (ppm)	LOAEL Serious (ppm)	Reference
ACUTE EXPOSURE						
Death						
Newt					20 ppm M LT_{50} = 255hrs.	Zaffaroni et al. 1978 Maneb
Systemic						
Mouse (Swiss albino)	1x			76 F (cellular proliferation as measured by an increase in DNA uptake of ^{3}H-thymidine in dermal cells)		Gupta and Mehrotra 1992 Mancozeb
Mouse (Swiss albino)	1x or 4x			76 F (maximal increase in ornithine decarboxylase activity compared to controls)		Gupta and Mehrotra 1992 Mancozeb
Dog (Mexican hairless X Beagle hybrid)	1x/d, 7d			M (1d post-treatment: mild thickening and hyperplasia of epidermis, when Maneb + UVA+B administered: hyperplasia increased to "moderate": pigmentation and hyperkeratosis observed;		Kimura et al. 1998 Maneb
Neurological						
Mouse (Swiss)	1x		1600M			Mitchell et al. 1989 Maneb
Mouse (Swiss)	once			32 M (taste aversion to saccharin)		Mitchell et al. 1989 Maneb
Mouse (Swiss)	1x			1600 M (3x increase in grouped activity over 4 hours [p<= 0.05])		Mitchell et al. 1989 Maneb
Mouse (Swiss)	1x			32 M (increased activity of grouped subjects 1.6-fold over water-dosed controls [p<=0.05])		Mitchell et al. 1989 Maneb

TABLE 2-5. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg)	LOAEL Less Serious (mg/kg)	LOAEL Serious (mg/kg)	Reference
INTERMEDIATE EXPOSURE						
Developmental						
Newt	24hr/d, 4d/wk; 15-85d				4 B (significant increase in malformed limbs in females, significant increase in delay in limb regeneration in both sexes)	Arias and Zavanella 1979 Maneb
Newt	5d/wk - up to 10wks (2-10wk)				4 ppm F (developmental delay; structural abnormality)	Zavanella et al. 1984 Maneb
Cancer						
Newt	19-23wks		4 B			Zavanella et al. 1979 Maneb
CHRONIC EXPOSURE						
Death						
Mouse (Swiss-albino	3x/wk; 60wks				76 F (death)	Shukla et al. 1990 Mancozeb
Systemic						
Mouse (Swiss-albino	3x/wk; 60wks			76 F (scaly skin, baldness at treatment site, thinning of skin, disappearance of fatty layer below skin)		Shukla et al. 1990 Mancozeb
Neurological						
Human	4hr/d;4d/wk; 4mo/yr;2yrs				5000 M (bradykinesia, postural tremor, resting tremor, slow gait, slurred speech)	Meco et al. 1994 Maneb
Cancer						
Mouse (Swiss-albino	3x/wk; 17wks				80 F (significant increase in benign tumors including squamous cell papillomas, keratocanthomas)	Shukla et al. 1988 Maneb

TABLE 2-5. Levels of Significant Exposure to Organic Manganese - Maneb & Mancozeb - Dermal (continued)

Species (Strain)	Exposure/ Duration/ Frequency (Specific Route)	System	NOAEL (mg/kg)	LOAEL			Reference
				Less Serious (mg/kg)	Serious (mg/kg)		
Mouse (Swiss-albino	3x/wk; 60wks				76 F	(increase in benign tumors including squamous cell papillomas, keratoacanthomas, and mixed-type tumors)	Shukla et al. 1990 Mancozeb

B = both sexes; d = days; (GW) = gavage-water; hr = hour(s); LT_{50} = lethal time for 50% of the individuals; M = males; mo = month(s); ppm = parts per million; 1x = once; x = times; wk = week(s); yr(s) = year(s)

No studies were located regarding the following health effects in humans or animals after dermal exposure to inorganic manganese.

2.2.3.1 Death

2.2.3.2 Systemic Effects

2.2.3.3 Immunological and Lymphoreticular Effects

2.2.3.4 Neurological Effects

2.2.3.5 Reproductive Effects

2.2.3.6 Developmental Effects

2.2.3.7 Genotoxic Effects

2.2.3.8 Cancer

Organic Manganese

2.2.3.1 Death

MMT. No studies were located regarding death in humans from dermal exposure to MMT.

Hinderer (1979) reported LD_{50} values for rabbits (strain and sex were unreported) that were administered varying doses of "neat" commercial MMT on abraded skin in the trunk area for 24 hours. These values, generated by 4 different laboratories, ranged from 140 mg/kg to 795 mg/kg. Although this dose range is wide, the author reported that it was analogous to the wide oral LD_{50} range given for the compound in other reports.

Maneb or mancozeb. No studies were located concerning death in humans following dermal exposure to maneb or mancozeb.

Doses of 16, 160, or 1,600 mg maneb/kg were not lethal to adult male Swiss mice, when applied to the shaved area of the back just caudal to the skull. Lethality was observed for a 96-hour period following 1 application of the compound (Mitchell et al. 1989).

Pacces Zaffaroni et al. (1978) investigated the percutaneous toxicity of maneb in solution to the adult newt, *Triturus cristatus.* When incubated in water containing 20, 40, 60, 80, or 100 ppm maneb, the newts

suffered deaths in a dose-dependent fashion, with 50% of the male newts dead in 8.4–8.8 hours at 100 ppm and in 255 hours at 20 ppm. The females were not as susceptible to the lethal action of the fungicide, with 50% dying in 28.5 hours at 100 ppm, and some newts still alive after 5 months at 20 ppm. No studies were located concerning death following dermal exposure of animals to mancozeb.

In a chronic carcinogenesis study in female Swiss mice, thrice weekly applications of technical grade mancozeb at a dose of 76 mg/kg active ingredient resulted in the increased mortality of treated subjects (Shukla et al. 1990). Eleven mice remained in a treatment group of 20 at 360 days of treatment; fewer than 6 animals remained at 420 days of treatment. The deaths were reported to be related to the dermal toxicity caused by the mancozeb.

2.2.3.2 Systemic Effects

Respiratory Effects.

MMT. No studies were located regarding respiratory effects in humans or animals following dermal exposure to MMT.

Maneb or mancozeb. There were a few case studies of adult males exposed to maneb or mancozeb while spraying fields or gardens (de Carvalho et al. 1989; Israel et al.1983; Koizumi et al. 1979; Mena et al. 1984). The exact exposure pathway in these reports is not specified, although inhalation and dermal exposure are stressed since the subjects did not consistently use protective measures when either mixing or spraying the pesticide. In two cases, oral exposure was also possible (de Carvalho et al. 1989; Koizumi et al. 1979). The systemic effects, including respiratory effects, resulting from the combined inhalation and dermal exposures are discussed in Section 2.2.1.2. For every subsequent systemic effect listed in this section, the reader will be referred to Section 2.2.1.2 for a discussion of the appropriate studies and observations listed in the available reports. A discussion of the systemic effects in these human case reports will not be repeated here.

Vascular congestion and inflammatory cells were present in the cavity and the lung wall in 16% of newts exposed percutaneously to 40–100 ppm maneb for up to several days in an acute toxicity study (Pacces Zaffaroni et al. 1978).

Cardiovascular Effects.

MMT. No studies concerning cardiovascular effects following dermal exposure to MMT in humans or animals were located.

Maneb or mancozeb. Refer to Section 2.2.1.2 for a discussion of cardiotoxicity in humans following dermal exposure to fungicides.

Gastrointestinal Effects.

MMT. No studies were located regarding gastrointestinal effects in humans following dermal exposure to MMT. Hinderer (1979) observed bloody diarrhea in rabbits exposed dermally to MMT; the compound was obtained as commercial grade, "neat," and applied to shaved skin for 24 hours. No histopathology was performed to ascertain the presence of lesions on the gastrointestinal tract.

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 for a discussion of gastrointestinal effects in humans following potential dermal exposure to maneb or mancozeb. No studies were located regarding gastrointestinal effects in animals following dermal exposure to maneb or mancozeb.

Hematological Effects.

MMT. No studies were located regarding hematological effects in humans or animals following dermal exposure to MMT.

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 for a discussion of hematological effects in humans following dermal exposure to maneb or mancozeb. No studies were located regarding hematological effects in animals following dermal exposure to maneb or mancozeb.

Musculoskeletal Effects.

MMT. No studies regarding musculoskeletal effects in humans or animals following dermal exposure to MMT were located.

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 for a discussion of musculoskeletal effects in humans following dermal exposure to maneb or mancozeb. Arias and Zavanella (1979) and Zavanella et al. (1984) have reported severe skeletal deformities in newts with amputated limbs that were incubated in varying concentrations of maneb. However, these experiments were performed as a model for developmental toxicity of environmental pollutants; therefore, these reports are discussed fully in Section 2.2.3.6 . No studies were located regarding musculoskeletal effects following dermal exposure to mancozeb in animals.

Hepatic Effects.

MMT. Hinderer (1979) observed that rabbits that underwent dermal application of a commercial "neat" solution of MMT for 24 hours on shaved skin had discoloration of the liver and swollen liver. No histopathology was performed.

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 for a discussion of hepatic effects in humans following dermal exposure to maneb or mancozeb. Pacces Zaffaroni et al. (1978) observed that some newts dermally exposed to maneb at concentrations ranging from 20–100 ppm had livers with vacuolar degeneration of the hepatocytes and more frequently, marked sinusoid congestion, often with hemorrhage. No other studies were located concerning hepatic effects in animals following dermal exposure to maneb and no studies involving dermal mancozeb exposure in animals were located.

Renal Effects.

MMT. Hinderer (1979) observed that rabbits that underwent dermal application of a commercial "neat" solution of MMT for 24 hours on shaved skin had discoloration of the kidneys and swollen and congested kidneys. No histopathology was performed.

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 for a discussion of renal effects in humans following dermal exposure to maneb or mancozeb. Pacces Zaffaroni et al. (1978) observed that some newts dermally exposed to maneb at concentrations ranging from 20–100 ppm had kidneys with varying degrees of alterations. The early signs of maneb lesions were observed at the glomerular level after approximately 10 hours of incubation. Proteinaceous material was observed in the Bowman's spaces and the tubular lumen. Red cells and leukocytes were indicative of severe glomerular lesions. In animals

dying after 2 or more days (60 ppm maneb and greater), tubular degeneration and focal areas of necrosis with pycnotic nuclei and karyorrhexis were reported. Most of the pathologic lesions were found in the proximal convoluted tract. The authors reported the lack of a dose-response relationship between maneb and kidney damage. No other studies were located concerning renal effects in animals following dermal exposure to maneb and no studies involving dermal mancozeb exposure in animals were located.

Endocrine Effects.

MMT. No studies were located regarding endocrine effects in humans or animals following dermal exposure to MMT.

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 regarding endocrine effects in humans following potential dermal exposure to maneb or mancozeb. Thyroid hyperplasia was observed in 3 female newts dermally exposed to 20 ppm maneb for 5 months (Pacces Zaffaroni et al. 1978).

Dermal Effects.

MMT. No studies were located regarding dermal effects in humans following dermal exposure to MMT. Hinderer (1979) observed that rabbits exposed dermally to commercial "neat" MMT on shaved skin for 24 hours developed edema and erythema. Further dermal irritation tests performed showed that MMT is a moderate skin irritant. Campbell et al. (1975) exposed male albino rats dermally to MMT for 24 hours on closely clipped dorsolateral aspects of the trunk that were either abraded or allowed to remain intact; skin reactions were evaluated and scored at 24 hours and again 48 hours later. By comparing skin reactions following exposure to a test rating that categorized irritancy levels, MMT was determined to be safe for intact or abraded skin contact. However, the authors note that MMT in concentrated form is absorbed through the skin, and dermal absorption or interactions with other materials or factors were not incorporated into their study.

Maneb or mancozeb. Maneb and mancozeb have been found to cause allergic contact dermatitis, a delayed (type IV immune system response) hypersensitivity reaction. After sensitivity to a particular allergen develops, subsequent contact to minute doses of the antigen elicits an acute response. While allergic and irritant contact dermatitis usually have indistinguishable clinical characteristics, the former

reaction is a true allergy whereas the latter reaction is one whose intensity is proportional to the dose applied (Rice and Cohen 1996).

Patch test concentrations that may be used for maneb and mancozeb range from 0.2-1% in petrolatum, respectively, reported in a review of patch testing procedures (Fisher 1983). Patch test concentrations used in human case studies that have yielded positive results range from 0.2 to 1. % for maneb (Adams and Manchester 1982; Crape et al. 1990; List and Carragheen 1985; Magnesia et al. 1988; Nader et al. 1979) and 0.002 to 2% for mancozeb (Alive and Elsner 1997; Kleibl and Rá…ková 1980; Oakley 1988).

The ability of maneb and mancozeb to cause allergenicity have been evaluated in animal studies. Matsushita et al. (1976) evaluated the allergenicity of maneb, mancozeb, and zineb and their related compounds using the guinea pig maximization test. After animals were sensitized via high dose exposure (5% intradermal concentration, 25% topical concentration), topical concentrations of 2.0 or 0.5% were applied to 10 female Hartley guinea pigs per group and the results reported as an allergenicity rating ranging from weak (grade I) to extreme (grade V) 24 and 48 hours later. Maneb and mancozeb applied at both 2.0 and 0.5% concentrations elicited grade V responses in 100% of the animals at both 24 and 48 hours post-challenge. Therefore, Matsushita et al. (1976) showed maneb and mancozeb to be strong sensitizers in guinea pigs. However, a similar guinea pig maximization test performed by Obama (1996) with 2.0 and 0.2% topical
challenge concentrations of maneb found allergenicity ratings of only II (mild) for maneb; the author attributes differences from Matsushita's results to a difference in species and the condition of the experiment.

Allergic contact dermatitis caused by maneb or mancozeb exposure has been described in several case reports (maneb: Adams and Manchester 1982; Crape et al. 1990; Magnesia et al. 1988; Nader et al. 1979; mancozeb: Burry 1976; Crape et al. 1990; Alive and Elsner 1997; Kleibl and Rá…ková 1980; Koch 1996; List and Carragheen 1985; Oakley 1988). In these studies, dermal exposure occurred as a result of treating wheat and barley seeds with mancozeb (Burry 1976), planting vine seedlings, potatoes, or wheat and barley seeds previously treated with mancozeb (Burry 1976; Kleibl and Rá…ková 1980), spraying a combination of pesticides as an apple orchardist (Oakley 1988), working in a vineyard irrigated with a mancozeb-containing fungicide (List and Carragheen 1985), handling ornamental plants treated with maneb (Nader et al. 1979), and working in a home garage in which a lawn fungicide containing maneb was stored (Adams and Manchester 1982). Allergic contact dermatitis has also been reported under the

following scenarios in which the exact compound or compounds of exposure are uncertain: spraying a combination of pesticides as an apple orchardist (unspecified combination of dithiocarbamates; orchardist showed positive patch test reactions to mancozeb and zineb, both containing zinc ethylene-BIS-dithiocarbamate; Oakley 1988), working in a fungicide factory (Magnesia et al. 1988), working on a chemical factory's packing line (Alive and Elsner 1997), and handling ornamental plants treated with dithiocarbamates (Crape et al. 1990; Nader et al. 1979). In some of the cases caused by occupational exposure, the workers had worn recommended protective gear (Burry 1976; Oakley 1988). No information about doses required to elicit sensitivity to maneb or mancozeb were available.

One case of photoallergic contact dermatitis was reported in which mancozeb was responsible for the sensitization; despite avoiding further contact with mancozeb, the man's severe eczema was still observed 5 months later after a 20-minute exposure to sunlight (Higo et al. 1996). In another case of mancozeb-induced allergic contact dermatitis, a vineyard worker showed positive reactions to both patch and photopatch tests with both maneb and mancozeb (List and Carragheen 1985). She had worked in a vineyard irrigated with two fungicides, one containing phenarimol, and the other containing cymozanil and mancozeb. It was considered an unusual case because of the pellagra-like dermatitis and its appearance on vitiliginous areas (areas of irregular depigmentation).

Dermal effects noted in these case reports include: an arm rash and inflammation of the eyelids; papulo-vesicular sheeted dermatitis of the face, hands, and front chest (Kleibl and Rá…ková 1980), severe dermatitis on both exposed and, to a lesser degree, covered areas (Burry 1976), eczematous lesions on arms, hands, fingers, and face; watering eyes and blocked nose; and acute papular and vesicular eczema (Nader 1979), recurrent, blistering dermatitis on arms, trunk, and face (Adams and Manchester 1982), contact dermatitis of backs and palms of hands, sides of fingers; itchy red scaly trunk; acute relapsing contact dermatitis of face and upper arms (Magnesia et al. 1988), erythematous vesicular eruption on palms resembling dyshidrotic eczema with remission and recurrence (Crape et al. 1990), allergic contact dermatitis of face, lower neck, forearms (Alive and Elsner 1997), and severe eczema predominantly on sun-exposed areas (Higo et al. 1996).

In spite of Matsushita's (1976) conclusion from guinea pig maximization tests that maneb and mancozeb are strong sensitizers, several authors note that their sensitization potential appears to be low, given the widespread use of these fungicides and the low frequency of reports of allergic skin reactions in humans (Alive and Elsner 1997; Kleibl and Rá…ková 1980; List et al. 1987). In one study, (Kleibl and Rá…ková

1980), the authors based this conclusion upon the fact that three cutaneous allergic reactions to dithiocarbamates were reported in Czechoslovakia in three years. Therefore, it is suggested that people showing sensitivity to maneb and mancozeb may be a relatively small subpopulation.

Cross reactions between chemicals, which may occur if chemicals share similar functional groups important to the formation of complete allergens, can make the control of allergic contact dermatitis difficult; improvement will only occur if the known allergen and all potentially cross-reacting chemicals are avoided (Rice and Cohen 1996). Matsushita et al. (1976) have studied cross-sensitization between dithiocarbamates and their related compounds using the guinea pig maximization test, as described above. Cross reactions among maneb, mancozeb, and zineb were found to be extreme in guinea pigs (Matsushita et al. 1976). Obama (1996) reported a mild degree of cross reaction between maneb and benomyl from their guinea pig maximization tests.

Patch testing in some human case studies show some cross-reactivity between various dithiocarbamate compounds and other chemically-similar compounds. Burry et al. (1976) reported cross reactions between Mankobunt (active ingredient mancozeb) and Zineb 65 (active ingredient polymeric zinc ethylene 1,2-bisdithiocarbamate). However, Nader et al. (1979) found no regular pattern of cross reactions in their patch test series that included maneb, zineb, and a series of compounds with a dithiocarbamate group. They could not demonstrate a cross reaction to the closely related zinc-ethylene-BIS(dithiocarbamate). Cross sensitization among maneb, mancozeb, and tetramethylthiuram has also been reported (List and Carragheen 1985). Possible cross reactions between mancozeb and the dithiocarbamates maneb, nabam, propineb, and zineb have also been observed (Koch 1996). Finally, Alive and Elsner (1997) note potential cross reactivity between mancozeb and azamethiphos, an organophosphorus compound. In this case, a chemical factory worker was employed on the packing line of an insecticide containing azamethiphos when his reaction occurred; he had been working on the packing line of a mancozeb-containing fungicide 2 months prior to this reaction and showed positive reaction to patch testing with mancozeb.

In addition to dermal effects due to allergic contact dermatitis and cross-reactivities between maneb or mancozeb and other compounds, dermal effects of maneb and mancozeb not attributable to allergies were found in animals. Exposure daily for 1 week to 0.0003 mg/kg maneb on the backs of hairless dogs resulted in slight thickening and hyperplasia of the epidermis on the first day following the end of treatment (Chimaera et al. 1998); effects were slightly more severe when the compound was administered in conjunction with irradiation. When UNA and UVB rays were administered at a dose of 35 kJ/m^2 for

UNA and 5 kJ/m^2 for UVB, the dogs suffered slight epidermal thickening and moderate hyperplasia, and also exhibited epidermal pigmentation and hyperkeratosis. These effects were notable within 1 day following cessation of treatment; however, inflammation was reported within the 14 days following the end of treatment, with a severe inflammatory reaction reported at 14 days post-dosing. Histological changes reported at this time with maneb treatment alone were a slight epidermal thickening with inter- and intracellular edema, with moderate epidermal degeneration. The dermis also exhibited a slight vasodilation with moderate cellular infiltration. When mancozeb was administered with irradiation, the effects were also more severe at 14 days post-dosing. The epidermis had slight thickening with moderate inter- and intracellular edema, moderate pigmentation, and marked degeneration. The dermis showed moderate vasodilation with marked cellular infiltration (Chimaera et al. 1998).

Female adult Swiss mice dermally treated to 76 mg/kg mancozeb thrice weekly for up to 60 weeks in a carcinogenesis study exhibited scaly skin, followed by total baldness at the site of treatment (2 cm^2 shaved area on the dorsal side) (Shukla et al. 1990). The skin became thin as a result of continued treatment, and showed a black metallic luster with a nearly complete disappearance of the fatty layer below the skin.

Newts dermally exposed to maneb concentrations from 20–100 ppm appeared to be grossly edematous; skin sections showed an increase in serous secretory material that accumulated between the dermis and epidermis, and frequently, under the superficial horny layer (Pacces Zaffaroni et al. 1978).

A single dermal dose of 80 mg mancozeb/kg (administered in DMSO) increased the ornithine decarboxylase (ODC) activity in the skin of female Swiss mice (Gupta and Mehrotra 1992). Maximal enzyme activity was reached after a 5 hour application of the fungicide. The increase in ODC activity was dose-dependent, and was 3-, 6-, 4-, and 3-fold higher than DMSO controls when given at 40, 80, 160, or 400 mg/kg, respectively. Cycloheximide was found to inhibit the increase in ODC, suggesting that the mancozeb caused an increase in protein synthesis. Dosing mice once with 80 mg mancozeb/kg resulted in an increase in DNA synthesis, as measured by ^{3}H-thymidine incorporation, that reached a maximum of approximately 110% of the level of the DMSO control, at 24 hours post treatment. The significance of this finding to dermal toxicity is unknown.

Ocular Effects.

Inorganic Manganese

No studies were located regarding ocular effects in humans or animals following dermal exposure to inorganic manganese.

Organic Manganese

MMT. Hinderer (1979) performed a standard Draize irritation test with commercial "neat" MMT in rabbits and found the compound not to be an eye irritant.

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 for a discussion of ocular effects in humans that were potentially dermally exposed to these fungicides. No studies were located regarding ocular effects in animals following dermal exposure to maneb or mancozeb.

Body Weight Effects.

Inorganic Manganese

No studies were located regarding body weight effects in humans or animals following dermal exposure to inorganic manganese.

Organic Manganese

MMT. Rabbits exposed dermally to commercial "neat" MMT exhibited slight body weight loss, although the actual amount was not reported (Hinderer 1979).

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 for a full discussion of body weight effects in humans following potential dermal exposure to maneb or mancozeb. No studies were located concerning body weight effects in animals following dermal exposure to these fungicides.

Metabolic Effects.

Inorganic Manganese

No studies were located regarding metabolic effects in humans or animals following dermal exposure to inorganic manganese.

Organic Manganese

MMT. No studies were located regarding metabolic effects in humans or animals following dermal exposure to MMT.

Maneb or mancozeb. The reader is referred to Section 2.2.1.2 for a full discussion of the available reports concerning metabolic effects in humans following potential dermal exposure to maneb or mancozeb. No studies were located regarding metabolic effects in animals following dermal exposure to these compounds.

2.2.3.3 Immunological and Lymphoreticular Effects

Inorganic Manganese

No studies were located regarding immunological and lymphoreticular effects following dermal exposure to inorganic manganese in either humans or animals.

Organic Manganese

MMT. No studies regarding immunological and lymphoreticular effects following dermal exposure to MMT in humans or animals were located.

Maneb or mancozeb. The reader is referred to section 2.2.1.3 for a full discussion of these effects following potential dermal exposure to maneb or mancozeb. No studies were located regarding immunological or lymphoreticular effects in animals following dermal exposure to these compounds.

2.2.3.4 Neurological Effects

Inorganic Manganese

No studies were located regarding neurological effects following dermal exposure to inorganic manganese in either humans or animals.

Organic Manganese

MMT. Rabbits exposed to "neat" commercial grade MMT on shaved areas of their trunks for 24 hours experienced the following reported symptoms: polypnea, vocalization, excitation, ataxia, tremors, cyanosis, and convulsions (Hinderer 1979).

Maneb or mancozeb. For a discussion of neurological effects in humans potentially exposed to maneb or mancozeb through a dermal route, refer to Section 2.2.1.4.

The behavioral effects of dermal exposure to maneb were studied in adult male Swiss mice (Mitchell et al. 1989). Five mice per dose group were dermally exposed to either 16, 160, or 1,600 mg/kg maneb in a conditioned taste aversion assay. Aversion to a saccharin solution was tested 24 hours following the dermal application of the compound to a shaved area on the mouse's back, immediately caudal to the skull. Maneb application did not affect taste aversion compared to water controls. Activity was measured for both ambulatory and nonambulatory activity: ambulatory was when a subject sequentially disrupted at least two adjacent infrared beams in an activity monitor; nonambulatory activity was when a subject sequentially broke the same beam. When given at a dose of 1,600 mg/kg, maneb increased the activity levels of a tested group (5 subjects) by 3-fold over controls. When the mice were tested individually, however, there were no activity increases, even at the highest dose. The authors suggest that the increase in group activity may have been due to ingestion of the compound by reciprocal licking of the mice in the exposed group in light of the lack of effect in individual animals. Interestingly, the authors also observed a 1.6-fold increase in group activity compared to controls in mice orally dosed once with 32 mg maneb/kg (Mitchell et al. 1989).

2.2.3.5 Reproductive Effects

Inorganic Manganese

No studies were located regarding reproductive effects in humans or animals following dermal exposure to inorganic manganese.

Organic Manganese

No studies were located regarding reproductive effects in humans or animals following dermal exposure to organic manganese.

2.2.3.6 Developmental Effects

Inorganic Manganese

No studies were located regarding reproductive effects in humans or animals after dermal exposure to inorganic manganese.

Organic Manganese

MMT. No studies were located in humans or animals concerning developmental effects following dermal exposure to MMT.

Maneb or mancozeb. No studies were located in humans concerning developmental effects following dermal exposure to maneb or mancozeb.

Maci and Arias (1987) immersed unincubated chicken eggs in solutions of Maneb 80 (1.5, 4.5, 13.6, or 41 mM in tap water) for 30 seconds. One control group of eggs was immersed only in tap water; a second control group was immersed in the inert ingredients found in the maneb formulation. The eggs were then blotted and incubated for 19 days. After opening, the contents were inspected for survival and for external and visceral malformations. Parts of the embryos were fixed and skeletons were stained and examined for bone abnormalities. Histology was performed on selected embryos.

Only treatment at the highest dose reduced the number of live embryos a significant amount ($p<0.05$) (73.6% survival) when compared to the control incubated with inert ingredients (82.9%). Maneb caused a significant increase in malformations at every dose ($p<0.05$ or better) with the majority of defects reported as unilateral limb defects consisting of contracted and often shortened lower limbs. Bent tibias, tibiotarsus, and phalanges were also reported (Maci and Arias 1987).

Munk and Schulz (1989) performed a subsequent egg incubation study in an attempt to evaluate the potential teratogenic effect of maneb. The author remarked on the differences between their study and the one done earlier. These differences included the following: inclusion of an untreated control group; candling the eggs on day 8 and day 16 to analyze for early and late fetal death; and allowing the incubated eggs to hatch on their own. Two maneb dose groups were used: 6 mM and 36 mM. This study reported fertility rates ranging from 96.2 (water control) to 98% (untreated control and low dose group). Early embryonic deaths in the groups indicated they might have resulted from dipping: the untreated control had a rate of 2.2%, compared to 4.0% in the water control, 3.5% in the inert ingredients control, and 6.3 and 5.4% in the low- and high-dose groups, respectively (both of which were statistically significantly different than the untreated control group, $p<0.01$). There were no differences in late embryonic deaths between any of the groups. There were also no statistically significant differences in the number of hatched living chicks as a percentage of fertile eggs. The incidence of hatched chicks with externally visible abnormalities was low, with a range of 1.44–2.33%, with no statistically significant differences among the groups. The authors suggest that the omission of an untreated control group in the Maci and Arias (1987) study, as well as the analysis of eggs that had not hatched on their own, may have biased the teratogenicity results in the earlier study. The authors state that the undipped control group is critical to the study, because dipping of the egg increases shell permeability and facilitates the entrance of bacteria. Further, the authors state that improper handling conditions, such as unfavorable incubation conditions and unfavorable humidity, can increase the incidence of malformed embryos. The high incidence of lower limb deformities in the embryos in the Maci and Arias (1987) study could be attributable to misinterpretation of compacted limbs forced by the limited space within the egg (Munk and Schulz 1989). Although the data presented in the Maci and Arias (1987) and (Munk and Schulz 1989) studies are equivocal, the increased controls in the latter study, along with the methodology that allowed full development of the offspring, indicate that maneb is not a developmental toxin at the concentrations used in this model system.

Two studies used the regenerating limbs of the adult newt as a model for developmental effects caused by dermal exposure to maneb (Arias and Zavanella 1979; Zavanella et al. 1984). Both studies involved

bilateral amputation of the forelimbs of male and female newts through the distal third of the humerus. The newts were then maintained at appropriate temperature in 4 ppm active ingredient of maneb solution. The older study (Arias and Zavanella 1979) only used two exposure groups: newts exposed to maneb, and control newts kept in tap water. Exposure continued for 85 days post-amputation, with selected newts being sacrificed starting on the 15th day post-amputation. Fresh solutions of the fungicides were made twice a week. Delayed regenerative growth in the maneb-exposed newts was evident up to the 5th week post-amputation in females and until the 6th week post-amputation in the males. The differences were statistically significant in the females after 3 weeks ($p<0.05$), increasing in significance after 4 and 5 weeks ($p<0.001$). In males, the differences were significant at the 5th week ($p<0.001$) and 6th week ($p<0.01$). In the maneb-exposed newts, vascular dilatation and extensive hemorrhages were frequently seen through the undamaged skin. Distal deformities were the most common abnormality occurring in the treated newts, with arthrogryposis observed in all of the maneb-exposed newts with the autopodium being consistently twisted dorsally. This effect was only observed in some of the newts by the late-digit stage. Of nine newts of each sex analyzed for skeletal effects, all had skeletal malformations. Most had deformed phalanges, with distal phalanges most often affected, being hook-shaped and sometimes kinky. More rarely, deformed metacarpals were found.

In the later study (Zavanella et al. 1984), three groups of female newts were employed: water control, control group incubated in dispersing agents within the maneb formulation (sodium lignin sulfonate and *n*-butylnaphthalene sulfonate), and the 4 ppm maneb active ingredient group. The exposure and amputation conditions were the same as in the earlier study, except that this study was started in the spring, whereas the earlier study had been started in the fall. The newts were incubated 5 days/week and then fed over the weekend, out of the solution to avoid ingestion of the fungicide. Two to four animals were killed per week up until the 10th week of the experiment. Hyperemia was observed in the maneb-exposed newts, as well as hemorrhages, similar to the previous study. Regenerating growth of the amputated limb was significantly slowed in treated animals, whereas it was faster in the control group incubated in inert ingredients; the difference in speed of regeneration between tap water and inert ingredient control groups was statistically significant from week 4 to week 6 ($p<0.01$). The growth differences between the maneb and water control groups was significant from week 4 to week 8 ($p<0.001$). The maneb-treated newts had significantly delayed growth compared to the inert ingredient group at week 3 ($p<0.01$). Out of ten limbs from each group analyzed for skeletal defects at the end of the experiment, the tap water control had six limbs with slight deformities, one with a severe deformity; the inert ingredient control had three limbs with slight deformities, two with severe deformities; the maneb group had zero limbs with slight deformities,

and ten limbs with severe deformities. Limb abnormalities noted in the control groups included radial and ulnar reduction, absence of metacarpals, supernumerary carpals, and fused or missing phalanges. Minor limb abnormalities were also noted, such as the absence of one to three distal phalanges, or incurved distal phalanges. Severe proximal and distal abnormalities were observed in all maneb-treated newts, including large preaxial cartilaginous nodules in the elbow region, spike-like outgrowths from the anterior side of the humerus, and deformities of radius and ulna, usually involving shortening. Distal abnormalities included supernumerary carpals and metacarpals, and most frequently, missing phalanges. Histological observation of newts incubated in inert ingredients showed that development was indistinguishable from the normal pattern observed in the tap water controls. Histological analysis of maneb-exposed newts showed enlargement of the blood vessels and blood extravasation were observed. In those animals sacrificed 2–3 weeks post-amputation, extensive hemorrhagic areas often caused disruption of the blastema into small irregular clumps of mesenchymal cells. Also, disruption or distortion of the cartilaginous elements by these hemorrhagic foci could be observed. Soft tissues, especially muscle tissue, were reported as poorly developed in the regenerating limbs of maneb-treated newts.

2.2.3.7 Genotoxic Effects

No studies were located regarding genotoxic effects in humans or animals following dermal exposure to inorganic or organic manganese.

Other genotoxicity studies are discussed in Section 2.5.

2.2.3.8 Cancer

Inorganic Manganese

No studies were located regarding carcinogenic effects in humans or animals after dermal exposure to inorganic manganese.

Organic Manganese

MMT. No studies were located regarding carcinogenic effects in humans or animals after dermal exposure to MMT.

Maneb or mancozeb. No studies were located regarding carcinogenic effects in humans following dermal exposure to maneb or mancozeb.

Two studies were performed to investigate the activity of mancozeb as a tumor promotor. In the first study, adult female Swiss mice were dosed with a single dose of 52 µg 7,12-dimethylbenz[a]anthracene (DMBA) on a 2 cm^2 shaved area of the interscapular region (Shukla et al. 1988). This initiated region was then promoted with a thrice-weekly application of 5 µg of 12-*O*-tetradecanoylphorbol-13-acetate (TPA; dissolved in acetone) or 80 mg mancozeb (dissolved in dimethylsulfoxide, DMSO)/kg body weight. One group of animals was dosed with acetone and then mancozeb as the promotor. Applications of promotors continued for 17 weeks during which time the mice were observed for gross and morphological changes. The acetone:mancozeb group did not develop any tumors over the 17-week treatment period. The DMBA:mancozeb group did not develop any tumors until the 13th week of the treatment, when 4/14 mice exhibited tumors; by the 15th week, 8 out of 13 mice developed tumors, and by the end of the study, all 13 surviving mice had developed tumors. The positive control DMBA:TPA group developed tumors with 100% frequency by the 7th week of the study. All of the tumors on the dorsal skin were benign and included squamous-cell papillomas, keratoacanthomas, and mixed-type tumors.

The second study evaluated the complete carcinogenic potential of dermally-applied mancozeb, again in female Swiss albino mice (Shukla et al. 1990). The mice were dosed with technical grade mancozeb at a dose of active ingredient of 76 mg/kg, applied in DMSO to the shaved area of the interscapular region of the back 3 times per week for 60 weeks. The mice were observed for clinical signs and were necropsied at the end of the study. In the mancozeb group, the first tumor was observed after 217 days; after 48 weeks of treatment, 5/14 mice had developed tumors, while after 60 weeks, 2/6 of the surviving mice had developed tumors. Five out of 20 animals total in the mancozeb group developed tumors, compared to the 12/20 mice administered Benz(a)pyrene alone (5 µg) on an identical dosing schedule. Acetone, DMSO, and untreated controls did not develop any tumors. The tumors were similar to the previous study: all were benign in nature and included squamous-cell papillomas, keratacanthomas, and mixed-type tumors.

Newts exposed dermally to maneb concentrations in water ranging from 0.4–4.0 ppm for 5 months did not develop any tumors (Zavanella et al. 1979).

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2.2.4 DIAGNOSTIC USES

Manganese is a paramagnetic element that can contain up to five unpaired electrons in its ionic form. The unpaired electrons can facilitate T1 relaxation (in MRI) by interacting with hydrogen nuclei of water molecules (Earls and Bluemke 1999). This T1 relaxation provides a contrast in signal during MRI from normal cells and tumor cells because normal cells will take up the metal, whereas the cancerous cells take up little or no manganese (Toft et al. 1997a). The Mn^{2+} ion is the ion of choice because it is most readily found in the body. However, because increased amounts of other sources of Mn^{2+}, especially $MnCl_2$, were found to have a high acute toxicity (as discussed in the previous sections), it is necessary to chelate the Mn^{2+} ion with another molecule that might decrease the toxic nature of the free ion. One such chelate is the fodipir molecule, or dipyridoxal diphosphate. The result is mangafodipir, manganese (II)-*N,N'*-dipyridoxylethylendiamino-*N,N;*-diacetate-5,5'-bis(phosphate), or manganese dipyridoxal diphosphate, MnDPDP. This clinical agent is primarily used in the detection of hepatobiliary tumors, as it is preferentially taken up by parenchymatous cells. However, as other organs have parenchymatous cells, the compound is also useful in the detection of kidney, pancreas, and adrenal gland tumors (Earls and Bluemke 1999).

This section will discuss the adverse effects of administration of mangafodipir. This section will not discuss the efficacy of mangafodipir as a contrast agent in the identification of abdominal cancer. Because this compound is used primarily in the detection of liver and other parenchymatous tumors, it is found exclusively in hospitals and other clinical settings. It is only administered intravenously, therefore all subsequent studies discussed entail an intravenous exposure route. Because the toxicity of mangafodipir is mediated by manganese, the doses will be in mg manganese/kg body weight, rather than in terms of the parent compound.

2.2.4.1 Death

There are no reports of lethality in humans following administration of mangafodipir.

Administration of mangafodipir can occur either all at once (bolus) or over a specific timed period necessary to give the entire amount of a precalculated dose (slow infusion). The latter method has been found to be better tolerated in a clinical setting (Bernardino et al. 1992; Lim et al. 1992; Padovani et al. 1996).

Mangafodipir was found to cause lethality in both sexes of Swiss-Webster mice with a LD_{50} of 2,916 mg manganese/kg after slow infusion of 15 seconds (Larsen and Grant 1997). The compound had a LD_{50} of 103 mg/kg in both sexes of the same rodent when administered in a bolus dose (Larsen and Grant 1997), showing the increased toxicity in the bolus administration. When given as a slow infusion over 5 minutes in both sexes of the CD-1 mouse, the compound had a LD_{50} value of 157 mg/kg, and when given at a rate of 1.2 mL/sec in BOM:NMRI male mice, the LD_{50} was 211 mg/kg. In another study, the LD_{50} in both sexes of the Swiss-Webster mouse was found to be 290 mg/kg, when given as a slow infusion over approximately 2.5 minutes (Elizondo et al. 1991). One male and one female beagle dog given a single slow infusion (lasting ~110 seconds) of 160 mg/kg mangafodipir, as well as the one male given 120 mg/kg, died prior to the second day of the experiment; the remaining female given 120 mg/kg was sacrificed due to a moribund condition on day 3 of the experiment (Larsen and Grant 1997). Dogs of both sexes given 83 or 99 mg/kg survived the 14-day observation period. A single slow infusion (lasting 5 minutes) at a dose of 160 mg/kg did not result in lethality in the Sprague-Dawley rat (Larsen and Grant 1997).

Death was not observed in Sprague-Dawley rats administered 9 doses of 16 mg manganese/kg/day as mangafodipir given over 3 weeks (Elizondo et al. 1991; Larsen and Grant 1997). Moribund condition prompted the sacrifice of one male and one female beagle dog on days 12 and 21, respectively, of a 21-day exposure period in which the animals were administered 5.4 mg manganese (as mangafodipir)/kg/day, whereas a lower dose of 1.6 mg/kg/day did not result in death or sacrifice of any treated dogs (Larsen and Grant 1997). Moribund condition also prompted the sacrifice of a single male cynomolgus monkey on day 18 of a mangafodipir-dosing regimen involving 16 mg manganese/kg/day doses also given 3 times/week for 3 weeks (Larsen and Grant 1997). The authors did not indicate the precise cause of lethality in the sacrificed dogs; however, they noted the dogs' livers showed histological signs of cholangiohepatitis, fibroplasia, bile duct proliferation, and hepatocyte necrosis, with cortical tubular necrosis in the kidneys. The sacrificed monkey had a serum chemistry profile indicative of renal failure and associated liver toxicity.

2.2.4.2 Systemic Effects

Respiratory Effects.

No reports were located concerning respiratory effects in humans following dosing with mangafodipir.

A single dose of 160 mg manganese/kg as mangafodipir in Sprague-Dawley rats of both sexes resulted in dyspnea (Larsen and Grant 1997).

Cardiovascular Effects.

Mangafodipir, when administered to humans in timed doses in clinical studies has resulted in transient facial flushing and increased blood pressure at doses as low as 0.2 mg manganese/kg (facial flushing) (Bernardino et al. 1992; Lim et al. 1992; Padovani et al. 1996; Wang et al. 1997).

Slow infusion of mangafodipir at doses of 16.5 mg manganese/kg resulted in no cardiotoxicity in mongrel dogs of either sex (Karlsson et al. 1997). The dogs suffered from medically-induced acute ischaemic heart failure; cardiotoxicity was measured as the depression of cardiovascular function, with specific measured endpoints being aortic pressure, pulmonary artery pressure, right atrial pressure, cardiac output, and heart rate (Karlsson et al. 1997). Sprague-Dawley rats suffered no cardiotoxicity (as measured by histomorphological evaluation) after a single administration of mangafodipir at doses as high as 63 mg/kg (Larsen and Grant 1997).

Rats administered 9 doses (3 times per week for 3 weeks) of 16 mg manganese/kg did not suffer any adverse cardiovascular effects as measured by histomorphological analyses (Larsen and Grant 1997). Twenty-one days of daily administration of 5.4 mg manganese/kg in beagle dogs resulted in reduced heart rate by the end of the treatment (Larsen and Grant 1997). Cynomolgus monkeys administered 16 mg/kg for 3 days/week for 3 weeks resulted in flushing of the face, but no other measured cardiovascular effects (Larsen and Grant 1997).

Gastrointestinal Effects.

Incidences of gastrointestinal effects in humans following injection with mangafodipir have been limited to rare complaints of nausea or vomiting that are short-lived (15 seconds to 5 minutes in length) and not dose- or administration-dependent (bolus vs. infusion) (Bernardino et al. 1992; Lim et al. 1991; Padovani et al. 1996; Wang et al. 1997). A dose of 81 mg manganese/kg as mangafodipir in beagle dogs of both sexes resulted in vomiting, diarrhea, and decreased food consumption (Larsen and Grant 1997).

Vomiting was observed in cynomolgus monkeys of both sexes after administration of 9 doses of 16 mg manganese/kg, given 3 times/week for 3 weeks (Larsen and Grant 1997). No other gastrointestinal effects in animals were reported.

Hematological Effects.

No hematological changes (versus pretreatment values) were noted in 3 different studies that included 13 healthy males (Wang et al. 1997), 54 healthy males (Lim et al. 1991), or 96 human volunteers of both sexes with known or suspected focal liver tumors (Bernardino et al. 1992) administered up to 1.4 mg manganese/kg as mangafodipir (either via bolus or slow infusion).

A single dose of 63 mg manganese/kg as mangafodipir in both sexes of Sprague-Dawley rats resulted in no adverse hematological effects (Larsen and Grant 1997). Intermediate studies of adverse effects were also negative. Doses as high as 16 mg/kg given 3 times/week for 3 weeks to Sprague-Dawley rats (Elizondo et al. 1991; Larsen and Grant 1997) or cynomolgus monkeys, or 5.4 mg/kg in beagle dogs dosed daily for 21 days, failed to induce any adverse hematological effects (Larsen and Grant 1997).

Musculoskeletal Effects.

No reports of musculoskeletal effects in humans or animals following mangafodipir administration were located.

Hepatic Effects.

Blood chemistry analyses revealed no significant changes in liver enzymes in several human volunteers, either with or without tumors, given mangafodipir at doses up to 1.4 mg manganese/kg (Bernardino et al. 1992; Lim et al. 1991; Wang et al. 1997). Three individuals dosed with 0.55 mg manganese/kg, and one dosed with 1.4 mg/kg had increased serum alanine aminotransferase; however, there was no dose response with these incidences and the maximum increase in the enzyme was to 70 International Units (IU)/l (the upper limit of the normal range is 45 IU/l) (Lim et al. 1991).

A single dose of up to 63 mg manganese/kg administered to both sexes of Sprague-Dawley rats did not produce any adverse hepatic effects as observed by histomorphological analyses (Larsen and Grant 1997). The administration of 9 total doses of mangafodipir, 3 per week, at 16 mg manganese/kg/day per dose, resulted in an increased incidence (relative amount unreported) in hepatic microgranulomas in female Sprague-Dawley rats but no effect on liver enzymes as measured by serum chemistry (Elizondo et al. 1991; Larsen and Grant 1997). Twenty-one daily doses of 1.6 mg/kg/day resulted in an increase in serum enzymes (alanine aminotransferase, ornithine carbamyl transferase, glutamine dehydrogenase, alkaline phosphatase, gamma-glutamyl transferase), as well as bilirubin and cholesterol, in both sexes of beagle dogs, while a higher dose of 5.5 mg/kg/day resulted in increased liver enzymes and liver weight and changes in liver pathology (cholangiohepatitis, fibroplasia, bile duct proliferation, and hepatocyte necrosis) (Larsen and Grant 1997). The authors noted that altered serum albumin:globulin ratios and increased prothrombin time were indicative of decreased liver protein synthesis. When dogs at this high dose were allowed a 4 week recovery period, healing of the liver was observed; specific measures of healing were not provided, although resolution of lesions in other affected organs, such as the kidneys, was mentioned. The authors also noted that increased serum levels of liver enzymes, and decreased liver protein synthesis were reversible effects in dogs allowed a recovery period. Doses of 0.54 mg/kg/day did not have any effect on the liver (Larsen and Grant 1997). In both sexes of the cynomolgus monkey, 9 total doses of 16 mg/kg/day given 3 times/week for 3 weeks, resulted in increases in liver enzymes (alanine aminotransferase, gamma-glutamyl transferase), as well as increases in bilirubin and relative liver weights in males, and focal hepatitis/cholangiolitis in one male at the end of the dosing period. When the monkeys were given a 2-week recovery period following a 3-week administration of the highest dose, only 1 male had a liver lesion, which was in the process of healing. Doses of 1.6 mg/kg/day in this primate did not cause any adverse hepatic effects (Larsen and Grant 1997).

Renal Effects.

Administration of mangafodipir at up to 1.4 mg manganese/kg in a few human studies has not resulted in any adverse renal effects as measured by blood chemistry or urinalysis (Bernardino et al. 1992; Wang et al. 1997).

Single doses of mangafodipir up to 63 mg manganese/kg given to Sprague-Dawley rats did not cause renal effects as measured by blood chemistry, urinalysis, gross necropsy, and histopathology (Larsen and Grant 1997). Sprague-Dawley rats of both sexes given 9 doses (thrice weekly for 3 weeks) of 16 mg/kg manganese did not show any adverse renal effects as measured by urinalysis, blood chemistry, and histomorphological analysis (Elizondo et al. 1991; Larsen and Grant 1997). Daily administration of mangafodipir over 21 days in both sexes of the beagle dog at concentrations up to 6 mg/kg resulted in cortical tubular necrosis of the kidneys at this highest dose, as well as decreased glomerular filtration rate, as indicated by high serum carbamide and creatinine levels. There were no measurable effects at 1.6 mg/kg or lower (Larsen and Grant 1997). Administration of 9 doses of mangafodipir, also given thrice weekly for 3 weeks, at individual concentrations of 16 mg manganese/kg to cynomolgus monkeys of both sexes resulted in increased kidney weights and enzymes, as well as creatinine, urea, and other inorganic ions. Doses of 1.6 mg/kg over the same time period did not result in any adverse effect (Larsen and Grant 1997).

Endocrine Effects.

No studies were located regarding endocrine effects in humans or animals following administration of mangafodipir.

Dermal Effects.

No studies were located regarding dermal effects in humans or animals following intravenous administration of mangafodipir.

Ocular Effects.

No studies were located concerning ocular effects in humans following administration of mangafodipir.

Cynomolgus monkeys administered 9 individual doses at 16 mg/kg over 3 weeks and beagle dogs given up to 6 mg/kg daily for 21 days did not have any adverse ocular effects from the mangafodipir treatment (Larsen and Grant 1997).

Body Weight Effects.

No reports were located concerning body weight effects in humans following mangafodipir dosing.

Mice given acute doses of mangafodipir as high as 275 mg manganese/kg and rats administered a dose of 160 mg/kg did not suffer any body weight effects (Larsen and Grant 1997).

Rats (Elizondo et al. 1991; Larsen and Grant 1997) and monkeys (Larsen and Grant 1997) administered 9 doses of mangafodipir over 3 weeks at doses as high as 16 mg manganese/kg did not have any treatment-related effects on body weight. Dogs administered 21 daily doses of the compound suffered decreased body weight (unspecified decrease) at 5.4 mg/kg, but no effect at 1.6 mg/kg (Larsen and Grant 1997). There were no significant treatment-related adverse effects on body weight of male and female rats or female rabbits used in reproductive studies with mangafodipir (Blazak et al. 1996; Grant et al. 1997; Treinen et al. 1995), except for a transient decrease in body weight during weeks 2–5, 9, and 10 in male rats administered 6 mg manganese/kg/day for 85 days (Grant et al. 1997). The authors noted that the decrease was significant when compared to controls, but did not report actual data.

Metabolic Effects.

No studies were located regarding metabolic effects in humans or animals following administration of mangafodipir.

2.2.4.3 Immunological and Lymphoreticular Effects

No studies were located regarding immunological or lymphoreticular effects in humans following exposure to mangafodipir.

Injection of mangafodipir 3 times/week for 3 weeks in Sprague Dawley rats at doses of 1.6, 6.3, or 16 mg manganese/kg resulted in eosinophilia in females only at the highest dose, but had no effect in males. The

authors stated they are unsure of the clinical importance of this effect as it was only seen at repeated high doses (Larsen and Grant 1997). Daily dosing of mangafodipir in beagle dogs of both sexes at doses of 1.6 mg manganese/kg for 21 days resulted in a decrease in eosinophils and an increase in toxic neutrophils (absolute amounts not reported) (Larsen and Grant 1997). A lower dose of 0.54 mg/kg had no immunological effect.

2.2.4.4 Neurological Effects

No statistically significant increases in adverse neurological effects in humans following mangafodipir administration were reported. In one study, 4 subjects given doses ranging from a low of 0.17 to a high of 1.4 mg/kg complained of light-headedness or dizziness (Lim et al. 1992). Five patients out of 96 administered mangafodipir complained of a headache following dosing; only 2 of these 5, given varying doses of mangafodipir ranging from 0.17 to 1.4 mg manganese/kg, could be attributed to the contrast agent (Bernardino et al. 1992). No other neurological effects were reported in human studies.

Single doses of mangafodipir ranging from 8.3 to 275 mg manganese/kg in mice, and a single dose of 160 mg/kg in rats, resulted in decreased activity and abnormal gait and stance (Larsen and Grant 1997). Mongrel dogs infused once with mangafodipir at doses of 0.55, 3.3, or 16.5 mg manganese/kg did not have any treatment-related changes in plasma catecholamines or physiological signs of sympathetic activation as compared to the undosed controls (Karlsson et al. 1997). In a separate study, beagle dogs receiving either single doses ranging from 83 to 160 mg/kg or 21 daily doses at 5.4 mg manganese/kg suffered decreased appetite as measured by decreased food consumption; when the dogs were allowed a recovery period following the repeated dosing, the food consumption normalized within the first 2–3 days (Larsen and Grant 1997).

Rats and monkeys administered 9 doses of up to 16 mg/kg each did not have any observable neurotoxic effects (Larsen and Grant 1997).

Grant et al. (1997) did observe behavioral changes in the pups of Sprague-Dawley dams exposed to 0, 0.6, 1.1, or 2.2 mg manganese/kg on gestation days 6–17. Although no significant effects were observed at the lowest dose, the exposed pups suffered a significant decrease in grasp/holding time and a 10–11% decrease in body weight at postnatal days 4 and 7 at the 1.1 mg/kg dose. At the highest dose, pup weight was significantly decreased at postnatal days 4, 7, 14, and 21; performance on grasp/holding, negative geotaxis, and surface righting tests was also significantly impaired. In addition, postnatal survival was decreased on

days 0–4 (56 vs. 95.9% in the control group) and 4–21 (78.9 vs. 100% in the control group) at the highest dose (Grant et al. 1997).

Current studies do not provide evidence on the potential for neurotoxicity following clinical exposure to mangafodipir. In general, studies on neurological effects in humans or animals following mangafodipir exposure did not involve a long observation period. Because deposition of manganese in the brain can be significantly delayed following exposure, it is possible that the studies to date were terminated prior to the onset of potential neurotoxicity. However, neurotoxicity in humans or animals has not been reported following single exposures to manganese, even at high doses. Studies on toxicokinetics of other manganese compounds also indicate that a single exposure is not likely to result in significant neurological effects. For further information on distribution, refer to Section 2.3 Toxicokinetics.

2.2.4.5 Reproductive Effects

No studies were located regarding reproductive effects in humans following administration of mangafodipir.

A single dose of 160 mg/kg in male Sprague-Dawley rats resulted in no adverse effects in testes as measured by organ weight and histomorphological analysis (Larsen and Grant 1997).

Male Sprague-Dawley rats dosed 9 times in 3 weeks with 16 mg manganese/kg as mangafodipir suffered a decrease in absolute testes weights, but no relative decrease in weight and no histomorphological effects (Larsen and Grant 1997).

Injection of pregnant Sprague-Dawley rats with up to 4.4 mg manganese/kg as mangafodipir, on gestational days 6–8, 9–11, 12–14, or 15–17 (all during organogenesis) resulted in no evidence of reproductive toxicity as measured by pregnancy rate, numbers of corpora lutea, implantations or resorptions (Treinen et al. 1995). Further, daily intravenous administration of doses up to 2.2 mg manganese/kg throughout gestational days 6–17 did not result in any significant changes in pregnancy rate, corpora lutea, implantations, or resorptions (Treinen et al. 1995). However, Grant et al. (1997) observed a >50% rate of post-implantation loss in pregnant Sprague-Dawley rats administered 2.2 mg manganese/kg as mangafodipir during gestation days 6–17. Doses of 0.6 and 1.1 mg/kg resulted in postimplantation loss rates that were similar to that of the control group. There were no obvious differences in compound administration or animal husbandry between the two studies that would indicate why such disparate results would occur.

similar to that of the control group. There were no obvious differences in compound administration or animal husbandry between the two studies that would indicate why such disparate results would occur. Intravenous dosing of New Zealand white rabbits with up to 1.1 mg manganese/kg/day on gestation days 6–17 did not cause reproductive toxicity in one study (Grant et al. 1997), but a dose of 3.3 mg manganese/kg/day during gestation days 6–18 in the same species resulted in a significant increase (3-fold) in post-implantation loss (Blazak et al. 1996). This latter dose corresponds to a 12-fold increase over the one-time human clinical dose (Earls and Bluemke 1999).

Mangafodipir dosing in female Sprague-Dawley rats for 22 total days, starting prior to conception and ending on the 7th day of gestation at a dose of up to 6 mg manganese/kg, did not result in any adverse reproductive effects (Grant et al. 1997).

Male Sprague-Dawley rats dosed for 84–85 days with 0, 0.6, 2, or 6 mg manganese/kg as mangafodipir did not show any signs of reproductive toxicity as measured by histomorphological analyses. Although absolute testes weights in the intermediate dose group were reduced compared to controls, relative weights were not, and in the absence of histopathological findings, this reduction is not considered an adverse effect. The treated rats were bred with females to determine if mangafodipir dosing had any effect on fertility. Pregnancy rates, and the number of corpora lutea, implantations, or resorptions were unaffected by parental treatment (Grant et al. 1997).

2.2.4.6 Developmental Effects

No studies were located regarding developmental effects in humans following intravenous exposure to mangafodipir.

Treinen et al. (1995) tested the sensitivity of different gestational periods to the administration of mangafodipir in Sprague-Dawley rats. Pregnant rats were dosed with 0, 1.1, 2.2, or 4.4 mg manganese/kg on 3 consecutive days: gestation days 6–8, 9–11, 12–14, or 15–17. The 1.1 mg/kg dose given on days 15–17 resulted in a significant increase in skeletal malformations in fetuses (10/113 fetuses vs. 0/106 in the control group; $p<0.05$). A higher dose of 2.2 mg/kg also caused a significant increase in malformations when given on gestational days 12–14 (10 out of 104 fetuses affected) and days 15–17 (21/143) (both $p<0.05$), and the 4.4 mg/kg dose caused increases in malformations when given on days 9–11(5/83), 12–14 (45/128), and 15–17 (98/129) (all $p<0.05$). The malformations seen in this study included angulated or

irregularly shaped clavicle, femur, fibula, humerus, ilium, radius, tibia, ulna, and/or scapula (Treinen et al. 1995).

The offspring of Sprague-Dawley rats dosed with 0, 0.1, 0.3, or 1 mg manganese/kg as mangafodipir daily throughout gestation days 6–17 had a significant increase ($p<0.05$) in abnormal limb flexures (38/270 fetuses affected) and skeletal malformations (141/270 fetuses affected) only at the highest dose (Treinen et al. 1995). These malformations included the same ones listed for the segmented teratology study above. In a separate experiment evaluating the teratology of mangafodipir administration on gestation days 6–17 in pregnant Sprague-Dawley rats, Treinen et al. (1995) observed a significant increase ($p<0.05$) in skeletal malformations in offspring of rats dosed with 2.2 mg manganese/kg (86/92 fetuses affected) compared to controls. In both the segmented and continuous teratology studies no maternal toxicity was observed.

Fetuses from Sprague-Dawley females dosed with 0, 0.6, 1.1, or 2.2 mg manganese/kg on gestation days 6–17 exhibited a statistically significant increase in wavy ribs at 0.6 mg/kg (20.5% of the viable fetuses impacted vs. 0.7% at the control dose; $p<0.05$). At the intermediate dose, there was a statistically significant increase in the number of fetuses with abnormalities (20 out of 159 viable fetuses) including distortion or misshaping of one or more of the following bones: humerus, radius, ulna, scapula, clavicle, femur, tibia, and fibula; in addition, 56.6% of the viable fetuses had wavy ribs and the fetuses weighed 14% less than controls ($p<0.05$). At 2.2 mg/kg, there was a significant decrease in fetal viability (56% decrease; $p<0.05$), a greater increase in fetuses with abnormalities (45 out of 64 viable fetuses,) and a greater percentage (85.9%) with wavy ribs (Grant et al. 1997). These effects were observed in the absence of maternal toxicity. By contrast, when the mangafodipir was administered for 22 days prior to conception and up to gestation day 7 in the same species at doses of 0, 0.6, 2, and 6 mg manganese/kg/day, no adverse effects on the number of viable fetuses, fetal weight, or the number of fetuses with abnormalities were reported (Grant et al. 1997). These teratogenic studies indicate that developmental toxicity resulting from mangafodipir dosing is highly dependent on the time-frame of administration.

Grant et al. (1997) also observed behavioral changes in the offspring of Sprague-Dawley dams administered 0, 0.6, 1.1, or 2.2 mg manganese/kg on gestation days 6–17. The exposed pups suffered a significant decrease in grasp/holding time and a 10–11% decrease in body weight at postnatal days 4 and 7 at the 1.1 mg/kg dose, but no significant effects at the lower dose (Grant et al. 1997). At the highest dose, pup weight was significantly decreased at postnatal days 4, 7, 14, and 21, and performance on grasp/holding, negative geotaxis, and surface righting tests was significantly impaired. In addition, postnatal survival was

decreased on days 0–4 (56 vs. 95.9% in the control group) and 4–21 (78.9 vs. 100% in the control group) at the highest dose (Grant et al. 1997). These effects occurred at doses that did not cause observable maternal toxicity.

Mangafodipir administration in New Zealand white rabbits at doses of 0, 0.3, 0.55, or 1.1 mg manganese/kg on gestation days 6–18 resulted in incomplete ossification of the sternebrae at 1.1 mg/kg in one study (Grant et al. 1997), but no significant effects on fetotoxicity or fetal weight; this dose did not result in any maternal toxicity. In a separate study, mangafodipir at doses as high as 3.3 mg manganese/kg in the same strain of rabbit for the same period of exposure did not result in any significant increases in external, skeletal, or visceral malformations in a separate teratology study (Blazak et al. 1996). This dose did result in an 11% decrease in fetal weight (although this value was not statistically significant in the study, it is considered a significant developmental effect) and a 20% decrease in the number of viable fetuses (also not statistically significant). It is not readily apparent why two studies with similar dosing regimens would obtain such conflicting results. A comparison between rat and rabbit gestational studies indicates that the rabbit is a much less sensitive model for reproductive and developmental toxicity induced by mangafodipir.

2.2.4.7 Genotoxic Effects

There were no studies located regarding genotoxicity in humans or animals following intravenous administration of mangafodipir.

Other genotoxicity studies are discussed in Section 2.5.

2.3 TOXICOKINETICS

Manganese is required by the body and is found at low levels in virtually all diets. Regardless of manganese intake, adult humans generally maintain stable tissue levels of manganese. The basis for this homeostatic mechanism is the regulation of absorption (EPA 1984a) as well as regulation by excretion. Manganese absorption occurs primarily by the oral and inhalation routes, via the gastrointestinal tract after ingestion and via the alveolar lining after inhalation. Some inhaled manganese may also be swallowed with the mucus and, thus, will be absorbed from the gastrointestinal tract.

Manganese is normally found in human tissue, blood, serum, and urine. Oral exposure to manganese can result in manganese increases in all tissues. The major route of manganese excretion is via the bile although some excretion does occur in urine, milk, and sweat (EPA 1993b). Tissue distribution of manganese usually reflects chronic exposure, not acute exposure.

2.3.1 Absorption

2.3.1.1 Inhalation Exposure

Inorganic Manganese

No studies were located regarding the absolute amount of manganese that is absorbed by humans or animals after inhalation exposure to manganese dusts. In general, the extent of inhalation absorption is a function of particle size, because size determines the extent and location of particle deposition in the respiratory tract. Particles that are deposited in the lower airway are probably mainly absorbed, while particles deposited in the upper airways may be moved by mucociliary transport to the throat, where they are swallowed and enter the stomach. This process has been found to account for clearance of a significant fraction of manganese-containing particles initially deposited in the lung (Drown et al. 1986–intratracheal rat study; Mena et al. 1969–oral human study; Newland et al. 1987–inhalation monkey study). Thus manganese may be absorbed both from the lungs and in the gastrointestinal tract following inhalation of manganese dust. However, the relative amounts absorbed from each site are not accurately known.

Tjälve et al. (1996) investigated the uptake of manganese in brain regions of weanling male Sprague-Dawley rats (150 g) following intranasal administration of 4 µg/kg ^{54}Mn. Whole body autoradiography of the rats at different time points revealed that the olfactory bulb contained the vast majority of measured manganese at 1, 3, and 7 days post-dosing (90, 69, and 47%, respectively) with values decreasing to a low of 16% at 12 weeks. The total amount of absorbed label was not reported. Significant uptake of manganese by other brain regions was not observed until the third day, when the basal forebrain, cerebral cortex, hypothalamus, and striatum had 21, 2, 3, and 1% of the measured label, respectively. Maximum uptake of the label by these regions was observed at 7 days post-dosing, with percentage of label in the basal forebrain reaching 28%, and the other organs containing amounts slightly more than double their 3-day values. By contrast, liver and kidneys each contained approximately 1% of the measured manganese at 1, 3, and 7 days, with values decreasing consistently to 12 weeks (Tjälve et al. 1996).

Roels et al. (1997) measured blood manganese levels in 3-month-old rats following intratracheal administration of 1.22 mg manganese/kg as either $MnCl_2$ or MnO_2. Manganese levels in blood following $MnCl_2$ dosing reached a maximal level of 7,050 ng/100mL at 30 minutes post-dosing (first time point measured). By contrast, the maximal blood manganese level of 1,760 ng/100 mL was not reached until 168 hours following MnO_2 administration. The relative uptake and distribution of manganese in the brain regions of 3-month-old rats were studied following administration of the same inorganic manganese compounds (Roels et al. 1997). In this experiment, concentrations of 1.22 mg manganese/kg were administered once weekly for 4 weeks. At the end of the regimen, there was a 68% increase in blood manganese after dosing with $MnCl_2$, but an increase of only 41% following exposure to MnO_2 (a rate of 100% absorption of the dose was assumed). Further, following manganese levels were significantly increased over control levels in the cerebellum, striatum, and cortex, with the striatum containing the highest level of manganese (124% over control levels). Similar results were seen after exposure to MnO_2, with significant increases in manganese levels observed in cerebellum, cortex, and striatum; striatum also contained the highest amount of manganese, but the relative differences between the three brain regions were not as different as with $MnCl_2$. These data indicate that manganese is more readily absorbed via inhalation in a more soluble chemical form. Together, the Tjälve et al. (1996) and Roels et al. (1997) studies indicate that absorption via the olfactory mucosa is a potential contributor to brain manganese deposition in rodents.

Organic Manganese

No studies were located regarding the absorption of organic manganese compounds following inhalation exposure in either humans or animals.

2.3.1.2 Oral Exposure

Inorganic Manganese

The amount of manganese absorbed across the gastrointestinal tract in humans is variable but typically averages about 3–5% (Davidsson et al. 1988, 1989; Mena et al. 1969). Data were not located on the relative absorption fraction for different manganese compounds, but there does not appear to be a marked difference between retention of manganese ingested in food (5% at day 10) or water (2.9% at day 10) (Davidsson et al. 1988, 1989a; Ruoff 1995). In humans, manganese absorption tends to be greater from

$MnCl_2$ (in demineralized water) than from foods (labeled intrinsically or extrinsically with ^{54}Mn); however, the biological half-life of manganese from either $MnCl_2$ or food is the same (EPA 1995b; Johnson et al. 1991). In human adults, supplementation of the diet with $MnSO_4$ for 12–35 weeks at a level approximately 2 times the normal dietary intake caused a 30–50% decrease in absorption of a tracer dose of $^{54}MnCl_2$ (Sandstrom et al. 1990).

Roels et al. (1997) noted that in 3-month-old male rats, gavage administered $MnCl_2$ (24.3 mg manganese/kg) reached a maximal level in blood, 7.05 µg/100 mL, within the first 30 minutes post-dosing (first time point measured), whereas manganese from MnO_2, administered in the same fashion, did not reach a maximal level in blood of 900 ng/100 mL until 144 hours (6 days) post-dosing. Following 4 weekly gavage doses of $MnCl_2$ at 24.3 mg manganese/kg per dose, significant increases in manganese concentration were observed in blood and the cerebral cortex, but not cerebellum or striatum, as compared to controls; for identical doses of MnO_2, manganese levels were significantly increased only in blood. The lack of significant increase in manganese levels in any brain region following administration of the dioxide is likely due to the delayed uptake of manganese in the blood.

One study showed that, in full-term infants, manganese is absorbed from breast milk and cow's milk formulas that were either unsupplemented or supplemented with iron, copper, zinc, and iodine (Dorner et al. 1989). Manganese intake was greater in the formula-fed infants than in the breast-fed infants due to the higher manganese content of the formula. However, breast-fed infants retained more of their daily intake of manganese (40%) than did the formula-fed infants (20%). It must be noted that the full-term infants evaluated in this study were 2–18 weeks old, and the data did not stratify intake and retention amounts by age. Further, the data did not indicate if there were similar proportions of manganese taken up from breast milk as compared to the formulas. A study by Davidson and Lönnerdal (1989) demonstrated the *in vitro* receptor-mediated uptake of manganese from lactoferrin; the authors speculated that this may lead to the absorption of manganese from breast milk in human infants.

There is some evidence to suggest that manganese absorption is age-dependent. Dorner et al. (1989) have shown that infants, especially premature infants, retain a higher proportion of manganese than adults. Animal studies also support this finding. For example, Rehnberg et al. (1980, 1981, 1982) dosed 1 day-old rat pups with up to 214 mg manganese/kg/day (as Mn_3O_4) for up to 224 days, then measured manganese concentrations in tissues. The authors noted that intermediate and chronic exposure of rats to Mn_3O_4 in water or food resulted in much larger increases in tissue levels in young rats (1–15 days in

intermediate studies, 24–40 days in chronic study) than in older rats. These increases in neonates were judged to be due to the neonates' greater absorption of manganese as a result of a slower rate of transport through the gut (Rehnberg et al. 1985). Similar results have been reported in rats exposed to $MnCl_2$ (Kostial et al. 1978). However, such age-dependent differences in tissue retention of manganese could also be due to differences in excretory ability (Cotzias et al. 1976; Miller et al. 1975) or to age-related changes in dietary intake levels of iron and manganese (Ballatori et al. 1987). Dorner et al. (1989) found that both pre-term and full-term infants had active excretion of manganese; in fact, some infants had negative manganese balances. Animal studies show that absorption and/or retention of manganese is higher in neonates, but returns to the level of older animals at approximately post-gestational day 17–18 (Kostial et al. 1978; Lönnerdal et al. 1987; Miller et al. 1975; Rehnberg et al. 1981). Available studies (Dorner et al. 1989) do not provide adequate data to determine when this transition takes place in human infants.

One of the key determinants of absorption appears to be dietary iron intake, with low iron levels leading to increased manganese absorption. Mena et al. (1969) administered oral ^{54}Mn and ^{39}Fe to subjects with iron-deficiency anemia (ranging in age from 13 to 44 years old) and measured Mn and Fe uptake with whole-body autoradiography. The uptake of manganese by anemic subjects was 7.5% while in non-anemic subjects, it was 3.0%. This is probably because both iron and manganese are absorbed by the same transport system in the gut. The activity of this system is inversely regulated by dietary iron and manganese intake levels (Chandra and Tandon 1973; Diez-Ewald et al. 1968; Rehnberg et al. 1982; Thomson et al. 1971). Interaction between iron and manganese occurs only between nonheme iron and manganese. Davis et al. (1992a) demonstrated that increasing dietary intakes of nonheme iron, but not heme iron, depressed biomarkers of manganese status, i.e., serum manganese concentrations and lymphocyte manganese-dependent superoxide dismutase activity.

Studies of oral absorption of manganese in animals have yielded results that are generally similar to those in humans. Manganese uptake in pigs, which have similar gastrointestinal tracts to humans, has been measured using labeled manganese administered orally (Finley et al. 1997). The mean absorption rates for different times post-dosing were 5% 1–6 hours post-dosing, 7% 6–12 hours post-dosing, and 3.8% 12–24 hours post-dosing. Gastrointestinal uptake of $MnCl_2$ in rats has been estimated to be 2.5–8.2% (Davis et al. 1993; Pollack et al. 1965). Uptake is increased by iron deficiency (Pollack et al. 1965) and decreased by preexposure to high dietary levels of manganese (Abrams et al. 1976a; Davis et al 1992b). In a rat study, the intestinal transfer of the calcium ion and manganese ion was found to be competitive, and

the authors suggested that there is a common mechanism for their transfer in the intestines (Dupuis et al. 1992). High dietary intakes of phosphorus (Wedekind et al. 1991) and calcium (Wilgus and Patton 1939) have also been demonstrated to depress manganese uptake in chicks.

Manganese absorption has also been found to vary according to manganese intake; in rats whose diet was manganese deficient, absorption was at least two-fold higher than in rats whose diets contained an adequate amount of manganese (as manganese carbonate) (Davis et al. 1992b).

Two studies in suckling rat pups found differing absorptions of manganese from different milks and formulas. The first study (Lönnerdal et al. 1987) found that the percent of ^{54}Mn (added to the food source as an extrinsic label) retained (measured as whole-body retention) in 14-day-old pups fed breast milk, cow milk, cow milk formula, and soy formula, was 82, 90, 77, and 65%, respectively.

The latter study (Lönnerdal et al. 1994)found that 13-day-old rat pups fed ^{54}Mn (from $MnCl_2$ that was incubated with the food for at least 24 hours prior to feeding) in breast milk, cow milk, and several different manufacturer's cow milk formulas, had similar absorption values. These pups absorbed (measured as whole-body retention) 80% of the label from breast milk, 83% from cow milk, and 63–90% from the cow milk formulas, with the 2 lowest retention values being significantly lower than the others. In this latter study, manganese absorption from soy formulas was significantly lower than the other milks and formulas tested, ranging from 63–72%.

The inherent concentration of manganese in each of these food sources from the first study was 0.01, 0.04, 0.05, and 0.30 µg/mL, respectively. Therefore, when the retention of the label was multiplied by the actual manganese concentration of the food, the total amounts of absorbed manganese were 4, 18, 19, and 96.8 ng/dose fed, respectively. These data indicate that infants fed cow milk formula may retain 5 times more manganese, and infants fed soy formula may retain 25 times more manganese than breast-fed infants. Although the latter results differ significantly from those observed earlier, the researchers report that the similar relative values for manganese absorption were indicative of significant efforts made to optimize both the relative concentrations and the bioavailability of minerals and trace elements in the manufactured formulas.

Organic Manganese

MMT. No studies were located regarding absorption of manganese following oral exposure to MMT in either humans or animals. The available studies (Hanzlik et al. 1980; Hinderer 1979; Hysell et al. 1974; Komura and Sakamoto 1992) indicate absorption is occurring because toxicity is observed following MMT exposure; however, no absorption rates or relative amounts were provided in these studies.

Maneb or mancozeb. No studies were located regarding absorption of manganese in humans following oral exposure to maneb or mancozeb.

Two studies discuss the acute absorption of radiolabeled maneb in rodents. The first study (Brocker and Schlatter 1979) used unfasted adult female rats dosed with [^{54}Mn]maneb at a dose of 4–10 mg/kg. The rats were kept in metabolism cages which allowed the collection of respired air, urine, and feces for several hours post-dosing. The maneb was given alone or in conjunction with different metal compounds. Radioanalysis of excreta and selected tissues revealed that at 72 hours post-dosing, only 4–6% of the radioactivity was retained in the body with the majority of the label located within the liver and kidney. For 2 different chemical preparations of maneb, the recovery of label in feces was 94–96%, with the remainder in the urine. The respired air of two rats contained only 0.24 and 0.60% of the label, respectively. When molar excesses of the chloride salts of zinc, copper, iron, and mercury were added with the maneb, absorption was decreased to 0–5%, with residual levels in the liver reduced from a high value of $4.46 \pm 1.04 \times 10^{-3}$ (as a fraction of the labeled dose/g wet tissue) with maneb alone, to a low of $0.97 \pm 0.5 \times 10^{-3}$ with an 8-fold molar excess of $CuCl_2$.

Rats dosed with 100 mg/kg of [^{14}C] mancozeb for 7 days via gavage were sacrificed 24 hours after the last dose to determine the amount of label retained in the tissues. Analyses on material balance revealed that 0.96% of the label was retained in the carcass, 0.31% in the tissues, with the remainder collected in the feces and urine (Lyman 1971).

2.3.1.3 Dermal Exposure

Inorganic Manganese

The only available human study regarding dermal exposure to manganese discussed a case report of a man burned with a hot acid solution containing 6% manganese. The authors speculated that manganese absorption had occurred across the burn area (Laitung and Mercer 1983) because the man had slightly elevated urinary manganese levels (11–14 mg/L versus 1–8 mg/L). In most cases manganese uptake across intact skin would be expected to be extremely limited, although solutions of metal ions or complexes in the proper solvent (e.g., dimethylsulfoxide) will readily be absorbed through the skin.

Organic Manganese

No studies were located regarding absorption of organic manganese in humans or animals following dermal exposure. However, studies reporting systemic effects in animals following dermal exposure to organic manganese compounds indicate absorption has occurred.

2.3.2 Distribution

Manganese is a normal component of human and animal tissues and fluids. In humans, most tissue concentrations range between 0.1 and 1 µg manganese/g wet weight (Sumino et al. 1975; Tipton and Cook 1963), with the highest levels in the liver, pancreas, and kidney and the lowest levels in bone and fat (see Table 2-3). Manganese levels in the blood, urine, and serum of healthy, unexposed subjects living in the Lombardy region of northern Italy were 8.8±0.2 µg/L, 1.02±0.05 µg/L, and 0.6±0.014 µg/L, respectively (Minoia et al. 1990). Serum manganese concentrations in healthy males and females in Wisconsin were 1.06 µg/L and 0.86 µg/L, respectively (Davis and Greger 1992; Greger et al. 1990). Although precise inhalation exposure data were not available for humans, chronic occupational exposure studies have shown that higher levels of inhalation exposure generally correspond with higher blood or urine manganese levels for groups but that individual measurements may not correspond to individual exposure or be reliable exposure predictors (Abel-Hamid et al. 1990; Alessio et al. 1989; Jarvisalo et al. 1992; Roels et al. 1992; Siquiera et al. 1991).

Studies investigating manganese levels in human fetal tissues or fluids are very few. Widdowson et al. (1972) measured manganese in fetal livers from 29 unborn infants (ranging in gestational age from 20–41 weeks) and from 5 adults. The fetal manganese levels ranged from 0.09–0.23 mg/100g wet weight with a mean of 0.14 mg/100g wet weight, while the mean of the 5 adults was 0.18 mg/100g wet weight (range of values not reported). The highest fetal manganese value of 0.23 mg/100g wet weight was from 1 of the 2 infants at 41 gestational weeks of age when analyzed. The data indicate that fetal liver manganese levels throughout the latter half of gestation are comparable to those in the adult.

Concentrations of manganese also have been measured in the blood of pregnant women, as well as in the plasma of cord blood of preterm and full-term infants (Wilson et al. 1991). Manganese concentrations in full-term (37–42 weeks gestation) infants were 5.5+/-1.5 µg/L, slightly higher than the preterm (27–36 weeks gestation) infants' values of 5.0+/-1.1 µg/L, but the difference was not statistically significant. There were no correlations between the levels in infants and mothers. The higher manganese levels in cord blood of gestationally older infants, along with the higher manganese level in the oldest fetus from the Widdowson et al. (1972) study, suggest that manganese levels may rise slightly as the fetus approaches birth; however, there are inadequate data points to make a strong argument for this possibility. Serum manganese values of 180 healthy Venezuelan infants decreased consistently from a high value of 0.45 µg/L (mean of 22 infants) at 5 days of age to a low value of 0.29 µg/L (mean of 40 infants) at 12 months of age (Alarcón et al. 1996). The level of manganese at 12 months was the only measurement that was statistically different than the 5-day value. The values were not statistically different between the sexes. Rükgauer et al. (1997) obtained very different results in their analyses of serum manganese levels in German children, adolescents, and adults. The authors evaluated 137 children (aged 1 month to 18 years); the mean serum manganese level for all children was 1.4 µg/L (range 0.17–2.92 µg/L). When the children were separated by age, the serum manganese values were found to decrease from a mean value of 2.12 µg/L (age 0–1 year) to a minimum of 0.98 µg/L (age 14–18 yr). Adults (age 22–75 years) had a mean value of 0.79 µg/L. These data indicate that children had much higher manganese levels in serum than those levels shown by the other studies. It is unknown why this latter study indicates results that are vastly different from those reported in the earlier studies. Rükgauer et al. (1997) took precautions to prevent manganese contamination of their experimental materials during sampling and analysis. Also, the authors reported that the subjects were healthy and were not suffering from nutritional diseases or metabolic disorders and were not taking medicines containing trace elements. However, the children and adolescent subjects were chosen from a pediatric hospital after seeking medical attention on non-nutrition related matters. Therefore, this population may not be a representative sample of the general population. Animal

studies, by contrast, suggest that distribution of manganese in the infant and young child may be very different from the adult.

Levels in tissues from animals fed a normal diet are generally similar but, perhaps are slightly higher than those in humans (Fore and Morton 1952; Rehnberg et al. 1982). Levels of manganese in the milk of rats fed a normal diet averaged 0.054 µg/g (Miller et al. 1975). Data on changes in tissue levels following acute exposures to excess manganese are presented in exposure-specific subsections later in this chapter.

Manganese is also found in breast milk for the continuing metabolic nutrition of the infant. One study reported manganese concentrations from 82 normal, healthy French women of 12+/-5.6 µg/L at postpartum day 2 in human colostrum decreasing to 3.4+/-1.6 µg/L at postpartum day 6 in breast milk (Arnaud and Favier 1995). Another study reported an average manganese concentration in breast milk of 6.2 µg/L using 2,339 samples from mothers of 20 full-term and 6 preterm infants (Dorner et al. 1989). Collipp et al. (1983) have reported concentrations of manganese in breast milk of 10 µg/L. These reports, however, did not address the dietary manganese intake of the nursing mothers. It is unknown whether mothers exposed to increased concentrations of manganese have higher-than-usual levels of the metal in breast milk.

Manganese is distributed throughout all cells in the body; therefore, it is present in germ cells. However, existing studies in humans and animals are not sufficient to predict if distribution of excess manganese into germ cells might result in heritable genetic changes. Manganese is constantly present in human tissues and, therefore, is able to enter germ cells. One human study involving inhalation exposure to nickel and manganese observed chromosomal aberrations in welders working with these metals (Elias et al. 1989). However, the presence of nickel is a confounding factor, as it is known for causing chromosomal changes. Studies in animals are equivocal; there are not enough data to make predictions as to the likelihood for excess exposures of manganese to cause heritable genetic changes.

Concentrations of manganese in select human and animal tissues are presented in Table 2-6 and concentrations of manganese in plasma and serum in infants of differing ages and adults are presented in Table 2-7.

Table 2-6. Manganese Levels in Human and Animal Tissues

	Tissue concentrations (µg Mn/g wet weight)			
	Humans		Rats	Rabbits
Tissue	Tipton and Cook (1963)	Sumino et al. (1975)	Rehnberg et al. (1982)	Fore and Morton (1952)
Liver	1.68	1.2	2.6–2.9	2.1
Pancreas	1.21	0.77	No data	1.6
Adrenals	0.20	0.69	2.9	0.67
Kidney	0.93	0.56	0.9–1.0	1.2
Brain	0.34	0.30[a]	0.4	0.36
Lung	0.34	0.22	No data	0.01
Heart	0.23	0.21	No data	0.28
Testes	0.19	0.20	0.4	0.36
Ovary	0.19	0.19	No data	0.60
Muscle	0.09	0.09	No data	0.13
Spleen	0.22	0.08	0.3	0.22
Fat	No data	0.07	No data	No data
Bone (rib)	No data	0.06	No data	No data
Pituitary	No data	No data	0.5	2.4

[a]Average of cerebrum and cerebellum

Table 2-7. Manganese Levels in Human Serum/Plasma

Age	Concentration (µg/L) (mean±2SD)	
	Serum	Plasma
5 days[a]	0.45±0.12 (22)[c]	
1 month	0.41±0.11 (20)	
3 months	0.39±0.13 (22)	
5 months	0.39±0.10 (14)	
7 months	0.38±0.09 (20)	
10 months	0.37±0.11 (20)	
11 months	0.36±0.12 (22)	
12 months	0.29±0.10 (40)	
1 months–18 years[b]	1.4±1.25	
22–75 years		0.79±0.63

[a]Data from infants 5 days–12 months in age are from Alarcon et al. 1996. Data are from mixed-sex groups. No statistically significant differences in manganese concentrations were found between sexes.
[b]Data from Rukgauer et al. 1997.
[c]Value in parentheses is number of subjects.

SD = standard deviation

2.3.2.1 Inhalation Exposure

Inorganic Manganese

Following inhalation exposure of mice to manganese dust, for a short period of time the concentration of manganese in the lung is approximately proportional to the concentration of manganese in the air(Adkins et al. 1980c). However, as noted earlier, some of the particles that are deposited in the lung are transported to the gastrointestinal tract (Mena et al. 1969). The rate of particle transport from the lungs has not been quantified in humans, but half-times in animals range from 3 hours to 1 day (Adkins et al. 1980c; Bergstrom 1977; Newland et al. 1987).

The relative increases in tissue levels of manganese following inhalation exposure have not been thoroughly investigated. Increases of 20–60% in manganese levels in the kidney and spleen were noted in mice 24–48 hours after exposure to MnO_2 (Adkins et al. 1980c). Rats exposed to an aerosol containing 0.0003 mg $^{54}Mn/m^3$ for 1 hour had manganese levels in the liver, lung, kidney, and brain of 0.0495, 0.1366, 0.0141, and 0.0014 ng ^{54}Mn/organ, respectively, 5 days after exposure (Wieczorck and Oberdorster 1989b). Sheep exposed to welding fumes for 3 hours exhibited a 40-fold increase in lung manganese content (Naslund et al. 1990). Preferential accumulation of manganese in specific locations of the brain (including the caudate nucleus, globus pallidus, and the substantia nigra) was noted in 1 monkey exposed to an aerosol of $MnCl_2$ (20–40 mg/m^3) several hours/day for 3–5 months (Newland et al. 1989). This preferential uptake could play a role in the characteristic neurological effects of manganese (see Section 2.4).

Roels et al. (1997) investigated the distributional differences in rats exposed to manganese in two forms ($MnCl_2$ and MnO_2) administered via intratracheal injection (which mimics inhalation), by gavage (oral administration), and via intraperitoneal injection. When administered intratracheally once a week for 4 weeks, 1.22 mg manganese/kg as $MnCl_2$ resulted in a 68% steady-state increase in blood manganese concentration after the dosing period. This dose also resulted in significantly increased concentrations of manganese in the rat cerebellum (27% increase that approached statistical significance), striatum (205% increase), and cortex (48% increase), as compared to control rats.

When rats were administered the same amount of manganese under the same dosing regimen, with manganese in the form of MnO_2, similar, but less striking, results were observed (Roels et al. 1997).

Manganese concentrations in the blood were increased by 41%, and in the cerebellum, striatum, and cortex by 31, 48, and 34%, respectively, over the control rats.

Tjälve et al. (1996) investigated the distribution of manganese in brain tissues, liver, and kidneys of young male rats following intranasal injection of $^{54}MnCl_2$. Radiography data indicated that 1 day after dosing, the olfactory bulb contained 90% of the manganese (measured as µg/100g wet weight) in the measured tissues, while the basal forebrain contained 6% of the manganese. Concentrations of manganese in the basal forebrain increased to 21 and 28% of the measured total at 3 and 7 days post-dosing, respectively. Manganese in the cerebral cortex, hypothalamus, striatum, and hippocampus were also maximal at 7 days post-dosing. Manganese values in liver and kidneys were approximately 1% of the total measured for the first 7 days, and then decreased steadily until 12 weeks. These results were compared to distribution of manganese following i.p. injection, in which no brain region showed preferential distribution at 1, 7, or 21 days post-dosing (Tjälve et al. 1996). In another study,Gianutsos et al. (1997) found a dose-dependent accumulation of manganese in the olfactory bulb and tubercle following intranasal injection of $MnCl_2$ into one nostril. Injection of 200 µg manganese resulted in maximally elevated levels in the olfactory bulb (400% higher than the uninjected side) with levels in the tubercle half that in the bulb within 12 hours post-exposure; these levels remained elevated for 3 days. Two injections of 200 µg manganese doubled the level of manganese in the striatum compared to saline-injected controls; single doses did not increase tissue manganese levels. No other brain regions were noted and blood manganese levels were not changed with any treatment. These data indicate that the olfactory mucosa is an important pathway for distribution of manganese into the brain.

Vitarella et al. (2000) exposed adult rats to airborne doses of particulate manganese, as manganese phosphate, at 0, 0.03, 0.3, 3 mg manganese/m^3. The particles had a mean diameter of 1.5µm. Exposures lasted for 6 hours/day for either 5 days/week (10 exposures) or 7 days/week (14 exposures). The following tissues were analyzed for manganese content using neutron activation analysis: plasma, erythrocytes, olfactory bulb, striatum, cerebellum, lung, liver, femur, and skeletal muscle. Increased manganese concentrations were reported in olfactory bulb, lung, femur, and skeletal muscle following exposure to 3 mg/m^3 (after either dosing regiment); a lower dose of 0.3 mg/m^3 resulted in increased manganese concentrations in olfactory bulb, and lung (14 dose regimen only). Striatal manganese levels were increased at the two highest doses only after 14 days of exposure. However, concentrations in the cerebellum were similarly elevated, which was interpreted by the authors to indicate that accumulation of manganese was not selective for the striatum. Red blood cell and plasma manganese levels were increased only in rats exposed

to the highest dose for the 10-day exposure period. These data indicate that even at lower doses manganese can accumulate in the olfactory bulb. However, it must be noted that tissues were collected and analyzed at the cessation of exposure. It is not clear if the distribution of manganese would be similar if tissues had been analyzed at a later time.

Organic Manganese

MMT. No studies were located regarding distribution of manganese in human or animals following inhalation exposure of MMT.

Maneb or mancozeb. Fifty pesticide sprayers with potential inhalation exposure to maneb for at least 6 months showed neurological symptoms even though their blood manganese levels (7.7±3.1 ng/100mL) did not differ statistically from the unexposed control group (8.8±7.0 ng/100 mL) (Ferraz et al. 1988).

No studies were located regarding distribution of manganese in animals following inhalation exposure to maneb or mancozeb.

2.3.2.2 Oral Exposure

Inorganic Manganese

Excess manganese uptake has occurred in humans following oral exposure, presumably via the diet, when the individuals suffered from chronic liver disease or some other liver dysfunction (cirrhosis, portacaval shunt, etc.). In these instances, excess manganese was shown to accumulate in certain regions of the brain, as determined by T1-weighted MRI or neutron activation analysis (Devenyi et al. 1994; Fell et al. 1996; Hauser et al. 1994, 1996; Pomier-Layrargues et al. 1998; Rose et al. 1999; Spahr et al. 1996). These studies show that manganese preferentially accumulates in the basal ganglia, especially the globus pallidus, and the substantia nigra.

Rats given a single oral dose of 416 mg manganese/kg (body weight)/day (as $MnCl_2*4H_2O$) exhibited little tissue accumulation of manganese 14 days later (Holbrook et al. 1975). Studies in animals indicate that prolonged oral exposure to manganese compounds results in increased manganese levels in all tissues, but that the magnitude of the increase diminishes over time (Kristensson et al. 1986; Rehnberg et al. 1980,

1981, 1982). Table 2-5 provides illustrative data based on rats exposed to 214 mg manganese/kg(body weight)/day (as Mn_3O_4) for up to 224 days. As the data reveal, large increases in tissue levels of manganese compared to the controls occurred in all tissues over the first 24 days, but levels tended to decrease toward the control levels as exposure was continued. This pattern is thought to be due to a homeostatic mechanism that leads to decreased absorption and/or increased excretion of manganese when manganese intake levels are high (Abrams et al. 1976a; Ballatori et al. 1987; Mena et al. 1967). Davis et al. (1992b) and Malecki et al. (1996b) demonstrated that rats fed elevated levels of manganese for several weeks had increased tissue manganese concentrations, despite increased gut endogenous losses of manganese, as biliary manganese. This reflected several factors. Although the percentage of manganese absorbed decreased, the total amount of manganese absorbed increased when higher levels of manganese were fed. Moreover, although the total amount of manganese lost in bile increased when manganese intake increased, the percentage of manganese intake lost in bile remained constant at ~1% of manganese intake (Malecki et al. 1996b). Table 2-8 contains a summary of manganese levels measured in rat tissue.

A study measuring the retention of a single oral dose of radiolabeled manganese in adult and neonatal rats indicated that retention of the label 6 days after exposure was much greater in pups (67%) than in adults (0.18%); the addition of manganese to the animals' drinking water decreased radiolabel retention in pups and adults (Kostial et al. 1989).

The distributional differences in rats exposed to either $MnCl_2$ or MnO_2 by gavage was investigated by Roels et al. (1997). After administration of 24.3 mg manganese/kg as $MnCl_2$ once weekly for 4 weeks, the authors analyzed blood and brain concentrations of the metal. Manganese concentrations were significantly elevated in the blood (approximately 83% increase over controls) and the cortex of the brain (approximately 39% increase over controls). Gavage administration of MnO_2, by contrast, did not significantly increase the amount of manganese in blood or any section of the brain. In addition, administration of manganese as $MnCl_2$ by gavage caused roughly the same amount of increased manganese in the blood as intratracheal administration of manganese in the same form; it did not cause as significant an increase of manganese in the cortex (Roels et al. 1997). These data indicate that inhalation exposure to manganese in the form of $MnCl_2$ or MnO_2 causes accumulation of manganese in the brain more readily than oral exposure.

Acute manganese exposure in drinking water was found to alter brain regional manganese levels in neonatal rats; after 5 days of exposure, the highest level was in the striatum (12.05 µg/g wet weight) and

Table 2-8. Manganese Levels in Rat Tissue After Oral Exposure[a]

Tissue	Tissue concentration (% control)[b]		
	24 days	60 days	224 days
Liver	810	137	138
Kidney	430	102	128
Brain	540	175	125
Testes	260	125	100

[a]Adapted from Rehnberg et al. (1980)

[b]Values presented are the ratio (expressed as a percentage) of tissue levels of manganese in animals receiving 3,550 ppm manganese in the diet (as Mn_3O_4) compared to animals receiving a normal diet (50 ppm).

the lowest level was in the cerebral cortex (0.85 µg/g wet weight) (Chan et al. 1992). After 10 days, the highest concentrations were in the pons and medulla and the lowest were in the hypothalamus. Regional manganese differences were less pronounced in weanling and adult rats. A study by Lai et al. (1991) confirms that intermediate exposure to manganese in drinking water increases brain manganese concentrations; rats exposed from conception to 120 days at 0.04 or 0.4 mg manganese/kg/day had mean brain manganese levels of 0.36–0.72 µg/g in the low-dose animals and 0.62–1.35 µg/g in the high-dose animals, compared to 0.21–0.38 µg/g in controls.

In a dietary study, elevated manganese levels were found in the organs of male mice fed $MnCl_2$, manganese acetate, $MnCO_3$, or MnO_2 at 284 mg manganese/kg/day for 100 days; levels of manganese in the liver and kidney were significantly higher in the animals exposed to manganese acetate or $MnCO_3$ than in those exposed to $MnCl_2$ or MnO_2 (Komura and Sakamoto 1991). In a 1993 NTP study, mice and rats chronically fed $MnSO_4$ generally exhibited elevated tissue levels of manganese; the manganese levels in the liver and kidney were higher than the levels in the brain.

Organic Manganese

MMT. No studies were located concerning disposition of manganese in humans or animals following oral exposure to MMT.

Maneb or mancozeb. No studies were located regarding disposition of manganese in humans with potential oral exposure to maneb or mancozeb.

Rats administered radiolabeled maneb via gavage for 7 days at a daily dose of 100 mg/kg had 0.31% of the label in organ and tissue samples, 0.96% of the label retained in the carcass, and 0.18% originating from intestinal washings as measured 1 day after the last dose. When tissue concentrations were reported in relative amounts of the label as maneb, the distribution of the compound in the tissues were as follows: thyroid, 865 mg/kg; kidney, 51.6 mg/kg; liver, 24.8 mg/kg; spleen, 10.7 mg/kg; heart, 8.2 mg//kg; fat, 7.4 mg/kg; muscle, 6.8 mg/kg; and brain, 2.2 mg/kg (Lyman 1971).

Mice that ate chow containing maneb for 12 months at a dose of 24 mg manganese/kg did not show increases in manganese concentrations (as compared to the controls who ate regular chow) in the brain, thyroid, spleen, and bone (Komura and Sakamoto 1992b). The maneb-exposed mice had increased

concentrations of manganese in the following organs: kidney, 12.5 vs. 5 µg manganese/g wet weight; liver, 10.5 vs. 4 µg/g; sublingual gland, 7 vs. 2 µg/g; pancreas, 4 vs. 3 µg/g; prostate gland, 7 vs. 2 µg/g; lung, 4 vs. 1 µg/g; muscle, 1.5 vs. 0.5 µg/g (all were statistically significant at the $p<0.01$ level, except the prostate which was $p<0.05$).

No other studies on disposition of maneb or mancozeb in animals were located.

2.3.2.3 Dermal Exposure

Inorganic Manganese

No studies were located regarding tissue distribution of manganese in humans or animals after dermal exposure to inorganic manganese.

Organic Manganese

No studies were located regarding tissue distribution of manganese in humans or animals after dermal exposure to organic manganese.

2.3.2.4 Other Routes of Exposure

Inorganic Manganese

No studies were located regarding tissue distribution of inorganic manganese in humans after exposure via other routes of exposure.

A number of studies have been conducted that investigated various facets of the distribution of inorganic manganese in animal models. The studies utilized a number of routes of administration, and the results suggested that route may play an important role in distribution. In an intraperitoneal study performed in monkeys, manganese was reported in all tissues studied. The highest levels were found in the pancreas, liver, and kidney, and the lowest levels were found in the blood; levels in the central nervous system were found to decrease more slowly than those in other tissues (Dastur 1971). Calves injected intravenously with ^{54}Mn were found to have 3-fold higher liver manganese concentrations and 13-fold higher pancreatic

manganese concentrations than calves fed manganese (Carter et al. 1974). Davis et al. (1993) observed that rats injected intraportally with free ^{54}Mn or ^{54}Mn complexed with transferrin and rats injected intraperitoneally with free ^{54}Mn accumulated more manganese in the pancreatic tissue and less in the liver than those rats that were either fed ^{54}Mn or injected intravenously in the portal vein with an albumin-^{54}Mn complex. The similarity in the distribution of the injected manganese-albumin complex and the free manganese in the diet when compared to the distribution of manganese when it was administered by other routes or complexed with other proteins suggests that the route of administration and type of complexed protein may cause differences in the transport of manganese in the sera.

Roels et al. (1997) studied the effect of intraperitoneal administration of $MnCl_2$ and MnO_2 on distributional differences of manganese in rats. Doses of 1.22 mg manganese/kg as $MnCl_2$ given once per week for 4 weeks resulted in significant increases (when compared to controls) in blood (approximately 60%), striatum (34%) and cortex (36%) concentrations of manganese; no changes were observed in the cerebellum. Identical dosing of rats with MnO_2 resulted in significant increases in manganese levels in blood (79%), cerebellum (40%), striatum (124%), and cortex (67%) over those in controls. These data indicate that administration of MnO_2 by this route resulted in greater accumulation of manganese in the brain than did $MnCl_2$.

The distribution of manganese in the brain was investigated using Cebus (Newland and Weiss 1992; Newland et al. 1989) and Macaque (Newland et al. 1989) monkeys given intravenous injections of $MnCl_2$ that reached a cumulative dose of 10–40 mg manganese/kg. Magnetic resonance images indicated a darkening of the globus pallidus and substantia nigra consistent with an accumulation of manganese in these areas (Newland and Weiss 1992; Newland et al. 1989). Substantial accumulation of manganese was also noted in the pituitary at low cumulative doses (Newland et al 1989). London et al. (1989) reported a rapid localization of manganese in the choroid plexus observed on MRI; similarly, radiotracer studies of manganese injected into the intracerebroventricular space revealed that radiolabeled manganese was located in the choroid plexus within 1 hour and was located in the rat dentate gyrus and CA3 of the hippocampus 3 days post-dosing (Takeda et al. 1994).

Organic Manganese

MMT. No studies were located regarding disposition of MMT in humans following other routes of exposure.

Young adult male rats were administered MMT dissolved in propylene glycol via s.c. injection at a dose of 1 mg manganese/kg (McGinley et al. 1987). Control rats received vehicle alone. The rats were sacrificed 1.5, 3, 6, 12, 24, 48, or 96 hours post-injection. Levels of manganese in the control animals were measured in the blood (0.09±0.01 mg/kg), lung (1.51±0.22 mg/kg), liver (2.49±0.36 mg/kg), kidney (1.29±0.23 mg/kg), and brain (0.45±0.01 mg/kg). These values were assumed by the authors to originate from the feed given to the rats and were subtracted from similar values analyzed for MMT-treated rats to determine the amount of manganese in these tissues and fluids that originated from MMT. Maximum accumulation of MMT-derived manganese was measured 3 hours after dosing and was found primarily in the following 4 tissues: lung (~9 mg/kg); kidney (3.9 mg/kg); liver (2.75 mg/kg); and blood (~0.75 mg/kg). Concentrations of manganese in these 4 tissues was still elevated (~1 mg/kg) at 96 hours post-dosing. Brain manganese concentrations were not significantly elevated over control levels in MMT-treated animals (McGinley et al. 1987).

Gianutsos et al. (1985) administered 0, 11, or 22 mg manganese/kg as MMT (dissolved in propylene glycol) to male adult mice via s.c. injection to determine distribution of manganese. Control mice received vehicle alone. Mice were sacrificed at different time points after dosing. The experiment was divided into an acute study (one dose) or a "chronic study" (ten doses). The brain manganese level 24 hours after the single dose of MMT at 11 mg/kg was 0.93±0.07 µg/g; the value after 22 mg/kg was 1.35±.09 µg/g. Both values were significantly different from the control value of 0.61±0.08. The brain manganese level in the mice administered 10 doses of 11 mg/kg each was 1.37±0.27 µg/g; after 10 doses of 22 mg/kg the value was 3.33±0.15 µg/g; both were significantly greater than the control value of 0.64±0.06 µg/g, and were significantly different than the levels reported after the acute exposure. Manganese levels in the brains of mice given a single dose of MMT at 22 mg manganese/kg were compared with those following injection of the same manganese dose as $MnCl_2$; mice were sacrificed at different time points from 1–24 hours post-dosing. The brain manganese levels following MMT exposure increased from a low at 1 hour to a maximum at 24 hours of ~1.4 µg/g wet weight. The manganese level in brain after $MnCl_2$ exposure followed the same increasing trend over the 24 hour analysis period, but was higher at each time point, with a maximum value of >2.0 µg/g wet weight (Gianutsos et al. 1985).

Mangafodipir. Clinical studies involving cancer patients or healthy volunteers have analyzed the usefulness of mangafodipir as a contrast agent for the identification of certain abdominal tumors. Although these studies do not necessarily quantify the amount of manganese, or mangafodipir, in particular tissues, they are useful tools in identifying the location of the metal; also relative proportions of

manganese among two or more tissues that contain the metal can be observed by differences in signal from these imaging studies.

Several studies have shown the qualitative presence of manganese in the liver due to increased signal in that organ following mangafodipir administration of 0.17–0.83 mg manganese/kg upon T1-weighted MRI (Bernardino et al. 1992; Lim et al. 1992; Padovani et al. 1996; Wang et al. 1997). Two studies show that the human liver takes up more of the manganese from mangafodipir than any other organ: the signal from the liver was roughly 2 times the amount from the spleen after dosing with 0.55 mg manganese/kg (Lim et al. 1992); the liver signal after dosing with 0.55 mg manganese/kg had reached a 100% increase over baseline signal by 20 minutes following post-dosing, whereas the maximal signal from other organs was only 80% in the pancreas, ~30% in the spleen, ~90% in the renal medulla, and 50% in the choroid plexus, all at the same dose. The renal cortex was the only other tissue to reach a 100% increase over baseline signal at 0.55 mg manganese/kg. Dosing with 0.25 mg manganese/kg (the clinically used dose for current MRI testing of patients) resulted in a similar distribution pattern, although the signal was decreased compared to the higher dose. The signal from the renal cortex at the lower dose had a maximum of 80% over baseline, whereas the signal in the liver at this dose was ~75% of the baseline value (Wang et al. 1997).

Several studies have determined the distribution of manganese in tissues of animals following i.v. administration of mangafodipir. Grant et al. (1999) reported that in rats injected with 2 times the clinical dose of [^{54}Mn] mangafodipir (0.55 mg manganese/kg) the carcass retained 8% of the label, and the tissues retained 7% of the label; individual tissue concentrations of manganese were not reported.

Gallez et al. (1997) injected adult male mice once with 0.25 mg manganese/kg as [^{54}Mn] mangafodipir (clinical dose) and determined the tissue manganese content at time points ranging from 15 minutes to 3 months post-dosing. Brain concentration of ^{54}Mn did not reach a maximum value of 0.26±0.04 (value is the percent of injected dose/g tissue) until 24 hours post-dosing; this value was not different than the brain manganese content of mice injected with $MnCl_2$. This maximum value was still observed in the brain 2 weeks post-dosing, but measurements taken at 1 and 3 months post-dosing were below the detection limit. By contrast, manganese from $MnCl_2$ was still detectable, although not at maximal levels, at 3 months' time. Liver manganese reached a maximum value of 7.5±1.4 (% dose/g tissue) 15 minutes post-dosing and then decreased to below the detection limit 1 month later.

Male and female Sprague-Dawley rats injected with [^{54}Mn] mangafodipir at a dose of 5.5 mg manganese/kg had the following distribution of labeled manganese 30 minutes post-dosing (values are given in percent injected dose/g tissue): liver, 1.3; kidney, 1.2; heart, 0.25; spleen, 0.2; blood, 0.3; small bowel, 1.3; large bowel, 0.5; muscle, 0.1; and brain, negligible. Distribution of manganese in tissues of rats injected with labeled $MnCl_2$ was compared to the previous results, and for all tissues, the label was greater after administration with the chloride than from the mangafodipir, with the exception of kidney and large bowel, but these differences were not significant (Elizondo et al. 1991).

The distribution of label in male and female Sprague-Dawley rats injected with either [^{54}Mn] or [^{14}C] mangafodipir at a dose of 0.39 or 0.55 mg manganese/kg, respectively, was studied by Hustvedt et al. (1997). The plasma concentration of labeled manganese reached a peak of 10.2 µg/mL at 5 minutes post-dosing and was quickly distributed into the following organs (values given as µg equivalents of compound/g): pancreas, 10.2; liver, 4.0; kidneys, 3.6; testes/ovaries, 1.7; spleen, 1.0; heart, 0.9; and brain, 0.69. When the bile duct was cannulated, the distribution of an equivalent dose of mangafodipir showed an increased retention of labeled manganese in all organs but the brain (0.62): pancreas, 17.2; liver, 12.3; kidneys, 10.1; testes/ovaries, 5.6; small intestine, large intestine and heart, 2.1; and spleen, 1.9. By contrast, tissue retention of ^{14}C from radiolabeled mangafodipir was very low: pancreas, 0.016; liver, 0.045; kidneys, 0.067; testes/ovaries, 0.015; spleen, 0.023; small intestine, 0.012; large intestine, 0.019; heart, 0.017; and brain, 0.009. These data indicate that manganese dissociates from the fodipir moiety after mangafodipir administration and partitions into the tissues listed above.

The tissue distribution of normal and bile-cannulated dogs following administration of [^{54}Mn] or [^{14}C] mangafodipir was also studied (Hustvedt et al. 1997). Doses of 0.55 manganese/kg were used except for the normal dogs when the manganese was labeled; the dose in this case was 0.38 mg/kg. The general pattern of distribution of manganese and carbon was similar to that seen with rats, except the concentrations were increased in the dog. The values for normal dogs were taken 168 hours post-dosing for both forms of labeled mangafodipir; the bile-cannulated dogs were analyzed 24 hours post-dosing. The maximum concentration of ^{54}Mn in the plasma following dosing was 13.1 µg/mL at the end of the infusion period. The plasma concentrations declined rapidly with a terminal half-life of approximately 15 minutes. In the normal dog and bile-cannulated dog, the tissue distribution was as follows (the values for the bile-cannulated dog are given in parentheses; all values are in µg equivalents of compound/g): liver, 8.7 (79.8); pancreas, 8.1 (2.5); kidneys, 6.6 (37.5); bile, 5.9 (no sample); testes/ovaries, 2.2 (3.2); brain, 0.79 (1.1); spleen, 0.65 (26.6); and heart, 0.62 (3.1). The distribution of labeled carbon in normal (or bile-cannulated

dogs) was the following: kidneys, 0.79 (4.1); liver, 0.13 (0.48); bile, 0.059 (no sample); testes/ovaries, 0.05 (0.079); pancreas, 0.015 (0.11); heart, 0.015 (0.035); spleen, 0.007 (0.15); and brain, not detected (not detected). These data indicate that in the dog, as in the rat, the manganese cation is retained by the tissues, but the fodipir moiety is not.

Distribution of ^{54}Mn and ^{14}C following mangafodipir administration was also studied in the pregnant rat (Hustvedt et al. 1997). Whole body autoradiography of a section of the rat made at different time points revealed that the kidney had retained the highest amount of labeled manganese; later time points showed a distribution similar to those seen in the rat and dog studies mentioned previously with the pancreas and liver causing the most intense signal upon autoradiography. By 24 hours, fetal livers and bones were clearly seen, but placental radioactivity had decreased substantially. Fat deposits also contained a significant amount of the radioactivity at 24 hours. By contrast, radioactivity from labeled carbon in the mangafodipir was relatively uniformly distributed throughout the pregnant rat at 5 minutes and 1 hour post-dosing, with the highest levels in the kidneys. At 24 hours, virtually all tissues were indistinguishable from background.

The human distribution studies have involved much shorter observation times than the animal studies, with maximal increase in MRI signal in human studies observed in minutes following administration. These studies have shown the liver to accumulate the highest amount of manganese from the administered dose of mangafodipir. This is an important limitation since the brain, the primary target of manganese neurotoxicity, may not accumulate a significant amount of manganese until much later, possibly after the current experiments in humans and animals were truncated. Experiments in rats and dogs, both normal and bile-cannulated, indicate that the brain does not accumulate a significant amount of manganese following administration of mangafodipir at levels much higher than the recommended clinical dose of the agent (Hustvedt et al. 1997), even at 168 hours post-dosing in the dog. Gallez et al. (1997) reported that manganese accumulation in the brain of adult mice following injection of a clinical dose of mangafodipir did not reach maximal levels until 24 hours post-dosing. This would indicate that the human distribution studies were terminated prematurely. However, while brain accumulation of manganese following mangafodipir administration is similar to that from $MnCl_2$, the manganese is not present after 2 weeks, whereas manganese from the inorganic compound was present, although at a decreased amount, 3 months following dosing (Gallez et al. 1997). These data indicate that single, clinical doses of mangafodipir are not likely to cause persistent accumulation of manganese in the brain.

2.3.3 Metabolism

Manganese is capable of existing in a number of oxidation states, and limited data suggest that manganese may undergo changes in oxidation state within the body. Circumstantial support for this hypothesis comes from the observation that the oxidation state of the manganese ion in several enzymes appears to be Mn(III) (Leach and Lilburn 1978; Utter 1976), while most manganese intake from the environment is either as Mn(II) or Mn(IV) (see Chapter 5). Another line of evidence is based on measurements of manganese in tissues and fluids using electron spin resonance (ESR), which detects the unpaired electrons in Mn(II), Mn(III), and Mn(IV). When animals were injected with $MnCl_2$, levels of manganese increased in bile and tissues, but only a small portion of this was in a form that gave an ESR signal (Sakurai et al. 1985; Tichy and Cikrt 1972). This suggests that Mn(II) is converted to another oxidation state [probably Mn(III)], but it is also possible that formation of complexes between Mn(II) and biological molecules (bile salts, proteins, nucleotides, etc.) results in loss of the ESR signal without formal oxidation of the manganese ion.

Evidence by Gibbons et al. (1976) suggests that oxidation of manganese occurs in the body. It was observed that human ceruloplasmin led to the oxidation of Mn(II) to Mn(III) *in vitro*, and although the process was not studied *in vivo*, it is a likely mechanism for manganese oxidation in the blood. These authors also noted that manganese oxidation led to a shift in manganese binding *in vitro* from α_2-macroglobulin to transferrin and that *in vivo* clearance of Mn(II)-α_2-macroglobulin from cows was much more rapid than the clearance of Mn(III)-transferrin (Gibbons et al. 1976). This suggests that the rate and extent of manganese reduction/oxidation reactions may be important determinants of manganese retention and toxicity in the body.

As demonstrated in a study by Komura and Sakamoto (1991), tissue levels of manganese in rats were affected by the form in which the manganese was administered in the diet; levels of manganese were significantly higher in animals fed manganese acetate or $MnCO_3$ than in animals fed $MnCl_2$ or MnO_2. Roels et al. (1997) also found differences in manganese concentrations in blood and brain regions depending on the oxidation state of the metal.

Organic Manganese

MMT. Following i.v. administration in the male rat, MMT was metabolized to hydroxymethylcyclopentadienyl manganese tricarbonyl (CMT-CH_2OH) and carboxycyclopentadienyl manganese tricarbonyl (CMT-COOH), both of which are present in urine (Hanzlik et al. 1980b). Metabolites are also present in the bile, as indicated by the fecal recovery of ^3H from the ring structure in MMT following i.v. or i.p. administration of radiolabeled compound to rats (Hanzlik et al. 1980b; 1980a). After i.v. dosing of MMT in rats, 11% of the radiolabel was recovered in feces within 30 minutes (Hanzlik et al. 1980b). These metabolites have not been characterized; however, the administration of phenobarbitol to the rat doubled the biliary excretion of the metabolite (Hanzlik et al. 1980a).

In vitro studies showed that rat liver microsomes activated with NADPH and molecular oxygen metabolized MMT (Hanzlik et al. 1980b). Preliminary studies with pooled liver microsomes from 5–6 normal or phenobarbital-induced rats showed that reaction rates of metabolism were linear for the first 20 minutes. MMT and aminopyrine, a positive control compound that is metabolized exclusively by cytochrome P450, showed parallel responses to changes in incubation conditions (i.e. NADPH dependence, inhibition by carbon monoxide, induction by phenobarbital). Liver microsomes metabolized MMT with an estimated K_M of 78 µM and a V_{max} of 3.12 nmol/mg protein/min. When the studies were done with liver microsomes from phenobarbital-treated rats, the K_M remained the same, but the V_{max} doubled (Hanzlik et al. 1980b). Lung microsomes were equally capable of metabolizing MMT, but phenobarbital induction did not enhance the response.

Maneb or mancozeb. Studies show that maneb and mancozeb are metabolized to several compounds including ethylenethiourea (ETU), ethylene urea, and ethylenediamine (Jordan and Neal 1979; Lyman 1971) in plants and animals. ETU has been identified in the urine of occupationally-exposed sprayers of maneb and mancozeb (Kurttio and Savoleinen 1990; Kurttio et al. 1990). No studies were located concerning the metabolism of manganese originating from these fungicides in either humans or animals.

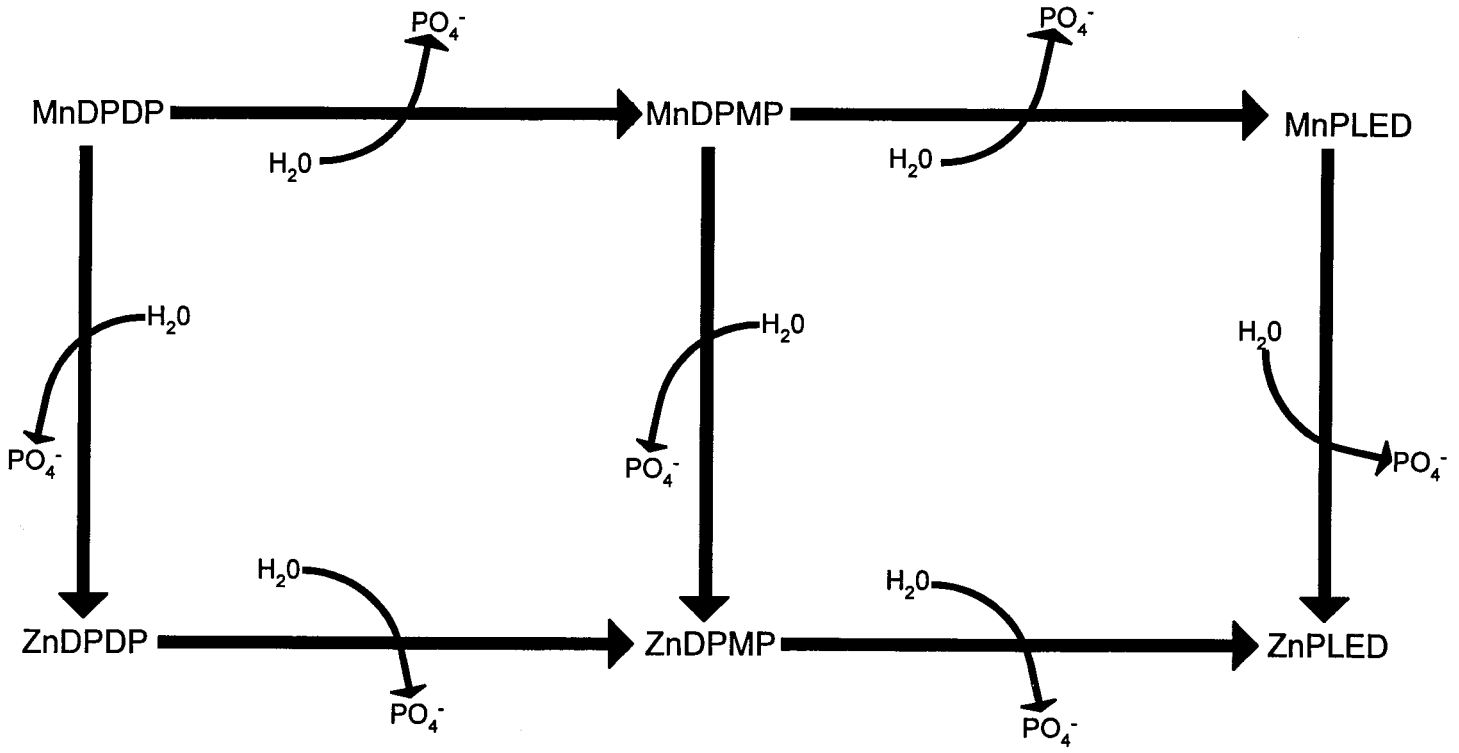
Mangafodipir. In humans, an infusion of the clinical dose of mangafodipir (MnDPDP) (5 µmol/kg, or 0.25 mg/kg) is rapidly dephosphorylated to manganese dipyridoxyl monophosphate (MnDPMP). This metabolite has been measured in human blood as quickly as 18 minutes after the beginning of infusion of the contrast agent, and is still measurable 1.3 hours after the start of the infusion (Toft et al. 1997a). MnDPDP was not observed in the blood after the first 18 minutes. The monophosphate then is fully

dephosphorylated to manganese dipyridoxyl ethylenediamine (MnPLED); this compound has been isolated in blood from 18 minutes after the start of an infusion until 40 minutes after the start. Transmetallation of either MnDPDP, MnDPMP, or MnPLED with zinc can occur, forming ZnDPDP, ZnDPMP, or ZnPLED. ZnDPDP has been identified in the bloodstream during the first 18 minutes of an infusion of 0.25 mg manganese/kg as MnDPDP. ZnDPMP has been detected in the blood from 18–40 minutes following the start of the infusion, and ZnPLED has been measured in the blood from 18 minutes to 8.33 hours following the start of the infusion. The major metabolite detected in urine was ZnPLED (Toft et al. 1997a). Figure 2-5 depicts the metabolism of mangafodipir in the human.

To study mangafodipir metabolism in the dog, Toft et al. (1997c) injected 3 male and female beagles with 0.55, 1.7, or 5.5 mg manganese/kg and took timed blood samples post-dosing to analyze for the presence of metabolites. Mangafodipir was rapidly metabolized by dephosphorylation and transmetallation at all three doses. After infusion with 0.55 mg/kg, MnPLED was the primary metabolite observed in the bloodstream 1 minute after the end of the infusion period, and MnDPDP was present at a concentration lower than the 5 metabolites. At 30 minutes post-dosing, ZnPLED was the main metabolite. However, at 5.5 mg/kg, MnPLED was the main metabolite at all sampling times (1, 5, and 30 minutes). The authors estimated that the ratio of manganese metabolites to zinc metabolites was 1, 2, and 3.5 at doses of 0.55, 1.7, or 5.5 mg manganese/kg, respectively; these data are consistent with the authors' hypothesis that the limited availability of free or loosely bound plasma zinc governs the initial transmetallation reaction (Toft et al. 1997c).

In vitro experiments with radiolabeled MnDPDP and whole blood or plasma from human donors indicate that mangafodipir undergoes a rapid transmetallation with zinc that is nearly complete within 1 minute after the start of incubation, followed by a relatively slow dephosphorylation process. The primary metabolite after a 90-minute incubation of whole blood with MnDPDP was MnDPMP, followed by CaDPDP/DPDP, Mn(III)DPDP (suggested as an artifact due to high pH and oxygen), and MnPLED. Experiments using ^{14}C-DPDP indicate that this chelate cannot enter red blood cells; therefore, the zinc contained within the cells is unavailable for binding to this compound. Binding of manganese ion to serum proteins was observed as well, indicating that dissociation of the metal from the chelate had occurred during incubation (Toft et al. 1997b).

Figure 2-5. Metabolism of MnDPDP[a]



[a]Adapted from Toft et al. 1997c

2.3.4 Excretion

In humans, absorbed manganese is removed from the blood by the liver where it conjugates with bile and is excreted into the intestine. Biliary secretion is the main pathway by which manganese reaches the intestines where most of the element is excreted in the feces (Bertinchamps et al. 1965; Davis et al. 1993; Malecki et al. 1996). However, some of the manganese in the intestine is reabsorbed through enterohepatic circulation (Schroeder et al. 1966).

Small amounts of manganese can also be found in urine, sweat, and milk (EPA 1993b). Urinary excretion of manganese by healthy males was 7.0 nmole/g creatinine (7.0 nmole = 385 ng = 0.385 µg) (Greger et al. 1990). Similarly, urinary manganese excretion by women was 9.3 nmole/day. Moreover, urinary excretion of manganese was not responsive to oral intake of manganese (Davis and Greger 1992). Dorner et al. (1989) showed that some infants fed breast milk and formula suffered negative manganese balances due to high fecal excretion. However, animal studies indicate that in the young, excretion is not well-developed and may result in increased retention of the element. For example, in mice, rats, and kittens, there is an almost complete absence of excretion during the neonatal period (Cotzias et al. 1976). However, data in neonatal rats indicate that manganese retention rates decrease to rates observed in adult animals. This is indirect evidence that excretion may mature during the end of the neonatal period though the exact time frame across species is unknown.

2.3.4.1 Inhalation Exposure

Inorganic Manganese

In humans who inhaled $MnCl_2$ or Mn_2O_3, about 60% of the material originally deposited in the lung was excreted in the feces within 4 days (Mena et al. 1969). Chronically exposed male workers were reported to have urine manganese levels that were significantly higher than unexposed persons; for example, male foundry workers had a mean manganese level of 5.7 µg/L compared to 0.7 µg/L in unexposed controls (Alessio et al. 1989). Other studies have reported significantly increased levels of urinary manganese in men occupationally exposed to airborne manganese dusts and fumes (Lucchini et al. 1994; Roels et al. 1987a, 1992). Mergler et al. (1994) did not report a significant difference in urinary manganese levels between the exposed and control groups in their occupational study. The differences in urinary excretion may be due to differences in duration or extent of exposure. A listing of these occupational studies that

measured exposure levels of manganese and the resultant levels of the metal in biological samples is provided in Table 2-9.

Rats exposed to either $MnCl_2$ or Mn_3O_4 by intratracheal instillation excreted about 50% of the dose in the feces within 3–7 days (Drown et al. 1986). Monkeys exposed to an aerosol of $^{54}MnCl_2$ excreted most of the manganese, with a half-time of 0.2–0.36 days (Newland et al. 1987). However, a portion of the compound was retained in the lung and brain. Clearance of this label was slower, occurring with half-times of 12–250 days. These data do not provide information on how much of the manganese excreted in the feces after inhalation exposure was first absorbed and then excreted via the bile versus the amount simply transported directly from the lung to the gastrointestinal tract where it may have been absorbed. Rat studies have demonstrated that urinary excretion of manganese 1 day following inhalation exposure was increased 200- and 30-fold when the animals were treated with the chelating agents 1,2-cyclohexylene-aminetetraacetic acid (CDTA) and diethylene triamine pentaacetic acid (DTPA), respectively, but fecal excretion was not altered (Wieczorek and Oberdörster 1989b).

Organic Manganese

No studies were located regarding excretion of manganese in either humans or animals following inhalation exposure to organic manganese.

2.3.4.2 Oral Exposure

Inorganic Manganese

Humans who ingested tracer levels of radioactive manganese (usually as $MnCl_2$) excreted the manganese with whole-body retention half-times of 13–37 days (Davidsson et al. 1989a; Mena et al. 1969; Sandstrom et al. 1986). The route of manganese loss was not documented but was presumed to be mainly fecal after biliary excretion. Serum manganese concentrations in a group of healthy men and women in Wisconsin were 1.06 µg/L and 0.86 µg/L, respectively (Davis and Greger 1992; Greger et al. 1990). Urinary excretion of manganese by men was 7.0 nmole/g creatinine (Greger et al. 1990). Similarly, urinary manganese excretion of women was 9.3 nmole/day. Moreover, urinary excretion of manganese was not responsive to oral intake of manganese (Davis and Greger 1992).

Table 2-9. Levels of Manganese in Exposed and Non-Exposed Workers

Occupational Study	Mean Age (years)	Mn in Air (mg/m^3)	Biological Samples	
			Mn-Blood ug/100ml	Mn-Urine ug/g creatinine
Roels et al. (1987b)				
Exposed	34.3 ±9.6	0.97# total dust	1.36* ±0.64 (1.22)**	4.76* (0.4
Non-Exposed	38.4 ±11.3		0.57* ±0.27 (1.59)**	0.30* (0.15)**
Roels et al.(1992)				
Exposed	31.3 ±7.4	0.179# respirable dust	0.81**	0.84**
Non-Exposed	29.3 ±8.0		0.68**	0.09**
Chia et al.(1993)				
Exposed	36.6 ±12.2	1.59* total dust	2.53**	6.1** (ug/liter)
Non-Exposed	35.7 ±12.1		2.33**	3.9** (ug/liter)

Table 2-9. Levels of Manganese in Exposed and Non-Exposed Workers (continued)

Occupational Study	Mean Age (years)	Mn in Air (mg/m^3)	Biological Samples	
			Mn-Blood ug/100ml	Mn-Urine ug/g creatinine
Mergler et al. (1994)				
Exposed	43.4 ±5.4	0.032# respirable dust	1.12* (1.03)**	1.07* (0.73)**
Non-Exposed	43.2 ±5.6		0.72* (0.68)**	1.05 (0.62)**
Lucchini et al (1999)				
Exposed	42.1 ±8.3	0.0967 respirable dust (CEI/yrs)	0.97* (0.92)**	1.81* (1.53)**
Non-Exposed	42.6 ±8.8		0.6* (0.57)**	0.67* (0.40)**

median
* arithmetic mean
**geometric mean

In a more recent study, young rats fed 45 mg manganese/kg/day were found to absorb 8.2% of the manganese ingested and to lose approximately 37% of the absorbed manganese through endogenous gut secretions (Davis et al. 1993).

Organic Manganese

MMT. The daily excretion of manganese from mice ingesting 11 mg manganese/kg as MMT in their daily diet was 5.4% of their daily intake (Komura and Sakamoto 1992).

No other studies were located regarding excretion of manganese from organic manganese compounds in either humans or animals.

2.3.4.3 Dermal Exposure

No studies were located regarding excretion of inorganic or organic manganese in humans or animals after dermal exposure to manganese.

2.3.4.4 Other Routes of Exposure

Inorganic Manganese

No studies were located regarding excretion of manganese by humans after exposure to inorganic manganese via other routes of exposure.

Rats exposed to $MnCl_2$ by intravenous injection excreted 50% of the dose in the feces within 1 day (Klaassen 1974) and 85% by day 23 (Dastur et al. 1971), indicating that biliary excretion is the main route of manganese clearance. Only minimal levels were excreted in urine (<0.1% of the dose within 5 days) (Klaassen 1974). Direct measurement of manganese levels in bile revealed concentrations up to 150-fold higher than in plasma, indicating the existence of either an active transport system (Klaassen 1974) or some sort of trapping mechanism (Tichy and Cikrt 1972). Based on the difference in blood levels following portal or femoral injection, Thompson and Klaassen (1982) estimated that about ⅓ of the manganese burden in blood is removed in each pass through the liver. Apparently some manganese can

cross directly from the blood to the bile (Bertinchamps et al. 1965; Thompson and Klaassen 1982), but most appears to be secreted into the bile via the liver (Bertinchamps et al. 1965).

The chemical state of manganese in bile is not known, but a considerable fraction is bound to bile components (Tichy and Cikrt 1972). This material is apparently subject to enterohepatic recirculation, since biliary manganese is reabsorbed from the intestine more efficiently than free Mn(II) (Klaassen 1974). The amount of manganese that contributes to total body burden following reabsorption from enterohepatic recirculation is not known.

While biliary secretion appears to be the main pathway by which manganese is excreted into the intestines, direct transport from blood across the intestinal wall may also occur (Bertinchamps et al. 1965; Garcia-Aranda et al. 1984). The relative amount of total excretion attributable to this pathway was not quantified by Bertinchamps, but it appears to be only a fraction of that attributable to biliary secretion (Bertinchamps et al. 1965).

Organic Manganese

Mangafodipir. Manganese originating from mangafodipir administered at clinical (0.25 mg/kg) and more than twice the clinical dose (0.55 mg/kg) is primarily excreted in the feces via the bile in both humans and animals (Grant et al. 1994; Hustvedt et al. 1997; Toft et al. 1997a; Wang et al. 1997). In contrast to the chelate, DPDP, manganese is incompletely cleared from the body 24 hours after administration, and roughly 7–8% of a dose is still retained in the body after 1 week (Hustvedt et al. 1997).

2.3.5 Physiologically Based Pharmacokinetic (PBPK)/Pharmacodynamic (PD) Models

Physiologically based pharmacokinetic (PBPK) models use mathematical descriptions of the uptake and disposition of chemical substances to quantitatively describe the relationships among critical biological processes (Krishnan et al. 1994). PBPK models are also called biologically based tissue dosimetry models.

PBPK models are increasingly used in risk assessments, primarily to predict the concentration of potentially toxic moieties of a chemical that will be delivered to any given target tissue following various combinations of route, dose level, and test species (Clewell and Andersen 1985). Physiologically based

pharmacodynamic (PBPD) models use mathematical descriptions of the dose-response function to quantitatively describe the relationship between target tissue dose and toxic end points.

PBPK/PD models refine our understanding of complex quantitative dose behaviors by helping to delineate and characterize the relationships between: (1) the external/exposure concentration and target tissue dose of the toxic moiety, and (2) the target tissue dose and observed responses (Andersen et al. 1987; Andersen and Krishnan 1994). These models are biologically and mechanistically based and can be used to extrapolate the pharmacokinetic behavior of chemical substances from high to low dose, from route to route, between species, and between subpopulations within a species. The biological basis of PBPK models results in more meaningful extrapolations than those generated with the more conventional use of uncertainty factors.

The PBPK model for a chemical substance is developed in four interconnected steps: (1) model representation, (2) model parametrization, (3) model simulation, and (4) model validation (Krishnan and Andersen 1994). In the early 1990s, validated PBPK models were developed for a number of toxicologically important chemical substances, both volatile and nonvolatile (Krishnan and Andersen 1994; Leung 1993). PBPK models for a particular substance require estimates of the chemical substance-specific physicochemical parameters, and species-specific physiological and biological parameters. The numerical estimates of these model parameters are incorporated within a set of differential and algebraic equations that describe the pharmacokinetic processes. Solving these differential and algebraic equations provides the predictions of tissue dose. Computers then provide process simulations based on these solutions.

The structure and mathematical expressions used in PBPK models significantly simplify the true complexities of biological systems. If the uptake and disposition of the chemical substance(s) is adequately described, however, this simplification is desirable because data are often unavailable for many biological processes. A simplified scheme reduces the magnitude of cumulative uncertainty. The adequacy of the model is, therefore, of great importance, and model validation is essential to the use of PBPK models in risk assessment.

PBPK models improve the pharmacokinetic extrapolations used in risk assessments that identify the maximal (i.e., the safe) levels for human exposure to chemical substances (Andersen and Krishnan 1994). PBPK models provide a scientifically-sound means to predict the target tissue dose of chemicals in humans who are exposed to environmental levels (for example, levels that might occur at hazardous waste sites)

based on the results of studies where doses were higher or were administered in different species. Figure 2-6 shows a conceptualized representation of a PBPK model.

If PBPK models for manganese exist, the overall results and individual models are discussed in this section in terms of their use in risk assessment, tissue dosimetry, and dose, route, and species extrapolations.

PBPK models for manganese are discussed below.

No PBPK models for manganese were located involving humans (adults or children) or animals.

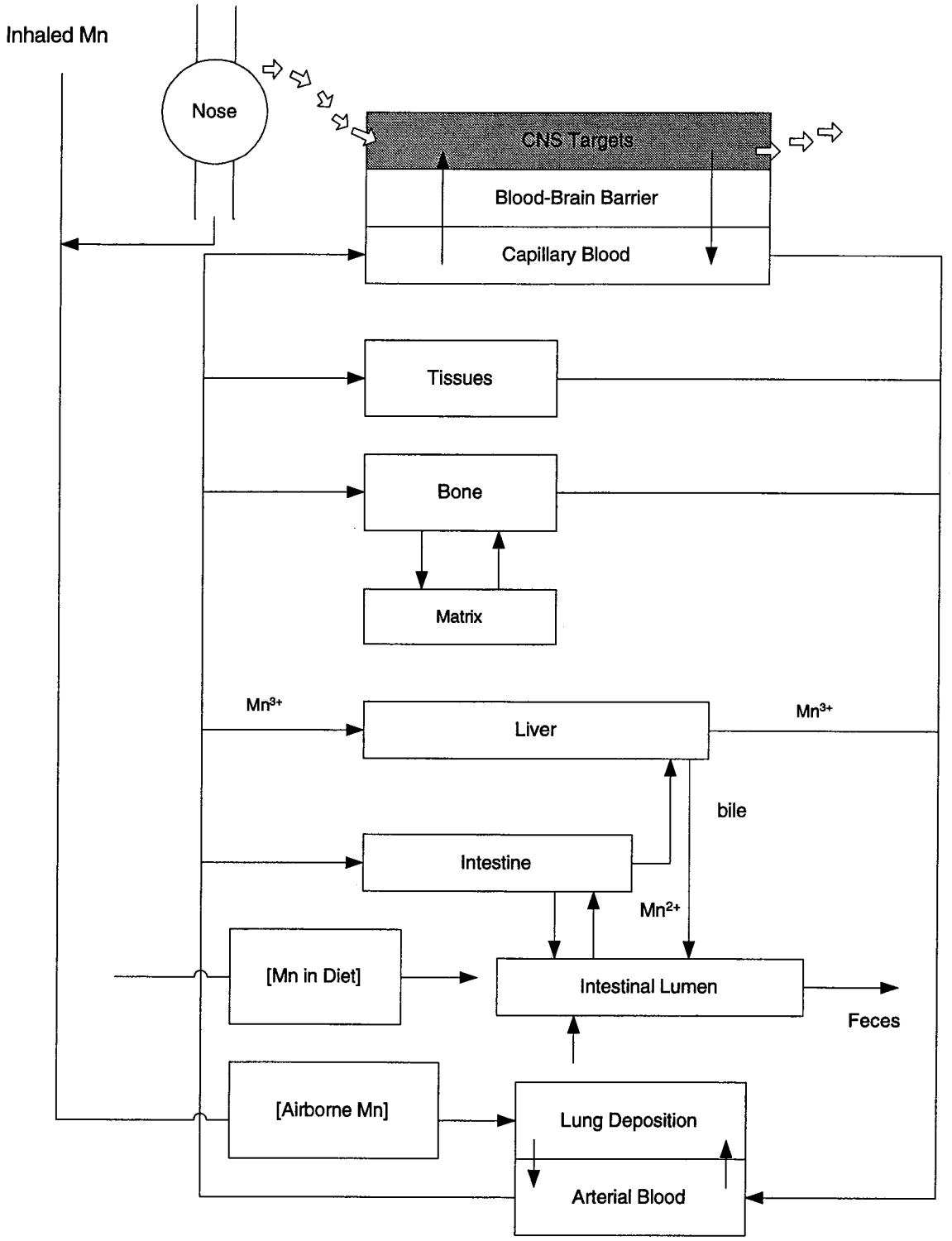
A qualitative PBPK model for manganese disposition in humans and animals has recently been developed by Andersen et al. (1999). This model represents the current understanding of manganese nutrition and toxicology; because several data gaps exist concerning manganese pharmacokinetics, this model is anticipated to change with time (Andersen et al. 1999). The model, shown in Figure 2-6, is currently not designed to be quantitative in nature. The authors indicate that several data gaps prevent such an evaluation of manganese uptake, distribution, and excretion. For instance, there are inadequate data concerning oxidation rates for manganese in blood, uptake rates of protein-bound forms by the liver, neuronal transfer rates within the central nervous system, and quantitative data on systems controlling manganese uptake via the intestines and liver (such as transport mechanism in the intestines) (Andersen et al. 1999). Based on available information about the distribution of manganese in brain tissues, this model might also include a separate compartment for the brain or brain regions versus a general CNS compartment. Differences in transport of different oxidation states of manganese into the brain have been reported (Murphy et al. 1991; Rabin et al. 1993). These differences may also be important parameters to consider in a PBPK model for manganese disposition.

2.4 MECHANISMS OF ACTION

2.4.1 Pharmacokinetic Mechanisms

Manganese absorption occurs primarily through the diet; however, absorption via the lungs can be significant for occupationally exposed persons or for those exposed to excess levels of airborne manganese, such as downwind of a manganese point source. Manganese absorption through the gut may occur through a nonsaturable simple diffusion process through the mucosal layer of brush border

Figure 2-6. Qualitative PBPK Model for Manganese



Source: Adapted from Andersen et al. 1999.

membranes (Bell et al. 1989) or via an active-transport mechanism that is high-affinity, low-capacity, and rapidly saturable (Garcia-Aranda et al. 1983). Manganese particles that are too large to enter the alveoli (larger than 10 microns in diameter) remain in the upper respiratory tract, where they are coughed up by mucociliary transport and swallowed. Differences in solubility of manganese compounds deposited in the alveolar regions may impact the rate at which manganese will be absorbed, but manganese is bioavailable when deposited in these regions (Drown et al. 1986).

Diets high in iron have been shown to suppress manganese absorption, and conversely, iron-poor diets increase manganese uptake (Lönnerdal 1994,1997). Phosphorus (Wedekind et al. 1991) and calcium (Wilgus and Patton 1939) have also been found to decrease manganese uptake.

A review article by Aschner and Aschner (1991) summarizes some of the available data regarding the distribution of manganese. Current knowledge suggests that dietary manganese, absorbed as Mn(II), enters portal circulation from the gastrointestinal tract and is bound to α_2-macroglobulin or albumin in the plasma. After traveling to the liver, the major portion of Mn(II) is secreted in the bile, but some may be oxidized by ceruloplasmin to Mn(III). The Mn(III) enters circulation conjugated with plasma transferrin; once this complex enters a neuron, it dissociates; from there the manganese is transported to axon terminals. For example, Sloot and Gramsbergen (1994) observed that radiolabeled manganese injected into the striatum or substantia nigra of rat brain is transported in an anterograde direction through both γ-amino-butyric acid-producing striato-nigral and dopaminergic nigro-striatal fibers.

Recent studies, however, argue for the transport of Mn(II) into the brain. For example, Murphy et al. (1991) measured the kinetics of manganese transport in the brains of adult male rats using a perfusion technique. The rats were infused with increasing concentrations of $[^{54}Mn]Cl_2$; blood and brain samples were analyzed for manganese at varying time points. The data indicated a saturable mechanism for transporting Mn(II) into the choroid plexus, and influx into the cerebral cortex was also near saturation at the highest plasma concentration of manganese used. Influx into other brain regions (e.g., caudate nucleus, hippocampus, hypothalamus) and cerebrospinal fluid (CSF) showed non-saturable transport of the cation. The authors suggested that the non-saturable transport into these brain regions resulted from passive diffusion of manganese down a concentration gradient from ventricular cerebrospinal fluid because some of these brain regions have components adjacent to the ventricles and manganese concentrations in these regions were below levels in the CSF. The authors also noted that at all plasma manganese concentrations tested (from 0.8 to 78 nmol/ml), the transfer coefficient for manganese uptake into the choroid plexus was

significantly higher than in any other area of the central nervous system. For example, at 0.08 nmol/ml, the transfer coefficients for the CSF and the choroid plexus were $16.2 \pm 2.43 \times 10^{-6}$ ml/sec*g and $23{,}800 \pm 2{,}910 \times 10^{-6}$ ml/sec*g, respectively. Even after correcting for differences in compartment size, influx of manganese into the choroid plexus was an order of magnitude greater than influx into CSF.

Rabin et al. (1993) also measured transport of $[^{54}Mn]Cl_2$ in adult rats using a similar technique. In this study, the authors used three perfusates (whole blood, plasma/serum, and saline) to determine brain uptake in environments that facilitated or prevented protein binding of the metal. The authors reported that uptake of manganese into the cortex, hippocampus, caudate nucleus and the choroid plexus was greater and more rapid when saline was used rather than with whole blood. When EDTA-saline was used as the perfusate, uptake was not significantly different than zero, indicating that divalent manganese was the form taken up by the brain. The transfer coefficients of Mn(II) from saline in the different regions of the brain (frontal, parietal, and occipital cortex regions; hippocampus; caudate nucleus; and thalamus-hypothalamus) ranged from $5–10 \times 10^{-5}$ ml/sec*g, whereas that of the choroid plexus was 727×10^{-3} ml/sec*g. The authors noted that the transfer coefficients were greater than that expected for passive diffusion and suggested a facilitated blood-brain barrier transport by a channel or carrier mechanism (Rabin et al. 1993). These findings of a rapid uptake mechanism and concentrated uptake into the choroid plexus are consistent with results reported by Murphy et al. (1991). Separate binding studies performed by the authors determined that albumin, transferrin, α_2-macroglobulin added to the manganese during perfusion significantly decreased brain uptake of the cation in all brain regions. The authors were uncertain whether Mn(II) in the form of low-molecular mass solutes was taken up at the blood-brain barrier. However, based on other literature and their own unpublished results, they suggest that the free ion is the species transported.

Other studies have also revealed the rapid appearance of manganese in the choroid plexus. Ingersoll et al. (1995) demonstrated that manganese levels in the lateral choroid plexus were 44 and 24 times higher than levels in cerebrospinal fluid (CSF), and blood, respectively, 4 hours after i.p. injection of 10 mg Mn/kg. However, manganese concentration in the choroid plexus did not change significantly following intrathecal administration of this same dose. This demonstrated that manganese in the blood could be sequestered by the choroid plexus, whereas little to no transfer of manganese from CSF to the choroid plexus occurred. Intrathecal administration of manganese increased manganese concentrations in all brain regions examined while there were only slight changes in brain manganese concentrations after i.p. administration. Moreover, intrathecal administration of manganese decreased spontaneous motor activity with no effect on motor activity following i.p. dosing. The authors suggested that these results indicated that the brain is

protected from high concentrations of manganese through sequestering in the choroid plexus, but this mechanism could become overwhelmed with rising levels of blood manganese such that manganese could then “leak’ from the choroid plexus into CSF and thereby enter the brain. This interpretation appears to be consistent with the findings of London et al (1989). In these studies, 50 and 100 mg Mn/kg manganese was administered i.p., doses 5- and 10-times that used by Ingersoll et al (1995). Using MRI images, these doses were shown to concentrate in the ventricles, the pineal gland, and the pituitary gland and the authors indicated that this high concentration of manganese appeared in the ventricular CSF because it crossed the barrier of the choroid plexus. Takeda et al. (1994) used autoradiography to also show that manganese in selected brain regions was taken up via the CSF from the choroid plexus.

2.4.2 Mechanisms of Toxicity

The central nervous system is the primary target of manganese toxicity. Although it is known that manganese is a cellular toxicant that can impair transport systems, enzyme activities, and receptor functions, the principal manner in which manganese neurotoxicity occurs has not been clearly established (Aschner and Aschner 1991).

Mn(III) has been found to be more cytotoxic to human neural cells as a manganese pyrophosphate complex (MnPPi) than as a manganese-transferrin complex (MnTf) (Suarez et al. 1995). Specifically, human neuroblastoma cells (cell line SH-SY5Y) grown in culture showed effects of cytotoxicity from 30 µM MnPPi but did not show the same drastic signs of cytoxicity from MnTf (membrane damage and cell granulation and aggregation) until concentrations of 60 µM were reached (Suarez et al. 1995). Both manganese complexes inhibited mitochondrial enzyme activity, but MnTf was slightly more toxic than MnPPi in this respect (Suarez et al. 1995).

Neuropathological changes are detectable in the basal ganglia of humans with manganism, and the specific area of injury appears to be primarily in the globus pallidus; the substantia nigra is sometimes affected, but generally to a lesser extent (Katsuragi et al. 1996; Yamada et al. 1986). Studies in nonhuman primates have produced similar findings (Newland et al. 1989, 1992). Limited evidence suggests that dopamine levels in the caudate nucleus and putamen are decreased in manganism patients (Bernheimer et al. 1973). Similarities in the behavior of manganism patients to those with Parkinson's disease has prompted some to refer to manganism as "manganese-induced Parkinsonism" or "Parkinson-like disease." Further, the two diseases do affect functional related regions of the brain, but Parkinsonism is believed to be due to the

selective loss of subcortical neurons whose cell bodies lie in the substantia nigra and whose axons terminate in the basal ganglia (which includes the caudate nucleus, the putamen, the globus pallidus, and other structures). These nigral neurons use dopamine as their neurotransmitter, and treatment of Parkinson patients with levo-dopa (the metabolic precursor to dopamine) often relieves some of the symptoms of Parkinson's disease (Bernheimer et al. 1973). Some investigators have reported that oral levo-dopa can temporarily improve symptoms of manganese-induced neurotoxicity (Barbeau 1984). However, most studies show that manganism patients typically do not respond to levo-dopa treatment (Calne et al. 1994; Chu et al. 1995; Huang et al. 1989), indicating that they have likely suffered degeneration of the receptors and neurons that normally respond to this neurochemical (Chu et al. 1995).

The precise biochemical mechanism by which manganese leads to this selective destruction of dopaminergic neurons is not known, but many researchers believe that the manganese ion Mn(II) enhances the autoxidation or turnover of various intracellular catecholamines, leading to increased production of free radicals, reactive oxygen species, and other cytotoxic metabolites, along with a depletion of cellular antioxidant defense mechanisms (Barbeau 1984; Donaldson 1987; Garner and Nachtman 1989b; Graham 1984; Halliwell 1984; Liccione and Maines 1988; Parenti et al. 1988; Verity 1999). It is important to note that oxidation of catechols is more efficient with Mn(III), than with Mn(II) or Mn(IV) (Arhibald and Tyree 1987). Formation of Mn(III) may occur by oxidation of Mn(II) by superoxide (O_2^-). In cases of exposure to Mn(VII), it is likely that a reduction to the Mn(II) or Mn(III) state occurs (Holzgraefe et al. 1986), but this has not been demonstrated.

Hussain et al. (1997) studied the effects of chronic exposure of manganese on antioxidant enzymes, including manganese superoxide dismutase (MnSOD). MnSOD is an antioxidant enzyme located primarily in the mitochondria that contains manganese as a functional component. MnSOD protects against oxidative injury by catalyzing the dismutation of superoxide (O_2^-), the univalent reduction product of dioxygen. Hussain et al. (1997) found that administration of 0, 1.1, and 2.2 mg manganese (as $MnCl_2$)/kg/day, 5 days/week for 3 months, resulted in increased MnSOD in the hippocampus, cerebellum, and brainstem. Other areas of the brain were not affected and other antioxidant enzymes, such as Cu,ZnSOD and glutathione peroxidase (GPx), were not increased. The researchers suggest that since a critical role of MnSOD is to protect against oxidative injury, the increase of this enzyme after manganese exposure may reduce the risk of oxidative stress induced by that exposure. Thus, this protective mechanism would have to be overwhelmed in cases of manganese toxicity. Additionally, the authors suggest that, since MnSOD was altered while Cu,ZnSOD and Gpx were unchanged, manganese may not

affect cytosolic enzymes like Cu,ZnSOD. In support of this point, the authors also mention other reports that suggest that these antioxidant enzymes are independently regulated (Mossman et al. 1996; Warner et al. 1993; Yen et al. 1996).

Supporting evidence for the hypothesis that high levels of manganese exerts neurotoxicity through oxidation is provided by Desole et al. (1994). The authors observed that 22 mg manganese (as $MnCl_2$)/kg/day administered orally in 6-month-old rats resulted in increased concentrations of DOPAC (an oxidation product of DA) and uric acid, but left DA levels unchanged. Daily doses of 44 or 66 mg manganese/kg/day resulted in significantly decreased concentrations of DA, glutathione, ascorbic acid, and DOPAC, and increased concentrations of uric acid in the rat striatum when compared to controls. The researchers also measured levels of these metabolites in the rat striatal synaptosomes, which were used as a model for neuronal terminals. Here, DA levels were unchanged at 22 mg manganese/kg/day but were decreased at the 2 highest doses. DOPAC levels remained constant at all three dose levels. Thus the DOPAC/DA ratio was significantly increased at 44 and 66 mg manganese/kg/day in the synaptosomes. While the authors suggest that these data support other findings that manganese oxidizes dopamine (Segura-Aguilar and Lind 1989), the decrease in DA could be the result of decreased production or release of the chemical, rather than increased oxidation. Catabolism of adenosine triphosphate (ATP) forms xanthine and hypoxanthine, both of which are metabolized by xanthine oxidase. The products of this metabolism are uric acid and superoxide radical anion (Desole et al. 1994). The increase in uric acid production in rat striatum following oral dosing with 44 or 66 mg manganese/kg, as $MnCl_2$, suggests that manganese induces oxidative stress mediated by xanthine oxidase. Desole et al. (1995) expanded their studies to investigate the protective effect of allopurinol, a xanthine-oxidase inhibitor, to 3-month-old rats exposed to manganese. In this study, allopurinol was administered by gavage at a dose of 300 mg/kg/day for 4 days. Manganese (87 mg/kg/day) was also administered by gavage, for 7 days, either alone or with allopurinol; the allopurinol decreased the striatal ratio of DOPAC and homovanillic acid (HVA) to dopamine. When given in conjunction with manganese, allopurinol antagonized the manganese-induced increase in DOPAC levels and the (DOPAC + HVA)/DA ratio. Together, the two studies suggest that manganese-induced oxidative stress through the formation of reactive oxygen species may be a mechanism for manganese neurotoxicity, and allopurinol may protect against this oxidative stress in the striatum and brainstem of young rats.

Experiments such as the one by Desole et al. (1994) indicate that overexposure of rats to manganese results in increased dopamine turnover in the rat striatum. However, patients with basal ganglia dysfunction

caused by manganese had normal striatal fluorodopa uptake on PET scan, indicating that the nigrostriatal pathway was intact (Wolters et al. 1989). Seven intravenous injections of $MnCl_2$ into Rhesus monkeys resulted in an extrapyramidal syndrome characterized by bradykinesia, facial grimacing, and rigity, with gliosis of the globus pallidus and the substantia nigra par reticularis (Olanow et al. 1996). Striatal dopamine and homovanillic acid levels were within normal ranges; yet there was clear evidence of manganese-induced neurotoxicity. Interestingly, none of the symptoms improved after levo-dopa administration, supporting findings in humans that manganism does not respond to levo-dopa treatment (Chu et al. 1995; Huang et al. 1989).

While there are a number of studies that support the hypothesis that manganese exerts its neurotoxicity through oxidation, a recent study has demonstrated atypical antioxidative properties of manganese in iron-induced brain lipid peroxidation and copper dependent low density lipoprotein conjugation (Sziráki et al. 1999). However, the underlying mechanisms of the antioxidant effects are not clear. In addition, Sziráki et al. (1999) do not discuss alternate mechanisms of manganese neurotoxicity. Recently, Brenneman et al. (1999) measured reactive oxygen species (ROS) in the brains of neonatal rats administered up to 22 mg manganese/kg/day for up to 49 days (dosing was only 5 days/wk from day 22-49). On PND 21, no increase in ROS was seen in the striatum, hippocampus, or hindbrain of exposed rats at any dose, compared to controls administered water only. In the cerebellum, ROS levels were significantly increased to the same extent at both dose levels, as compared to controls. Manganese levels were not increased significantly in the cerebellum at any dose level, but were increased in the striatum, and the rest of the brain at the high dose level, when measured at PND49. Mitochondrial manganese was not significantly elevated in the cerebellum or striatum, but was elevated in the rest of the brain at this high dose level, also at PND 49. These data do not support the hypothesis that oxidative damage is a mechanism of action in manganese-induced neurotoxicity in the rat.

Mn(II) may also be involved in neurotoxicity. The neurotoxicity of Mn(II) has been linked to its ability to substitute for Ca(II) under physiological conditions (Aschner and Aschner 1991), and the intestinal transfers of Ca(II) and Mn(II) have been shown to be competitive *in vivo* (Dupuis et al. 1992). Although the mechanism for Mn(II) transport into brain cells is uncertain, Mn(II) preferentially accumulates in the mitochondria in the areas of the brain that are associated with manganism and neurological symptoms. Manganese is taken up into mitochondria via the calcium uniporter, and once there, Mn(II) inhibits mitochondrial oxidative phosphorylation. Gavin et al. (1992) observed that Mn(II) can inhibit mitochondrial oxidative phosphorylation when incubating isolated mitochondria with Mn(II) at

concentrations higher than 1 µM. Recently, it has also been shown that intramitochondiral Mn(II) can inhibit the efflux of Ca(II), which may result in a loss of mitochondrial membrane integrity (Gavin et al. 1999). At the same time, intramitochondrial Mn(II) can also inhibit oxidative phosphorylation and decrease energy production. However, Brouillet et al. (1993) has suggested that the impaired oxidative metabolism induced by manganese is indirectly linked to an excitotoxic process that results in neuronal degeneration. Because manganese accumulates in the mitochondria and is associated with impaired energy production, these authors compared the effects of intrastriatal injection of manganese with effects produced by known mitochodral toxins, aminooxyacetic acid and 1-methyl-4-phenylpyridinium. Lesions produced by these compounds can be blocked through an inhibition of the glutamatergic *N*-methyl-*D*-aspartate (NMDA) receptor or by the removal of the cortical glutamatergic input into the striatum by decortication. Thus, these lesions are termed "excitotoxic lesions." It was shown that decortication or pre-treatment with the NMDA noncompetitive antagonist, MK-801, could reverse or ameliorate neurochemical changes induced by intrastriatal injection of manganese. These authors also showed that intrastriatal manganese treatment also interfered with energy metabolism, ATP concentrations were significantly reduced by 51% and lactate levels were increased by 97%. There is additional evidence that the glutamatergic excitatory system may play a role in manganese toxicity. Recent studies in genetically epilepsy-prone rats have suggested that there are abnormalities in manganese-dependent enzymes. Although the manganese-dependent enzymes are believed to be unrelated to seizure activity in these animals, it is suggested that there is a link between the low manganese concentrations in glial cells, and elevated glutamate levels due to low glutamine synthetase activity (Critchfield et al. 1993).

Manganese(II) (from $MnCl_2$) has also been shown to inhibit mitochondrial aconitase activity to a significant level in the frontal cortex of male rats dosed with 6 mg manganese/kg/day for 30 days (Zheng et al. 1998). Aconitase levels in striatum, hippocampus, and substantia nigra were decreased in treated rats, but not to a significant extent. Aconitase, which catalyzes the interconversion of L-citrate to isocitrate, via *cis*-aconitate, requires iron as a cofactor at its active center (Zheng et al. 1998). When the authors incubated brain mitochondrial fractions with Mn(II), aconitase activity was decreased; the addition of excess iron [Fe(II)] revived the enzyme activity. These data suggest that the similarity of manganese and iron facilitates their proposed interaction at the subcellular level; however, the data do not prove that Mn(II) is the form of manganese that is exerting the inhibitory effect.

Conversely, Suarez et al. (1995) did not observe cytotoxicity in cultured cells exposed to 100 µM Mn(II). The discrepancy noted in this study, and that of Gavin et al. (1992) may have occurred because of a

protective effect of the cell membrane; if the cell membrane protects the cytosol, which typically has a low manganese concentration, then the Mn(II) concentration may be too low to affect the mitochondria through uniport uptake (Suarez et al. 1995). Another explanation is that mitochondrial uptake of Mn(II) occurs, but toxic effects require that cells be exposed much longer than isolated mitochondria (Suarez et al. 1995). It has also been established that manganese accumulation in the brain varies between regions, particularly in developing animals; this region-specific accumulation may alter the metabolism and homeostasis of manganese (Chan et al. 1992). In addition, it has been demonstrated that the manganese concentration in the central nervous system (CNS), in particular the ventral mesencephalon, can be reduced by cocaine, a dopamine reuptake inhibitor, or by reserpine, a dopamine depleting agent (Ingersoll et al. 1999). This suggests that the dopamine reuptake carrier is linked to a transport mechanism for manganese.

In vitro studies of rat brain mitochondria have demonstrated that there is no apparent mechanism for Mn(II) clearance other than the slow Na^+ independent mechanism; it is suggested that Ca(II) and Mn(II) may accumulate in the brain mitochondria during manganese intoxication (Gavin et al. 1990). Other theories regarding the mode of neurotoxicity for manganese (and other metal ions) include toxicity caused by the formation of hydroxyl radicals during the manganese-catalyzed autooxidation of hydrazines (Ito et al. 1992).

It has been suggested that the mechanism of manganese neurotoxicity may in part involve complex interactions with other minerals (Lai et al. 1999). In a developmental rat model of chronic manganese toxicity, administration of manganese in drinking water was associated with increased levels of iron, copper, selenium, zinc, and calcium in various regions of the brain. Moreover, the subcellular distribution of various minerals were differentially altered following manganese treatment. Iron deficiency is associated with increased manganese burden in the central nervous system of rats, while administration of excess iron significantly decreases manganese uptake (Aschner and Aschner 1990). The biochemical mechanisms underlying the interactions between manganese and other minerals are unclear.

2.4.3 Animal-to-Human Extrapolations

As discussed in Section 2.2, the available literature on toxicological analysis of manganese in humans and animals is quite extensive. However, due to the wide dose ranges administered, the variety of responses, and the differences in measured endpoints, comparisons of effects across species is not straightforward.

Rodent models have primarily been used to study manganese neurotoxicity. These studies have reported mostly neurochemical, rather than neurobehavioral, effects (Brouillet et al. 1993; Chandra 1983; Chandra and Shukla 1978, 1981; Daniels and Abarca 1991; Deskin et al. 1980, 1981; Eriksson et al. 1987a; Gianutsos and Murray 1982; Parenti et al. 1986; Singh et al. 1979; Subhash and Padmashree 1991), as very few studies investigated neurobehavioral effects. It has been suggested that this focus may reflect difficulties in characterizing behavioral changes following basal ganglia damage in the rodent (Newland, 1999). Other techniques, such as those used to identify basal ganglia damage as a result of exposure to neuroleptics (Newland 1999) may be refined to further exploit the rodent model as a predictor of neurobehavioral change in the human. The usefulness of the rat model for manganese neurotoxicity is also limited because the distribution of manganese in brain regions is dissimilar to that of the human (Chan et al. 1992; Brenneman et al. 1999; Kontur and Fechter 1988; Pappas et al. 1997). Studies to date have used exposure routes such as i.v., i.p., or s.c., with few exceptions (Brenneman et al. 1999; Dorman et al. 2000; Lown et al. 1984; Morganti et al. 1985; Pappas et al. 1997). Future studies should focus on inhalation or oral pathways to model more accurately the toxicokinetics of occupational and environmental exposures to manganese.

The rabbit has also been used as a model for manganese toxicity in a few studies (Chandra 1972;Szakmáry et al. 1995). The only available neurotoxicity study using the rabbit (Chandra 1972) reported that the species, when dosed intratracheally with 253 mg manganese/kg body weight (inferred as a 1-time dose), developed hind-limb paralysis after an observation period of 18 months. The animals also exhibited wide-spread neuronal degeneration in the brain. This study suggests that rabbits and humans may be qualitatively similar in the manifestation of neurobehavioral effects. However, further studies are needed to determine if the two species manifest comparable symptoms within the same dose range.

The non-human primate has been a useful model for predicting neurotoxicity in the human as the monkey presents neurobehavioral symptoms very similar to the human (Eriksson et al. 1987b; Gupta et al. 1980; Newland and Weiss 1992; Olanow et al. 1996). Further, the monkey also undergoes neurochemical changes (Bird et al. 1984) as a result of manganese exposure. Studies have shown that monkeys exposed to manganese injected either intravenously or subcutaneously exhibit symptoms very similar to those observed in miners and others exposed to manganese, including ataxia, bradykinesia, unsteady gait, grimacing, and action tremor (Eriksson et al. 1992; Newland and Weiss 1992; Olanow et

al. 1996). In addition, monkeys exhibiting these effects show accumulation of manganese in the basal ganglia as observed by MRI (Eriksson et al. 1992; Newland and Weiss 1992), as do humans who are either exposed to, or are unable to clear, excess manganese (Devenyi et al. 1994; Fell et al. 1996; Hauser et al. 1994; Ono et al. 1995; Pomier-Layrargues et al. 1998; Rose et al. 1999; Spahr et al. 1996). However, primate studies showing these neurobehavioral effects have involved routes of administration that do not mimic environmental exposures, and although the effects in monkeys are qualitatively similar, it is currently unknown whether the effects are seen at the same dose metric as those in humans. Newland (1999) proposes using MRI techniques to relate the administration of certain amounts of manganese with a corresponding MRI signal in the brain and the resultant neurobehavioral effects. This technique might be very useful in developing a true dose-response relationship for manganese neurotoxicity in both the monkey and human.

2.5 RELEVANCE TO PUBLIC HEALTH

Issues relevant to children are explicitly discussed in 2.6 Children's Susceptibility and 5.6 Exposures of Children.

Overview.

Manganese is a naturally occurring element that exists in the environment primarily as a salt or an oxide of Mn(II) or Mn(IV). It is an essential nutrient for humans and animals and plays a role in bone mineralization, protein and energy metabolism, metabolic regulation, cellular protection from damaging free radical species, and the formation of glycosaminoglycans (Wedler 1994). Several enzyme systems (e.g., transferases, decarboxylases, hydrolases, dehydrogenases, synthetases, and lyases) have been reported to interact with or depend on manganese for their catalytic or regulatory function (Wedler 1994). Mitochondrial superoxide-dismutase, pyruvate carboxylase, and liver arginase are known manganese metalloenzymes (NRC 1989; Wedler 1994). There is limited evidence that some disorders or disease states in humans (e.g., amyotrophic lateral sclerosis, acromegaly, catabolic disease, and epilepsy) may be associated with an imbalance in tissue levels of manganese (Aihara et al. 1985; Carl and Gallagher 1994; Nagata et al. 1985; Tulikoura and Vuori 1986). However, research in this area is ongoing and inconclusive at this time. Although manganese intakes of many adult Americans are believed to be less than the Estimated Safe and Adequate Daily Dietary Intake (ESADDI), no large-scale deficiency has been

reported (Freeland-Graves 1994; NRC 1989). This may reflect, in part, the lack of adequate methods to monitor nutritional status in regard to manganese.

Manganese deficiency was inadvertently induced in one participant in a vitamin K study (Doisy 1973). Consumption of a diet low in manganese (0.35 mg manganese/day) and vitamin K resulted in a decreased level of clotting proteins, decreased serum cholesterol, reddening of black hair and beard, slowed growth of hair and nails, and scaly dermatitis. After vitamin K was returned to the diet, the subject continued to exhibit these symptoms; when manganese was returned to the diet the symptoms were eliminated. It was suggested that manganese completes biosynthesis of the glycoprotein clotting factors by activating the glycosyl transferases (Doisy 1973). Five of 7 men fed a manganese deficient diet (0.11 mg manganese/day) for 39 days exhibited dermatitis (*Miliaria crystallina*) at the end of the depletion diet; the dermatitis cleared rapidly when manganese was returned to the diet (Friedman et al 1987). From these studies, the minimum manganese requirement was estimated to be 0.74 mg/day (0.014 mg manganese/kg/day assuming 55 kg body weight). In a dietary study with 47 female subjects, it was estimated that 20% of the women consumed <1 mg of manganese daily (from 0.0078 to 0.015 mg manganese/kg/day, body weight = 60 kg) (Davis et al. 1992a). Lymphocyte manganese superoxide dismutase (MnSOD) levels and serum manganese levels were significantly elevated in women (body weight = 60 kg) who received manganese supplements in the diet at 15 mg/day (0.25 mg manganese/kg/day) for 119 days (Davis and Greger 1992).

Studies in humans indicate that manganese is an essential element (Doisy 1973; Friedman et al. 1987). However, a Recommended Dietary Allowance (RDA) has not been established for manganese because data have been insufficient to determine nutrient needs of healthy persons (NRC 1989). However, the Food and Nutrition Board of the National Research Council establishes ESADDI levels for an essential nutrient when information is sufficient to establish a range of requirements but insufficient for establishing an RDA.

The ESADDIs for manganese are as follows: 0.3–0.6 mg/day for infants from birth to 6 months; 0.6–1.0 mg/day for infants from 6 months to 1 year; 1.0–1.5 mg/day for children from 1 to 3 years; 1.0–2.0 mg/day for children from 4 to 6 years; 1.0–2.0 mg/day for children from 7 to 10 years; and 2.0–5.0 mg/day for adolescents (>11 years) and adults (NRC 1989). These provisional dietary intake

ranges approximate the quantity that is usually delivered via the diet, although individual intakes may be higher or lower (see Section 5.5 and Table 5-4). However, the Food and Nutrition Board has cautioned that upper levels of the ESADDIs should not be exceeded on a routine basis because toxic levels may be only several times the usual intake levels (NRC 1989).

Effects that have been associated with manganese deficiency in animals include impaired growth (Smith et al. 1944), skeletal abnormalities (Amdur et al. 1944; Hurley and Keen 1987; Strause et al. 1986), impaired reproductive function in females and testicular degeneration in males (Boyer et al. 1942), ataxia (Hurley et al. 1961), altered metabolism of carbohydrates (Baly et al. 1988; Hurley et al. 1984) and lipids (Abrams et al. 1976a), and increased oxidation of mitochondrial membranes (Malecki and Greger 1995). Reduced high density lipoprotein (HDL) cholesterol, HDL protein, and HDL apo E levels have been noted in manganese deficient rats; these changes have been attributed to decreased cholesterol synthesis and excretion (Davis et al. 1990; Kawano et al. 1987).

The precise biochemical basis of the nutritional requirement for manganese in animals is not known, but at least three enzymes (arginase, pyruvate carboxylase, and superoxide dismutase) appear to require manganese for their proper function (Borgstahl et al 1992; Fridovich 1974; Kuhn et al. 1995; Leach and Lilburn 1978; Utter 1976). Primarily as a result of studies in the avian liver, researchers have found that pyruvate carboxylase catalyzes the first step of carbohydrate synthesis from pyruvate in the liver and kidney; the enzyme may have other functions in the brain as well. Paynter (1980) and Brock et al. (1994) observed that liver arginase activity was depressed in manganese-deficient rats. Moreover, Brock et al. (1994) noted that the depressed arginase activity resulted in depressed plasma urea and elevated plasma ammonia concentrations among these manganese-deficient rats. Superoxide dismutase catalyzes the oxidation of superoxide to molecular oxygen and peroxide (Fridovich 1974). Davis et al. (1990, 1992b) and Paynter (1980) found greatly decreased heart superoxide dismutase activity in rats fed manganese-deficient diets. Malecki and Greger (1995) demonstrated that decreases in cardiac manganese-dependent superoxide dismutase activity were strongly correlated with increased conjugated diene concentrations (indicative of oxidative damage) in heart mitochondria of manganese-deficient rats.

Manganese may also serve as a cofactor in other enzymatic reactions but is not permanently associated with the enzymes. For example, under *in vitro* conditions, manganese can stimulate hundreds of enzymes including phosphatases, kinases, thioesterases, peptidases, dehydrogenases, decarboxylases, and glycotransferases (Utter 1976). Decreased activity of manganese-activated enzymes (i.e., glycosyltransferase

and xylosyltransferase, which are involved in proteoglycan synthesis in bone) during manganese deficiency, is believed to be the cause of bone disease in cattle, goats, pigs, and poultry. This bone disease is characterized by enlarged hock joints, shortened legs, lameness, and joint pain (Hurley and Keen 1987).

Although low levels of manganese intake are necessary for human health, chronic exposure to high manganese levels may be harmful. Reports of adverse effects resulting from manganese exposure in humans are associated primarily with inhalation in occupational settings. Neurological effects are the hallmark of excessive exposure to manganese. The threshold for early or preclinical neurological effects observed has not been clearly defined from available information. Recently, mathematical models have been used to estimate threshold levels from some of these occupational studies. These occupational exposures are presumed to be a source of manganese in addition to daily intake from food and water. It is noted that exposure levels at which these early, preclinical effects have been seen (Iregren 1990; Lucchini et al. 1995; Mergler et al. 1994; Roels et al. 1987a, 1992) are at least five times greater than estimates of typical daily dietary intakes. Recently, environmental exposures to airborne manganese (Bowler et al. 1999; Mergler et al. 1999) have been associated with similar preclinical neurological effects and mood effects as are seen in occupational studies (Lucchini et al. 1995; Mergler et al. 1994). In these environmental studies, effects were most significant in older men with elevated blood manganese concentrations. Acute or intermediate exposure to excess manganese also affects the respiratory system. Inhalation exposure to high concentrations of manganese dusts (specifically manganese dioxide [MnO_2] and manganese tetroxide [Mn_3O_4]) can cause an inflammatory response in the lung, which, over time, can result in impaired lung function. Lung toxicity is manifested as an increased susceptibility to infections such as bronchitis and can result in manganic pneumonia. Pneumonia has also been observed following acute inhalation exposures to particulates containing other metals. Thus, this effect might be characteristic of inhalable particulate matter and might not depend solely on the manganese content of the particle.

Several reports have been located concerning adverse effects after oral manganese exposure. These studies, including recent studies in children, have numerous limitations that preclude firm conclusions about the potential for adverse effects from exposures to excess manganese. However, collectively, these studies suggest that ingestion of water and/or foodstuffs containing increased concentrations of manganese may result in adverse neurological effects.

Immunological effects, characterized by suppression of T and B lymphocytes, have been observed in workers exposed to manganese by inhalation (Boshnakova et al. 1989), but because the workers in this

study were also exposed to known immunotoxicants, it is impossible to know whether exposure to manganese was also related to the effects.

There is indirect evidence that reproductive outcomes might be affected (decreased libido, impotence, and sexual dysfunction have been observed in manganese-exposed men). The available studies on the effect manganese has on fertility (as measured by birthrate) is inconclusive. Two studies in men occupationally exposed to manganese show adverse effects on reproductive parameters: one measured sexual dysfunction, the other measured semen and sperm quality, but neither measured birthrate in wives of affected workers. Impaired sexual function in men may be one of the earliest clinical manifestations of manganism, but no dose-response information is currently available, so it is not possible to define a threshold for this effect. There is a lack of information regarding effects in women since most data are derived from studies of male workers.

Developmental data in humans exposed to manganese are limited and consist mostly of reports of adverse pulmonary effects from inhaling airborne manganese dust and adverse neurological effects following ingestion exposure. Animal studies indicate that manganese is a developmental toxin when administered orally and intravenously, but inhalation data concerning these effects are scarce and not definitive.

Manganese is normally found in human tissue, and levels in the human body are regulated by a homeostatic mechanism. Human data indicate that manganese levels in the body may increase following inhalation or oral exposure; however, they do not necessarily correspond with exposure levels. Levels of manganese in animal tissue have also been found to increase following oral or inhalation exposure. Dermal exposure to inorganic manganese is not a concern since this form has not been found to enter the body through undamaged skin. Current studies involving potential or known dermal exposure to organic manganese compounds indicate that absorption occurs, although significant toxicity was only clearly demonstrated in newts exposed to maneb (Arias and Zavanella 1979; Pacces Zaffaroni et al. 1978; Zavanella et al. 1984). It is likely that manganese levels in tissues rise following dermal exposure to organic manganese compounds, but no quantitative studies have been performed to date. No data were identified regarding the absorption of organic manganese compounds in humans or animals following dermal exposure. Therefore, it is unknown whether dermal absorption of organic manganese compounds could contribute to manganese tissue levels.

The mechanism of the toxic action of inorganic manganese at the molecular level has not been completely explained, and the evidence for mutagenicity in humans is equivocal. No conclusive studies on the potential genotoxicity of manganese in humans exist. Manganese has been found to be clastogenic in mice following oral exposure. *In vitro* results of both human and animal genotoxicity studies have been mixed; there is evidence to suggest that manganese may be potentially genotoxic, but the data are not sufficient to reach a conclusion.

No studies were found that reported cancer in humans associated with inorganic manganese. Although no firm conclusions can be drawn from the mixed results in animal studies, there is little data to suggest that inorganic manganese is carcinogenic.

For most people, food is the primary source of manganese exposure. The Environmental Protection Agency (1984a) has estimated that the typical human intake of manganese is 0.00046 mg/day (air), 0.008 mg/day (water), and 3.8 mg/day (food). It should be noted that manganese is an essential element for human health, functioning as a cofactor in a number of enzymatic reactions. The National Research Council's Food and Nutrition Board has suggested 2–5 mg manganese/day as an Estimated Safe and Adequate Daily Dietary Intake for children aged 11 years to adults. Consequently, the typical human exposure to manganese is not of concern as confirmed by numerous human and animal studies. However, humans exposed to manganese by the inhalation route in occupational settings may develop neurological symptoms, and individuals working in or living near hazardous waste sites could potentially be exposed to manganese through airborne dusts or the contamination of water sources. The neurotoxic effects and respiratory tract damage following the inhalation of manganese-containing respirable dusts have been well documented. Also, although there are no conclusive data, the epidemiological data suggest an association between ingesting water or food containing elevated concentrations of manganese and the development of neurological symptoms (Goldsmith et al. 1990; He et al. 1994; Iwami et al. 1994; Kawamura et al. 1941; Kilburn 1987; Kondakis et al. 1989; Zhang et al. 1995). Therefore, since potentially high levels of manganese may be found at hazardous waste sites, adverse neurological effects in persons working or living at or near these sites would be of potential concern. Potentially high manganese levels are also associated with industrial sources of manganese dust and from auto emissions (in areas where MMT is used as a gasoline additive); therefore, adverse neurological effects may be of concern to those living near these sources.

This profile discusses both inorganic and organic forms of manganese, the latter including MMT, maneb, mancozeb, and mangafodipir. MMT primarily affects the lung, maneb and mancozeb primarily target the kidneys and central nervous system. Mangafodipir, when given for several days at doses much higher than those recommended for its clinical use, is primarily a liver and kidney toxin, but also induces adverse developmental effects. Aside from mangafodipir, none of the adverse effects resulting from exposure to the remaining organic compounds can be clearly associated with the presence of manganese. Because few of the studies involving exposure to organic manganese compounds, excepting those concerning mangafodipir, have focused on manganese as the active agent, a critical evaluation of effects common to both inorganic and organic manganese compounds is not straightforward.

Minimal Risk Levels for Manganese.

While manganese is beneficial or essential at low intake levels, inhalation or oral exposure to high levels can cause adverse effects.

Inhalation MRLs.

- A chronic inhalation MRL of 0.00004 mg/m^3 was derived. As discussed in Section 2.2.1, the most sensitive and most significant effects caused by inhalation exposure to manganese dusts in the air are neurological deficits with progressive increased injury with prolonged exposures. For chronic inhalation exposure, Roels et al. (1992) reported that battery plant workers exposed to MnO_2 dust at 0.046 to 10.84 mg manganese/m^3 (total dust) and 0.021 to 1.317 mg manganese/m^3 (respirable dust) for 5.3 years exhibited impaired visual reaction time, eye-hand coordination, and hand steadiness. The benchmark dose (BMD) approach was selected to provide a surrogate NOAEL from the Roels et al. (1992) study. The BMD method defines an adverse effect as a risk level of more than an established percentage above background (Malsch et al. 1994). This risk level is determined by estimating a lower confidence limit (e.g., 95%) on a dose corresponding to a predetermined increase (e.g., 10%) in the incidence of a particular adverse effect for quantal data or a relative percentage change compared to the control group when continuous data are available. Sufficient data on individual participants' exposure levels and test performance results were provided by the Roels et al. (1992) study to inform the development of a dose-response relationship and calculation of a benchmark dose (Clewell and Crump 1999). A NOAEL of 0.074 mg/m^3 (respirable dust) was estimated at the 95% confidence limit for an increased risk of 10% ($BMDL_{10}$) for the effects observed. A second BMD analysis was performed using data on exposure and effects from individual participants in the Iregren (1990) study, which reported that workers exposed to a median concentration of 0.14 mg/m^3 total manganese dust for 1–35 years had below-average scores in a number of neurobehavioral tests including reaction time and finger tapping. The $BMDL_{10}$ value for the Iregren (1990) study was 0.071 mg manganese/m^3 (respirable dust), based upon the reported observation that the respirable fraction ranged upwards to 80% of the total dust measured. This $BMDL_{10}$ value was comparable to the one derived using the Roels et al. (1992) database, although there were differences in work environment and duration of exposure. More recently, Gibbs et al. (1999) reported that exposure to 0.051 mg manganese/m^3

(median, respirable dust) was a NOAEL among workers at a metal producing plant when evaluated using both novel and older neurobehavioral test methods. However, individual exposure and test performance data from this study were not available to ATSDR for conducting a benchmark dose analysis. Nonetheless, the NOAEL reported by Gibbs et al. (1999) is consistent with the $BMDL_{10}$ values derived from raw data provided by Drs. Roels and Iregren. This comparability across these studies supports the NOAEL from the benchmark dose analysis and strengthens the scientific basis for its use in deriving the MRL. Based on this BMD value, a chronic inhalation MRL of 0.00004 mg manganese/m^3 (0.04 µg manganese/m^3) was derived using (1) an uncertainty factor of 10 for human variability, (2) factors of 5/7 and 8/24 to account for intermittent exposure (5 days/week, 8 hours/day), (3) an uncertainty factor of 10 to account for limitations in the inhalation database, including the lack of data on developmental effects and data on the potential for reproductive effects in women, and the potential for differences in toxicity from different forms of manganese and, (4) a modifying factor of 5 for the potential for increased susceptibility in children based upon differences in the pharmacokinetic handling of manganese in the young. The studies by Roels et al. (1992) and Iregren (1990) are supported by another study by Roels et al. (1987a), in which workers exhibited decreased performance in comparable neurobehavioral tests along with increased incidences of weakness and decreased hand steadiness after being chronically exposed to 1 mg/m^3 of manganese dusts. Mergler et al. (1994) found that workers in a manganese alloy plant, exposed to a geometric mean concentration of 0.04 mg manganese/m^3 (in respirable dust), had increased levels of blood manganese. Further, the exposed workers showed decreased performance on motor function tests, particularly tests requiring coordinated or sequential movements at maximum speed. The workers also showed lower cognitive flexibility and difficulty in set shifting. Lucchini et al. (1995) observed the effects of manganese exposure to workers in a ferroalloy plant after a brief (1–45 days) cessation from work. These workers had been exposed to manganese from 1–28 years, but recent concentrations of total manganese in dust of 0.0027–0.27 mg/m^3 had decreased over the last 10 years from much higher values. These workers exhibited significantly decreased performance in similar neurobehavioral tests as with the other studies, but the blood and urine manganese levels were positively correlated with manganese exposure, as estimated by a cumulative exposure index (CEI). Also, the CEI was negatively correlated with test performance. A more recent study by Lucchini et al. (1999) reported significant differences in performance on different neurobehavioral exams based on the CEI of the workers whose exposure was defined as low, medium, or high. Dose-effect relationships were noted between test performance and CEI values, but not with blood or urine manganese levels. Further, using a LOAEL-based approach the authors estimated an exposure threshold value of 96.71 µg/m^3 total dust, based on the CEI for the medium exposure group whose duration of exposure was 11.51 years. The respirable dust fraction for this study was 40-60%; therefore, the estimated threshold value would be 38.7 µg/m^3. Roels et al. (1999) also reported a more recent prospective study on the effect of decreasing occupational manganese exposure on neurobehavioral test performance. This study also involved low, medium, and high exposure groups in a ferroalloy plant in which exposure concentrations decreased dramatically from 1987-1995, especially for the years 1992-1995. Performance on eye-hand coordination tests was inversely correlated with manganese exposure dose, but not visual reaction time or hand steadiness. Although all groups increased in performance on the eye-hand coordination test, that of the high-exposure group reached a plateau, despite dramatic decreases in exposure in 1994, suggesting a permanent effect from manganese exposure on the ability to perform this test. In addition, when a group of exposed workers was retested after a 3 year lack of exposure to manganese, their performance on the visual reaction time and hand steadiness tests did not significantly improve, indicating a permanent deficit in some types of neurobehavioral performance. This study indicated that only conditions following low-level manganese exposure may show signs of improvement following manganese abatement.

Adverse effects may sometimes occur after exposures to manganese for only several months (Rodier 1955), but quantitative data are not available to derive either acute- or intermediate-duration inhalation MRLs.

Oral MRLs.

No oral MRLs were derived for acute-, intermediate-, or chronic-duration exposure. Quantitative data are not available to derive acute-duration oral MRLs. In the intermediate-duration study by Chandra and Shukla (1978), neuronal degeneration in neonatal rats, a serious effect, was reported after 1 mg manganese/kg/day as manganese chloride was administered. However, a NOAEL for decreased brain dopamine levels and enzyme levels was observed in adult rats also administered 1 mg manganese/kg/day as manganese chloride in the study by Deskin et al. (1980). LOAELs for alterations in dopamine and other brain neurotransmitter levels and enzyme activity were observed at 10–14 mg manganese/kg/day as manganese chloride (Bonilla and Prasad 1984; Deskin et al. 1980; Subhash and Padmashree 1991). These doses are the lowest tested in the intermediate-duration database and no threshold level for effects could be determined as the basis for an intermediate-duration MRL.

Data on the effects of manganese following chronic oral exposure are less extensive than those available on inhalation exposure and are not sufficient to derive a chronic oral MRL. However, the available literature reports do show that neurological effects similar to those seen after inhalation exposure may be anticipated following chronic oral exposure to levels above estimated daily intake. In most chronic oral studies examined (Holzgraefe et al 1986; Iwami et al. 1994; Kawamura et al. 1941; Kilburn 1987; Kondakis et al. 1989), there is uncertainty regarding the exposure level or whether the effects were solely attributable to manganese, so these studies are not suitable for the derivation of a chronic oral MRL value.

Two studies in children (He et al. 1994; Zhang et al. 1995) have shown that ingestion of well water containing at least 0.241 mg manganese/L (measured for 3 years) and food fertilized with sewage containing increased levels of manganese or the actual intake levels of food and drinking water containing excess manganese levels is associated with poorer performance in school and on the WHO core test battery neurobehavioral exams. Exposed children, aged 9–11, were compared to children of the same age who drank water with 0.04 mg manganese/L. Although manganese concentrations were measured for 3 years, the reports did not state how long the children were exposed to increased manganese. Neither study

reported total daily manganese intake in either exposed or control groups and thus neither was suitable for estimating a chronic oral MRL.

Another study involving adults (Vieregge et al. 1995) reported no difference in the neuromotor functioning of people chronically exposed to concentrations of manganese as high as 0.3 mg/L in drinking water, as compared to drinking water containing only 0.05 mg/L of the metal. The authors could not control for other sources of water ingested by the study populations, and blood manganese concentrations did not significantly differ between exposed and control populations. The highest level of manganese intake reported in this study is 0.6 mg/day (based on an assumed water intake of 2 L/day). This value, although higher than estimates of daily manganese intake from water in the U.S. (0.008 mg/day), is still well below the ESADDI for adults. Therefore, this study was also not appropriate for use to derive a chronic oral MRL.

The wide variability in human intake (from all sources) makes it difficult to determine normal human exposure compared to levels that might be harmful, particularly to those with potentially increased manganese susceptibility. No firm conclusions on a critical effect level versus essential dietary levels for manganese were considered possible. The upper range of the ESADDI (5 mg/day) was used to derive a provisional guidance value for total dietary intake of 0.07 mg/kg/day (5 mg/day divided by 70 kg, the weight of an adult) for ATSDR human health assessments until more current information on actual intake levels of manganese across environmental media can be obtained. In addition, information that provides a clearer understanding of the potential for harmful effects from known levels of exposure to excess manganese in the environment is needed. This guidance is necessary because of the prevalence of manganese at hazardous waste sites and the fact that manganese is an essential nutrient.

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

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

More detailed information on the adverse effects of inhalation and oral exposure to manganese is presented below.

Death. No studies have been identified that conclusively link inhalation exposure to manganese with death in humans. A single study was found evaluating the possibility of increased death in humans resulting from inhalation exposure to manganese (Hobbesland et al. 1997a). This occupational study found a slight increase in the number of deaths as a result of pneumonia in furnace workers who worked in plants manufacturing iron-manganese and silicomanganese alloys. The authors could not discount other causes of the pneumonia, however, such as correlated work exposures. A similar study with the same cohort (Hobbesland et al. 1997b) reported an increase in cardiac-related sudden death among furnace workers in the alloy plants. However, the authors stated that the link to manganese was speculative, as other factors (stress, heat, noise) could have caused the deaths.

There is one report of two people who died after ingesting manganese-contaminated well water (Kawamura et al. 1941), but there is considerable doubt that the deaths were due to manganese (see Section 2.2.2.4). Oral gavage administration of highly concentrated manganese in water (16,000–44,000 mg manganese/L) can cause death in animals (Kostial et al. 1978; Rehnberg et al. 1980; Smyth et al. 1969), and intravenous administration of 4.4 mg manganese (as $MnCl_2$)/kg/day to beagle dogs for 4 days resulted in the death of 1 out of 4 dogs, with 2 more being sacrificed as moribund (Khan et al. 1997). However, manganese given to animals in feed at similar levels was tolerated for up to 2 years. MMT was found to have an inhalation LD_{50} of 62 mg manganese/m^3 (Hinderer 1979) and an oral LD_{50} of 12.5 mg manganese/kg (50 mg MMT/kg) in the rat, when administered by gavage (Hanzlik et al. 1980). The inhalation dose required to cause death in this species is 12 times the allowable exposure level in occupational environments. The concentration resulting in death from oral exposures is not expected in the environment or near hazardous waste sites given current or future predictions concerning MMT use (see Chapter 5). A single dose of maneb at 32 mg/kg did not cause death in adult male Swiss mice (Mitchell et al. 1989). Deaths from acute exposures to mancozeb have resulted from doses as high as 11,250 mg/kg. For mangafodipir, lethality is strain and administration specific. Lethality from mangafodipir is generally achieved with a lower dose when given as a bolus injection, rather than a slow infusion; acute LD_{50} values range from 103 mg manganese/kg in male and female mice (bolus) to 2,916 mg/kg , also in mice (infusion) (Larsen and Grant 1997). These findings indicate that manganese has low acute toxicity by both the oral and inhalation routes and that ambient manganese levels in the water or air are not expected to be of concern. Death is not expected to result from ingestion or inhalation of

manganese from excess exposure levels which may be encountered in the environment or near hazardous waste sites.

Systemic Effects. The few available inhalation and oral studies in humans and animals indicate that inorganic manganese exposure does not cause significant injury to heart, stomach, blood, muscle, bone, liver, kidney, skin, or eyes (Hejtmancik et al. 1987a, 1987b [oral]; Shiotsuka 1984 [inhalation]; Ulrich et al. 1979b [inhalation]). However, if manganese is in the (VII) oxidation state (as in potassium permanganate), then ingestion or dermal contact may lead to severe corrosion at the point of contact (Southwood et al. 1987). Manganese has been found to have an affinity for melanin (it was found to bind to beef eye, human hair, and synthetic dopamine melanin) although functional damage has not been reported (Lydén et al. 1984).

The majority of oral and inhalation studies for selected organic manganese compounds (MMT, maneb and mancozeb) in humans and animals indicate that these compounds do not cause significant injury to heart, stomach, blood, muscle, bone, skin or eyes. Injection studies in humans using mangafodipir at clinical doses show that the compound does not have significant systemic toxicity. At increased doses in animals, the compound does not cause significant injury to blood, muscle, bone, skin or eyes.

Respiratory Effects. Inhalation exposure to manganese dusts often leads to an inflammatory response in the lungs of both humans and animals. This generally leads to an increased incidence of cough and bronchitis (Lloyd Davies 1946; Roels et al. 1987a; WHO 1987) and can lead to mild-to-moderate injury of lung tissue (Lloyd Davies 1946; Shiotsuka 1984; Suzuki et al. 1978; Zaidi et al. 1973) along with minor decreases in lung function (Roels et al. 1987a). In addition, susceptibility to infectious lung disease may be increased (Adkins et al. 1980b; Maigetter et al. 1976), leading to increased pneumonitis and pneumonia in some manganese-exposed worker populations (Lloyd Davies 1946; Lloyd Davies and Harding 1949). These effects have been reported primarily in workers exposed to fairly high concentrations of manganese dusts in the workplace, although there are some data that indicate that, in populations living and attending school near ferromanganese factories, there was an increased prevalence of respiratory effects (Kagamimori et al. 1973; Nogawa et al. 1973; WHO 1987). The risk of lung injury in people exposed to the levels of manganese typically found in the general environment is expected to be quite low (EPA 1985d). However, exposure to manganese-containing dusts from factories, mining operations, automobile exhaust or other sources may be of concern.

It should be noted that these effects on the lung are not unique to manganese-containing dusts but are produced by a variety of inhalable particulate matter (EPA 1982). On this basis, it seems most appropriate to evaluate the risk of inflammatory effects on the lung in terms of total suspended particulate matter (TSP) or particulate matter smaller than 10 μm in diameter (PM_{10}), as well as the concentration of manganese in the air (EPA 1985d).

Studies involving controlled inhalation exposures in humans or animals to MMT are not available because the compound breaks down readily in light to form inorganic manganese compounds (Lynam et al. 1999). Rats exposed to high concentrations of car exhaust containing oxidation products from MMT-containing fuel exhibited labored breathing (Hinderer 1979). MMT administered via gavage in oil to rats at a doses of 20–38 mg Mn/kg or by injection at doses ranging from 0.5–9 mg Mn/kg typically cause dark red lungs that show extensive mottling, hemorrhage, edema, and congestion of the alveoli with proteinaceous fluid (Clay and Morris 1989; Cox et al. 1987; Hanzlik et al. 1980; Hinderer 1979; Hysell et al. 1974). Injected doses of 1.3–2.1 mg Mn/kg in the rat, 30 mg Mn/kg in the mouse, and 45 mg Mn/kg in the hamster have resulted in necrosis of the Clara cell in the bronchioles within 1 day of injection. This is followed by proliferation of Clara cells and type II epithelial cells (Hakkinen and Haschek 1982; Haschek et al. 1982; Verschoyle et al. 1993). An increase in protein levels in broncheoalveolar lavage fluid (BALF) is a hallmark of MMT-induced respiratory toxicity (Clay and Morris 1989; McGinley et al. 1987), except for the specific case of the protein CC16, so named because it is produced by the Clara cells. This protein decreases in both BALF and serum following MMT exposure (Halatek et al. 1998; Bernard and Hermans 1997). The reduction is thought to be due to decreased synthesis and/or secretion of the protein due to the loss of the producing cells (Halatek et al. 1998).

Maneb and mancozeb are not associated with respiratory toxicity following inhalation, oral, or dermal exposures. Mangafodipir has caused dyspnea in rats at acute doses of 160 mg manganese/kg (Larsen and Grant 1997). Exposure to maneb or mancozeb at concentrations that might be found in the environment or at hazardous waste sites are unlikely to result in any adverse respiratory effects in humans. Although MMT is a potent respiratory toxin when injected, the adverse toxicity following inhalation exposure to MMT is unknown. Further, exposure to this compound is expected to be limited to inhalation exposure of its combustion products and would be similar to those discussed for inorganic manganese oxides.

Cardiovascular Effects. Three studies reported adverse cardiovascular effects after occupational exposure to manganese. Saric and Hrustic (1975) observed a lower mean systolic blood pressure in male workers at a ferromanganese plant. Manganese concentrations in the plant ranged from 0.4 to 20 mg/m^3, but specified data on exposure levels were lacking. More recently, Jiang et al. (1996a) observed a significant increase in the incidence of low diastolic blood pressure with a mean manganese concentration of 0.13 mg/m^3 in male and female workers at a manganese processing plant. Hobbesland et al. (1997b) reported a significant increase in sudden death in ferromanganese/silicomanganese furnace workers during active-time, especially when compared to non-furnace workers. Causes of death included all natural causes, but focused primarily on cardiotoxicity. The authors cautioned that the link between sudden death from cardiotoxicity and manganese exposure was speculative.

A few animal studies reported no adverse cardiovascular effects, after oral exposure, based on gross and histological examination (Hejtmancik 1987a, 1987b; NTP 1993). One study involving intravenous administration of 4.4 mg manganese as $MnCl_2$/kg/day to beagle dogs found that this dose of manganese resulted in hypotension and tachycardia (Khan et al. 1997). These effects were considered, by the authors, to be secondary to severe hepatotoxicity. Another study investigated the cardiotoxicity of manganese using isolated rat hearts infused with increasing concentrations of $MnCl_2$ (Brurok et al. 1997). Manganese caused a decrease in contractile function and heart rate with EC_{50} values of 0.7 and 28 µg manganese (as $MnCl_2$), respectively. These effects were rapidly reversed upon manganese washout, except at very high concentrations (90 µg manganese). The significance of available information about cardiovascular effects in humans is difficult to interpret and the results from animal studies do not provide definitive evidence for manganese-induced cardiotoxicity. Therefore, the potential for adverse cardiovascular effects in the general population of people exposed to inorganic manganese near hazardous waste sites does not appear to be of great concern at this time.

MMT is not associated with cardiotoxicity. Inhalation exposure to this compound is likely to be primarily to its combustion products. It is currently unknown what the levels of exposure would be should MMT undergo widespread use in the U.S. Therefore, the potential for cardiotoxicity resulting from increased exposure to manganese from this source is not believed to be a concern at this time.

Potential myocardial ischemia resulted from a 2–day exposure to 229 mg maneb/kg in an adult male farmer (Koizumi et al. 1979); however, a similar exposure to 15,700 mg/kg manzidan (maneb and zineb combined) resulted in no clinical signs of cardiotoxicity. Another acute exposure to a much lower dose

(1.1 mg maneb/kg/day) also resulted in no signs of cardiotoxicity. Although the data are equivocal, they indicate that low levels of maneb do not appear to pose a significant risk of cardiotoxicity to humans. Data are very limited on the levels of manganese-containing pesticides that might be encountered at hazardous waste sites, or persisting at other environmental locations. Thus, it is unknown if levels of these pesticides present in the environment might be associated with adverse cardiac effects.

Mangafodipir causes facial flushing (from vasodilation) in both humans and animals following i.v. exposure (Dearls and Bluemke 1999; Larsen and Grant 1997). No adverse effects on cardiac function were observed in clinical trials, volunteer studies, and studies using dogs with induced heart failure (Earls and Bluemke 1999; Elizondo et al. 1991; Karlsson et al. 1997; Wang et al. 1997). One study reported a decrease in heart rate in dogs administered a dose that was 20 times the recommended clinical dose (Larsen and Grant 1997). The data indicate that individuals who might have limited exposure to mangafodipir at clinical doses (e.g. liver cancer patients) need not be concerned about cardiotoxicity arising from the compound.

Gastrointestinal Effects. No information concerning gastrointestinal effects in humans following inhalation exposure to inorganic manganese was available. A single human case report indicated that ingestion of manganese in the form of potassium permanganate caused local corrosion but no systemic toxicity (Southwood et al. 1987). The few animal studies available reported adverse gastrointestinal effects including hyperplasia and erosion of the forestomach after oral exposure to inorganic manganese in mice but not in rats (Hejtmancik 1987a, 1987b; NTP 1993). The significance of these effects is unknown since they occurred at such a low incidence in only one species and only after exposure to fairly large doses. Gavage exposure to MMT at lethal doses of 20–37.5 mg Mn/kg resulted in discolored and spotted intestinal tracts, fluid-filled with thin walls (Hinderer 1979; Hysell et al. 1974). Exposures of farmers and pesticide sprayers to maneb at doses ranging from 1–229 mg/kg/day for 1–2 days have resulted in nausea, vomiting, and diarrhea (de Carvalho et al. 1989; Koizumi et al. 1979). The relevance of the data on inorganic manganese to humans is unknown. The data from oral exposures to organic manganese indicate that short-term exposures to very high doses, such as in the workplace, may be of concern.

Hematological Effects. In studies of occupationally exposed workers to both inorganic and organic manganese, no adverse hematological effects of inhalation exposure have been reported. No information was available concerning hematological effects (red blood cell count, etc.) in humans following oral exposure to inorganic manganese. A few animal studies reported no adverse effects after inhalation

exposure (EPA 1977; Shiotsuka 1984; Ulrich et al. 1987b), but some alterations in hematological parameters (including decreases in red blood cell, leukocyte, and neutrophil counts) were reported in animals following oral exposure (Hejtmancik 1987a, 1987b; Komura and Sakamoto 1991; NTP 1993). The biological significance of these effects were unclear as the hematological effects varied according to species, duration of exposure, and form of manganese administered, and the authors did not consider these effects to be clearly compound-related. The available human data in combination with the limited evidence for hematological effects demonstrated by animal models suggests that hematological effects would be of little concern to the general population or to persons exposed near hazardous waste sites.

Musculoskeletal Effects. No information was available concerning musculoskeletal effects in humans following exposure to inorganic manganese. A few animal studies reported no adverse musculoskeletal effects after oral exposure (Hejtmancik 1987a, 1987b; NTP 1993). Exposure to 229 mg maneb/kg/day for 2 days in a farmer resulted in muscular weakness (Koizumi et al. 1979), while another man exposed to 15,700 mg maneb and zineb combined for 2 days suffered tonic and clonic convulsions and slight hemiparesis (Israeli et al. 1983a). A mill worker exposed to 16,000 mg maneb/kg for 16 hours/week for 8 months during a 2-year period developed a resting tremor in all 4 limbs and in his lips; this effect was secondary to neurological toxicity (Meco et al. 1994). Hind limb paralysis was observed in male rats given mancozeb by gavage at doses of 375 mg/kg (Kackar et al. 1997a; 1997b; Trivedi et al. 1993). These effects may also have been secondary to neurotoxicity. The data concerning musculoskeletal effects in humans or animals following exposure to manganese (either inorganic or organic) is limited and involves exposures to relatively high doses. Therefore, it is not possible to make a prediction concerning the likelihood of musculoskeletal effects arising from exposure to manganese at hazardous waste sites or in environmental settings. It is not possible to predict if environmental levels of manganese would cause musculoskeletal effects either directly or as a secondary effect to neurotoxicity. However, these effects would be of concern to individuals exposed to increased concentrations of manganese originating from hazardous waste sites or other areas.

Hepatic Effects. Little information was available regarding hepatic effects in humans. Liver function studies and serum levels of hepatic enzymes have been found to be normal in occupationally exposed workers with chronic inhalation exposure to inorganic manganese (Mena et al. 1967). No information was available regarding oral exposure to inorganic manganese in humans. Animal studies (both inhalation and oral exposure) indicate possible slight effects such as minor histological changes and liver weight changes (NTP 1993; Ulrich et al. 1979b; Wasserman and Wasserman 1977); however, these may be considered

adaptive changes. Rodents given MMT orally by gavage at doses of 20-38 mg Mn/kg have severe liver toxicity including mottling; scattered hepatocytes with cytoplasmic vacuoles; acute centrolobular passive congestion; hepatic parenchymal necrosis and leukocytic infiltration; extensive cytoplasmic vacuolar change (Hanzlik et al. 1980; Hinderer 1979; Hysell et al. 1974). However, the evidence across several studies suggests a potential concern for these effects in humans, although it is not believed to be of any great concern because ingestion of MMT from the environment is not considered to be a primary pathway of exposure. Although the doses resulting in these toxic effects are not much larger than the amount of manganese found in the diet, the effects are not necessarily associated with manganese, but with MMT itself. Therefore, there is no evidence to suggest that hepatic effects would be of great concern to the general population or to persons exposed to inorganic or organic manganese near hazardous waste sites. Exposure to very large amounts of maneb or mancozeb following acute exposures has not been associated with any significant adverse hepatic effects. Exposure to mangafodipir at i.v. doses up to 20 times the recommended clinical dose has resulted in increases in liver enzymes and liver histopathology in dogs and monkeys (Larsen and Grant 1997), but clinical and volunteer trials in humans have not reported any adverse hepatic effects at doses as high as 2 times the clinical dose (Elizondo et al. 1991; Wang et al. 1997). Therefore, exposure to maneb or mancozeb at concentrations that may be encountered at hazardous waste sites or used around the home (with adequate protection) are not expected to be a concern.

Renal Effects. Little information on inorganic manganese was available regarding renal effects in humans. Abnormalities were not observed in the urine of workers chronically exposed to manganese by the inhalation route (Mena et al. 1967), but other renal parameters have not been studied. No information was available regarding oral exposure in humans or inhalation exposure in animals to inorganic manganese compounds. Some oral studies in animals reported no adverse effects in rats or mice (Gray and Laskey 1980; Hejtmancik 1987a, 1987b), although one study found that the severity of endogenous nephropathy was increased in male rats with 2-year oral exposures (NTP 1993). The adverse renal effects noted in the animal models are gender and species specific and are too limited to draw firm conclusions regarding renal effects in humans, particularly in the absence of human data.

Gavage dosing of rats at 20–38 mg Mn/kg (as MMT) resulted in the following signs of renal toxicity: vacuolar degeneration of proximal convoluted tubules; hyaline droplet change; and distention of the glomerular space and tubule lumens with a granular, basophilic substance (Hanzlik et al. 1980; Hysell et al. 1974). However, oral exposure to MMT is not expected to be a prominent pathway of exposure to this

compound and gavage dosing is not representative of exposures that might occur at or around hazardous waste sites. Acute exposure to doses of maneb 1–229 mg maneb/kg has been associated with toxicity and renal failure (de Carvalho et al. 1989; Koizumi et al. 1979). However, with appropriate clinical treatment, the symptoms of renal toxicity subsided and histopathological analyses revealed no significant remaining cellular damage (de Carvalho et al. 1989). Exposure to mangafodipir at doses up to 20 times the clinical dose have resulted in kidney toxicity, but these signs abated upon removal of the compound in an adequate clearing period (Larsen and Grant 1997). Environmental levels of MMT, maneb, and mancozeb are unknown; therefore, it is uncertain if exposures to these compounds at these locations would result in kidney toxicity. There is little expectation that mangafodipir would be found in the environment; therefore, kidney toxicity from environmental exposure to mangafodipir should not be a great concern.

Endocrine Effects. Most human studies did not measure endocrine parameters. One inhalation study of chronically-exposed workers reported elevated levels of serum prolactin and cortisol (Alessio et al. 1989); another human study involving a shorter exposure reported no changes in FSH, LH, or prolactin levels (Roels et al. 1992). Smargiassi and Mutti (1999) reported elevated serum prolactin levels in a subset of 20 ferromanganese workers in a follow-up study to a previous occupational investigation. Serum prolactin levels were significantly elevated over earlier levels, taken 5 years previous, which were themselves significantly higher than control levels. Exposure to airborne manganese was believed to be consistent over that time period, with exposure levels not decreased. No animal endocrine studies with inhalation exposures were located. Short-term animal studies and some of the long-term animal studies were negative for endocrine effects following oral exposure to manganese (NTP 1993). One intermediate study investigated endocrine effects on male rats dosed intraperitoneally with 6.6 mg manganese/kg/day as $MnCl_2$ (Hong et al 1994). The rats in this study exhibited significant decrease in body weight, as well as a decrease in circulating testosterone, and a significant increase in substance P in the hypothalamus and neurotensin in the pituitary. Two other studies in rats reported that Mn_3O_4 in food, given at a dose of 350 mg manganese/kg/day for 224 days (starting on day 1 of gestation and continuing for 224 days); (Laskey et al. 1982) and 214 mg manganese/kg/day given up to 28 days (Laskey et al. 1985), resulted in reduced testosterone levels in male rats. The biological significance of this effect is unknown since the decrease had no result on fertility and in the latter study (Laskey et al. 1985), there were no observed effects on the hypothalamus or pituitary.

Thyroid function tests were normal for a farmer that 6 months prior had been exposed for 2 days to 229 mg maneb/kg; no thyroid tests had been performed just after the exposure occurred (Koizumi et al. 1979).

Intermediate and chronic dosing of mancozeb by gavage to rats at doses as low as 375 mg/kg resulted in decreased circulating thyroxine (T_4) levels and increased thyroid:body weight ratios after 30 days exposure, and resulted in decreased thyroid peroxidase (at 1,125 mg/kg); hypertrophy and hyperplasia of follicular cells with loss of colloid were also observed (Kackar et al. 1997b; Trivedi et al. 1993). Doses of 750 and 1,125 mg/kg mancozeb resulted in significant inhibition in thyroid radioiodine uptake after 90 days exposure in the chronic (360 day) study (Kackar et al. 1997b). Maneb injected i.p. into rats at doses of 5–40 mg/kg decreased cold-stimulated thyroid stimulating hormone (TSH), but had no effect on TSH-stimulated thyroid releasing hormone, TRH (Laisi et al. 1985). Maneb dosing also had no effect on circulating T_3 or T_4 levels.

Although the studies in humans indicate a general lack of significant effect, the studies in animals indicate that exposure to inorganic manganese can result in decreases in reproductive hormones, with no apparent effect on fertility. Although other reproductive effects have been observed in humans as a result of manganese exposure, the data are not conclusive. It is not clear that exposures to inorganic manganese at hazardous waste sites or in the environment would result in significant endocrine effects. Animal studies with manganese pesticides reveal an effect on the hypothalamic-pituitary axis, resulting in significant hormonal changes. However, the functional or clinical effects of these hormonal changes to the normal functioning of the species investigated is unknown. The only study that investigated endocrine function in a human exposed to manganese pesticides did so well after the acute exposure event and reported no effect. It is not clear whether the levels of organic manganese compounds that might be encountered at hazardous waste sites or in the environment would result in adverse endocrine changes.

Dermal Effects. No information was available regarding dermal effects from inorganic manganese exposure in humans. Animal studies reported no adverse dermal effects in rats or mice orally dosed with manganese (Hejtmancik 1987a, 1987b; NTP 1993). Dermal absorption of inorganic manganese would be expected to be minimal, unless manganese complexes or ions were dissolved in the appropriate solvent. However, there are no studies available that examine the effects of dermal exposure to inorganic manganese. Dermal effects observed after dermal exposure to MMT are limited to two animal studies. These data indicate that MMT is either a moderate skin irritant (Hinderer 1979) or a compound safe for intact or abraded human skin contact (Campbell et al. 1975). MMT in concentrated form is absorbed through the skin (Campbell et al. 1975). However, no studies were identified regarding dermal absorption of MMT, any subsequent health effects from such absorption, or contribution to manganese tissue levels. Allergic contact dermatitis has been reported in people handling organomanganese pesticides (maneb and

mancozeb) and pesticide-treated plants, but dermal penetration, potential contribution to manganese levels in the body, and any further health effects were not considered in these studies (Adams and Manchester 1982; Burry 1976; Crippa et al. 1990; Iliev and Elsner 1997; Kleibl and Rá…ková 1980; Koch 1996; Lisi and Caraffini 1985; Manuzzi et al. 1988; Matsushita et al. 1976; Nater et al. 1979; Oakley 1988). Dermal application of maneb at a dose equivalent to 0.0003 mg/kg on the skin of a hairless dog resulted in moderate dermatotoxicity, characterized by slight to moderate changes to the skin. These changes included epidermal edema, degeneration, and hyperplasia, with vasodilation and cellular infiltration of the dermis (Kimura et al. 1998). Mancozeb induced ornithine decarboxylase activity in mouse skin in a dose-dependent fashion; further, the pesticide increased the rate of DNA synthesis (Gupta and Mehrotra 1992). However, maneb and mancozeb are readily degraded in the environment and exposure to the parent compound is not likely for the general population.

Ocular Effects. No information was available regarding ocular effects in humans. Two animal studies reported no significant ocular effects from oral inorganic manganese exposure (Hejtmancik 1987a, 1987b). Airborne exposures of rats to high (unreported) doses of MMT resulted in conjunctivitis (Hinderer 1979). Maneb, mancozeb and mangafodipir are not associated with ocular toxicity. Therefore, there is no evidence to suggest that ocular effects would be of concern to the general population or to persons exposed near hazardous waste sites.

Body Weight Effects. The only information available regarding body weight effects in humans was from 1 instance of body weight increasing 11% following edema prompted by renal failure in a man acutely exposed to 1.1 mg maneb/kg (de Carvalho et al. 1989). These limited data indicate that exposure to organic manganese does not appear to cause weight reductions in humans.

In some animal studies, significantly decreased body weights were reported, although in some of these studies, the manganese dose resulted in decreased consumption of diet due to taste aversion; for example, in a 14-day rat study at 1,300 mg manganese/kg/day (as $MnSO_4$), body weight gains were decreased by 57% for males and 20% for females (NTP 1993). In a 14-day mouse study (NTP 1993) with $MnSO_4$, although some exposed groups had significantly reduced body weight gains compared to controls, the authors noted that no conclusions could be made regarding the effects of the metal on this parameter due to poor randomization at study initiation. Ulrich et al. (1979b) observed no effect on body weight in rats that inhaled Mn_3O_4 at concentrations up to 1.1 mg manganese/m^3 continuously for 9 months and Shiotsuka (1984) observed no effect on body weight in male and female rats that inhaled MnO_2 at up to 138 mg

manganese/m^3 for 6 hours/day for 10 days. Orally administered $MnCl_2$ given to pregnant rats by gavage in water on gestational days 6–17 had no observable effects on the body weights of the dams (Grant et al. 1997). Brenneman et al. (1999) and Dorman et al. (2000) reported a <10% decrease in body weight in PND 49 and PND 21 rats, respectively, who were administered 22 mg manganese/kg/day (as MnCl2) daily (the Brenneman study only dosed the neonates 5 days/wk from day 22–49). The earlier study only reported significant decreases in body weight on day 49, although body weights were recorded twice a week; the latter study reported body weight differences in exposed rats from PND 11–21. Because weight effects have not been reported in humans and since body weight changes have not been consistently reported in animal studies, there is little evidence to suggest that adverse body weight effects would be of concern to the general population or persons exposed near hazardous waste sites.

Exposure to MMT has been associated with decreases in body weight when given acutely or chronically via diet or gavage (Hinderer 1979; Komura and Sakamoto 1992). Other studies have not seen an a similar effect (Hanzlik et al. 1980). Due to the lack of data on levels of inorganic or organic manganese compounds in the environment and at hazardous waste sites, it is unknown whether body weight effects would be of great concern to the general public.

Metabolic Effects. Acute exposure of 2 men to maneb at concentrations of 1.1 or 229 mg/kg resulted in metabolic acidosis (de Carvalho et al. 1989; Koizumi et al. 1979). No other information on potential metabolic effects of inorganic or organic manganese was located. Due to the limited information concerning metabolic effects resulting from exposure to manganese pesticides and the wide range of doses that resulted in effects, it is unclear what effects might occur to the population at large who may be exposed to current levels of these compounds at hazardous waste sites or in the environment.

Immunological and Lymphoreticular Effects. One study was located regarding immunological effects in humans following inhalation exposure to manganese. Male welders exposed to manganese by inhalation exhibited suppression of the T and B lymphocytes, but there were confounding factors in this study including exposure to other known immunotoxicants (Boshnakova et al. 1989). Animal studies of manganese exposure by the oral route have reported decreased lymphocyte and neutrophil counts in males and decreased total leukocytes in females (NTP 1993). Male rats fed 32–520 mg manganese/kg/day for 13 weeks had decreased lymphocytes at $130 mg manganese/kg/day, while total leukocyte count was decreased in females fed $155 mg manganese/kg/day (NTP 1993). Studies in animals exposed to $MnCl_2$ by intraperitoneal or intramuscular injection indicate that manganese can influence several immunological

cell types. For example, manganese treatment stimulates macrophage and natural killer cell activity in mice, possibly by the increased production of interferon (Rogers et al. 1983; Smialowicz et al. 1985, 1987). Manganese also alters the responsiveness of lymphoid cells to mitogens and inhibits antibody production in response to a T-dependent antigen (Hart 1978; Lawrence 1981; Srisuchart et al. 1987). The limited evidence for T and B cell suppression in conjunction with the animal studies makes it difficult to judge whether these manganese-dependent modulations in immune cell activity are likely to result in clinically significant impairment of the immune function in the general population or in persons exposed near hazardous waste sites. This is an area of potential concern. However, studies in animals indicate that impaired immune function is not responsible for the increased sensitivity to lung infection discussed above (Adkins et al. 1980c). Human exposure to an acute dose of 1.1 mg maneb/kg did not result in any measured immunological effects (de Carvalho et al. 1989).

Mangafodipir administration in animals has been associated with slight eosinopenia and eosinophilia and reduction of toxic neutrophils following exposure to doses several times the recommended clinical dose (Larsen and Grant 1997). No adverse immunological effects have been observed in clinical trials (Earls and Bluemke 1999). Immunological effects are not a concern for individuals exposed to mangafodipir.

No information was available regarding lymphoreticular effects in humans or animals.

Neurological Effects. There is clear evidence from studies of humans exposed to manganese dusts in mines and factories that inhalation of high levels of manganese can lead to a series of serious and ultimately disabling neurological effects (Emara et al. 1971; Rodier 1955; Saric et al. 1977a; Schuler et al. 1957). This disease, termed manganism, typically begins with feelings of weakness and lethargy. As the disease progresses, a number of other neurological signs may become manifest. Although not all individuals develop identical signs, the most common are a slow and clumsy gait, speech disturbances, a masklike face, and tremors. There is evidence that indicates that the neurological symptoms may be improved in some cases (Shuqin et al. 1992; Smyth et al. 1973); in most cases, however, the symptoms were found to be irreversible, persisting for many years after exposure ceases (Cotzias et al. 1968). In addition, a syndrome of psychological disturbances (hallucination, psychosis) frequently emerges, although such symptoms are sometimes absent (e.g., Cook et al. 1974). As the disease progresses, patients develop severe hypertonia and muscle rigidity and may be completely and permanently disabled (Rodier 1955).

Subclinical neurological effects have been observed in several occupational studies (Chia et al. 1993, 1995; Crump and Rousseau 1999; Iregren 1990; Lucchini et al. 1995, 1999; Mergler et al. 1994; Roels et al. 1987, 1992, 1999; Wennberg et al. 1991). These effects included decreased performance on neurobehavioral tests; significantly poorer eye-hand coordination, hand steadiness, and reaction time; poorer postural stability; and lower levels of cognitive flexibility. One occupational study reported a lack of significant neurological effects (Gibbs et al. 1999). In addition, a study on environmental manganese sources, including a manganese alloy plant as a potential point source, indicates that both men and women are adversely affected by nonoccupational exposure to manganese as evidenced in performance on neurobehavioral tests and increased neuropsychiatric symptoms (Baldwin et al. 1999; Beuter et al. 1999; Bowler et al. 1999; Mergler et al. 1999). In these studies, a blood manganese level-age interaction was observed, with the poorest performance occurring among those older than 50 years-of-age and those with the highest blood manganese levels (Mergler et al. 1999).

There is limited evidence that oral exposure to manganese leads to neurological effects similar to those reported for inhalation exposure (Banta and Markesbery 1997; Cawte et al. 1987; Goldsmith et al. 1990; He et al. 1994; Kawamura et al. 1941; Kilburn 1987; Kondakis et al. 1989; Zhang et al. 1995). Although it was established in the Kawamura et al. (1941), Kilburn (1987), Kondakis et al. (1989), and Goldsmith et al. (1990) studies that the persons studied exhibited neurological symptoms resembling manganism and were exposed to excess manganese, there were numerous limitations in these studies and the data were not sufficient to conclude that the symptoms could be attributed solely to manganese exposure. Although there are limitations to interpreting their results, He et al. (1994) and Zhang et al. (1995) are among the first authors to evaluate neurological effects in children that ingested excess manganese from their environment.

Inhalation or oral studies in animals exposed to manganese have sometimes revealed biochemical or neurobehavioral evidence of neurological effects (Ali et al. 1983a; Bird et al. 1984; Bonilla and Prasad 1984; Chandra 1983; Chandra and Shukla 1978, 1981; Deskin et al. 1980, 1981; Eriksson et al. 1987a; Gray and Laskey 1980; Komura and Sakamoto 1991; Kristensson et al. 1986; Morganti et al. 1985). Signs of impaired motor function similar to those seen in humans are typically not seen in rodents. Although decreased motor activity (Gray and Laskey 1980; Komura and Sakamoto 1991) has been observed at high oral doses (280 mg manganese/kg/day for 100 days), motor deficits similar to clinical effects in humans are seldom observed in rodents and tend to be transient effects (Kristensson et al. 1986). These data indicate that rodent studies may be more beneficial for evaluating the neurochemical changes and understanding the mechanism of neurotoxicity that may occur as a result of manganese exposure. Newland (1999) suggests

that rodent models are useful for assessing manganese effects on the CNS when appropriate behavioral endpoints are measured.

Primate models tend to exhibit neurological effects typical of those seen in occupational workers exposed to manganese (Newland 1999). Newland and Weiss (1992) dosed Cebus monkeys intravenously in intermittent administrations throughout the day with either 5 or 10 mg manganese/kg/day (cumulative dose was equivalent to 50–60 mg/kg/day) for 180–280 days. The authors observed that a single intravenous dose of 5 or 10 mg/kg produced a large increase in the number of incomplete responses to a device requiring physical exertion. Action tremor was evident in the animals at cumulative doses of 40 mg/kg or higher. The tremor was irreversible in two of three animals studied. Onset of these behavioral effects corresponded with an increase in the manganese content of the global pallidus and the substantia nigra as measured by MRI. Monkeys exposed to doses as low as 4.4 mg manganese/kg/week (as $MnCl_2$) by intravenous injection for 7 weeks exhibited bradykinesia, rigidity, facial grimacing, and abnormal posturing of hind limbs, all characteristic of manganese neurotoxicity (Olanow et al. 1996). Onset of the neuromuscular deficits corresponded with damage to the globus pallidus and the substantia nigra. Although the exposure thresholds leading to effects appear different, these studies confirm that nonhuman primates can develop neurological and neurobehavioral symptoms similar to those in humans, and indicate the monkey is a good model for human neurological effects.

It has been reported that manganese neurotoxicity has clinical similarities to Parkinson's disease because patients exhibit an extrapyramidal syndrome (masked facies or facial grimacing, resting tremor in limbs or tremor upon extension, bradykinesia, stooped posture, and shuffling gait, often accompanied by propulsion or retropulsion). However, significant differences between Parkinsonism and manganism do exist (Calne et al. 1994; Chu et al. 1995). For example, manganism patients present a hypokinesia and tremor that is different from Parkinson's patients (Barbeau 1984). In addition, manganism patients sometimes have psychiatric disturbances early in the disease, have a propensity to fall backward when pushed, have less frequent resting tremor, more frequent dystonia, a "cock-walk", and a failure to respond to dopamino-mimetics (Calne et al. 1994; Chu et al. 1995). In humans and nonhuman primates, manganese accumulates and causes neurochemical and neuropathological changes predominantly in the globus pallidus. The precise biochemical mechanism behind the neurotoxicity is not elucidated, but several possibilities have been proposed. These include enhancement of the oxidation of dopamine and other catecholamines, which increases production of free radicals and reactive oxygen species, thereby causing oxidative stress and damage to the cell, following the depletion of cellular antioxidant defense systems (Donaldson 1987;

Liccione and Maines 1988; Parenti et al. 1988). Other hypotheses involve damage predominant to the mitochondria (Gavin et al. 1992).

Reliable dose-response data on the inhalation exposure levels leading to neurological injury in humans are not extensive, but recent epidemiological data indicate that concentrations of manganese in respirable dust ranging from 0.027–0.215 mg/m^3 (geometric mean values of respirable dust) and 0.14–1.59 mg manganese/m^3 in total dust in the workplace can result in measurable neurological effects. Inhalation exposure of 0.215 mg manganese/m^3 (respirable dust) may produce preclinical signs of neurological change in some people (Roels et al. 1992). Using benchmark dose analysis (BMD), a surrogate NOAEL (the $BMDL_{10}$) of 0.074 mg/m^3 was estimated from individual exposure and neurobehavioral response data provided by the authors of Roels et al. (1992). This NOAEL was the basis for deriving an MRL for chronic inhalation exposures of 0.00004 mg manganese/m^3 (0.04 µg manganese/m^3), as described in footnote "c" in Table 2-1. Another $BMDL_{10}$ was developed using individual exposure and neurobehavioral response data supplied by Dr. Anders Iregren from his study (Iregren 1990). The $BMDL_{10}$ derived from Dr. Iregren's study (0.071 mg/m^3) was comparable to the $BMDL_{10}$ developed from Roels et al. (1992).

While manganism is clearly associated with chronic inhalation exposure to high levels of manganese dust, there is only limited evidence that oral exposure is of concern. There are a few reports of manganism-like symptoms in people who ingested unusually high levels of manganese, but all of these reports have limitations that make data interpretation difficult. For example, Kawamura et al. (1941) reported an outbreak of a disease in a small group of people exposed to approximately 14 mg/L of manganese in their drinking water (corresponding to a dose of about 0.4 mg manganese/kg/day). While many of the symptoms were similar to those associated with inhalation exposure, there were a number of aspects in the incident which suggest that manganese was not solely responsible (see Section 2.2.2.4).

In a more recent study, Kondakis et al. (1989) reported an increased prevalence of neurological signs in the elderly residents of two towns in Greece where drinking water contained elevated levels of manganese (0.2–2 mg/L, corresponding to doses of up to about 0.06 mg manganese/kg/day). This study suggests that above-normal oral exposure to manganese might be of concern; however, there are a number of limitations to this study that make this conclusion uncertain. Although no details were reported regarding which neurological signs or symptoms were increased, a weighting factor assigned to each neurological symptom was based on its diagnostic value for Parkinsonism. However, the clinically significant differences between manganism and Parkinsonism make it difficult to judge whether the differences were due to

effects characteristic of manganism or to nonspecific parameters. More emphasis on those symptoms reported in manganese-exposed miners (e.g., sleep disorders, emotional lability, weakness/fatigue, and irritability) would have made the weighting scheme more valuable. In another report (Holzgraefe et al. 1986), one man, who accidentally ingested $KMnO_4$ for several months, later developed some manganism-like symptoms. However, since only one person was involved and absorption may have been markedly increased by direct damage to the stomach, it is difficult to draw conclusions regarding the levels associated with neurological risks from ingestion of Mn(II) compounds.

One report in Japanese adults (Iwami et al. 1994) linked the ingestion of food relatively high in manganese (equivalent to eating ~6 mg/day), in conjunction with low concentrations of magnesium in drinking water, with increased incidences of motor neuron disease. Two studies in children (He et al. 1994; Zhang et al. 1995) showed that those who ingested drinking water with increased concentrations of manganese (0.241 mg/L or higher) and who ate food containing high manganese levels for at least 3 years had observable neurological effects. Children exposed to excess manganese performed more poorly in school and on neurobehavioral tests as compared to control children who drank water containing only 0.04 mg manganese/L. These studies indicate that observable preclinical neurological effects can be seen in humans who have ingested manganese in increased amounts. However, no study gave clear dose levels associated with an effect while taking into account confounding factors or clear evidence for duration of exposure. Although neurological effects are a potential concern, the current evidence does not allow the estimation of levels in the environment that may be of potential harm. It is not possible to determine if manganese levels at or near hazardous waste sites would be associated with neurological effects.

Two studies have found higher manganese levels in the hair of learning disabled children than in nondisabled children (Collipp et al. 1983; Pihl and Parkes 1977). Two other studies found increased manganese concentrations in children who performed more poorly in school and on neurobehavioral tests following over-exposure to manganese in water and food (He et al. 1994; Zhang et al. 1995). The route of excess exposure may be through ingestion of increased amounts, metabolic disturbances, improper balance of other nutrients (such as iron), or decreased ability to clear manganese. However, studies involving increased manganese content in hair have drawbacks in that environmental contaminants and the distance from the scalp that the hair is cut can skew results, and some studies suggest that manganese may have an affinity for darker colored hair (Lydén et al. 1984; Sturaro et al. 1994). Other factors, such as lead, might have been involved in the adverse outcome (Pihl and Parkes 1977). Collectively these studies suggest a link between excess manganese and learning deficits, but there are numerous limitations and no firm

conclusions can be drawn. Further studies must be done to better evaluate the relationship between excess manganese and learning impairment.

Gottschalk et al. (1991) suggested an association between violent behavior and excess manganese exposure in prison subjects. The prisoners did have a significantly higher hair manganese content than the control population, but the data are inconclusive because manganese might not have been the only factor contributing to the violent behavior. Also, the highest concentrations of manganese in hair samples were within control ranges reported by Kondakis et al. (1989) in their study of excess manganese exposure from drinking water. As discussed previously, this study (Gottschalk et al. 1991) is only suggestive of an association between manganese and violent behavior because of the confounding factors such as the limitations of using hair concentrations as indicators of body burden and the possible presence of other contributing factors.

Fell et al. (1996) studied a group of 57 children receiving parenteral nutrition, 11 of whom had a combination of hypermanganesemia and cholestasis. Four of these 11 patients died; the 7 survivors had whole blood manganese concentrations ranging from 34–101 µg/L. Four months after reduction or removal of manganese from the supplementation, the blood concentration of manganese decreased by a median of 35 µg/L. Two of the seven survivors had movement disorders, one of whom survived to have a MRI scan. The scan revealed bilateral symmetrically increased signal intensity in the globus pallidus and subthalamic nuclei. These signals were also observed in five other children—one from the original group exhibiting cholestasis with hypermanganesemia and five more given parenteral nutrition chronically with no liver disease.

Studies in animals exposed to manganese by the oral route, and for MMT and maneb via oral and injection exposures, have revealed biochemical changes in various neurotransmitter levels, especially in the region of the basal ganglia (Bonilla and Prasad 1984; Chandra 1983; Dorner et al. 2000; Eriksson et al. 1987a; Gianutsos and Murray 1982; Komura and Sakamoto 1994; Serif et al. 1984). Altered behavior (Chandra 1983; Gray and Laskey 1980; Maraud et al. 1988; Sobotka et al. 1971) or minor motor dysfunction (Gupta et al. 1980; Kristensson et al. 1986) have also been observed occasionally as evidence of neurological impairment. Although rodents exposed to manganese generally do not develop the striking bradykinesia, ataxia, and hypertonicity characteristic of manganism in humans, the clinical syndrome has been reported in monkeys (Eriksson et al. 1987b; Newland et al. 1989; Olanow et al. 1996; Suzuki et al. 1975), and the

effects in rodents are at least qualitatively similar to the biochemical and functional changes in humans exposed by the inhalation route.

In adult animals, these effects are usually seen at doses of 40–400 mg manganese/kg/day (see Table 2-2), although Bonilla and Prasad (1984) noted an effect in rats exposed to 14 mg manganese/kg/day. Taking a dose of 14 mg manganese/kg/day as the LOAEL for these changes (which do not appear to be associated with any visible dysfunction) would correspond to a dose of about 980 mg/day in an adult human. Since typical dietary intake via food provides about 4 mg/day (see Section 5.5), environmental levels would have to be very high to yield an intake level that would approach this dose. There is some evidence available suggesting that nonhuman primates may be a more appropriate animal model than rodents. Newland and Weiss (1992) observed reductions in effortful responding in Cebus monkeys administered a cumulative i.v. dose of 50–60 mg/kg/day (divided into several doses of 5-10 mg/kg given throughout the day) for 180–280 days. A single intravenous dose of 5 or 10 mg/kg produced a significant decrease in effortful response to a device requiring physical exertion. Further, action tremor was evident in the animals at cumulative doses of 40 mg/kg or higher. Olanow et al. (1996) observed severe neurological signs in 3 Rhesus monkeys at intravenous doses as low as 4.4 mg/kg (equivalent to #79.2 mg/day, once per week, or 11.3 mg/day averaged over the 7-day week) given once per week for 7 weeks. Studies in neonatal rats indicate biochemical changes in the brain may be produced by doses of 1–22 mg manganese/kg/day (Chandra and Shukla 1978; Deskin et al. 1980; Dorner et al. 2000). When 1 mg manganese/kg/day is the LOAEL in neonatal rats, then the corresponding dose in a human infant (5–10 kg) would be 5–10 mg/day. The Food and Nutrition Board of the NRC (1989) recommended that infants (aged 0–0.5 year and 0.5–1 year) consume 0.3–0.6 mg manganese/day and 0.6–1.0 mg manganese/day, respectively. These range recommendations are based on analyses of pooled human milk samples. Lönnerdal (1997) reported that manganese concentrations of formulas sold in the United States ranged from 30–75 µg manganese/L. Thus a 10-kg infant consuming 1 L of some of these formulas could consume as much as 75 µg manganese/day or 7.5 µg manganese/kg/day. This level is still well below the LOAEL level for neonatal rats.

Chronic occupational exposure to maneb has resulted in significant increases in complaints of headache, nervousness, memory complaints, and sleepiness (Ferraz et al. 1988), and has also resulted in few cases of more severe effects, such as knee jerk with ankle jerks (Koizumi et al. 1979), or rigidity with cogwheeling (Ferraz et al. 1988), that are similar to those observed in manganism cases. One case of extremely high exposure involved a mill worker who was exposed to maneb for 4 days per week, 16 weeks per year for

2 years, at a daily dose of 16,000 mg/kg (Meco et al. 1994). The man developed a mild tremor with associated numbness in his right leg 2 years after the last exposure; this progressed, and despite treatment with amantadine for extrapyramidal syndrome, the man's symptoms worsened with tremor spreading to all limbs. Ten years after the first tremor appeared the man exhibited a mild, generalized bradykinesia and rigidity, postural tremor in the right limbs, slowness of gait, mild hypomimia, and slurred speech. Brain MRI revealed normal basal ganglia with no hyperintensity of signal that would indicate manganese deposition. Exposure of an adult male to 15,700 mg manzidan (maneb and zineb)/kg/day for 2 days resulted in tonic and clonic convulsions and unconsciousness (Israeli et al. 1983a). Oral and dermal dosing of adult mice with 32 or 1,600 mg maneb/kg, respectively, resulted in activity increases, and for oral dosing, behavioral changes in the form of flavor aversion (Mitchell et al. 1989).

Although some of the neurochemical and behavioral changes in animals dosed with MMT or maneb (Komura and Sakamoto 1992; Serio et al. 1984; Sobotka et al. 1972) resemble those changes induced by inorganic manganese, the mechanism of action of organomanganese-induced neurotoxicity has not been identified. One potential mechanism for dithiocarbamate neurotoxicity is the compound's disruption of the transmembrane proton gradient in brain synaptic vesicles (Vaccari et al. 1999). It is believed that the dithiocarbamates share toxicity through structural similarities (Komulainen and Savolainen 1985). If so, the manganese moiety may likely be immaterial to the induction of the adverse effect. Soleo et al. (1996) observed that concentrations of 8 and 40 FM mancozeb significantly decreased dopamine and GABA (γ-amino-butyric acid) uptake in a coculture of dissociated mesencephalic-striatal cells in a dose-dependent fashion. Identical concentrations of zineb (dithiocarbamate pesticide with only the zinc metal, not manganese) had very comparable results. These data suggest that the dithiocarbamate moiety is responsible for the decrease in observed neuron viability, and may be the causative factor in any neurological deficits in pesticide-exposed individuals.

The World Health Organization (WHO 1981) estimated that the typical human exposure level via inhalation for the general population in areas without manganese-emitting industries is <2 µg/day and that in industrial areas it could reach 4–10 µg/day. While data from animals suggest that typical human exposure levels are not of concern to either adults or infants, it must be remembered that animals (with the possible exception of nonhuman primates) do not appear to be as sensitive to manganese as humans, possibly due to pharmacokinetic differences. Thus, there is considerable uncertainty in using data from rodent models to estimate a no-effect oral exposure level in humans or to evaluate the potential for adverse

effects in humans exposed near a hazardous waste site. Nevertheless, neurological effects are a potential concern for people exposed to excess manganese in the environment.

Reproductive Effects. Decreased libido and impotence are frequently observed in male workers exposed to high levels of manganese dusts in the workplace (Emara et al. 1971; Mena et al. 1967; Rodier 1955). The number of children born to occupationally exposed males may also be lower than average; in a study by Lauwerys et al. (1985), the number of children sired by manganese-exposed males during their exposure was significantly lower than the control value. However, results by Gennart et al. (1992) indicate that inhalation of manganese dust at a concentration of 0.71 mg/m^3 in an occupational environment had no effect on fertility. Jiang et al. (1996b) reported increased sexual dysfunction and decreased libido in male workers exposed to manganese dusts lower than current limits (0.145 mg manganese (total dust)/m^3). Further, Wu et al. (1996) reported increased semen liquefaction time, decreased sperm count and viability in manganese-exposed miners and welders who inhaled either dusts or fumes, with a higher proportion of miners suffering adverse effects than the other groups. Although effects on libido and sexual performance are at least partly
neurological in origin, the recent human and animal studies indicate that manganese is damaging to the testes.
In young male rats and mice exposed to manganese (as Mn_3O_4) orally, growth and maturation of the testes and other reproductive tissues were retarded (Gray and Laskey 1980); this was thought to be due to decreased testosterone secretion by Leydig cells (Laskey et al. 1982, 1985). However, sperm counts do not appear to be affected (Laskey et al. 1982), and there is conflicting evidence for altered sperm morphology (Hejtmancik et al. 1987a, 1987b; Joardar and Sharma 1990).

A much more striking effect has been reported in rabbits exposed to manganese by intratracheal instillation (manganese particles may be absorbed from the lower airway and manganese particles deposited in the upper airways may be moved by mucociliary transport to the throat, where they are swallowed and enter the stomach) (Chandra et al. 1973; Seth et al. 1973). A single dose of 160 mg manganese/kg (as MnO_2) resulted in a slow degeneration of the seminiferous tubules over a period of 1–8 months. This was associated with loss of spermatogenesis and complete infertility (Chandra et al. 1975; Seth et al. 1973). Similar degenerative changes in testes have been reported in rats and mice following intraperitoneal injection of $MnSO_4$ (Chandra et al. 1975; Singh et al. 1974) and in rabbits following intravenous injection of $MnCl_2$ (Imam and Chandra 1975). It is not clear why testicular damage is more severe in some cases than in others, but it could be due to toxicokinetic differences between oral and parenteral exposures or

from differing biokinetics due to differing manganese compounds. Although direct damage to the testes has not been reported in humans following manganese exposure, current studies in men do not investigate this possibility; observations from animal studies suggest structural damage in the reproductive organs might also be of concern in humans. Reproductive effects have been shown to be of concern in occupationally-exposed males and could be of potential concern to men living or working near hazardous waste sites. The evidence does not suggest that ambient exposure levels would be of concern to the general population.

Information on reproductive effects of inhaled manganese in females is limited. Female mice exposed to MnO_2 by inhalation for 18 weeks had an increased number of pups per litter, perhaps as the result of the beneficial effects of manganese (Lown et al. 1984).

No effects on litter size, ovulations, resorptions, or fetal deaths were detected in rats exposed to up to 3,500 mg manganese/kg/day (as Mn_3O_4 in the diet), but pregnancies were significantly decreased at this dose (Laskey et al. 1982). However, because manganese was administered to both males and females in this study, it is impossible to determine which sex the manganese was primarily affecting. Manganese, as $MnCl_2$, administered by gavage to rats at a concentration of 22 mg manganese/kg/day on gestation days 6–17 (Grant et al. 1997), or administered *ad libitum* in drinking water at a concentration of 620 mg/kg/day during the entire gestational period (Pappas et al. 1997), did not cause reproductive toxicity. However, manganese at 33 mg/kg/day (as $MnCl_2$) given by gavage in water to pregnant rats throughout gestation (Szakmáry et al. 1995) caused a significant increase in postimplantation loss and resulted in a significant increase in structural abnormalities in the resultant pups. However, these abnormalities were resolved in pups allowed to grow to 100 days of age. Manganese injected subcutaneously into mice at 14 mg/kg/day (as $MnCl_2$) on gestation days 9–12 (Colomina et al. 1996) also caused a significant increase in postimplantation loss. In another reproductive study in mice (Sánchez et al. 1993), $MnCl_2$ caused significant postimplantation loss in pregnant mice when injected subcutaneously on days 6–15 of gestation at a much lower concentration of 1.1 mg manganese/kg/day. By contrast, $MnCl_2$ injected intravenously at a similar concentration of 1.7 mg manganese/kg/day into pregnant rats on days 6–17 of gestation did not cause any observable maternal toxicity or fetal resorptions or deaths (Grant et al. 1997), but did result in an increase in postimplantation loss when administered identically at a concentration of 2.2 mg /kg/day in rats on the same gestational days (Treinen et al. 1995).

These studies indicate that inorganic manganese, as $MnCl_2$, is quantitatively and qualitatively similar in its toxicity to both mice and rats, and that time of administration may influence the toxicity of the compound. These studies show that oral routes that mimic a human's intake of manganese do not result in observable reproductive toxicity in rodents even at very high daily doses (Grant et al. 1997), but when the manganese is administered all at once by bolus dosing or by injection, much lower concentrations are required to achieve significant toxicity (Szakmáry et al. 1995). The effects seen in gavage and parenteral studies (Szakmáry et al. 1995) may be due to circumvention of the body's homeostatic control of the metal. It is unclear what relevance these types of studies will have to humans. It is unknown if the levels of excess manganese encountered at hazardous waste sites or in the environment would pose a reproductive risk to the general public, although the collective data from animal studies do not indicate there is great cause for concern.

Reproductive studies of organic manganese compounds in animals are limited to the manganese pesticides and mangafodipir. Studies in animals exposed to maneb indicate that reproductive effects are generally observed only after administration of high doses and at certain times during gestation. When fed to laying hens in mash for 7 days at 180 mg/kg, maneb had no effect on egg production (Weppelman et al. 1980). Maneb administered by gavage-dosing (in water) to pregnant rats at doses of 400, 600, or 800 mg/kg, on either gestation day 11 or 13 resulted in reproductive toxicity only at the highest dose and only when given on day 11 (Petrova-Vergieva and Ivanova-Tchemishanska 1973). The only observed effect was a significant increase in resorptions. When slightly smaller doses of 100, 200, or 400 mg maneb/kg/day (also in water) were administered similarly, and the exposure days were expanded to days 6–15, no reproductive toxicity was observed (Petrova-Vergieva and Ivanova-Tchemishanska 1973). In a similar study, Chernoff et al. (1979) dosed pregnant rats with maneb in water at 0, 100, 190, or 380 mg/kg in the rat, and 0, 300, 600, or 1,200 in the mouse on gestation days 7–16. Maneb resulted in a dose-dependent decrease in maternal weight gain in the rat, but not the mouse; all doses caused an increase in liver:body weight ratios, although this was probably an artifact of the lower weight gain in the rat. No other effects were noted. In a second identical study with rats that were allowed to give birth, maneb at doses up to 380 mg/kg did not affect parturition or litter size (Chernoff et al. 1979).

Maneb did not induce any reproductive toxicity when dosed in water via gavage to mice at up to 1,200 mg/kg/day on gestation day 9 or 13 (Larsson et al. 1976), but resulted in a significant decrease in mice mating efficiency due to maternal mortality, occurrence of stillborn litters, and nonpregnant females, when given at 960 mg/kg/day in carboxymethylcellulose on gestation days 6–15 (Beck 1990). When given

in water to pregnant rats at 340, 655, or 1,200 mg/kg/day on gestation day 11, however, the compound induced a significant amount of resorptions, with 13.3% at the lowest, 56% at the intermediate, and 40% at the highest dose (Larsson et al. 1976). In contrast, mancozeb given in water to rats did not increase resorptions significantly over controls even at the highest dose of 1060 mg/kg/day. In a follow-up study, maneb given in water to pregnant rats on gestation day 11 caused a dose-dependent increase in resorption, with respective rates being 5.3 and 12% at 340 or 655 mg/kg, respectively (Larsson et al. 1976). When zinc acetate was added to the gavage mixture at concentrations of 15, 30, or 60 mg/kg (maneb was given at 640 mg/kg in this series of tests), the resorption rate went from 8.7 to 10.8 to 3.6% with increased zinc concentration, respectively. However, when the maneb dose was increased to 1,180 mg/kg and zinc acetate was increased to 108 mg/kg, the resorption rate climbed to 14.7%, indicating that the protective effect of zinc had been exceeded by the large maneb dose (Larsson et al. 1976).

Mancozeb at a dose of 375 mg/kg/day given by gavage to male rats for 180 days, reduced the gonadal activities of acid phosphatase and succinate dehydrogenase, increased the levels of alkaline phosphatase and lactate dehydrogenase, and decreased the relative weight of the epididymis (Kackar et al. 1997a).

Mangafodipir has been reported to affect reproduction by increasing post-implantation loss in rats when administered at a dose 8 times the clinical dose (2.2 mg manganese/kg/day) when given on gestation days 6–17 (Grant et al. 1997), and when given at 12 times the clinical dose (3.3 mg manganese/kg/day) during the same gestational period in rabbits (Blazak et al. 1996). No reproductive toxicity was observed in the rat at doses 4 times the clinical dose, or in the rabbit at 8 times the clinical dose.

The reproductive toxicity of maneb and mancozeb has been observed only after administration of high concentrations (typically higher than 330 mg/kg). The studies with zinc acetate suggest that chelation of the body's endogenous zinc may mediate the compound's reproductive effects. However, these studies show that manganese pesticides are toxic to the reproductive system by different pathways with similar effects in different rodent species. The high doses of maneb or mancozeb required to achieve adverse effects after gavage dosing indicate that reproductive toxicity may not be a concern for persons exposed to these compounds in the environment unless that exposure was to a great excess of these fungicides. Due to the limited number of people exposed to mangafodipir (people with or suspected to have abdominal tumors) and the high doses of compound that were required to induce reproductive toxicity in animal

models, there does not appear to be a concern that exposure to mangafodipir will result in reproductive toxicity in humans.

Developmental Effects. The effects of excess manganese on human fetal development have not been thoroughly investigated. Incidences of stillbirths and malformations have been studied in an Australian aboriginal population living on an island where environmental levels of manganese are high (Kilburn 1987). However, data from a suitable control group are lacking, and the study population is so small that it is not possible to judge if the incidence of developmental abnormalities is higher than average.

Only one study was located concerning behavioral effects in rodent pups following gestational exposure to airborne inorganic manganese by the dams (Lown et al. 1984). Unfortunately, this study does not provide conclusive evidence of exposure-induced behavioral effects.

Two studies in school children (He et al. 1994; Zhang et al. 1995) showed that over-exposure to manganese in drinking water and food was associated with poorer performance in school and on neurobehavioral tests as compared to non-exposed children. The exposed and control children in these studies were reportedly well-matched and the reports indicated that hair manganese was inversely related to performance on neurobehavioral exams and in school. However, there were several limitations in the reporting of the study, including the lack of analysis for lead or mercury, which are neurotoxins, and the lack of definitive exposure characterization.

Fell et al. (1996) studied 57 children receiving parenteral nutrition, 11 of whom had a combination of hypermanganesemia and cholestasis. Of these eleven, four died; the 7 survivors had blood manganese concentrations ranging from 34–101 µg/L that decreased by a median of 35 µg/L within 4 months following removal of manganese from the food source. An MRI scan of one of the children with a movement disorder revealed increased signal intensity in the globus pallidus and subthalamic nuclei. Similar hyperintense MRI signals were also seen in five other children, one with cholestasis and four more with no liver disease. The data indicate that the body can develop an increased manganese body burden (as evidenced by the increased manganese deposition in the brain) in spite of an apparently healthy liver. an inability to clear manganese from the body was not necessary to accumulate an excess level of manganese within the body.

There are several studies concerning developmental toxicity to animals from oral exposure to manganese. Gray and Laskey (1980) fed neonatal mice up to 1,050 mg manganese/kg/day as Mn_3O_4 in food for 40 days (beginning at day 15 of lactation). The treatment resulted in decreased growth and weight of the male reproductive organs, including the testes, seminal vesicles, and preputial gland. Neonatal rats given $MnCl_2$ in drinking water for 44 days at a dose of 150 mg manganese/kg/day developed a transient ataxia on days 15–20 of the treatment and had decreased levels of homovanillic acid in the hypothalamus and striatum on day 15 but not 60 (Kristensson et al. 1986). Neonatal rats given bolus doses of $MnCl_2$ in water of 1 mg manganese/kg/day for 60 days suffered neuronal degeneration and increased monoamine oxidase on days 15 and 30 of the study, but did not show any clinical or behavioral signs of neurotoxicity (Chandra and Shukla 1978). Similar neurochemical changes were seen in two studies in which $MnCl_2$ was administered to neonatal rats from day 1–24 post-birth (Deskin et al. 1980, 1981). In the first study, a dose of 10 mg manganese/kg/day caused decreased levels of dopamine, but not norepinephrine, in the hypothalamus, whereas a dose of 20 mg/kg/day caused a decrease of tyrosine hydroxylase activity and an increase in monoamine oxidase activity in the hypothalamus. In the second study, the authors found that a 20 mg manganese/kg/day dose resulted in increased serotonin in the hypothalamus and decreased acetylcholinesterase in the striatum. However the authors did not indicate that the acetylcholinesterase decrease was important given other mechanisms involved in the metabolism of this neurochemical. In a chronic study, Lai et al. (1984) observed that 40 mg manganese/kg/day given in drinking water to developing rat pups resulted in small decreases in choline acetyltransferase in the cerebellum and midbrain of the 2 month old rat. The treatment did not affect the regional distribution of glutamic acid decarboxylase, acetylcholinesterase, or NAD-linked isocitric dehydrogenase (although this latter enzyme was decreased transiently at a higher manganese concentration).

Pappas et al. (1997) administered up to 620 mg manganese/kg/day as $MnCl_2$ in drinking water to pregnant rats throughout gestation, up to weaning of the pups (postnatal day 22) and throughout postnatal day 30. The authors found that this dose resulted in a transient decrease in body weight on postnatal days 9–24 and a transient increase in activity on postnatal day 17. Brain neurochemistry and physiology were unaffected and the exposed rats did not perform differently than unexposed controls on performance tests. In a similar study, Kontur and Fechter (1988) observed that treating pregnant rats with $MnCl_2$ in drinking water at concentrations up to 1,240 mg manganese/kg/day on gestation days 0–20 did not result in changes in norepinephrine or dopamine in the fetal brain, nor did it affect the development of a startle response. No other neurological endpoints were measured. In another study, Ali et al. (1985) observed significant increases in brain dopamine and norepinephrine levels and a decrease in brain serotonin levels in pups of

male and female rats treated with $MnCl_2$ at 420 mg manganese/kg/day in drinking water for 118 days prior to mating, and throughout mating and gestation. In an older study with identical study design, Ali et al. (1983a) found delayed air righting reflexes in the offspring of rats exposed to 240 mg manganese ($MnCl_2$ in drinking water)/kg/day. Grant et al. (1997) observed no treatment-related effects in rat pups when dams were gavaged on gestational days 6-17 with 22 mg manganese chloride/kg/day.

More recently, Brenneman et al. (1999) and Dorman et al. (2000) studied brain neurochemistry and behavioral changes in neonatal and adult (Dorman et al. 2000) rats administered 0, 11, or 22 mg manganese/kg/day (by mouth or gavage in water) for up to 49 days. The earlier study reported a slight, but significant decrease in body weight in high dose rats on PND49 (last day of study), while the latter reported significant body weight decrease (5%) in high-dose rats from PND21. The earlier study reported no changes in motor activity except an increase in spontaneous motor activity on PND21, while the latter study reported a significant amplitude in the response to acoustic startle measured on day 21. No effects on body weight or behavior were observed in adults in the Dorman et al. (2000) study. These effects are consistent with those of Pappas et al. (1997), although the significance of the findings to human neurological health following manganese exposure is unclear. Although behavioral changes in rats are not clearly analogous to neurobehavioral changes observed in humans, these studies suggest that some neurological effects might occur in the developing animal and are thus of potential concern.

Maneb and mancozeb produced teratogenic effects in rat pups when dams were given a single gavage dose (Larsson et al. 1976; Petrova-Vergieva and Ivanova-Tchemishanska 1973). Structural abnormalities observed in 41 rat fetuses were only seen in 1 of 2 studies at a dose of 340 mg/kg; these effects were short tail (41), umbilical hernia (1), and open eye (1). However, structural abnormalities observed at the next higher dose, 655 mg/kg included 95 malformed fetuses. The primary malformations included short tail (95), adactyly or oligodactyly (77), cleft palate (55), bradygnathia (47), low external ears (52), and hydrocephaly (81) (Larsson et al. 1976). Zinc acetate had a protective effect for the development of malformations when administered at 30 and 60 mg/kg. In a separate study, slightly higher maneb doses of 1,600 or 3,200 mg/kg, given on day 11 of gestation, but not day 13, resulted in significant increases in late fetal deaths in gavage-dosed rats. Doses of 800 mg/kg and higher caused significant increases in structural malformations in the fetuses after exposure on either day, with the latter resulting in slightly higher numbers (Petrova-Vergieva and Ivanova-Tchemishanska 1973).

Administration of 960 mg/kg maneb to pregnant mice on gestation days 6–15 also resulted in fetal mortality and minor structural malformations, such as head and neck shape and bent tails (Beck 1990). Postnatal effects of pups born to dams gavage dosed each day with 0, 190, or 380 mg maneb/kg on gestation days 7–15 were limited in females to a transient significant increase in weight after birth, and in males to a delay in eye opening (Chernoff et al. 1979). When the daily gestational exposure to maneb doses as high as 400 mg/kg/day was shifted to days 2–21, no significant changes in late deaths or malformed fetuses were observed (Petrova-Vergieva and Ivanova-Tchemishanska 1973).

Neonatal rats ingesting 11 mg/kg/day mancozeb in chow fed to their dams pre-weaning and to them for 24 weeks post-weaning had a slightly higher incidence of pancreatic acinar cell hyperplasia after induction with nitrosomethylurea (NMU) than control rats. Further, exposed rats developed dysplastic foci of the pancreas and 5/14 developed pancreatic carcinoma *in situ* (Monis and Valentich 1993).

Maneb at aqueous concentrations of 5 ppm resulted in delayed development of amputated forearms in male and female newts, with reduced melanogenesis and malformations of regenerating limbs (Arias and Zavanella 1979; Zavanella et al. 1984). However, in light of the relevant pathways of pesticide exposure in humans, the relevance of this model system to human development is not clear.

Subcutaneous injection of pregnant mice with 1.1 mg manganese/kg/day, as $MnCl_2$, on gestation days 6–15 resulted in delayed ossification of the sternebrae in the pups (Sánchez et al. 1993). Similar injection of pregnant mice with 14 mg manganese/kg/day, as $MnCl_2$, on gestation days 9–12 resulted in a significant decrease in pup weight and delayed ossification of occipital parietal bones, asymmetrical sternebrae, and disunion of xyphoides (Colomina et al. 1996). A third study showed that intraperitoneal injection of pregnant mice with 12.5 mg manganese/kg/day, as $MnSO_4$, on days 8–10 of gestation resulted in exencephaly and embryolethality (Webster and Valois 1987). Two studies involving pregnant rats intravenously injected with $MnCl_2$ at 1.7 (Grant et al. 1997) and 1.1 (Treinen et al. 1995) mg manganese/kg/day on gestation days 6–17 reported significantly increased skeletal abnormalities, increased rib irregularities, and reduced ossification of the sternebrae and skull, as well as a significant decrease in fetal body weight. These studies strongly suggest that adverse developmental effects may occur if maternal exposure to manganese is high. Taken together, the studies from humans and animals suggest that high levels of manganese intake might lead to developmental effects. Evidence from animal studies indicate that the skeletal system is especially targeted, but the data are too limited to draw firm conclusions. Limitations in the data include equivocal results based on the day of exposure during gestation and the

limitations in reports of developmental studies in humans (such as mixed exposure to several pesticides including maneb and mancozeb). Although it is currently not possible to discern whether similar effects might occur in persons exposed to excess manganese levels possibly present at hazardous waste sites or in the environment, this is a potential concern.

Mangafodipir, injected into pregnant rats at doses of 2.2 mg manganese/kg also caused significantly increased skeletal and rib abnormalities, reduced ossification of certain bones, and a significant decrease in fetal body weight (Grant et al. 1997; Treinen et al. 1995). Exposure to mangafodipir should not be a concern for developmental toxicity given the high doses of the compound required to cause adverse developmental effects.

Genotoxic Effects. There is some evidence from a study on occupationally exposed welders that manganese may cause chromosomal aberrations; the welders were exposed to other potentially toxic compounds including nickel (known to cause chromosomal aberrations) and iron, therefore the observed increase in chromosomal aberrations cannot be attributed solely to manganese (Elias et al. 1989). Mutagenicity studies in both bacteria and mammalian strains are equivocal. While manganese sulfate was shown to not be mutagenic to Salmonella typhimurium strains TA97, TA98, TA100, TA1535, or TA1537 either in the presence or absence of S9 from Aroclor 1254-induced liver from rats or Syrian hamsters (Mortelmans et al. 1986), it was shown to be mutagenic to strain TA97 elsewhere (Pagano and Zeiger 1992). In yeast (S. cerevisiae strain D7), a fungal gene conversion/reverse mutation assay indicated that manganese sulfate was mutagenic (Singh 1984). Manganese chloride was reportedly not mutagenic in S. typhimurium strains TA98, TA100, and TA1535, but it was mutagenic in strain TA1537, and conflicting results were obtained for TA102 (Wong 1988; De Méo et al. 1991).

In vitro assays in mammalian cells also gave conflicting results concerning manganese mutagenicity. Manganese chloride produced gene mutations in cultured mouse lymphoma cells (Oberly et al. 1982). The compound also caused DNA damage in human lymphocytes in vitro using the single-cell gel assay technique in the absence of metabolic activation, but caused no DNA damage when S9 was present (De Méo et al. 1991). Manganese sulfate induced sister chromatic exchange in Chinese hamster ovary (CHO) cells in both the presence and absence of S9 from Aroclor 1254-induced rat liver (Galloway et al. 1987). In a separate assay, manganese sulfate also induced chromosomal aberrations in CHO cells in the absence of S9 but not in its presence (Galloway et al. 1987). By contrast, manganese chloride was not clastogenic in cultured FM3A cells in the absence of metabolic activation (Umeda and Nishimura 1979), although the

compound did cause chromosomal aberrations in root tip cells of Vicia faba (Glass 1955; 1956). Potassium permanganate caused chromosomal aberrations in FM3A cells (Umeda and Nishimura 1979), but not in a primary culture of cells from Syrian hamster embryos when tested in the absence of metabolic activation (Tsuda and Kata 1977). Manganese chloride caused cell transformation in Syrian hamster embryo cells (Casto et al. 1979). A list of in vitro study results is given in Table 2-10.

Manganese chloride did not produce somatic mutations in Drosophila melanogaster fruit flies in one study (Rasmuson 1985), and manganese sulfate did not induce sex-linked recessive lethal mutations in germ cells of male D. melanogaster (Valencia et al. 1985).

In vivo assays in mice showed that oral doses of manganese sulfate or potassium permanganate caused micronuclei and chromosomal aberrations in bone marrow (Joardar and Sharma 1990). In contrast, oral doses of manganese chloride did not cause chromosomal aberrations in the bone marrow or spermatogonia of rats (Dikshith and Chandra 1978). A list of in vivo study results is given in Table 2-11.

The results of in vitro studies show that at least some chemical forms of manganese have mutagenic potential. However, as the results of in vivo studies in mammals are inconsistent, no overall conclusion can be made about the possible genotoxic hazard to humans from exposure to manganese compounds.

Genotoxicity data concerning MMT or maneb were not available. Mancozeb was not found to be cytogenetic following gavage exposure to male mice at doses as high as 4050 mg/kg as measured by meiotic chromosome analysis and micronucleus test (Vasudev and Krishnamurthy 1994). Mancozeb was found to be genotoxic to human lymphocytes in culture only at cytotoxic doses (Perocco et al. 1989). Another study showed that mancozeb exposure increased chromosomal aberration frequency in a dose-dependent manner at concentrations higher than 4 Fg/mL in human lymphoctyes *in vitro* and increased clastogenicity in rat bone marrow cells cultured after a single i.p. injection of 0.002 and 0.004 mg/kg (Georgian et al. 1983). The authors (Georgian et al. 1983) also noted that chronic exposure to mancozeb in food at 0.0014 mg/kg/day for 280 days resulted in an increased percentage of aberrant bone marrow cells, genome breaks per cell, and gaps per cell. Mangafodipir was not found to be significantly genotoxic in a series of *in vivo* and *in vitro* test systems; cell transformation data were equivocal (Larsen and Grant 1997). As with inorganic manganese, the data concerning mancozeb are suggestive of genotoxicity, but do not permit an evaluation of the risk to humans posed by excess exposure to this compound.

Table 2-10. Genotoxicity of Manganese *In Vitro*

Species (test system)	Compound	End point	Strain	Results: With activation	Results: Without activation	Reference
Inorganic Manganese Compounds						
Prokaryotic organisms:						
Salmonella typhimurium (plate incorporation assay)	$MnCl_2$	Gene mutation	TA98	–	–	Wong 1988
			TA102	–	–	
			TA1535	–	–	
			TA1537	–	+	
	$MnSO_4$	Gene mutation	TA98, TA100, TA1535, TA1537, TA97	–	–	Mortelmans et al. 1986
Salmonella typhimurium (preincubation assay)	$MnSO_4$	Gene mutation	TA97	No data	+	Pagano and Zeiger 1992
	$MnCl_2$	Gene mutation	TA102	No data	+	DeMéo et al. 1991
			TA100	No data	–	DeMéo et al. 1991
	$MnCl_2$	Gene mutation	TA102	No data	+	DeMéo et al. 1991
			TA100	No data	–	DeMéo et al. 1991
Photobacterium fischeri (bioluminescence test)	$MnCl_2$	Gene mutation (restored luminecence)	Pf-13 (dark mutant)	No data	+	Ulitzur and Barak 1988
Escherichia coli	$MnCl_2$	Gene mutation	KMBL 3835	No data	+	Zakour and Glickman 1984

Table 2-10. Genotoxicity of Manganese *In Vitro* (continued)

Species (test system)	Compound	End point	Strain	Results: With activation	Results: Without activation	Reference
Bacteriophage (*E. coli lysis*)	$MnSO_4$	Gene mutation	T4	No data	+	Orgel and Orgel 1965
Bacillus subtilis (recombination assay)	$MnCl_2$ $Mn(NO_3)_2$ $MnSO_4$ $Mn(CH_3COO)_2$ $KMnO_4$	Inhibition of growth in recombination deficient mutant (Rec^-) compared to wild type (Rec^+)	M45 (Rec^-)	No data + 	+ + + –	Nishioka 1975
B. subtilis (recombination assay)	$MnCl_2$ $Mn(NO_3)_2$ $Mn(CH_3C00)_2$	Inhibition of growth in recombination deficient mutant (Rec^-) compared to wild type (Rec^+)	M45 (Rec^-)	No data –	– –	Kanematsu et al. 1980
Eukaryotic organisms:						
Fungi:						
Saccharomeyces cerevisiae	$MnSO_4$	Gene conversion, reverse mutation	D7	No data	+	Singh 1984
Mammalian cells:						
Mouse lymphoma cells	$MnCl_2$	Gene mutation	L5178Y TK+/-	No data	+	Oberley et al. 1982
Syrian hamster embryo cells	$MnCl_2$	Enhancement of SA7 transformation		No data	+	Casto et al. 1979

Table 2-10. Genotoxicity of Manganese *In Vitro* (continued)

Species (test system)	Compound	End point	Strain	Results: With activation	Results: Without activation	Reference
Human lymphocytes (Single-cell gel assay)	$MnCl_2$ 1991	DNA damage	lymphoc^{yte}	–	+	DeMéo et al.
Chinese hamster ovary cells	$MnSO_4$	Chromosomal aberrations/ Sister chromatid exchange		+	+	NTP 1993
Organic Manganese Compounds						
Prokaryotic organisms:						
Escherichia coli and *Salmonella typhimurium*	MnDPDP	Gene mutation	*E. coli:* WP_2uvrA^- *S. typhimurium*: TA100, TA1535, TA98, TA1537	–	–	Larsen and Grant 1997
Eukaryotic organisms:						
CHO cells	MnDPDP	Forward mutation		–	–	Larsen and Grant 1997
CHO-WBL cells	MnDPDP	Chromosomal aberration		–	–	Larsen and Grant 1997

Table 2-10. Genotoxicity of Manganese *In Vitro* (continued)

Species (test system)	Compound	End point	Strain	Results		Reference
				With activation	Without activation	
Human peripheral blood lymphocyes	Mancozeb	Genotoxicity via sister chromatid exchange		–	–	Perocco et al. 1989
Human lymphocytes	Mancozeb	Aberrant cells, Chromosomal breaks and gaps		No data	+	Georgian et al. 1983

– = negative result; + = positive result; (+) = weakly positive result; CHO = Chinese hamster ovary; DNA = deoxyribonucleic acid; $Mn(CH_3COO)_2$ = manganous acetate; $MnCl_2$ = manganous chloride; MnDPDP = mangafodipir; $Mn(NO_3)_2$ = manganous nitrate; $MnSO_4$ = manganous sulfate; Rec = recombination

Table 2-11. Genotoxicity of Manganese *In Vivo*

Species (test system)	Compound	Exposure End point	Route	Results	Reference
Inorganic Manganese Compounds					
Nonmammalian systems:					
Drosophila melanogaster	$MnSO_4$	Sex-linked recessive lethal	Feeding	–	Valencia et al. 1986
			Injection	–	
Drosophila melanogaster	$MnCl_2$	Somatic mutation	Soaking larvae	–	Rasmuson 1985
Mammalian systems:					
Albino rat (bone marrow cells) (spermatogonial cells)	$MnCl_2$	Chromosomal aberrations	Oral	–	Dikshith and Chandra 1978
Albino mouse	$MnSO_4$	Chromosomal aberrations	Oral	+	Joardar and Sharma 1990
	$KMnO_4$	Chromosomal aberrations	Oral	+	
Organic Manganese Compounds					
Mammalian systems:					
Wistar rat	Mancozeb	Chromosomal breaks, polyploidy, aberrant cells	Oral	+	Georgian et al. 1983
Wistar rat	Mancozeb	Aberrant cells, chromosomal breaks and gaps	I.P.	+	Georgian et al. 1983

Table 2-11. Genotoxicity of Manganese *In Vivo* (continued)

Species (test system)	Compound	Exposure Endpoint	Route	Results	Reference
Mammalian systems (continued):					
Mouse	Mancozeb	Chromosomal aberrations	Oral	+	Sobti et al. 1987
Swiss albino mouse	Mancozeb	Chromosomal aberrations	Oral	–	Vasudev and Krishnamurthy 1994
Human	Mancozeb, Maneb	Chromosomal translocations, Sister chromatid exchanges	Dermal	+	Steenland et al. 1997
Human (blood lymphocytes)	Mancozeb	Isochromatid breaks, gaps, and aberrant cells	Inhalation	+	Jablonicka et al. 1989

– = negative result; + = positive result; $KMnO_4$ = potassium permanganate; I.P.= intraperitoneal; $MnCl_2$ = manganous chloride; $MnSO_4$ = manganous sulfate

Cancer. Information on the carcinogenic potential of manganese is limited, and the results are equivocal. Inhalation exposure of humans to manganese dusts has not been identified as a risk factor for lung cancer, although intraperitoneal injection of mice with $MnSO_4$ led to an increased incidence of lung tumors (Stoner et al. 1976). Preliminary data from a 1987 study indicate that chronic oral exposure of rats to $MnSO_4$ may lead to increased incidence of pancreatic tumors (adenomas plus carcinomas), but this effect was quite small and was not dose responsive (Hejtmancik et al. 1987a). In mice, chronic oral exposure to $MnSO_4$ resulted in a small increase in pituitary adenomas in females in one study (Hejtmancik et al. 1987b). Chronic oral exposure of mice and rats to manganese as $MnSO_4$ resulted in a marginally increased incidence of thyroid gland follicular cell adenomas in mice; however, no evidence for cancer was noted in either sex of rats. NTP considered that there was equivocal evidence of carcinogenic activity based on a marginally increased incidence of thyroid gland follicular adenoma and a significantly increased incidence of follicular cell hyperplasia (NTP 1993). Repeated intramuscular injection of rats and mice with suspensions of metallic manganese or MnO_2 did not result in tumors at the injection site or elsewhere (Furst 1978). Repeated injections of MMT did not enhance tumor formation in mice (Witschi et al. 1981), but mancozeb was found to be both a weak initiator of benign tumors of mixed morphology after repeated doses of 80 mg/kg (Shukla et al. 1990), as well as a tumor promoter on mouse skin at the same dose level (Shukla et al. 1988). Gavage dosing of rats with 80 mg/kg resulted in promoting effects of nitrosomethylurea-induced pancreatic hyperplasias (Monis and Valentich 1993). Although limited evidence was found in the literature concerning the carcinogenesis of maneb or mancozeb, ETU, a primary metabolite of both fungicides, is a known thyroid carcinogen in the rodent (NTP 1990). It is plausible that any carcinogenesis arising from exposure to these fungicides is a result of ETU, and not the parent compound. The equivocal carcinogenesis data reported for rodents and the paucity of evidence from other species and from human data suggest that the potential for cancer in humans is low, although the data are inadequate to reach a firm conclusion. EPA has categorized manganese as a Group D substance ("not classifiable" with regard to human carcinogenicity) (EPA 1993b).

2.6 CHILDREN'S SUSCEPTIBILITY

This section discusses potential health effects from exposures during the period from conception to maturity at 18 years of age in humans, when all biological systems will have fully developed. Potential effects on offspring resulting from exposures of parental germ cells are considered, as well as any indirect effects on the fetus and neonate due to maternal exposure during gestation and lactation. Relevant animal and in vitro models are also discussed.

Children are not small adults. They differ from adults in their exposures and may differ in their susceptibility to hazardous chemicals. Children's unique physiology and behavior can influence the extent of their exposure. Exposures of children are discussed in section 5.6 Exposures of Children.

Children sometimes differ from adults in their susceptibility to hazardous chemicals, but whether there is a difference depends on the chemical (Guzelian et al. 1992; NRC 1993). Children may be more or less susceptible than adults to health effects and the relationship may change with developmental age (Guzelian et al. 1992; NRC 1993). Vulnerability often depends on developmental stage. There are critical periods of structural and functional development during both pre-natal and post-natal life and a particular structure or function will be most sensitive to disruption during its critical period(s). Damage may not be evident until a later stage of development. There are often differences in pharmacokinetics and metabolism between children and adults. For example, absorption may be different in neonates because of the immaturity of their gastrointestinal tract and their larger skin surface area in proportion to body weight (Morselli et al. 1980; NRC 1993); the gastrointestinal absorption of lead is greatest in infants and young children (Ziegler et al. 1978). Distribution of xenobiotics may be different; for example, infants have a larger proportion of their bodies as extracellular water and their brains and livers are proportionately larger (Widdowson and Dickerson 1964; Foman et al. 1982; Owen and Brozek 1966; Altman and Dittmer 1974; Foman 1966). The infant also has an immature blood-brain barrier (Adinolfi 1985; Johanson 1980) and probably an immature blood-testes barrier (Setchell and Waites 1975). Many xenobiotic metabolizing enzymes have distinctive developmental patterns and at various stages of growth and development, levels of particular enzymes may be higher or lower than those of adults and sometimes unique enzymes may exist at particular developmental stages (Leeder and Kearns 1997; Komori 1990; Vieira et al. 1996; NRC 1993). Whether differences in xenobiotic metabolism make the child more or less susceptible also depend on whether the relevant enzymes are involved in activation of the parent compound to its toxic form or in detoxification. There may also be differences in excretion, particularly in the newborn who has a low glomerular filtration rate and has not developed efficient tubular secretion and resorption capacities (Altman and Dittmer 1974; West et al. 1948; NRC 1993). Children and adults may differ in their capacity to repair damage from chemical insults. Children also have a longer lifetime in which to express damage from chemicals; this potential is particularly relevant to cancer.

Certain characteristics of the developing human may increase exposure or susceptibility while others may decrease susceptibility to the same chemical. For example, the fact that infants breathe more air per

kilogram of body weight than adults may be somewhat counterbalanced by their alveoli being less developed, so there is a disproportionately smaller surface area for absorption (NRC 1993).

Inorganic Manganese

Children as a group have typically not been studied for the adverse effects of overexposure to inorganic manganese. Manganese results in adverse respiratory effects, as well as neurological effects; the latter effects have been the most investigated. As discussed previously, manganism has typically been observed in occupational settings, as in manganese miners, or in isolated cases of extreme exposure to inhaled or ingested manganese. In general, these exposure scenarios do not pertain to children. Reports do exist, however, of incidences of overexposure to inorganic manganese resulting in respiratory illness. Two studies exist that investigated increased respiratory complaints and symptoms at a junior high school situated 100 meters from a manganese alloy plant in Japan (manganese concentrations in total dust at a 200 meter perimeter around the plant were 0.004 mg/m^3 (3.7 $\mu g/m^3$)) (Kagamimori et al. 1973; Nogawa et al. 1973). The initial study showed that the incidences of self-reported respiratory illnesses among children in the exposed school were much higher than those of a control school 7 km away from the plant (Nogawa et al. 1973). Further, evaluations of respiratory fitness showed significant decreases in several parameters. When the installation of dust catchers resulted in a decreased manganese concentration in total dust, complaints of illness decreased, and the test results improved (Kagamimori et al. 1973). These respiratory effects were not unique from those observed in adults exposed to airborne manganese. Further, it was not reported if other compounds were present in the dust generated by the plant which might have contributed to or caused the reported illnesses. It is possible that these effects might have been triggered by the dust and were not specific to manganese.

Studies on potential neurological effects in children from inhalation exposure to excess inorganic manganese are limited. One study showed that a native population living on an island with rich manganese deposits suffered increased neurological disorders and incidences of birth defects (Kilburn 1987); their exposure was most likely via inhalation and oral routes. This study involved small sample sizes and lacked exposure concentrations and a suitable control group; these limitations preclude ascribing these effects to manganese alone.

Children who have been exposed to elevated levels of inorganic manganese presumably through diet (either a normally ingested diet or through total parenteral nutrition, TPN) have shown signs of motor

disorders (e.g. dystonia, dysmetria, propulsion, retropulsion, poor check response bilaterally) similar to those observed in cases of frank manganism (Devenyi et al. 1994; Fell et al. 1996). In a few of the cases, the presence of liver dysfunction indicated a decreased ability to clear excess manganese (Devenyi et al. 1994; Fell et al. 1996), but some of the children with apparently normal livers also exhibited motor disorders (Fell et al. 1996). Several children also exhibited hyperintense signals on MRI resulting from increased exposure to manganese due to cholestatic end-stage liver disease (Devenyi et al. 1994) and from high concentrations of the element in TPN, either in the presence (Fell et al. 1996) or absence (Fell et al. 1996; Ono et al. 1995) of liver disease. The Ono et al. (1995) study involved a child on TPN for more than 2 years; although this child did have increased blood manganese and hyperintense signals in the basal ganglia as shown by MRI, the authors did not report any observable signs of neurotoxicity. A similar lack of observable neurotoxicity was reported in two siblings fed TPN with high manganese concentrations (0.2 mmol Mn/kg/day) for several months (the brother for 63 months total starting at age 4 months; the sister for 23 months total starting at age 1 month) (Kafritsa et al. 1998). Both children had elevated blood manganese levels and showed hyperintense signals in the basal ganglia (especially the globus pallidus and subthalamic nuclei) on MRI. Reduction of manganese concentration in the TPN resulted in a gradual loss of signal on MRI analysis (becoming comparable to normal scans) and decrease in blood manganese levels as measured in three subsequent annual exams. These equivocal results indicate that there are considerable differences in susceptibility to the neurotoxic effects of excess manganese in children.

Two studies indicate that oral exposure to excess inorganic manganese also results in measurable signs of preclinical neurotoxicity in children. These studies show that children who drank water containing manganese at average concentrations of at least 0.241 mg/L (Zhang et al. 1995) and who ate food with increased manganese content (He et al. 1994) for 3 years performed more poorly in school (as shown by mastery of their native language, mathematics, and overall grade average) and on the WHO neurobehavioral core test battery than those students who drank water with manganese #0.04 mg/L. These neurobehavioral tests are among those administered to workers occupationally exposed to manganese to determine the presence of early neurological deficit (Chia et al. 1993; Iregren 1990; Lucchini et al. 1995; Mergler et al. 1994; Roels et al. 1987a, 1992). These concentrations are much lower than the ones to which adults were exposed in the Kondakis et al. (1989) study. In this study, ingestion of drinking water with excess manganese (1.8–2.3 mg/L) was linked to the onset of unspecified neurological symptoms in an aged population (average age, over 67 years). Though there are limitations, this and other environmental studies in adults (Baldwin et al. 1999; Beuter et al. 1999; Goldsmith et al. 1990; Kawamura et al. 1941; Kondakis et al. 1989; Mergler et al. 1999) and two studies in children (He et al. 1994; Zhang et al. 1995)

indicate that both adults and children can manifest similar neurological deficits that are potentially linked to ingesting excess manganese. However, these reports are lacking well characterized and quantitative exposure data that would indicate whether children and adults experience neurological effects at the same or different exposure levels. Existing studies do not allow estimations of the quantitative susceptibility of children to the preclinical effects of excess manganese exposure. They do indicate, though, that children can develop symptoms of neurotoxicity after oral exposure to manganese that are similar to those effects seen in adults environmentally or occupationally exposed to the metal. Further, these studies indicate that neurological effects may be a concern for children exposed to excess manganese from the environment or from a hazardous waste site.

The investigations by He et al. (1994) and Zhang et al. (1995) showed that children with poorer school performance had higher manganese hair content than children from the control area. Other studies have found that manganese levels in hair are higher in learning disabled children than in normal children (Collipp et al. 1983; Pihl and Parkes 1977). The route of excess exposure is not known but it is presumed to be mainly oral. These observations are consistent with the possibility that excess manganese ingestion could lead to learning or behavioral impairment in children. However, an association of this sort is not sufficient to establish a cause-effect relationship since a number of other agents, including lead, might also be involved (Pihl and Parkes 1977).

Developmental studies in animals following inhalation exposure to manganese are sparse. One study exists (Lown et al. 1984) in which pregnant mice were exposed to a high concentration of airborne manganese or filtered air for 17 days preconception and then exposed to either the same concentration of manganese or filtered air postconception. Their pups were then fostered to adult females who had experienced the same inhalation exposures as the mothers (no manganese exposure, pre-or post-conception exposure, or both). The pups of exposed mothers had decreased body weight but exhibited no differences in activity compared to pups from mothers exposed to air, irrespective of exposure history. The paucity of data concerning potential developmental effects in animals following inhalation exposure makes the extrapolation of potential effects in humans experiencing the same exposure difficult.

Oral studies in animal models, whether involving the dosing of pregnant dams or sucklings, reveal a variety of neurochemical and physiological changes as a result of manganese exposure. The majority of studies have involved $MnCl_2$. One study in rats reported that pups exposed *in utero* 11 days during gestation to a relatively low concentration of $MnCl_2$ (22 mg/kg; by gavage in water) did not have any

observable decrease in weight gain, nor any gross or skeletal malformations upon necropsy (Grant et al. 1997). Another study (Szakmáry et al. 1995) which also administered $MnCl_2$ in water by gavage to pregnant rats at the slightly higher concentration of the 33 mg manganese/kg/day throughout the entire gestation period reported a delay of skeletal and organ development as well as an increase in skeletal malformations, such as clubfoot, in unborn pups. These malformations, however, were self-corrected in pups allowed to grow to 100 days of age. In addition, the same dose and route did not result in any observable developmental toxicity in the rabbit (Szakmáry et al. 1995). Rat pups exposed during gestation and after birth to manganese at relatively high concentrations of 120–620 mg/kg in drinking water suffered no observable adverse effects at the low dose and only transient adverse effects (decrease in weight and hyperactivity) at the high dose (Pappas et al. 1997). Similar transient body weight decreases and increases in motor activity were observed in neonatal rats administered 22 mg manganese (as $MnCl_2$)/kg/day, by mouth or gavage, for up to 49 days (Brenneman et al. 1999; Dorman et al. 2000).

Rat pups from a generational study in which the male and female parents were exposed to 240–715 mg manganese/kg/day (as $MnCl_2$ in drinking water) in either a diet adequate or deficient in protein (Ali et al. 1983a) suffered a delayed air righting reflex (independent of protein content of diet) and showed significant alterations in the age of eye opening and development of auditory startle when produced by parents fed low-protein diets with 240 mg manganese/kg/day in water. Kontur and Fechter (1988) administered up to 1,240 mg manganese/kg/day as $MnCl_2$ in drinking water to pregnant rats during days 0–20 of gestation. Although the authors found increased manganese levels in the fetus, there were no measurable effects on dopamine or norepinephrine turnover in the pup brain, or in the development of a startle response. In a more recent study, an increased amplitude in acoustic startle reflex was observed at PND21 in neonatal rats administered 22 mg manganese chloride/kg/day by mouth from PND1-PND21 (Dorman et al. 2000). Significant increases in brain dopamine and DOPAC concentrations in select brain regions in these animals as well as increased brain manganese concentrations were reported. This study demonstrated that neonates treated with manganese showed neurological changes whereas no effects were observed in the adult animals treated similarly. Jarvinen and Ahlstrom (1975) fed pregnant rats varying doses of $MnSO_4$ in food for 8 weeks prior to and during gestation. Fetuses taken at 21 days did not show gross abnormalities but did have significantly increased body burdens of manganese from mothers fed 187 mg/kg/day.

Neonatal rats given $MnCl_2$ in drinking water for 44 days at a dose of 150 mg manganese/kg/day developed a transient ataxia on days 15–20 of the treatment and had decreased levels of homovanillic acid in the

hypothalamus and striatum on day 15 but not day 60 (Kristensson et al. 1986). Neonatal rats given bolus doses of $MnCl_2$ in water of 1 mg manganese/kg/day for 60 days suffered neuronal degeneration and increased monoamine oxidase on days 15 and 30 of the study, but did not show any clinical or behavioral signs of neurotoxicity (Chandra and Shukla 1978). Similarly, neonatal rats given bolus doses of $MnCl_2$ in 5% sucrose at doses of 0, 1, 10, or 20 mg manganese/kg/day for 24 days after birth showed decreased levels of dopamine, but not norepinephrine, in the hypothalamus (Deskin et al. 1980); doses of 20 mg/kg/day caused a decrease of tyrosine hydroxylase activity and an increase in monoamine oxidase activity in the hypothalamus. In a follow-up study, Deskin et al. (1981) gave 0, 10, 15, and 20 mg manganese/kg/day (as $MnCl_2$ in 5% sucrose by gavage) to neonatal rats from birth to age 24 days. The authors found that the highest dose resulted in increased serotonin in the hypothalamus and decreased acetylcholinesterase in the striatum. However, the authors did not indicate that the acetylcholinesterase decrease was important given other mechanisms involved in the metabolism of this neurochemical.

Several studies evaluated the effects of manganese in the diet on reproductive development in the pre-weanling rodent. Gray and Laskey (1980) fed mice 1,050 mg manganese/kg/day as Mn_3O_4 in the diet beginning on postnatal day 15 and continuing for 90 days. The manganese caused decreased growth of the testes, seminal vesicles, and preputial gland. Later studies evaluated the effect of excess manganese via the diet and gavage on development of the rat (Laskey et al. 1982, 1985). These studies reported that 350 mg manganese/kg/day (as Mn_3O_4 in food fed to pregnant rats and resulting male offspring for a total of 224 days) (Laskey et al. 1982) or 214 mg manganese/kg/day (as Mn_3O_4 by gavage in water given for 28 days) (Laskey et al. 1985) reduced testosterone levels in developing rats.

Studies involving intravenous or subcutaneous exposure routes of pregnant dams indicate that doses of $MnCl_2$ as low as 1.1 mg manganese/kg/day administered on gestation days 6–17 in the rat (Grant et al. 1997; Treinen et al. 1995) and 14 mg/kg/day administered on gestation days 9–12 in the mouse (Colomina et al. 1996) can result in decreased fetal body weight and skeletal abnormalities. The data indicate that animals may suffer adverse developmental effects after inhalation, oral, and intravenous exposures of their pregnant mothers, but results are mixed. Taken together, the evidence from environmental studies in humans and studies in animals suggest that younger children can be affected by exposures to excess manganese. Only one study is available that compared the incidence of adverse neurological effects in neonates and adults exposed to excess manganese. Additional information is needed to further evaluate potential differences in susceptibility to manganese-induced effects in young and older animals and to possibly characterize this difference quantitatively.

Organic Manganese

No studies currently exist on the health effects arising in children as a result of exposure to organic manganese. Therefore, predictions concerning potential effects must be made from extrapolations from existing animal studies.

Weanling mice who ingested 11 mg manganese/kg/day as MMT for 12 months exhibited a significant increase in spontaneous activity at day 80, but no other behavioral differences throughout the exposure period (Komura and Sakamoto 1992b). Concentrations of certain neurotransmitters and dopamine metabolites were modified in different brain regions, but the relationship to manganese levels in the affected regions was weak to none (Komura and Sakamoto 1994).

Maneb administered via gavage in water to pregnant rats at doses as high as 380 mg/kg/day on gestational days 7–15 did not result in any developmental or behavioral effects (measured as development of startle reflex, air righting and activity in an open field), in exposed male pups compared to controls, except for a significant retardation in eye opening (Chernoff et al. 1979). However, cumulative doses of 2 and 20 mg/kg, administered to nursing dams in the chow for 28 days, significantly affected exploratory behavior in the 30-day-old male pup (Sobotka et al. 1971); further, these same doses caused a positive effect on latent learning in the adult rat, also exposed only during pre-weaning. Adult rats exposed during pre-and post-weaning to a cumulative dose of 135 mg/kg/maneb also exhibited increased learning. Rats exposed only post-weaning to 5.75, 11.5, or 115 mg/kg cumulative dose, did not exhibit increased learning. Changes in neurochemical levels were also observed in select brain regions of exposed rats, but these were only weakly linked to maneb exposure.

Single doses of maneb at 1,600 and 3,200 mg/kg administered to pregnant rats via gavage in water on gestation day 11, but not gestation day 13, resulted in significant increases in fetal deaths (Petrova-Vergieva and Ivanova-Tchemishanska 1973). An increased incidence of grossly malformed fetuses with severe structural defects was seen at doses of 800, 1,600 and 3,200 mg/kg when given to pregnant dams on both gestation days 11 and 13, with more fetuses affected on the latter day at the 800 and 3,200 mg/kg doses. By contrast, when the authors dosed pregnant rats with up to 400 mg/kg/day of maneb by gavage in water on gestation days 2–20, no increases in late fetal deaths or malformed fetuses were observed (Petrova-Vergieva and Ivanova-Tchemishanska 1973). It is not clear why a single dose of 3,200 mg/kg, given on 1 of 2 gestational days, would result in gross fetal malformations while a cumulative dose of

7,200 mg/kg, given over 18 days of gestation and encompassing the time points in the single-dose study, would not result in similar structural deformities.

In a similar developmental study involving exposure to either maneb and mancozeb on gestational day 11, Larsson et al. (1976) reported that doses of maneb at 655 and 1,200 mg/kg caused malformations, including skeletal deformations, in surviving rat pups; by contrast, mancozeb at 580 and 1,060 mg/kg resulted in increased hemorrhages, but not malformations. The lowest doses of either pesticide did not result in negative effects. The authors theorized that maneb was chelating endogenous maternal zinc which is necessary for proper skeletal development. Malformations in pups exposed to 640 mg/kg maneb showed a decrease in the incidence of malformations that was dependent on zinc-acetate dose. However, a dose of 108 mg/kg zinc acetate was unable to protect against the malformations induced by 1,110 mg/kg maneb (Larsson et al. 1976).

Chernoff et al. (1979) administered maneb via gavage in water at doses of 0, 100, 190, and 380 mg/kg/day to pregnant rats and mice on gestational days 7–16. The 380 mg/kg/day dose resulted in significant decreases in rat fetal weight and in caudal ossification centers; this dose also resulted in significant numbers of rat fetuses with hydrocephaly. The mice were slightly more sensitive to the decrease in ossification in that all doses resulted in a significant decrease in ossification centers. However, the mice did not suffer body weight decreases or hydrocephalus as a result of exposure. Delayed bone ossification was also seen in fetuses of pregnant mice dosed by gavage with 960 mg maneb/kg/day by gavage in carboxymethylcellulose on gestation days 6–15 (Beck 1990). This dose also resulted in a significant increase in fetal mortality, and minor abnormalities (head and nasal shape, spine curvature, and extranumerary ribs).

Dietary exposure to mancozeb while nursing and maintenance on a mancozeb-containing diet (100 mg mancozeb/kg diet) for 24 weeks following injection with the carcinogen nitrosomethylurea (NMU) at 50 mg/kg induced the formation of focal acinar cell hyperplasia of the pancreas that was slightly higher than control rats with no mancozeb exposure following NMU injection (Monis and Valentich 1993). Further, 36% of the mancozeb-exposed rats developed carcinoma *in situ*.

Developmental studies in rats involving i.v. exposure of pregnant dams to mangafodipir during organogenesis (days 6–17) indicate that the compound targets the skeletal system, resulting in irregularly shaped bones at doses as low as 1 mg manganese/kg/day (Grant et al. 1997; Treinen et al. 1995). Further,

application of specific doses of the compound during segmented time periods in organogenesis causes the same skeletal defects (Treinen et al. 1995). Interestingly, when the compound is administered from 22 days prior to conception until gestation day 7, at up to 6 mg manganese/kg/day, no developmental effects were observed (Grant et al. 1997). These data further indicate that animals developing during organogenesis are particularly susceptible to developmental toxicity from mangafodipir exposure. Further, behavioral changes and significant decreases in body weight were observed in rat pups delivered from dams dosed with 1.1 mg manganese/kg/day while decreased survival was observed in pups from dams given 2.2 mg manganese/ kg/day on gestation days 6–17.

In contrast to the rat, available studies suggest that the rabbit is far less susceptible to the developmental effects of mangafodipir. One study reported only decreased ossification in fetal sternebrae at 1.1 mg manganese/kg/day when given to dams on gestation days 6–17 (Grant et al. 1997); a similar study in the same species reported no observable developmental toxicity at 2.2 mg manganese/kg/day, but a significant decrease in fetal weight and viable fetuses, with no skeletal abnormalities, at a dose of 3.3 mg manganese/kg/day also given during organogenesis (Blazak et al. 1996).

In total, these developmental studies indicate that organic manganese can induce adverse developmental effects in the unborn and young, with effects ranging from slight biochemical changes in the brain to structural changes to changes in functional development. However, the majority of studies have involved very high exposure doses; the pesticide studies in particular have used doses that are much higher than would be expected to occur as residues on foods that were sprayed with these compounds (de Carvalho et al. 1989; Israeli et al. 1983a; Koizumi et al. 1979).

Although Chernoff et al. (1979) reported decreased exploratory activity in 30-day-old rat pups after a low cumulative dose of 2 mg maneb/kg, older pups were not tested to determine if the effects were long-lasting. Six-month old rats exposed to the same amount of maneb were shown to have increased learning as a result of the maneb exposure, although this was shown with a different technique that is not directly comparable to the one used with the younger pups. The developmental toxicity of elemental manganese has been shown in large part by comparison studies between $MnCl_2$ and mangafodipir (Blazak et al. 1996; Grant et al. 1997; Treinen et al. 1995). While these studies have provided much information as to the targeted teratogenicity of manganese during organogenesis, they have generally involved i.v. exposures, which are not particularly relevant to the general population. In addition, it is not believed that mangafodipir should be a concern for children's health, as children are not expected to be exposed to this contrast

agent. Further, it is likely that the majority of women who may be exposed to mangafodipir are beyond child-bearing age, since clinical subjects with suspected liver tumors that merit use of the compound to assist in diagnosis are often over 50 years old (mean values; Bernardino et al. 1992). Should child-bearing women be exposed to the compound in a clinic environment, the doses required to induce developmental toxicity in animals greatly exceed the clinical dose (Blazak et al. 1996; Grant et al. 1997; Treinen et al. 1995).

The pharmacokinetics of manganese in infants is known to be different than in adults. Balance studies, although limited, show that there is high retention of manganese during the neonatal period (Dorner et al. 1989). Formula-fed infants had an apparent manganese absorption of around 20% (Davidsson et al. 1988; 1989), compared to absorption in adults, which is shown to be around 3–5% (Mena et al. 1969). The increased absorption may be a compensatory mechanism due to the low concentration of manganese in mother's milk (Collipp et al. 1983; Dorner et al. 1989; Lönnerdal 1987) and to the increased metabolic needs of infants as compared to adults, since manganese is required for adequate bone mineralization, as well as for connective tissue synthesis (Hurley and Keen 1987). Alternatively, the increased absorption may be due to decreased excretion in the very young (Kostial et al. 1978; Lönnerdal et al. 1987; Miller et al. 1975; Rehnberg et al. 1981); although at least one study indicates that both pre-term and full-term infants actively excrete manganese (Dorner et al. 1989). Some studies have indicated that infants, who acquire all of their manganese in the first 4 months of life from human milk or milk formulas, ingest very different amounts of manganese due to the differing manganese content of these food sources. More specifically, studies showed that due to the low manganese concentration of human milk (4–10 µg/L) and its higher concentration in cow's milk formulas (30–75 µg/L) and soy formulas (100–300 µg/L) (Dorner et al. 1989; Lönnerdal 1987), more manganese was absorbed from the formula (with absorption rate from all sources being roughly equal). Recent changes in nutritional status of infant formulas have resulted in a more balanced absorption of manganese from human milk and cow's milk formulas (~80–90%), with absorption of manganese from soy milk formulas being slightly lower (~70%; Lönnerdal et al. 1994). However, given the existing differences in inherent manganese concentrations between the different food sources, reports still suggest that infant intake of manganese from milk formulas is 10–50 times that of a breast-fed infant (Lönnerdal 1997). Animal studies show that absorption and/or retention of manganese is similar to that of older animals at approximately post-gestational day 17–18 (Kostial et al. 1978; Lönnerdal et al. 1987; Miller et al. 1975; Rehnberg et al. 1981). However, when this transition takes place in human infants has not been clearly defined.

Animal studies also show increased absorption of manganese in the young. For example, Kostial et al. (1989) found that rat pups retained a greater proportion (67%) of a single oral dose of radiolabeled manganese than adult rats (0.18%). Bell et al. (1989) found that manganese absorption in rat pups (using isolated brush border membrane vesicles from the intestine) is nonsaturable and appears to occur primarily by diffusion. In the older rat, however, a high affinity, low capacity, active-transport mechanism for manganese absorption appears to be present (Garcia-Aranda et al. 1983).

Several elements, including iron (Davis et al. 1992a), phosphorus (Wedekind et al. 1991), and calcium (Wilgus and Patton 1939) are known to decrease manganese absorption in adults and animals. Iron-poor diets result in increased manganese absorption in humans (Mena et al. 1969) and in rats (Pollack et al. 1965). These interactions have not been studied in infants or children, but are expected to occur.

Manganese is known to cross the placenta and has been detected in cord blood in healthy full-term and pre-term infants. It is unknown whether mothers exposed to increased concentrations of manganese will pass on toxic amounts of the metal to their unborn children via the blood. However, as manganese is an essential nutrient and is part of the human body at all times, it is expected to be found in all tissues and fluids of the infant. Manganese is also naturally found in breast milk (typical concentrations in mature milk range from 4–10 µg/L) (Collipp et al. 1983). No studies exist concerning breast milk concentrations of mothers exposed to increased concentrations of manganese. In addition, there are no studies in animals measuring these endpoints. It is unclear if manganese stored in the brain, bone, or in another depot, in excess amounts, could be mobilized to affect a developing fetus. However, one study by Jarvinen and Ahlstrom (1975) showed that pregnant rats fed 94 mg manganese (as $MnSO_4$)/kg/day for 8 weeks accumulated the metal in their livers in contrast to non-pregnant females. Further, at a daily dose of 187 mg/kg/day, increased manganese concentrations were found in 21-day old fetuses. These data suggest that homeostatic control of pregnant mothers regulated the distribution of the metal at lower concentrations, but this control was circumvented at high daily concentrations, resulting in liver excesses and distribution in the developing fetus. Although the fetuses in this study showed no physical abnormalities, no neurochemical or neurobehavioral studies were performed to determine potential adverse effects on these relevant endpoints.

Transferrin is one of the proteins responsible for binding and transporting both iron and manganese throughout the body. One study (Vahlquist et al. 1975) reported no correlation between infant cord blood and maternal blood transferrin levels. The same study reported an increase in plasma transferrin from

1.68+/-0.60 mg/mL in blood from infants at 6 weeks of age, to a peak of 2.60+/-0.27 mg/mL at 10 months, with values stabilizing at these adult levels throughout 16 years of age. The authors did not comment as to the statistical difference, if any, of these values.

There are no established biomarkers consistently used as indicators for overexposure to manganese in either adults or children. Elevated blood concentrations and hyperintense signals in the globus pallidus on T1-weighted MRI have been observed in children with increased exposure to manganese (Devenyi et al. 1994; Fell et al. 1996; Kafritsa et al. 1998; Ono et al. 1995). However, the same limitations of these indicators of overexposure in adults (wide range of blood manganese in normal populations, high cost and low availability of MRI) apply to children. Blood manganese has generally been poorly related to current levels of exposure or cumulative exposure index (Smargiassi and Mutti 1999). Elevated blood manganese alone does not constitute an adequate indicator of manganese overexposure. There are no pediatric-specific biomarkers of exposure or effect. See Section 2.8.1 for further information.

Studies suggest that children may differ from adults in their susceptibility to the toxic effects of manganese due to toxicokinetic differences, i.e., increased absorption and/or retention. Qualitative similarities exist between respiratory and neurological effects seen in adults and children suffering from extreme manganese exposure. While infant and animal studies indicate that the young have an increased uptake of manganese, and distribution of the element in certain tissues may differ with age, studies that reveal quantitative levels of manganese associated with discrete frank effects in both adults and children are lacking. The studies to date (namely absorption, distribution and excretion studies in animals) suggest a pharmacokinetic susceptibility to manganese that is different in children than in adults.

2.7 ENDOCRINE DISRUPTION

Recently, attention has focused on the potential hazardous effects of certain chemicals on the endocrine system because of the ability of these chemicals to mimic or block endogenous hormones, or otherwise interfere with the normal function of the endocrine system. Chemicals with this type of activity are most commonly referred to as endocrine disruptors. Some scientists believe that chemicals with the ability to disrupt the endocrine system are a potential threat to the health of humans, aquatic animals, and wildlife. Others believe that endocrine disrupting chemicals do not pose a significant health risk, particularly in light of the fact that hormone mimics exist in the natural environment. An example of natural hormone mimics are the isoflavinoid phytoestrogens (Adlercreutz 1995; Mayr et al. 1992; Livingston 1978). These

compounds are derived from plants and are similar in structure and action to endogenous estrogen. While there is some controversy over the public health significance of endocrine disrupting chemicals, it is agreed that the potential exists for these compounds to affect the synthesis, secretion, transport, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis, reproduction, development, and/or behavior (EPA 1997). As a result, endocrine disruptors may play a role in the disruption of sexual function, immune suppression, and neurobehavioral function. Endocrine disruption is also thought to be involved in the induction of breast, testicular, and prostate cancers, as well as endometriosis (Hoel et al. 1992; Giwercman et al. 1993; Berger 1994).

Inorganic Manganese

Studies of endocrine effects in humans following manganese exposure are very limited. Alessio et al. (1989) reported the elevation of serum prolactin and cortisol in chronically-exposed workers, while no changes in prolactin, FSH, or LH levels were observed in an occupational study involving shorter exposure periods (Roels et al. 1992). Lucchini et al. (1995) reported elevated serum prolactin levels in ferromanganese workers; 20 of those workers still showed elevated prolactin levels 5 years later after exposure to consistent levels of airborne manganese (Smargiassi and Mutti 1999). In fact, the serum prolactin levels had increased significantly over the previous values. Although these changes are minor, changes in prolactin secretion may have effects on different physiological functions, including loss of libido and impotence in men, and infertility and change in menstrual cycle in women.

No studies of endocrine effects in animals following airborne manganese exposure were located. Short-term animal studies and some of the long-term animal studies were negative for endocrine effects following oral exposure to manganese (NTP 1993). One intermediate study reported a decrease in circulating testosterone and a significant increase in substance P in the hypothalamus and neurotensin in the pituitary in rats dosed intraperitoneally with 6.6 mg manganese/kg/day as $MnCl_2$ (Hong et al 1994). Two other studies in rats reported that Mn_3O_4 in food, given at a dose of 350 mg manganese/kg/day for 224 days (starting on day 1 of gestation and continuing for 224 days) (Laskey et al. 1982) and 214 mg manganese/kg/day given up to 28 days (Laskey et al. 1985), resulted in reduced testosterone levels in male rats. The biological significance of this effect is unknown because the decrease had no result on fertility in the latter study (Laskey et al. 1985), and there were no observed effects on the hypothalamus or pituitary.

Organic Manganese

A few studies currently exist that indicate that maneb or mancozeb may have endocrine disrupting capability. In only 1 case report of human exposure to maneb was thyroid function checked and the analyses were not performed until 6 months following the exposure; they were normal (Koizumi et al. 1979). Two studies were performed that evaluated the effect of oral gavage exposure to mancozeb on the thyroid gland of the rat, and another investigated the thyroid toxicity of injected maneb. Intermediate and chronic dosing of mancozeb by gavage to rats at doses as low as 375 mg/kg resulted in decreased circulating T_4 levels and increased thyroid:body weight ratios after 30 days' exposure, and resulted in decreased thyroid peroxidase (at 1,125 mg/kg) (Kackar et al. 1997b; Trivedi et al. 1993). Doses of 750 and 1,125 mg/kg mancozeb resulted in significant inhibition in thyroid radioiodine uptake after 90 days' exposure in the chronic (360 day) study (Kackar et al. 1997b). Maneb injected i.p. into rats at doses of 5–40 mg/kg decreased cold-stimulated thyroid stimulating hormone (TSH), but had no effect on TSH-stimulated thyroid releasing hormone, TRH (Laisi et al. 1985). Maneb dosing also had no effect on circulating T_3 or T_4 levels.

2.8 BIOMARKERS OF EXPOSURE AND EFFECT

Biomarkers are broadly defined as indicators signaling events in biologic systems or samples. They have been classified as markers of exposure, markers of effect, and markers of susceptibility (NAS/NRC 1989).

Due to a nascent understanding of the use and interpretation of biomarkers, implementation of biomarkers as tools of exposure in the general population is very limited. A biomarker of exposure is a xenobiotic substance or its metabolite(s), or the product of an interaction between a xenobiotic agent and some target molecule(s) or cell(s) that is measured within a compartment of an organism (NAS/NRC 1989). The preferred biomarkers of exposure are generally the substance itself or substance-specific metabolites in readily obtainable body fluid(s) or excreta. However, several factors can confound the use and interpretation of biomarkers of exposure. The body burden of a substance may be the result of exposures from more than one source. The substance being measured may be a metabolite of another xenobiotic substance (e.g., high urinary levels of phenol can result from exposure to several different aromatic compounds). Depending on the properties of the substance (e.g., biologic half-life) and environmental conditions (e.g., duration and route of exposure), the substance and all of its metabolites may have left the body by the time samples can be taken. It may be difficult to identify individuals exposed to hazardous substances that are

commonly found in body tissues and fluids (e.g., essential mineral nutrients such as copper, zinc, and selenium). Biomarkers of exposure to manganese are discussed in Section 2.8.1.

Biomarkers of effect are defined as any measurable biochemical, physiologic, or other alteration within an organism that, depending on magnitude, can be recognized as an established or potential health impairment or disease (NAS/NRC 1989). This definition encompasses biochemical or cellular signals of tissue dysfunction (e.g., increased liver enzyme activity or pathologic changes in female genital epithelial cells), as well as physiologic signs of dysfunction such as increased blood pressure or decreased lung capacity. Note that these markers are not often substance specific. They also may not be directly adverse, but can indicate potential health impairment (e.g., DNA adducts). Biomarkers of effects caused by manganese are discussed in Section 2.8.2.

A biomarker of susceptibility is an indicator of an inherent or acquired limitation of an organism's ability to respond to the challenge of exposure to a specific xenobiotic substance. It can be an intrinsic genetic conditions or other characteristic or a preexisting disease that results in an increase in absorbed dose, a decrease in the biologically effective dose, or a target tissue response. If biomarkers of susceptibility exist, they are discussed in Section 2.10, Populations That Are Unusually Susceptible.

2.8.1 Biomarkers Used to Identify or Quantify Exposure to Manganese

Manganese can be measured with good sensitivity in biological fluids and tissues (see Section 6.1), and levels in blood, urine, feces, and hair have been investigated as possible biomarkers of exposure. As a group, workers exposed to a mean concentration of 1 mg manganese/m^3 had higher levels of manganese in the blood and the urine than unexposed controls (Roels et al. 1987b). The group average levels in blood appeared to be related to manganese body burden, while average urinary excretion levels were judged to be most indicative of recent exposures. A study by Lucchini et al. (1995) is the only evidence that suggests that blood and urine levels were correlated with manganese exposure on an individual basis. This study differed from others in that it involved exposure to MnO_2 and measured adverse effects in workers after exposure ceased, whereas other studies involved current exposures, and some, like Roels et al. (1987b) involved exposure to numerous manganese compounds (salts and oxides). The findings of Lucchini et al. (1995) suggest that blood and urine levels of manganese, on an individual basis, are positively correlated with exposure levels in the few weeks following cessation of exposure. In a study of chronically exposed workers who were evaluated while exposure was ongoing, Lucchini et al. (1999) found a positive

correlation between manganese levels in total dust and in blood of exposed workers. This correlation did not exist for cumulative exposure index and blood levels of the metal.

Other studies have indicated that on an individual basis, the correlation between the level of workplace exposure and the levels in blood or urine is not a reliable predictor of exposure (Jarvisalo et al. 1992; Roels et al. 1987b, 1992; Smyth et al. 1973). However, two studies (Jarvisalo et al. 1992; Roels et al. 1992) suggest that blood and urinary manganese levels may be used to monitor group exposure, such as exposure in an occupational setting. Also, a study (Siquiera et al. 1991) of ferromanganese workers indicated that exposed workers had elevated levels of plasma and urinary urea and decreased levels of urinary calcium, HDL cholesterol, and plasma inorganic phosphate. The study authors concluded that measurement of these parameters may be useful in the early detection of manganese poisoning. Although manganese may play a role in a metabolic pathway or other biological function involving these products, it is unclear what physiological significance these parameters have as related to manganese toxicity. There was no significant correlation between fecal excretion of manganese and occupational exposure to the metal (Valentin and Schiele 1983). A recent study on environmental exposure to manganese (Baldwin et al. 1999)in southwest Quebec, Canada, indicates that significantly higher levels of blood manganese are correlated with high levels of airborne manganese. In this study, air samples were taken in four geographic areas around a former ferroalloy plant (point source for airborne manganese). The air samples, which were for total dust and PM_{10} levels, were taken for 3 consecutive days in the summer. Using a geometric algorithm , 297 blood manganese values from nearby residents in 7 postal zones were separated into two geographical areas corresponding to the point source. Higher blood manganese values in men and women were located in the geographic area with the higher airborne manganese values. It is notable that the air samples taken were limited in number and were taken only in the summer. However, the authors mentioned that the data were consistent with samples taken in an adjacent urban area and were consistent with potential exposure sources. Further, at the time of sampling, the ferroalloy plant was not in use and exposure data indicated that airborne levels of manganese decreased dramatically at a point 25 kilometers downwind of the plant after the plant closed (Zayed et al. 1994). Thus, manganese exposure of the population in the Baldwin et al. (1999) study is likely to have been greater in the past; current blood manganese levels may be analogous to those observed in occupational workers undergoing a forced layoff (Lucchini et al. 1995). These data, combined with the occupational studies, indicate that there may be a plateau level of homeostatic control of the metal. At low levels, blood manganese concentrations would be related to intake from food, water, and air; large differences in individual blood manganese levels would

be observed. At high exposure levels, such as in occupational environments, this plateau may be reached, or exceeded.

These data also indicate that blood manganese levels can be an indicator of exposure to environmental manganese. These data indicate that manganese in blood or urine may be useful in detecting groups with above-average current exposure, but that measurements of manganese in these body fluids in individuals may only be related to exposure dose after the exposure has ceased.

In addition to individual variability, another factor that limits the usefulness of measuring manganese in blood, urine, or feces as a measure of excess manganese exposure is the relatively rapid rate of manganese clearance from the body. As discussed in Section 2.3, excess manganese in blood is rapidly removed by the liver and excreted into the bile, with very little excretion in urine (Klaassen 1974; Malecki et al. 1996b). Thus, levels of manganese in blood or urine are not expected to be the most sensitive indicators of exposure.

Serum prolactin (PRL) has been shown to be a possible biomarker of manganese action of dopamine neurotransmission (Smargiassi and Mutti 1999). Manganese acts on the tuberoinfundibular dopaminergic system which exerts tonic inhibition of PRL secretion. Serum PRL levels observed in workers occupationally exposed to manganese were shown to be consistent with mechanistic studies as they were distinctly higher than unexposed workers. It is still unclear whether or not serum PRL levels indicate recent or cumulative exposure. The value of PRL as a biomarker is called into question by the Roels et al. (1992) study in which serum PRL levels were not increased in workers chronically exposed to airborne manganese.

Lymphocyte manganese-dependent superoxide dismutase activity increases with increased manganese uptake (Yiin et al. 1996). It has been suggested that this enzyme, in conjunction with serum manganese levels, may be helpful in assessing low and moderate levels of manganese exposure (Davis and Greger 1992; Greger 1999). MnSOD has been shown to be elevated in women ingesting 15 mg of supplemental manganese/day, while levels have been shown to be depressed in the heart and liver of manganese deficient animals. MnSOD is important as a possible biomarker because its levels can be related to oxidative damage. Its sensitivity as a biomarker depends on factors that induce oxidative stress or effect manganese bioavailability including diets high in polyunsaturated fatty acids and strenuous physical exercise (Greger 1999).

Brain MRI scans and a battery of specific neurobehavioral tests (Greger 1998) may be useful in assessing excessive manganese exposure even among industrial workers exposed to airborne manganese (Nelson et al. 1993). These scans also have been successfully used to identify accumulation of manganese in the brains of children exposed to excess manganese (Devenyi et al. 1994; Fell et al. 1996; Ono et al. 1995). Levels in feces could be useful in evaluating relatively recent high-level exposures but would not be expected to be helpful in detecting chronic low-level exposures. These methods are potentially useful biomarkers, but require additional evaluation to determine their validity.

While it is well established that exposure to excess manganese can result in increased tissue levels in animals, the correlations among exposure levels, tissue burdens, and health effects have not been thoroughly investigated in humans or animals. Also, since homeostatic mechanisms largely prevent fluctuations of manganese concentration in whole blood and since manganese is mainly excreted by the biliary route, it is not believed possible to identify a biological marker to assess the intensity of exposure or concentration in the target organ (Lauwerys et al. 1992). As noted by Rehnberg et al. (1982), manganese levels in tissues are subject to homeostatic regulation via changes in absorption and/or excretion rates. While exposure to very high levels may overwhelm these mechanisms, continuous exposure to moderate excesses of manganese does not appear to cause a continuous increase in tissue levels (Rehnberg et al. 1982). Moreover, even if tissue levels are increased in response to above-average exposure, levels are likely to decrease toward the normal level after exposure ceases. For example, the level of manganese in the brain of a subject with severe manganism was not different from the normal level (Yamada et al. 1986). For these reasons, measurement of tissue levels of manganese at autopsy or possibly biopsy may be of some value in detecting current exposure levels but is not useful in detecting past exposures. Evaluation of manganese exposure by analysis of tissue levels is also not readily applicable to living persons except through the collection of biopsy samples.

Magnetic resonance imaging (MRI) has been used to track manganese distribution in the brains of Cebus monkeys (Newland and Weiss 1992; Newland et al. 1989) and humans (Wolters et al. 1989). In addition, it has been used to assay hyperintense signaling in the globus pallidus and other brain areas of individuals with chronic liver disease (Devenyi et al. 1994; Hauser et al. 1994, 1996; Pomier-Layrargues et al. 1998; Spahr et al. 1996), individuals on chronically-administered TPN (Kafritsa et al. 1998; Nagatomo et al. 1999; Ono et al. 1995), and those with symptoms characteristic of manganism (Nelson et al. 1993). Although data addressing the sensitivity and specificity of MRI as a biochemical indicator for body burden or exposure are not currently available, the technique is being used to identify individuals who are likely to

have increased stores of manganese in brain and potentially in other tissues, as well. For example, the hyperintense signaling in the brain is typically coincident with elevated blood manganese levels (Devenyi et al. 1994; Hauser et al. 1994, 1996; Kafritsa et al. 1998; Nagatomo et al. 1999; Ono et al. 1995; Pomier-Layrargues et al. 1998; Spahr et al. 1996). Neutron activation has been shown to be a possible means of *in vivo* measurement of manganese in the liver and possibly other tissues and organs, including the brain (Arnold et al. 1999; Rose et al. 1999). Minimum detection levels are low enough to distinguish between normal and elevated concentrations.

Scalp hair has also been investigated as a possible biomarker of manganese exposure. While some studies have found a correlation between exposure level and manganese concentration in hair (Collipp et al. 1983), use of hair is problematic for several reasons. For example, exogenous contamination may yield values that do not reflect absorbed doses, and hair growth and loss limit its usefulness to only a few months after exposure (Stauber et al. 1987). Manganese has also been reported to have a strong affinity for pigmented tissues (Lydén et al. 1984), and Hurley and Keen (1987) and Sturaro et al. (1994) have reported that manganese concentrations in hair vary with hair color. Further, hair may be contaminated by dye, bleaching, or other materials. Thus, it is not surprising that other studies have found no correlation between individual hair levels and the severity of neurological effects in manganese-exposed persons (Stauber et al. 1987). A study that investigated the correlation between potentially toxic metal content in hair and violent behavior found an association between manganese and violent behavior, but it was not conclusively established that manganese was the causative factor (Gottschalk et al. 1991). He et al. (1994) observed that poor performance in school and on neurobehavioral tests was inversely correlated with hair levels of manganese. The manganese exposure in this study was via drinking water and certain foods. Several studies have found that manganese levels in hair are higher in learning disabled children than in nondisabled children (Collipp et al. 1983; Pihl and Parkes 1977). The route of excess exposure is not known but is presumed to be mainly oral. However, an association of this sort is not sufficient to establish a cause-effect relationship since a number of other agents, including lead, might also be involved (Pihl and Parkes 1977).

Ethylene thiourea (ETU) is readily detected in the urine of humans and animals exposed to maneb and mancozeb (Kurttio and Savolainen 1980; Kurttio et al. 1980; Lyman 1971). However, there is no clear dose-response relationship between urinary levels of ETU and the amount of fungicide to which a person or animal has been exposed, and despite the long estimated half-life of ETU in urine (estimated at between 32–37 hours and 100 hours; Kurttio and Savolainen 1980; Kurttio et al. 1980), its presence is still

indicative only of recent exposures. In addition, ETU is a metabolite of many dithiocarbamate pesticides, so the presence of ETU does not conclusively prove exposure to maneb or mancozeb. The presence of ETU in urine can only be used as a qualitative indicator of potential exposure. Finally, ETU is not an ideal choice as a biomarker of exposure because it is a suspected carcinogen. Preliminary experiments have been done investigating the potential for using ETU-hemoglobin (ETU-Hb) adducts as a biomarker of exposure (Pastorelli et al. 1995). Adducts were isolated from the blood of rats dosed with 62.5–500 mg ETU/kg. Acid hydrolysis of the protein regenerated ETU, which could then be quantitated. The dose-response was linear up to 250 mg ETU/kg. The technique was then used on an exposed population of workers in a pesticide plant, 40% of whom had ETU-Hb adducts.

Clara cell protein CC16 is a potential biomarker for exposure to MMT, because the protein decreases in both BALF and serum following MMT exposure (Halatek et al. 1998; Bernard and Hermans 1997), possibly due to decreased synthesis and/or protein secretion due to loss of producing cells (Halatek et al. 1998). The protein can be quantified in serum or urine, but no dose-response studies on the potential biomarker have been performed.

There are no known biomarkers of exposure that are specific for children; any biomarkers applicable for use in adults should be applicable for children. For example, manganese-induced hyperintense signals on MRI have been seen in children (Devenyi et al. 1995; Kafritsa et al. 1998; Ono et al. 1995) as well as adults (Hauser et al. 1994, 1996; Nagatomo et al. 1999; Pomier-Layrargues et al. 1998; Spahr et al. 1996).

2.8.2 Biomarkers Used to Characterize Effects Caused by Manganese

The principal adverse health effects associated with exposure to manganese are respiratory effects (lung inflammation, pneumonia, reduced lung function, etc.) and the neurological syndrome of manganism and preclinical neurological effects. Although the respiratory effects are similar in many different exposure studies (Kagimimori et al. 1973; Lloyd Davies 1946; Nogowa et al. 1973), there are no specific biomarkers of effect other than reduced lung function. The fully developed disease can be diagnosed by the characteristic pattern of symptoms and neurological signs (Mena et al. 1967; Rodier 1955), but the early signs and symptoms are not specific for manganese. Careful neurological and psychomotor examination in conjunction with known exposure to manganese may be able to detect an increased incidence of preclinical signs of neurological effects in apparently healthy people (Iregren et al. 1990; Roels et al. 1987a). However, these signs are not sufficiently specific for preclinical effects of manganese to reliably identify whether an

individual has been exposed to excess levels for a prolonged period. In addition, no biochemical indicator is currently available for the detection of the early neurotoxic effects of manganese. There are no specific biomarkers that would clearly indicate long-term exposure to excess manganese.

Idiopathic Parkinsonism and manganism can be difficult to distinguish due to some similarity in the symptoms (Kim et al. 1999). Idiopathic Parkinsonism is marked by neurodegeneration in the dopaminergic nigrostriatal pathway, while manganism induced damage occurs postsynaptic to the nigrostriatal system. Postiron emission tomography (PET) with ^{18}F-dopa afforded a differentiation between manganism and idiopathic Parkinsonism in isolated patients with manganese exposure by indexing the integrity of the dopaminergic nigrostriatal pathway.

Measurement of altered levels of dopamine and other neurotransmitters in the basal ganglia has proven to be a useful means of evaluating central nervous system effects in animals (e.g., Bonilla and Prasad 1984; Eriksson et al. 1987a, 1987b), and these changes are often observed before any behavioral or motor effects are apparent (Bird et al. 1984). No noninvasive methods are currently available to determine whether there are decreased dopamine levels in the brain of exposed humans, but decreased urinary excretion of dopamine and its metabolites has been noted in groups of manganese-exposed workers (Bernheimer et al. 1973; Siqueira and Moraes 1989). However, the relationship between manganese effects on peripheral versus central dopamine levels has not been clearly defined, and given the lack of change in dopamine content in substantia nigra of humans exposed to manganese, the relevance of the animal studies to central nervous system disorder is questionable.

Smargiassi et al. (1993) evaluated platelet monoamine oxidase (MAO) and serum dopamine β-hydroxylase (DBH) activities in 11 men occupationally exposed to manganese via inhalation in a ferroalloy plant. Exposed workers, in general, had lower MAO activities, but similar DBH activities, in comparison to 15 nonexposed control males. However, a positive dose-effect relationship was observed in the exposed group between a Cumulative Exposure Index (CEI) and DBH activity ($r^2 = 0.40$, $p<0.05$). The CEI took into account the average annual respirable or total manganese concentrations in dust, the ventilation characteristic of each working area, the number of years each worker spent in a given area, and all the areas a worker had been during his job history. The authors proposed that DBH, which is an expression of catecholamine release, might be increasing dose-dependently in response to reduced turnover of MAO. The authors cautioned however, that while the data appear interesting, they should be investigated in a larger study population, with careful analysis of possible confounding factors (Smargiassi et al. 1993).

Reduced urinary excretion of 17-ketosteroids (perhaps as a consequence of decreased testosterone production) has been noted in many patients with neurological signs of manganism (Rodier 1955), but it has not been determined whether this change is detectable prior to the occurrence of neurological effects. Although the urinary excretion of manganese is generally not related to oral manganese intake, Davis and Greger (1992) have suggested that the concentration of manganese in serum, combined with lymphocyte manganese-dependent superoxide dismutase activity, may be helpful in assessing low and moderate levels of manganese exposure. Manganese superoxide dismutase is activated by manganese, thus it is sensitive to the overall manganese balance. Therefore, increased manganese concentrations will effect an increased manganese superoxide dismutase level. There is no clear link between activity of superoxide dismutase and the harmful effects of manganese. Therefore, the potential usefulness of this technique as a biomarker of effect requires further evaluation.

The Clara cell protein CC16 is a potential biomarker for pulmonary effects from exposure to MMT (Bernard and Hermanns; Halatek et al. 1998). Damage of Clara cells by MMT causes a significant reduction in the levels of this protein in the BALF, but does not affect its level in serum. The protein can be quantified in serum or urine as well. However, no dose-response studies on the potential biomarker have been performed. Further, the protein has only been studied following i.p. administration of MMT. It is unknown if CC16 levels will change following other exposure pathways.

For more information on biomarkers for renal and hepatic effects of chemicals see ATSDR/CDC Subcommittee Report on Biological Indicators of Organ Damage (1990) and for information on biomarkers for neurological effects see OTA (1990).

2.9 INTERACTIONS WITH OTHER SUBSTANCES

There is clear evidence from studies in animals that the gastrointestinal absorption (and hence the toxicity) of manganese is inversely related to dietary iron concentrations. That is, high levels of nonheme iron lead to decreased manganese absorption and toxicity, and low levels of iron lead to increased manganese absorption
and toxicity (Chandra and Tandon 1973; Davis et al. 1992a, 1992b; Diez-Ewald et al. 1968; Rehnberg et al. 1982). Conversely, high levels of dietary manganese lead to decreased iron absorption (Davis et al. 1992; Diez-Ewald et al. 1968; Rossander-Hulten et al. 1991; Thomson et al. 1971). Short-term effects of this sort are believed to be the result of kinetic competition between iron and manganese for a limited

number of binding sites on intestinal transport enzymes (Thomson et al. 1971), while longer term effects of iron deficiency or excess are thought to be due to adaptive changes in the level of intestinal transport capacity (Cotzias 1958). The studies reporting competition between iron and manganese in absorption clearly indicate the impact an iron-poor diet will have on manganese uptake in the human (Chandra and Tandon 1973; Davis et al. 1992a, 1992b; Diez-Ewald et al. 1968; Mena et al. 1969; Rehnberg et al. 1982; Thomson et al. 1971). Further, competition between manganese and iron at the blood-brain barrier has been reported (Aschner and Aschner 1990), indicating that excesses of either metal will affect the brain distribution of the other. Johnson and Korynta (1992) found that, in rats, dietary copper can also decrease manganese absorption and increase manganese turnover; dietary ascorbate supplementation had minimal effects on manganese absorption. However, there is insufficient information to determine the significance of these observations for health effects in humans exposed to copper and manganese by the oral route.

Mn(II) pretreatment reduces Cd(II)-induced lethality (Goering and Klaassen 1985). Cadmium has been noted to have an inhibitory effect on manganese uptake (Gruden and Matausic 1989). In addition, manganese appears to be capable of increasing the synthesis of the metal-binding protein metallothionine (Waalkes and Klaassen 1985). Data from a study by Goering and Klaassen (1985) suggest that manganese pretreatment increases the amount of Cd^{+2} bound to metallothionine, thereby decreasing hepatotoxicity due to unbound Cd^{+2}. The significance of these observations for health effects in humans exposed to cadmium and manganese by the oral or inhalation routes is not clear.

High dietary intakes of phosphorus (Wedekind et al. 1991) and calcium (Wilgus and Patton 1939) were shown to depress manganese utilization in chicks. Low levels of calcium and iron may act synergistically to effect manganese toxicity by increasing absorption, but it is not known whether ensuring iron plus calcium sufficiency will reduce the toxic effects of manganese once it has been absorbed (Cawte et al. 1989). Thus, the importance of these observations to humans exposed to manganese by the oral or inhalation routes is not clear.

Ethanol has been suspected of increasing the susceptibility of humans to manganese toxicity (e.g., Rodier 1955), but evidence to support this is limited. Singh et al. (1979) and Shukla et al. (1976) reported that concomitant exposure of rats to ethanol and manganese (as $MnCl_2$ in drinking water) led to higher levels of manganese in the brain and liver than if manganese were given alone; the higher levels were accompanied by increased effects as judged by various serum or tissue enzyme levels (Shukla 1978). Although the

authors referred to these effects as "synergistic," the data suggest that the effects were more likely additive. Based on the report in humans and evidence in animals, the effects of manganese on humans may be enhanced by the consumption of ethanol, but additional investigation is needed.

There is some evidence from a study in animals that chronic administration of drugs such as chlorpromazine (an antipsychotic) results in increased levels of manganese in the brain, including the caudate nucleus (Weiner et al. 1977). Chronic chlorpromazine treatment sometimes results in tardive dyskinesia, and manganese deposition in the brain might contribute to this condition. It has not been determined whether excess manganese exposure increases the risk of chlorpromazine-induced dyskinesia.

Intramuscular injection of animals with metallic nickel or nickel disulfide (Ni_3S_2) normally leads to a high incidence of injection-site sarcomas, but this increased incidence is reduced when the nickel is injected along with manganese dust (Sunderman et al. 1976). The mechanism of this effect is not clear, but natural killer cell activity normally undergoes a large decrease following nickel injection, and this is prevented by the manganese (Judde et al. 1987). However, the significance these observation have for human health effects resulting from exposure to nickel and/or manganese by the oral or inhalation routes is not clear.

One study has found that allopurinol, when administered orally to rats, antagonized the oxidative effects of manganese in the striatum and brainstem (Desole et al. 1994). The authors suggest that allopurinol, a xanthine oxidase inhibitor, may exert its protective effect by inhibiting both dopamine oxidative metabolism and xanthine oxidase-mediated production of reactive oxygen species.

2.10 POPULATIONS THAT ARE UNUSUALLY SUSCEPTIBLE

A susceptible population will exhibit a different or enhanced response to manganese than will most persons exposed to the same level of manganese in the environment. Reasons include genetic make-up, developmental stage, age, health and nutritional status (including dietary habits that may increase susceptibility, such as inconsistent diets or nutritional deficiencies), and substance exposure history (including smoking). These parameters may result in decreased function of the detoxification and excretory processes (mainly hepatic, renal, and respiratory) or the preexisting compromised function of target organs (including effects or clearance rates and any resulting end-product metabolites). For these reasons we expect the elderly, with declining organ function, and the youngest of the population, with

immature and developing organs, will generally be more vulnerable to toxic substances than healthy adults. Populations who are at greater risk due to their unusually high exposure are discussed in Section 5.7.

A number of researchers have observed that there is a wide range in individual susceptibility to the neurological effects of inhaled manganese dusts (Rodier 1955; Schuler et al. 1957; Smyth et al. 1973; Tanaka and Lieben 1969). For example, Rodier (1955) reported that the majority of manganism cases in miners occurred after 1–2 years of exposure to the metal, with only 6 cases observed occurring with 1–3 months exposure. Schuler et al. (1957) showed that in his group of miners, the average time for manifestation of manganism was 8 years, 2 months, with a minimum exposure of 9 months required for symptoms to present. However, the reason for this variable susceptibility is not clear. One likely factor is a difference in work activities and level of exertion. Another is that rates of manganese absorption and/or excretion can vary widely among individuals (Saric et al. 1977a). These toxicokinetic variations may be due to differences in dietary levels of iron and differences in transferrin saturation (Chandra and Tandon 1973; Davis et al. 1992a, 1992b; Mena et al. 1969; Thomson et al. 1971), to differences in dietary levels of other metals (Chowdhury and Chandra 1987; Gruden and Matausic 1989) or of calcium (Cawte et al. 1989), or to different levels of alcohol ingestion (Schafer et al. 1974). Another factor that might be relevant is dietary protein intake: low-level protein intake appears to increase the effect of manganese on brain neurotransmitter levels in exposed animals (Ali et al. 1983a, 1983b, 1985). However, a genetic basis for the wide difference in susceptibility cannot be ruled out.

One group that has received special attention as a potentially susceptible population is the very young. This is mainly because a number of studies indicate that neonates retain a much higher percentage of ingested or injected manganese than adults, both in animals (Keen et al. 1986; Kostial et al. 1978; Rehnberg et al. 1980) and in humans (Zlotkin and Buchanan 1986). The basis for high manganese retention in neonates is not certain but is presumably a consequence of increased absorption (Mena et al. 1974; Rehnberg et al. 1980) and/or decreased excretion (Kostial et al. 1978; Miller et al. 1975; Rehnberg et al. 1981), possibly because maternal milk is low in manganese (Ballatori et al. 1987). Regardless of the mechanism, the result of the high retention is increased levels of manganese in the tissue of exposed neonatal animals (Miller et al. 1975; Rehnberg et al. 1980, 1981), especially in the brain (Kontur and Fechter 1985, 1988; Kostial et al. 1978; Kristensson et al. 1986; Miller et al. 1975; Rehnberg et al. 1981). This increase has caused several researchers to express concern over possible toxic effects in human infants exposed to manganese in formula (Collipp et al. 1983; Keen et al. 1986; Zlotkin and Buchanan 1986). At least one recent report indicates that an infant's rate of absorption of manganese from infant

formulas, cow's milk, and breast milk is similar (Lönnerdal et al. 1994), resulting mainly from recent modifications to formulas to optimize the bioavailability of several essential minerals. There is some limited evidence that prenatal or neonatal exposure of animals to elevated levels of manganese can lead to neurological changes in the newborn (Ali et al. 1983a; Chandra and Shukla 1978; Deskin et al. 1980, 1981; Dorman et al. 2000; Kristensson et al. 1986), others have either not observed any neurochemical or neurophysiological effects in young animals exposed to excess manganese or the effects have been transient (Kontur and Fechter 1988; Kostial et al. 1978; Pappas et al. 1997). Currently, there is only one report that indicates that neonatal animals showed adverse neurological effects at a dose of manganese that had no effect on adults (Dorman et al. 2000). Brain concentrations of manganese were elevated in the neonates, but not in the adult animals given comparable doses of manganese for similar durations. The concern is that the young may be more susceptible due to increased absorption and/or retention and the potential toxicity from higher circulating levels of the metal. A few studies have reported increased blood and brain levels of the metal, either because of an inability to clear manganese due to chronic liver disease (Devenyi et al. 1994) or to an excess in parenteral nutrition (Kafritsa et al. 1998; Ono et al. 1995). However, observable neurological signs associated with manganese toxicity were only reported in the case of chronic liver disease (Devenyi et al. 1994). Although data suggest that children, particularly infants, are potentially more susceptible to the toxic effects of manganese, available evidence indicates that individual susceptibility varies greatly. Current information is not sufficient to quantitatively assess how susceptibility in children might differ from adults.

Elderly people might also be somewhat more susceptible to manganese neurotoxicity than the general population. Neurological effects were observed in older persons consuming manganese levels similar to levels found in U.S. surface and groundwaters (Deveral and Mellard 1988; EPA 1984; Kondakis et al. 1989). The neurological effects observed in a group of families exposed to manganese in their drinking water were reportedly more severe among the older persons whereas there was little effect in the youngest (Kawamura et al. 1941). Further, occupational studies indicate that older workers represent the largest numbers of manganese poisoning cases (Rodier 1955; Tanaka and Lieben 1969). More recent occupational (Crump and Rousseau 1999; Gibbs et al. 1999) and environmental (Mergler et al. 1999) manganese exposure studies indicate that increasing age was a factor in poorer performance on certain neurobehavioral tests. For example, Beuter et al. (1999) and Mergler et al. (1999) reported that performance on tests that required regular, rapid, and precise pointing movements was significantly decreased in exposed individuals, especially in those 50 years of age and over with high blood manganese levels. These reports suggest that older persons may have a greater susceptibility to adverse effects from

inhaled or ingested manganese. One factor that could contribute to this increased susceptibility is a loss of neuronal cells due to aging or to accumulated neurological damage from other environmental neurotoxicants (Silbergeld 1982). Homeostatic mechanisms might become less effective in aged populations which leads to higher tissue levels of manganese following exposure (Silbergeld 1982).

Mena et al. (1969) noted that the oral absorption of manganese was increased in individuals with iron-deficiency anemia. Altered nutritional status might be another predisposing factor. The inverse relationship of manganese absorption and iron-status has also been reported in animal models (Davis et al 1992a, 1992b). It has been suggested that anemic persons may be more susceptible to the toxic effects of manganese because of enhanced absorption of iron and manganese through similar uptake mechanisms (Cotzias et al. 1968). Baldwin et al. (1999) reported an inverse relationship between serum iron and blood manganese levels in individuals environmentally exposed to airborne manganese.

Another group of potential concern is people with liver disease. This is because the main route of manganese excretion is via hepatobiliary transport (see Section 2.3.4), so individuals with impaired biliary secretion capacity would be expected to have a diminished ability to handle manganese excesses. In support of this hypothesis, Hambidge et al. (1989) reported that in a group of infants and children receiving parenteral nutrition, children with liver disease had higher average plasma concentrations of manganese than children without liver disease. Devenyi et al. (1994) also observed increased blood manganese concentrations, abnormal MRI scans indicative of increased manganese in the brain, and dystonia similar to that of patients with manganism, in an 8-year-old girl suffering from cholestatic liver disease. Hauser et al. (1994) reported increased blood and brain manganese in two patients with chronic liver disease and one with cirrhosis of the liver and a portacaval shunt. All three exhibited some form of neuropathy, including postural tremor of the upper extremities and a general lack of alertness, along with failure to concentrate and follow simple commands. In a later study, Hauser et al. (1996) did not observe movement disorders, but did observe the increased blood manganese concentrations and abnormal MRI scans in a group of adults with failing livers. Other studies have shown the link between increased deposition of manganese in the blood and/or the brains of humans with cirrhosis of the liver or chronic liver disease (Pomier-Layrargues et al. 1998; Rose et al. 1999; Spahr et al. 1996).

Patients on parenteral nutrition may be at risk for increased exposure to manganese. Forbes and Forbes (1997) observed that 31 of 32 adults treated with TPN due to intestinal failure had increased manganese concentrations in their blood. Nagatomo et al. (1999) observed elevated blood manganese levels and

hyperintense signals in the basal ganglia upon T1-weighted MRI in two elderly patients receiving TPN. Both patients exhibited severe symptoms associated with manganese exposure (masked facies, marked rigidity, hypokinesia). When manganese supplementation in the TPN was reduced, the blood and brain levels returned to normal.

Children receiving parenteral nutrition have also been shown to have increased blood manganese concentrations with accompanying hyperintense signals in the globus pallidus as observed by MRI (Fell et al. 1996; Kafritsa et al. 1998; Ono et al. 1995). Fell et al. (1996) studied a group of 57 children receiving parenteral nutrition, 11 of whom had a combination of hypermanganesemia and cholestasis. Four of these 11 patients died; the 7 survivors had whole blood manganese concentrations ranging from 34–101 µg/L. Four months after reduction or removal of manganese from the supplementation, the blood concentration of manganese decreased by a median of 35 µg/L. Two of the seven survivors had movement disorders, one of whom survived to have a MRI scan. The scan revealed bilateral symmetrically increased signal intensity in the globus pallidus and subthalamic nuclei. These signals were also observed in five other children—one from the original group exhibiting cholestasis with hypermanganesemia and five more given parenteral nutrition chronically with no liver disease. These results indicate that the cholestatic condition is not necessary for manganese to accumulate in the brain. A supporting study is provided by Ono et al. (1995) who observed increased blood manganese concentrations and hyperintense signals on MRI in the brain of a 5-year-old child on chronic parenteral nutrition due to a gastrointestinal failure. Five months after the manganese was removed from the parenteral solution, blood manganese levels returned to normal, and the brain MRI scans were almost completely free of abnormal signals. Further, the authors reported no neurological effects from exposure to manganese. Kafritsa et al. (1998) reported results similar to those of Ono et al. (1995). In the latter study, 2 siblings, one 9 and the other 2 years old, had been administered TPN chronically since the ages of 4 and 1 month(s), respectively. While elevated blood and brain manganese levels were reported (via laboratory analyses and MRI), no adverse neurological or developmental effects were observed. Once the manganese supplementation was reduced, the MRI signals abated, and the blood manganese levels returned to a normal range.

Although human interindividual variability is great concerning the ability to tolerate excess amounts of manganese in the body, these data indicate that, in general, children and the elderly may be more susceptible than young and middle-aged adults due to differential toxicokinetics and potential adverse effects superimposed on normal decline in fine motor function with age.

With respect to the respiratory effects of inhaled manganese (e.g., bronchitis, pneumonitis), people with lung disease or people who have exposure to other lung irritants may be especially susceptible. This is supported by the finding that the inhalation of manganese dusts by manganese alloy workers caused an increased incidence of respiratory symptoms (e.g., wheezing, bronchitis) in smokers but not in nonsmokers (Saric and Lucic-Palaic 1977b).

2.11 METHODS FOR REDUCING TOXIC EFFECTS

This section will describe clinical practice and research concerning methods for reducing toxic effects of exposure to manganese. However, because some of the treatments discussed may be experimental and unproven, this section should not be used as a guide for treatment of exposures to manganese. When specific exposures have occurred, poison control centers and medical toxicologists should be consulted for medical advice. The following text provides specific information about treatment following exposures to manganese: WHO/IPSC. 1999. Concise International Chemical Assessment Document 12: Manganese and its compounds. World Health Organization/Inter-Organization Programme for the Sound Management of Chemicals.

2.11.1 Reducing Peak Absorption Following Exposure

There is substantial evidence to indicate that an interaction between iron and manganese occurs during intestinal absorption (Chandra and Tandon 1973; Diez-Ewald et al. 1968; Keen and Zidenberg-Cher 1990; Mena et al. 1969; Rehnberg et al. 1982). Cawte et al. (1989) cite low levels of iron and calcium as "synergistic factors" that impact on the toxic effects associated with manganese exposures. In a dietary study investigating the effects of copper, iron, and ascorbate on manganese absorption in rats, these substances were all found to influence manganese absorption, depending in part on their relative concentrations (Johnson and Korynta 1992).

Evidence from these reports suggests that it may be possible to reduce the uptake of manganese and thereby circumvent the potential for toxic effects caused by current and future exposure to excess manganese through specific dietary supplementation. For example, sufficient iron or calcium stores, as opposed to a deficiency in these or other minerals, may reduce manganese absorption, and thus reduce potential toxicity. It is not known whether ensuring iron and calcium sufficiency will reduce the toxic

effects of manganese once it has been absorbed into the body because information on critical levels of manganese at target sites is not available.

2.11.2 Reducing Body Burden

Inhaled manganese is readily absorbed by the lungs, although some may be retained there. Larger particles of dust containing manganese may be transported by mucociliary transport from the throat to the gut (Drown et al. 1986). Manganese in the gut may be directly absorbed either by a simple diffusion process (Bell et al. 1989) or by a high-affinity, low-capacity, active-transport mechanism (Garcia-Aranda et al. 1983). Once in the plasma, manganese is reportedly transported by transferrin; however, information on the mechanism of uptake in extrahepatic tissues is limited (Keen and Zidenberg-Cher 1990).

Chelation therapy with agents such as EDTA (ethylenediaminetetraacetic acid) may alleviate some of the neurological signs of manganism, but not all patients show improvement, and some of the improvement may not be permanent (Cook et al. 1974; Ellenhorn and Barceloux 1988). Nagatomo et al. (1999) recently reported the use of Ca-EDTA treatment to reduce the body burden of two elderly patients with increased blood and brain levels of manganese. These patients exhibited masked facies, hypokinesia, and rigidity that are among the clinical signs of manganese poisoning. The potential use of calcium disodium ethylenediaminetetracetate ($CaNa_2$ EDTA) for the management of heavy metal poisoning was investigated in dogs by Ibim et al. (1992). $CaNa_2$ EDTA-treated dogs (without excess manganese exposure) were found to have decreased manganese levels in their hair. It is possible that the decrease was partially associated with mobilization and redistribution of this element from storage as well as from soft tissues. The authors, however, cautioned that the use of $CaNa_2$ EDTA could adversely affect the metabolism of manganese. Cyclohexylene-aminotetraacetic acid (CDTA) and dimercaptol-1-propanesulphonic acid sodium salt (DTPA) were shown to decrease tissue manganese content in rats following inhalation exposure, but it is unknown whether the effects of manganese were alleviated (Wieczorek and Oberdorster 1989a, 1989b).

A study in monkeys reported a long half-life of manganese in the brain following inhalation exposure (Newland et al. 1987). Given that neurotoxicity is of concern with manganese exposure, knowledge of the mechanisms behind this longer half-life in the brain may be central to the development of mitigation methods. Newland et al. (1987) reported that this long half-life reflected both redistribution of manganese from other body depots and a slow rate of clearance from the brain. A later study reported that elevated

levels in the brain persisted after inhalation exposure (due to redistribution), whereas for subcutaneous exposure, levels declined when administration was stopped (Newland et al. 1989). The authors observed that the accumulation of manganese in the brain was preferential in specific regions but was unrelated to the route of exposure (Newland et al. 1989). They also reported that there are no known mechanisms or "complexing agents" that have been shown to remove manganese from the brain.

Few data are available regarding the reversibility of the neurological injury produced by prolonged excess manganese exposure. The effects are thought to be largely irreversible, and treatment for manganese intoxication is mainly supportive (Ellenhorn and Barceloux 1988). However, some evidence indicates that recovery may occur when exposure ceases (Smyth et al. 1973). Anti-Parkinsonian drugs, such as levo-dopa, have been shown to reverse some of the neuromuscular signs of manganism (Ejima et al. 1992; Rosenstock et al. 1971), but these drugs can produce a variety of side effects, and reports have indicated that they are not effective in improving the symptoms of neurotoxicity in manganism patients (Calne et al. 1994; Chu et al. 1995; Cook et al. 1974; Ellenhorn and Barceloux 1988; Haddad and Winchester 1990; Huang et al. 1989). Para-aminosalicylic acid was used successfully to treat two patients who exhibited neurological signs of manganese poisoning; one person made an almost complete recovery and the other was significantly improved. The mechanism for this treatment is unknown (Shuqin et al. 1992). Parenti (1988) has proposed the use of antioxidants such as vitamin E, but the effectiveness of this treatment has not been further evaluated.

2.11.3 Interfering with the Mechanism of Action for Toxic Effects

The oxidation state of manganese may influence both its retention in the body (see Section 2.3.3) and its toxicity (see Section 2.4). Therefore, it is possible that interference with the oxidation of manganese could be a method for preventing manganese cellular uptake and toxicity. Regarding retention, one study suggests that clearance is much more rapid for divalent manganese than for trivalent manganese (Gibbons et al. 1976). Regarding neurotoxicity, Mn(III) appears to be more efficient in enhancing the oxidation of catechols than either Mn(II) or Mn(IV) (Archibald and Tyree 1986). Thus it is plausible that reducing the formation of Mn(III) could possibly both enhance elimination and prevent neurotoxicity, but no studies were located that evaluate this theory.

Ceruloplasmin is involved in the oxidation of iron and has also been involved in the oxidation of divalent manganese ion to the trivalent state (Gibbons et al. 1976). Selective inhibition of this oxidative function may be a method of mitigating the toxic effects of exposure to manganese. However, inhibition of the oxidation of manganese might also result in adverse effects on transport and cellular uptake of other essential metals, especially iron. Furthermore, it is not completely clear how the oxidation state of manganese is related to its normal function in neural cells or how this role is altered in manganese toxicity. Both Mn(II) and Mn(III) have been reported as components of metalloenzymes (Keen and Zidenberg-Cher 1990; Leach and Lilburn 1978; Utter 1976).

Manganese has been shown to catalyze the oxidation of dopamine *in vitro;* Cawte et al. (1989) reported that the toxicity induced by manganese resulted from the depletion of dopamine and the production of dopamine quinone and hydrogen peroxide through this mechanism. Antioxidants were tested for their ability to inhibit the dopamine oxidation induced by manganese, and it was found that ascorbic acid and thiamine completely inhibited dopamine oxidation both in the presence and absence of manganese. The report did not include data on background oxidation levels nor on the extent of dopamine oxidation in the absence of manganese. Results from treatment with antioxidants were viewed as evidence for their use in mitigating the adverse effects of manganese. However, because dopamine oxidation was inhibited to some degree in the absence of manganese, these data could alternately be interpreted as suggesting a more complex mechanism than the direct action of manganese for inducing dopamine oxidation and subsequent cell toxicity. Further investigation of the inhibition of manganese oxidation as a possible mitigation method should be preceded by additional studies to elucidate the role of manganese in its various oxidation states in normal neuronal cell metabolism and to determine whether oxidative stress is a primary mechanism for neurotoxicity mediated by manganese exposure.

2.12 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of manganese is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of manganese.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

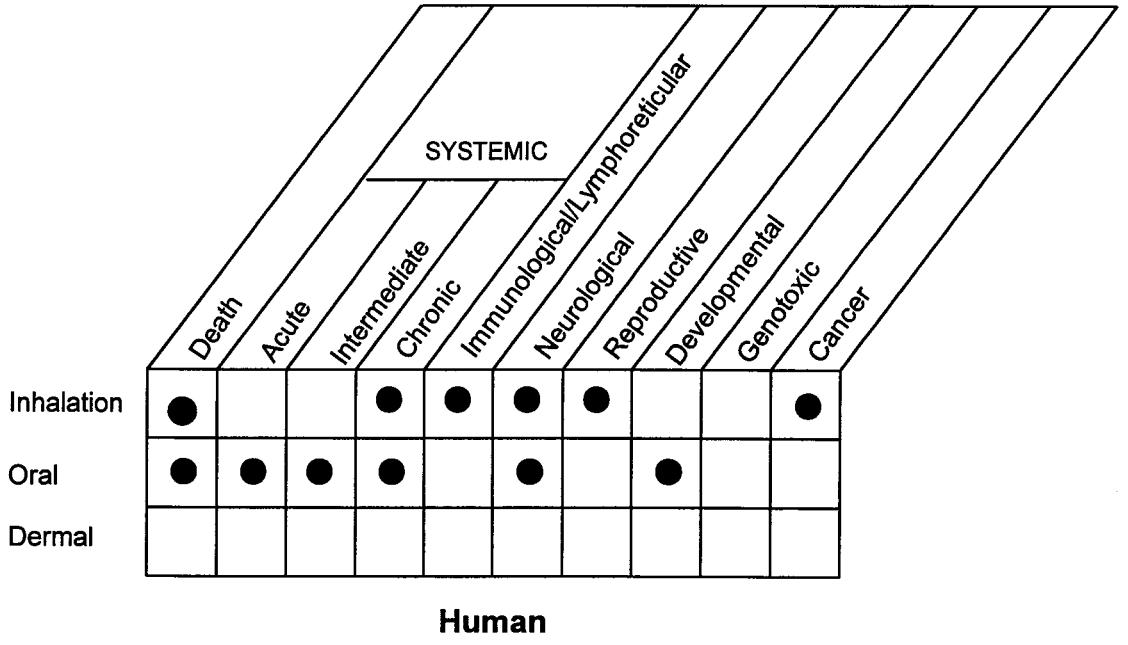
2.12.1 Existing Information on Health Effects of Manganese

The existing data on health effects of inhalation, oral, and dermal exposure in humans and animals to inorganic manganese and the organic manganese compounds maneb and mancozeb are summarized in Figures 2-7 and 2-8, respectively. The purpose of each figure is to illustrate the existing information concerning the health effects of manganese. Each dot in the figure indicates that one or more studies provide information associated with that particular effect. The dot does not necessarily imply anything about the quality of the study or studies, nor should missing information in this figure be interpreted as a "data need." A data need, as defined in ATSDR's Decision Guide for Identifying Substance-Specific Data Needs Related to Toxicological Profiles (ATSDR 1989), is substance-specific information necessary to conduct comprehensive public health assessments. Generally, ATSDR defines a data gap more broadly as any substance-specific information missing from the scientific literature.

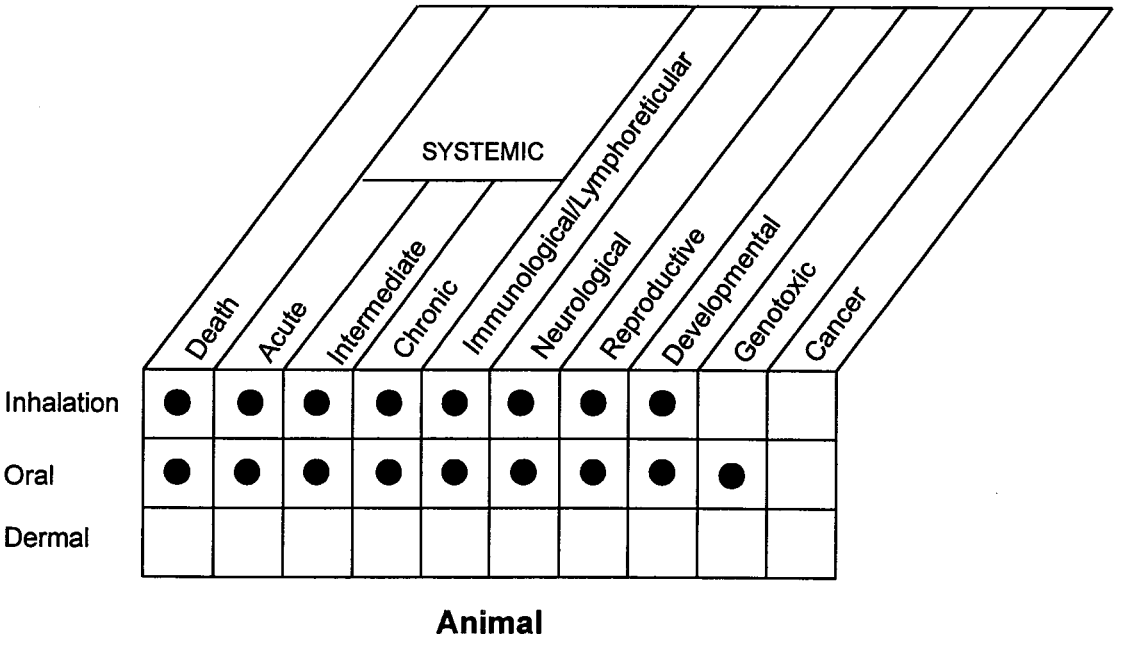
As the upper part of Figure 2-7 reveals, studies in humans exposed to inorganic manganese have focused mainly on intermediate and chronic inhalation exposure and the resulting neurological effects. There are several reports of humans exposed by ingestion and these too have focused on neurological effects. Reproductive effects have been studied in men exposed to manganese by inhalation, but other effects have generally not been formally investigated.

Inorganic manganese toxicity has been investigated in numerous animal studies, both by the oral and the inhalation routes. These studies have included most endpoints of potential concern. The dermal route for inorganic manganese has not been investigated. This lack of toxicological data makes it difficult to determine if dermal contact would be of concern. However, dermal contact to the organic compounds is expected to occur mainly in occupational settings, and organic compounds are degraded to some extent in the environment. Thus, dermal effects from organic manganese compounds are not expected to be of great concern for the general population or to persons near hazardous waste sites.

FIGURE 2-7. Existing Information on Health Effects of Inorganic Manganese



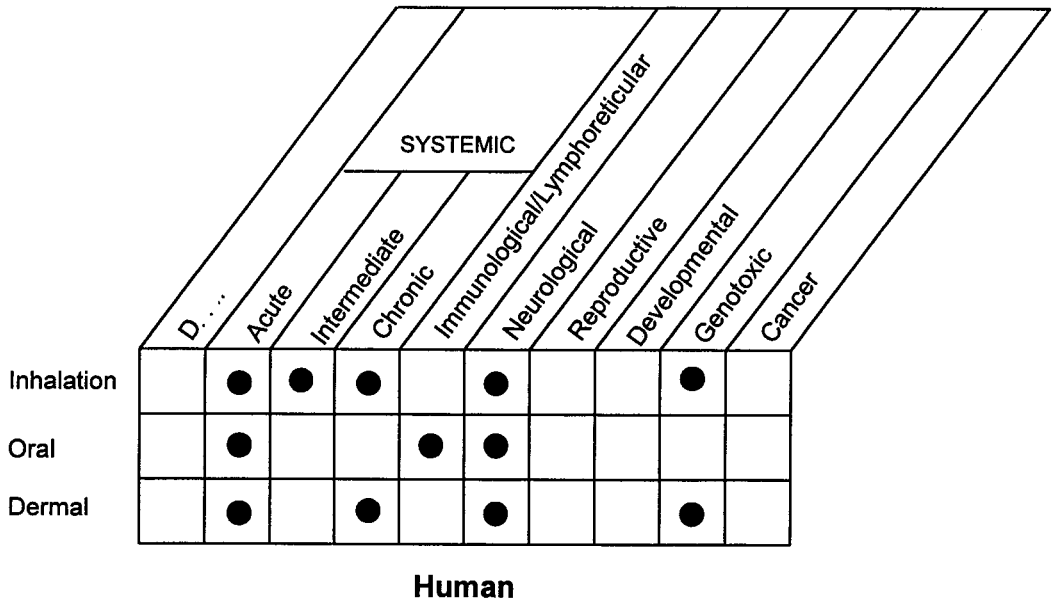
Human



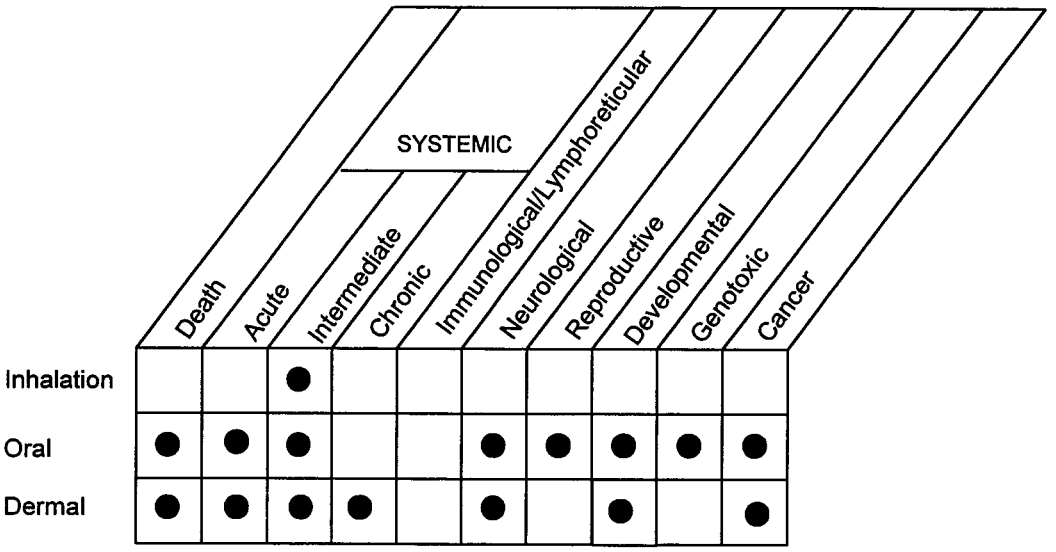
Animal

● Existing Studies

FIGURE 2-8. Existing Information on Health Effects of Organic Manganese --- Maneb & Mancozeb



Human



Animal

● Existing Studies

2.12.2 Identification of Data Needs

Presented below is a brief review of available information and a discussion of research needs. Although data are lacking, dermal studies to inorganic manganese are not discussed since there is no evidence that this exposure pathway is a human health concern.

Acute-Duration Exposure. Studies in animals and humans indicate that inorganic manganese compounds have very low acute toxicity by any route of exposure. An exception is $KMnO_4$, which is an oxidant that can cause severe corrosion of skin or mucosa at the point of contact (Southwood et al. 1987). Acute inhalation exposure to high concentrations of manganese dusts (MnO_2, Mn_3O_4) can cause an inflammatory response in the lung, which can lead to impaired lung function (Maigetter et al. 1976; Shiotsuka 1984). However, this response is characteristic of nearly all inhalable particulate matter (EPA 1985d) and is not dependent on the manganese content of the particle. Large oral doses of highly concentrated solutions of manganese salts given by gavage can cause death in animals (Holbrook et al. 1975; Kostial et al. 1978; Smyth et al. 1969), but oral exposures via food or water have not been found to cause significant acute toxicity (Gianutsos and Murray 1982; Hejtmancik et al. 1987a, 1987b). Since the acute database is incomplete and studies demonstrating a dose-response are not available, an acute MRL was not derived. In order to derive acute MRL values, further studies would be helpful to define the threshold for adverse effects following acute exposure to manganese. However, any MRL derived for the oral route would have to take into consideration that manganese is an essential nutrient.

Acute duration exposure studies in animals exposed to MMT via inhalation, or via a dermal pathway are lacking. The dermal pathway is very important, because MMT in gasoline that may be spilled on the skin could penetrate and become absorbed. Although the photolability of the compound is an important obstacle for any animal study, carefully planned and executed analyses of the toxicity of this compound to animal models through these exposure pathways are needed.

Case studies in men have provided some information as to the systemic toxicity following acute exposures to high doses of maneb and mancozeb (de Carvalho et al. 1989; Israeli et al. 1983a; Koizumi et al. 1979). However, these studies involve a wide range of doses, and incomplete analysis of potential systemic effects. Acute studies involving high-dose exposures of these pesticides in animals are generally lacking and would be helpful in discerning target organs and dose-response effects. Further, comparison studies

using maneb, mancozeb, and structurally similar dithiocarbamate pesticides would help elucidate a mechanism of action for toxicity.

The likelihood for exposure to mangafodipir is small and clinical trials in humans have shown a great tolerance for a controlled exposure to the compound. Toxicity studies in several different animal species have been performed, including reproductive and developmental studies (and more specifically, teratogenic analysis). Although behavioral data in the young who have been exposed during gestation are relatively limited, human gestational exposure to this compound is not believed to be very likely. Reports of neurological effects have been limited to complaints of headaches in clinical trials. Further evaluation of these effects relative to the distribution of manganese to the brain during clinical use is warranted. Mangafodipir is administered intravenously, which bypasses homeostatic control of the compound. Although animal studies indicate that a single, clinical dose does not cause accumulation of manganese in the brain for longer than 2 weeks (Gallez et al. 1997), human studies have not monitored central nervous system distribution of manganese following mangafodipir injection for longer than half an hour (Lim et al. 1992). In addition, given the neurotoxic effects of excess manganese, evaluation of patients treated with mangafodipir for neurological sequelae are needed.

Intermediate-Duration Exposure. Intermediate-duration inhalation exposure of humans to manganese compounds can lead to central nervous system effects (Rodier 1955). However, reliable estimates of intermediate-duration NOAELs or LOAELs for neurotoxicity in humans are not available. Intermediate-duration inhalation studies in animals have yielded NOAEL and LOAEL values for biochemical and neurobehavioral effects (EPA 1977; Morganti et al. 1985; Ulrich et al. 1979a, 1979b), but the range of exposure levels associated with these effects is too wide (an order of magnitude) to define a threshold. Although neurological effects were observed in animals, symptoms characteristic of manganese toxicity (e.g., ataxia, tremor, etc.) are not typically observed in rodent species (with the exception of one study in which ataxia was seen only transiently) (Kristensson et al. 1986). Although other rodent studies indicated decreases in motor activity (Gray and Laskey 1980; Komura and Sakamoto 1991) or increased activity and aggression (Chandra 1983) or delayed reflexes (Ali et al. 1983a), the effects are not consistent and are observed over a wide dose range. For these reasons, it is concluded that these data are not sufficient to derive an intermediate-duration inhalation MRL. Epidemiological studies in occupationally exposed human populations that help define the intermediate-duration exposure levels that are associated with neurological effects would be valuable.

Intermediate-duration oral exposure of humans to manganese has been reported to cause neurotoxicity in two cases (Holzgraefe et al. 1986; Kawamura et al. 1941), but the data for quantitating exposure levels are too limited to define the threshold or to judge whether these effects were due entirely to manganese exposure. An epidemiological investigation of people who have ingested high levels of manganese may provide valuable information on the health risk of intermediate-duration oral exposure and may provide sufficient dose-response data from which to derive an MRL. Additional oral studies in animals including rodents may be valuable in revealing cellular and molecular mechanisms of manganese neurotoxicity; studies on nonhuman primates would probably be the most helpful in estimating a MRL because they appear to be the most suitable animal model for manganese-induced neurological effects comparable to effects observed in humans. However, any MRL derived for the oral route would have to take into consideration that manganese is an essential nutrient and account for manganese intake from daily dietary sources.

Intermediate-duration studies of inhalation and oral exposure to MMT in humans and animals are lacking. Animal studies of this duration evaluating systemic toxicity from exposure to MMT and typical environmental concentrations of its combustion products would be helpful to determine body burdens that might be anticipated for the general population in areas that use this compound. Further, these studies would be helpful in determining mechanisms of toxicity and expected adverse effects in exposed populations.

Intermediate studies involving exposure to maneb and mancozeb in humans or animals are also lacking. Due to seasonal use of these compounds, human exposure is often transient, lasting only 3–6 months per year for some occupationally-exposed groups (such as farmers and sprayers). Intermediate studies in humans and animals or evaluations of workers using these agents would contribute greatly to the database concerning systemic and other health effects. These studies might also aid in the identification of dose-response relationships for effects from exposures to these pesticides.

Due to the nature of mangafodipir administration, which typically occurs only once in a subject, no intermediate-duration studies in humans have been identified for this compound. Although there are a few intermediate-duration studies in animals (Grant and Larsen 1997; Grant et al. 1997; Treinen et al. 1995), they have focused primarily on reproductive and developmental effects. Studies of the potential neurological effects of exposure to this compound are lacking, although the reason for this may be due to the lack of evidence that the compound distributes in the central nervous system. As discussed previously,

the exposure to mangafodipir is expected to be very limited due to the compound's clinical use. There are no identified data needs for this compound.

Chronic-Duration Exposure and Cancer. Studies in humans make clear that the main health effect following chronic inhalation exposure is nervous system toxicity (Emara et al. 1971; Mena et al. 1967; Rodier 1955; Schuler et al. 1957; Smyth et al. 1973) although adverse respiratory effects are also seen (Abdel Hamid et al. 1990; Akbar-Khanzadeh 1993; Lloyd Davies 1946; Roels et al. 1987a). Two studies exist concerning adverse pulmonary effects in adolescents that attend school and live near a manganese-processing plant (Kagamimori et al. 1973; Nogawa et al. 1973). Available data (Iregren 1990; Roels et al. 1992) are sufficient to derive a chronic inhalation MRL of 0.00004 mg/m^3 using benchmark dose analysis (Crump and Clewell 1999). A recent study reported the lack of an effect on performance in neuropsychological examinations following chronic exposure to low levels of manganese (0.051 mg/m^3 median, respirable dust) (Gibbs et al. 1999). This NOAEL is comparable to the NOAEL estimated from benchmark analysis of the individual exposure and response data from studies by Iregren (1990) and Roels et al. (1992). Thus, there does not appear to be a need for additional epidemiological studies to evaluate the neurological effects of inhaled inorganic manganese. Recent studies evaluating the reversibility of neurological effects from inhaled manganese have reported that there was an improvement in performance in at least one test among those exposed to low levels of manganese. However, deficits in neurological function remained for those exposed to higher levels (Crump and Rousseau 1999; Roels et al. 1999). A significant finding discussed by Crump and Rousseau (1999) was that the neurological effects first observed among these workers had not worsened or progressed toward clinical signs of manganism. Additional studies involving follow-up evaluation of previously exposed occupational cohorts are needed to provide information on threshold levels that are correlated with observed preclinical effects.

Chronic inhalation studies in animal models (Bird et al. 1984; EPA 1977; Newland et al. 1989; Olanow et al. 1996) indicate that while non-human primates are very sensitive to the neurological effects of manganese at very low doses (depending on exposure route), rodent models do not exhibit the same neurological symptoms as humans and monkeys despite the administration of high doses through inhalation, oral, and intravenous exposure routes. Although there is an apparent difference in susceptibility, neurological effects have been observed in rodents treated with manganese. Additional studies in animals could be valuable to increase our understanding of the mechanism of manganese-induced disease and the basis for the differences between humans and animals.

Some data on neurological or other health effects in humans from chronic oral intake of manganese exist (Cawte et al. 1987; He et al. 1994; Kondakis et al. 1989; Vierrege et al. 1995; Zhang et al. 1995). The majority of these studies are limited by uncertainties in the exposure routes, total exposure levels, duration of exposure, or by the influence of other confounding factors.; none of these studies adequately assessed daily dietary manganese intake. Two of the most recent studies (He et al. 1994; Zhang et al. 1995) indicate concentrations of manganese in drinking water that may be associated with preclinical neurological effects in children, but the studies have several limitations. There are a few (mainly negative) chronic oral exposure studies in animals (Gupta et al. 1980; Hejtmancik et al. 1987a, 1987b; Lai et al. 1984; Nachtman et al. 1986), but these studies do not provide sufficient information to determine dose levels or effects of concern following chronic oral exposure. Based on the lack of conclusive evidence for adverse effects associated with chronic oral exposure to manganese in both humans and animals, no chronic oral MRL has been derived. The upper range of the estimated safe and adequate daily dietary intake of 5 mg/day (NRC 1989) has been adopted as a provisional guidance value (0.07 mg/kg/day) for oral exposure to manganese. This guidance is necessary because of the prevalence of manganese at hazardous waste sites and the fact that manganese is an essential nutrient. Additional chronic oral studies, especially epidemiological studies in populations exposed to high levels of either inorganic and organic manganese in the environment, particularly MMT and its combustion products in areas of high traffic density, would be valuable for evaluating the potential for adverse effects from oral exposure to excess manganese from the environment in addition to that ingested through dietary intake.

No studies or anecdotal reports were located that described cancer associated with exposure of humans to inorganic manganese. Chronic oral exposure of rats and mice to high doses of manganese sulfate has provided equivocal evidence of carcinogenic potential (NTP 1993), however the lack of evidence for the carcinogenic potential of manganese in humans and the equivocal evidence in animals suggest that the potential for cancer may be low. Further animal studies are not needed at this time.

MMT has not been found to induce tumor formation in rodents (Witschi et al. 1981) and additional studies measuring this endpoint are needed to corroborate the limited database. Gavage exposure to maneb in rats has resulted in promotion of NMU-induced pancreatic hyperplasia (Monis and Valentich 1993). Mancozeb was found to be both a weak initiator and promotor of benign tumors of mixed morphology after repeated doses (Shukla et al. 1990, 1988). Although these limited studies indicate a weak carcinogenic potential of the pesticides, it is known that ETU, a primary metabolite of both fungicides, is a thyroid carcinogen in the rodent (NTP 1990). It is possible that any carcinogenesis arising from exposure to these fungicides is a

result of ETU exposure, and not due to the parent compound. Additional chronic studies, in which the carcinogenesis of structurally similar dithiocarbamate pesticides were concomitantly assessed, would be helpful in identifying whether the parent compound or a metabolite is the active carcinogen and the organs affected. Though no studies of carcinogenesis involving mangafodipir exposure were identified, there are no data needs regarding this endpoint with this compound.

Genotoxicity. One study was located regarding the genotoxic effects of inorganic manganese in humans. An increase in chromosomal aberrations was observed in welders exposed to manganese; however, the welders were also exposed to nickel (known to cause chromosomal aberrations) and iron, so the observed increase could not be attributed solely to manganese (Elias et al. 1989). Some *in vivo* studies in fruit flies and rats have been negative (Dikshith and Chandra 1978; Rasmuson 1985; Valencia et al. 1985), but manganese has been found to be clastogenic in mice (Joardar and Sharma 1990). *In vitro* studies in bacteria, yeast, and cultured mammalian cells have yielded mixed, but mainly positive, results (Casto et al. 1979; De Méo et al. 1991; Joardar and Sharma 1990; Kanematsu et al. 1980; Nishioka 1975; NTP 1993; Oberley et al. 1982; Orgel and Orgel 1965; Singh 1984; Ulitzur and Barak 1988; Wong and Goeddel 1988; Zakour and Glickman 1984). Additional studies, especially in cultured mammalian cells, heritable cell types, or in lymphocytes from exposed humans, would be valuable in clarifying the genotoxic potential of manganese. As for organic manganese, no genotoxicity studies were located regarding MMT and studies measuring this endpoint are needed. Studies with mancozeb (Jablonická et al. 1989) or mixed dithiocarbamate (Steenland et al. 1997) have been suggestive of genotoxicity, but have been complicated by confounding factors or exposure to other pesticides. Additional *in vitro* and *in vivo* studies in animal models would be helpful to ascertain the genotoxic potential of maneb and mancozeb. Genotoxicity studies for mangafodipir have shown negative effects (Grant et al. 1997).

Reproductive Toxicity. Men who are exposed to manganese dust in workplace air report decreased libido and impotency (Emara et al. 1971; Mena et al. 1967; Rodier 1955), and may suffer from sexual dysfunction (Jiang et al. 1996b) and decreased sperm and semen quality (Wu et al. 1996). In addition, studies in animals indicate that manganese can cause direct damage to the testes (Chandra et al. 1973; Seth et al. 1973). While the Jiang et al. (1996b) study suggests testicular damage in occupationally exposed men, additional epidemiological studies involving these subjects or other exposed groups to more fully evaluate reproductive function would be valuable. Results from such studies may provide definitive exposure-response data on reproductive function (e.g., impotence, libido, and number of children).

Additional studies in animals are needed to determine whether the testes are damaged directly from exposure to manganese. Information on adverse reproductive effects in women is not available. Data from studies in female animals indicate that manganese can cause post-implantation loss when administered through both oral and subcutaneous exposure routes in female mice and rats (Colomina et al. 1996; Sánchez et al. 1993; Szakmáry et al. 1995; Treinen et al. 1995). To establish more clearly whether or not this is a human health concern, two types of studies would be valuable. First, single-generation reproductive studies of female animals exposed by the inhalation route could be done. Then, if strong evidence for concern is found in animals from these studies, epidemiological studies that included women and men exposed in the workplace would be valuable to assess the effects of manganese on reproductive function.

Developmental Toxicity. There are few human data on potential developmental effects of excess manganese. The incidences of stillbirths and malformations have been studied in an Australian aboriginal population living on an island where environmental levels of manganese are high (Kilburn 1987), but small population size and lack of data from a suitable control group preclude determining whether reported incidence of developmental abnormalities is higher than average. Two studies investigated neurobehavioral and school performances (He et al. 1994; Zhang et al. 1995) of children exposed to excess levels of manganese in water and food. However, these studies did not report data on either lengths of exposure to the metal or on excess manganese intake compared to control areas. Studies evaluating developmental effects with clear analysis of exposure levels and duration are needed to estimate dose-response relationships of manganese toxicity in children. Several developmental studies have been performed in animals, but they are limited to rodent species and have measured limited developmental endpoints. One study in pregnant mice that inhaled manganese resulted in decreased pup weight and a transient increase in activity (Lown et al. 1984). Other studies have indicated that oral exposure to manganese adversely affects reproductive development in male mice (Gray and Laskey 1980) and rats (Laskey et al. 1982, 1985). A single study on rats involving oral exposure indicated that manganese caused a transient decrease in pup weight and increased activity (Pappas et al. 1997). Another study involving gavage dosing reported skeletal abnormalities in unborn pups, but these effects were resolved in pups allowed to grow to 100 days of age (Szakmáry et al. 1995). Neurobehavioral effects have been shown in neonates given excess manganese orally from PND1-PND21 (Dorman et al. 2000). Several studies have shown neurochemical changes in offspring of dams exposed to increased manganese concentrations (Lai et al. 1991) or in neonatal animals dosed with excess manganese (Chandra and Shukla 1978; Deskin et al. 1980, 1981; Dorman et al. 2000). Other studies indicate that injected manganese is

more toxic to a developing fetus than inhaled or ingested manganese. Manganese injected subcutaneously or intravenously during the gestation period causes serious effects on skeletal development and ossification, but studies to date using this exposure pathway have not measured neurological deficits in pups or young rodents. The relevance to humans of results from these injection studies is unclear. The monkey is increasingly regarded as a more appropriate model for neurological endpoints, but recent monkey studies have used only adults, and no reproductive or developmental studies using these animals have been found. Also, monkey studies are extremely expensive and will be limited for this reason. Evaluation of appropriate endpoints in rodent assays by the oral and inhalation route are needed so that these models can be used to increase the body of knowledge of the developmental toxicity of manganese. Further, the one developmental study involving inhalation exposure (Lown et al. 1984) had many complications; additional studies involving neurobehavioral effects in animals following gestational and postnatal exposure to airborne manganese are necessary. A few developmental studies have involved sectioning fetuses to detect internal malformations (Blazak et al. 1996; Grant et al. 1997; Szakmáry et al. 1995; Treinen et al. 1995). However, these studies have primarily administered the manganese via *i.v.*, except for Szakmáry et al. (1995). Additional teratogenesis studies that assess bone malformations following inhalation and oral exposures using a wide range of doses are needed given that manganese overexposure affects the developing skeletal system (Blazak et al. 1996; Grant et al. 1997; Szakmáry et al. 1995; Treinen et al. 1995).

Immunotoxicity. Studies in animals indicate that injection or consumption of manganese compounds can cause significant changes in the functioning of several cell types of the immune system (NTP 1993; Rogers et al. 1983; Smialowicz et al. 1985, 1987). However, it is not known whether these changes are associated with significant impairment of immune system function. Further studies are needed to determine whether these effects also occur after inhalation exposure in animals or humans. If so, a battery of immune function tests would be valuable in determining if observed changes result in a significant impairment of immune system function.

Neurotoxicity. Studies in humans exposed to high levels of manganese dust in the workplace provide clear evidence that the chief health effect of concern following manganese exposure is injury to the central nervous system (Emera et al. 1971; Mena et al. 1967; Rodier 1955; Schuler et al. 1957; Smyth et al. 1973). Quantitative data on exposure levels for chronic durations are sufficient to identify a LOAEL for preclinical neurological effects (Iregren 1990; Roels et al. 1987a, 1992), and some of these data have been used to estimate a NOAEL using benchmark dose analysis (Iregren 1990; Roels et al. 1992). These

NOAEL estimates are comparable to a NOAEL for early neurological effects recently reported by Gibbs et al. (1999). Thus, no additional epidemiological studies to characterize effects in workers exposed to manganese via inhalation appear necessary at this time. Two recent studies investigated longitudinally whether manganese-induced preclinical effects in workers previously evaluated were reversible (Crump and Rousseau 1999; Roels et al. 1999). Improved performance was observed only in workers exposed to the lowest levels of manganese and effects in others neither improved nor worsened. High variability in the results of neurobehavioral testing from year-to-year was a limitation in the interpretation of results in one of these studies (Crump and Rousseau 1999). Also, the two studies reported conflicting findings on the effect aging the in workers may have had on their performance in certain tests. Additional follow-up studies are needed to further evaluate the reversibility of manganese-induced effects and define threshold exposure levels above which manganese-induced neurological effects are irreversible.

Studies of environmental exposure to airborne manganese report a correlation between high levels of the metal and increased blood manganese levels and subtle neurological effects, particularly in those over 50 years old (Baldwin et al. 1999; Mergler et al. 1999). These studies are also the first to study manganese exposures and potential adverse effects in women. More studies are needed that include analyses of both sexes and assess the relationship between environmental sources of excess manganese, altered manganese body burden, and the potential for adverse effects.

The evidence for neurotoxicity in humans following oral exposure to manganese is inconclusive due to several limitations in the majority of these reports (Holzgraefe et al. 1986; Kawamura et al. 1941; Kilburn 1987; Kondakis et al. 1989). One report in Japanese adults (Iwami et al. 1994) showed the link between eating food with concentrations of manganese on the high end of the normal range of a typical Western diet (5.79 mg manganese ingested per day) and low intake concentrations of magnesium associated with an increased incidence of motor neuron disease. Two studies in children (He et al. 1994; Zhang et al. 1995) indicated that those who ingested drinking water and who ate food with increased concentrations of manganese (0.241 mg/L or higher) for at least 3 years had measurable deficits in performance on certain tests of the WHO Core Test Battery, also used to assess neurobehavioral deficits in adults. In addition, the children exposed to manganese performed more poorly in school compared to non-exposed control students (who drank water with manganese concentrations no higher than 0.04 mg/L), as measured in mastery of Chinese, performance in mathematics, and in their overall grade average (Zhang et al. 1995). These studies show that both adults and children show adverse neurological effects from oral exposure to excess manganese. There are no existing studies showing adverse neurological effects in children as a

result of inhalation exposure to the airborne metal, either from locations near work sites or near hazardous waste sites.

There currently exists only one series of studies of potential neurotoxic effects of inhaled environmental manganese (Mergler et al. 1999; Baldwin et al. 1999; Beuter et al. 1999). These exposures most likely resulted from a point source, but the possible contribution of airborne manganese from MMT-gasoline exhaust cannot be excluded. These studies lend support to the possibility that the elderly may be a population susceptible to the neurotoxic effects of excess manganese exposure. Studies are currently needed to further investigate the potential for neurological effects in people, including children, who may have ingested excess amounts of excess manganese from sources in the environment. Clearly defined information on exposure levels and regular dietary intakes should also be captured. Further studies are needed to determine whether manganese from MMT and/or its unique combustion products contribute to airborne manganese concetrations that can be associated with adverse effects (e.g., respiratory or neurological effects).

There are two studies showing neurological effects following occupational exposures to manganese-containing pesticides (Ferraz et al. 1988; Meco et al. 1984). The Ferraz et al. (1988) study is negatively impacted by the likely exposure of the subjects to a wide variety of pesticides and the lack of a well-matched control group. Additional studies in occupationally-exposed persons with restricted exposure to only maneb and mancozeb are needed.

Studies in rodents and nonhuman primates indicate that oral intake of high doses of manganese can lead to biochemical and behavioral changes indicative of nervous system effects (Bonilla and Prasad 1986; Chandra 1983; Gupta et al. 1980; Kristensson et al. 1986; Lai et al. 1984; Nachtman et al. 1986), and this is supported by intravenous studies in monkeys (Newland and Weiss 1992). Rodents do not appear to be as susceptible to manganese neurotoxicity as humans; however, a study by Newland and Weiss (1992) indicates that Cebus monkeys would be a reasonable animal model. Further studies in animals may help determine the basis for the apparent differences in route and species susceptibility.

Additional studies in animals concerning the cellular and biochemical basis of manganese neurotoxicity, including a more detailed analysis of precisely which neuronal cell types are damaged and why, are needed. For example, Carl et al. (1993) have performed initial studies investigating the relationship between manganese and the major Mn(II) enzymes (arginase and glutamine synthetase) in epileptic and

induced seizures. Further studies may prove helpful in elucidating mechanism(s) of toxic action and could potentially lead to developing methods for mitigating adverse effects induced by manganese.

Epidemiological and Human Dosimetry Studies. As already noted, there are numerous epidemiological studies of workers exposed to manganese dusts in air, and the clinical signs and symptoms of the resulting disease are well established. However, these studies have only involved males and have only involved the inhalation route of exposure. Additional epidemiological studies on populations exposed to manganese dust in the workplace or local environments, e.g. such as near foundries, populations exposed to manganese emissions from MMT-burning automobiles, particularly those living in areas of high-traffic density, and populations exposed to above-average oral intakes (either through water and/or food) would be valuable in strengthening conclusions on dose-response relationships and no-effect exposure levels. This would be helpful in evaluating potential risks to people who may be exposed to above-average manganese levels near hazardous waste sites.

Epidemiological studies in occupationally-exposed pesticide workers typically involve exposure to a wide variety of pesticides. Therefore, it is impossible to ascribe the observed effects solely to maneb or mancozeb exposure. Additional epidemiological studies are necessary to more fully define the potential spectrum of systemic effects associated with these specific pesticides and to build a database from which dose-response relationships can be more fully defined.

Biomarkers of Exposure and Effect

Exposure. Studies in humans have shown that it is difficult to estimate past exposure to manganese by analysis of manganese levels in blood, urine, feces, or tissues (Roels et al. 1987b; Smyth et al. 1973; Valentine and Schiele 1983; Yamada et al. 1986). This is the result of several factors: (1) manganese is a normal component of the diet and is present in all human tissues and fluids, so above average exposure must be detected as an increase over a variable baseline; (2) manganese is rapidly cleared from the blood and is excreted mainly in the feces, with very little in the urine; and (3) manganese absorption and excretion rates are subject to homeostatic regulation, so above average exposures may result in only small changes in fluid or tissue levels. Probably the most relevant indicator of current exposure is manganese concentrations in tissues but at present this can only be measured in autopsy or biopsy samples. Studies on noninvasive methods capable of measuring manganese levels *in vivo*, either in the whole body or in specific organs (e.g., brain), would be very helpful in identifying persons with above average exposure.

Effect. The principal biological markers of toxic effects from manganese exposure are changes in the levels of various neurotransmitters and related enzymes and receptors in the basal ganglia (Bird et al. 1984; Bonilla and Prasad 1984; Eriksson et al. 1987a, 1987b). Noninvasive methods to detect preclinical changes in these biomarkers or in the functioning of the basal ganglia need to be developed to help identify individuals in whom neurological effects might result. Research to determine the correlation between urinary excretion levels of neurotransmitters, neurotransmitter metabolites, and/or 17-ketosteroids (Bernheimer et al. 1973; Rodier 1955; Siqueira and Moraes 1989) and the probability or severity of neurological injury in exposed people is also needed. Measurements of MnSOD as a biomarker of effect may also be helpful (Greger 1999), but there is a lack of information concerning the relationship of this enzyme to manganese toxicity.

Research in the use of Clara cell protein CC16 may be useful in identifying populations at risk from exposure to MMT; however, the majority of exposure to this compound is expected to arise from inhalation and ingestion of its combustion products. Therefore, increased use of MMT in gasolines necessitates the development of biomarkers of exposure to inorganic manganese compounds, as discussed previously. Further development of quantitative exposure to maneb and mancozeb, potentially through the use of ETU, or other metabolites in bodily fluids might be helpful in assaying exposure to these compounds. No data needs for biomarkers of effect or exposure are identified for mangafodipir.

Absorption, Distribution, Metabolism, and Excretion. The toxicokinetics of manganese absorption, distribution, and excretion have been studied in both humans and animals. The oral absorption rate is about 3–5% in humans (Davidsson et al. 1988, 1989a; Mena et al. 1969), but the rate may vary depending on age and dietary iron and manganese intake levels (Chandra and Tandon 1973; Diez-Ewald et al. 1968; Rehnberg et al. 1982; Thomson et al. 1971). Information is needed on the relative proportion of manganese that is absorbed via the gut following mucociliary transport of particles from the lung to the stomach. The oral absorption rate may depend on the chemical form of manganese ingested but data on this are sparse. Data on the differences in uptake as a function of chemical species (MnO_2, Mn_3O_4) and particle size would also be valuable in assessing human health risk from different types of manganese dusts.

Manganese appears to be distributed to all tissues, including the brain (Kristensson et al. 1986; Rehnberg et al. 1980, 1981, 1982). Recent data indicate that inhaled manganese is distributed more extensively to the brain than ingested manganese and that there are differences in distribution between different forms of

manganese ($MnCl_2$ compared to MnO_2) (Roels et al. 1997). Additional information concerning the differential distribution and toxicity of manganese in differing forms would help determine and quantify potential differences in toxic effects observed with various manganese compounds. Further studies would be valuable on the rate and extent of manganese uptake into the brain, since it is probably a critical step in manganese neurotoxicity. In addition, the metabolism of manganese (specifically, the degree and the rate of oxidation state interconversions) has not been thoroughly investigated. Data on this topic are needed to understand the mechanism of manganese toxicity and would help in evaluating the relative toxicity of different manganese compounds. Additional data on the interaction of manganese and other chemicals such as ethanol would also be valuable. Excretion of manganese is primarily through the feces (Drown et al. 1986; Klaassen 1974; Mena et al. 1969); because the rate of excretion is an important determinant of manganese levels in the body, further studies would be valuable on the biochemical and physiological mechanisms that regulate manganese excretion.

Additional studies are needed to more fully elucidate the pharmacokinetic mechanisms responsible for uptake, distribution, and excretion in humans and animals. Studies are also needed to determine the following: control rates and processes for uptake of ingested manganese by the intestines and liver, including uptake rates of protein-bound forms by the liver; oxidation rates of manganese in the blood and tissues; relative speciation of Mn(II vs. III) in blood transport mechanisms into the CNS, including transfer rates; competition between manganese and iron in terms of transport processes; and distribution following long-term exposures to assess potential storage depots.

Data on the pharmacokinetics of all organic manganese compounds, except mangafodipir, are lacking. Additional studies concerning absorption, distribution, metabolism, and excretion of MMT, maneb, and mancozeb, via inhalation, ingestion, and dermal exposures, would be very helpful.

Comparative Toxicokinetics. Exposure of animals, particularly rodent species, to manganese by either the oral or inhalation routes does not usually result in the appearance of the neurological symptoms characteristic of manganism in humans (Bird et al. 1984; Bonilla and Prasad 1984; Chandra 1983; EPA 1977; Gray and Laskey 1980; Lai et al. 1984; Nachtman et al. 1986; Ulrich et al. 1979a, 1979b). The reason for this apparent difference, both qualitative and quantitative, between humans and animals is not clear, but could be due, at least in part, to toxicokinetic differences in humans and animals with regard to manganese absorption, distribution, metabolism, and excretion. There does appear to be a qualitative

similarity in the responses of humans and nonhuman primates. Studies to compare manganese toxicokinetics in humans, nonhuman primates, and other species are needed.

Methods for Reducing Toxic Effects. The recommended methods for the mitigation of manganese toxicity (manganism) are mainly supportive (Ellenhorn and Barceloux 1988). Administration of anti-Parkinson drugs, such as levo-dopa, are of little use. Chelation therapy was reportedly effective in reducing some of the symptoms (Ellenhorn and Barceloux 1988; Haddad and Winchester 1990) but was not effective in all cases. Studies on the efficacy of newly developed methods to reduce the toxic effects of manganese are needed. The available data indicate that para-aminosalicylate was successfully used to treat neurological symptoms of manganese poisoning in two patients (Shuqin et al. 1992). The use of the antioxidant vitamin E has also been proposed to mitigate manganese-induced effects (Parenti 1988). Additional studies on the efficacy of these treatments are needed. Further evaluation for the mitigation of effects from excess exposure to manganese is also needed.

Methods for reducing toxic effects have not been identified for MMT. Methods for reducing toxic effects following exposure to maneb or mancozeb have been identified in the case studies; these methods are specific for the affected organ (de Carvalho et al. 1989; Israeli et al. 1983a; Koizumi et al. 1979). Further studies concerning methods for reducing toxic effects following exposure to these compounds would be helpful.

Children's Susceptibility. Children have been identified as a potentially susceptible population because of their high absorption and/or retention of manganese as compared to adults. Although some available studies indicate that tissue concentrations of human fetuses are comparable to adults, animal studies indicate that neonates retain higher tissue concentrations than adult animals. Researchers hypothesize that this increased retention of manganese may lead to neurotoxicity. Existing data indicate that the adverse neurological effects of manganese overexposure from intravenous and oral sources are qualitatively similar in children and adults. One study has reported that neonates are more susceptible to the effects of oral exposure to excess manganese than adults (Dorman et al. 2000). Additional quantitative information on the levels of manganese that result in adverse effects in children as compared to adults for inhalation, oral, and intravenous exposures are needed. Further, analysis of existing data from effects observed in the clinical setting might be helpful.

There are inadequate data on the pharmacokinetics of manganese in children. Although two studies provided typical serum manganese levels in differing ages of healthy children (Alarcón et al. 1996; Rükgauer et al. 1997), no studies have provided any data on distribution of manganese in infants or adolescents. Studies in animals, particularly nonhuman primates, are needed to clearly elucidate the pharmacokinetic handling of manganese in neonates and the young (absorption, metabolism, distribution, elimination). There are no PBPK models for children, embryos, fetuses and pregnant women, infants and lactating women, or adolescents. Such models would be very informative if they could assist in the identification of depots for manganese storage under conditions of excess exposure, as well as the nutritional needs of these age groups for the compound. One study was available that would provide information on the concentrations of manganese that might be found in the developing fetus of a highly-exposed mother (Jarvinen and Ahlstrom 1975). Further studies of this nature, especially those that measure neurological endpoints in live offspring following excess exposure, are needed. Similarly, data are needed to determine whether increased amounts of manganese might be present in the breast milk of a mother with significantly elevated blood or tissue manganese concentrations.

There are inadequate data to determine whether metabolism of manganese is different in children than in adults. Manganese is necessary for normal functioning of certain enzymes. However, there are no definitive data to indicate that children might need more manganese than adults for normal body processes. A few studies suggest that children may have a higher need for manganese than adults, based on the increased retention of manganese in the brains of certain neonatal animals, but this hypothesis has not been proven. Additional studies are necessary to determine the nutritional requirements of children for manganese.

Studies indicate that children exposed to increased concentrations of inorganic manganese, either via the diet, due to inability to clear the compound from the body or through parenteral nutrition, develop neurological dysfunction similar to that of adults (Devenyi et al. 1994; Fell et al. 1996; He et al. 1994; Zhang et al. 1995). Other data exist that indicate that children may not be as susceptible as adults to the adverse neurological effects of inorganic manganese (Kawamura et al. 1941), but the limitations in this report make predictions about susceptibility inconclusive. Additional animal studies comparing the potential for inorganic manganese to induce neurological effects in different age groups are needed to help understand the susceptibility of the young compared to adults.

The mechanism of action of inorganic manganese toxicity has not been identified. Studies in humans indicate that children and adults with increased manganese deposition in the globus pallidus and other basal regions suffer neuromuscular deficits. It has been suggested that manganese accelerates the autoxidation of catecholamines and contributes to oxidative stress in these affected regions of the brain. Further research is needed to more completely elucidate the mechanism of inorganic manganese toxicity.

There are no dependable biomarkers of exposure or effect that are consistently used in a clinical setting. However, MRI scans have been used in both adults and children to determine whether manganese is accumulating in certain brain regions. More data are needed to determine the sensitivity and specificity of this method.

Available data do not indicate that there are any interactions of manganese with other compounds that occur only in children. Interactions with compounds in adults are expected to also occur in children. Data concerning the significance of any interactions of manganese with other compounds are needed.

Studies of children's susceptibility to toxicity induced by organic manganese are generally lacking. With the exception of one behavioral study involving ingestion exposure of mice to MMT, and a few gestation studies with maneb and mancozeb, nothing is known concerning the effects of these compounds in the young. Mangafodipir has not been extensively studied in young animal models, either, but exposure of this compound by non-adults is not expected. Additional studies involving all endpoints discussed in this section that are relevant to inorganic manganese are needed for these organic manganese compounds.

Child health data needs relating to exposure are discussed in 5.8.1 Data Needs: Exposures of Children.

2.12.3 Ongoing Studies

A number of research projects are in progress investigating the health effects and the toxicokinetics of manganese. Projects sponsored by the federal government are summarized in Table 2-12.

Table 2-12. On Going Studies on Manganese

Investigator	Affiliation	Research description	Sponsor
A.J. Adler	Veterans Affairs Medical Center Brooklyn, NY	*In vitro* study Mn and superoxide dismutase activity	NS
S.F. Ali	Food and Drug Administration	Oxidative stress: A neurochemical mechanism of manganese-induced neurotoxicity	NS
A.B. Arquitt	Oklahoma State University	Mn levels in healthy women, effects of Zn supplementation on Mn levels	USDA
D.L. Baly	Rutgers University	Mn and carbohydrate metabolism in rats	USDA-CSRS
D.L. Baly	University of California	Effects of Mn on insulin gene expression and peripheral insulin action in adipose cells of rats	Agency: CRISP
J.P. Buchet	University Catholique, Belgium	Homovanillic acid excretion in the urine of Mn exposed workers	NS
G.F. Carl	Veterans Administration Medical Center, Augusta, GA	Lymphocyte and whole blood Mn levels in epileptics, diabetics, and alcoholics	Veterans Administration Wash., DC
G.F. Carl	Veterans Administration Medical Center, Augusta, GA	Relationship of epilepsy, seizures and Mn levels in rats	Veterans Administration Wash., DC
S.E. Chia	National University Singapore, Singapore	Human Mn exposure assessment using a computerized postural sway measurement system	NS
J.W. Critchfield	University of California	Dietary Mn and seizures in genetically epilepsy prone rats and their offspring	NS
E.J. Dowling	University of London, England	Human recombinant Mn superoxide dismutase and treatment of inflammation	NS
C. Fasolato	Max Planck Institute, Germany	Mechanism of Mn^{+2} influx into rat peritoneal mast cells	NS

Table 2-12. On Going Studies on Manganese (continued)

Investigator	Affiliation	Research description	Sponsor
J. Freeland-Graves	University of Texas	Development of enzyme-linked immunosorbent assay for Mn superoxide dismutase to be used to determine Mn requirements in the elderly	Agency: NRGO TEXR
M.S. Golub	University of California	Chronic oral toxicity of aluminum, manganese, and iron in mouse model	US Department HHS/ Public Health Service
M.S. Golub	University of Wisconsin	Dietary levels of Al, Mn, and Fe and the developing mouse brain	NS
J.M. Gorell	Henry Ford Health System	Epidemiological risk factors for Parkinsons disease	US Department HHS/ Public Health Service
J.L. Greger	University of Wisconsin	Dietary bioavailability and interaction between Mn, Al, and Fe	USDA
J.L. Greger	University of Wisconsin	Determination of human Mn requirements using rat studies; dietary Mn and Fe interaction	National Institute of Diabetes & Digestive & Kidney Diseases
M. Iszard	Xavier University of Louisiana, College of Pharmacy	Acute and intermediate-duration toxicity of manganese in rodents	ATSDR
D.J. Klimis-Tavantzis	University of Maine	*In vivo* lipoprotein metabolism and Mn in rats	NS
D.J. Klimis-Tavantzis	University of Maine	Dietary Mn and atherosclerosis in rats	USDA-CSRS
H. Komiskey	Xavier University of Louisiana, College of Pharmacy	Neurotoxicity of manganese in rats: uptake, tissue distribution, and neurotoxic effects of three manganese compounds	ATSDR
J. Komura	Hokuriku University Japan	Subcellular Mn distribution in the mouse brain	NS
B.P. Kreft	University of Bonn, Germany	Oral $MnCl_2$ and potential detection of hepatic tumors in rats	NS
T.K.C. Leung	Idaho State University	Postnatal development of Mn treated and normal rats	NS

Table 2-12. On Going Studies on Manganese (continued)

Investigator	Affiliation	Research description	Sponsor
W.K. Lin	State University of New York	Mn and process outgrowth in cultured rat pheochromocytoma cells	NS
B. Lönnerdal	University of California	Dietary factors and Mn absorption in humans	Agency: CRGO CALB
R. Lucchini	University of Brescia, Italy	Olfactory threshold and motor steadiness in ferroalloy production workers exposed to manganese oxides	NS
H. Mielke	Xavier University of Louisiana, College of Pharmacy	Multimedia study of manganese and nickel in rural and urban environments of New Orleans	ATSDR
P. Nikolova	Medical University Varna, Bulgaria	Mn and other essential trace elements metabolism in rats	NS
E. Oriaku	Florida A&M University, College of Pharmacy and Pharmaceutical Sciences	The effects of manganese exposure on reactive oxygen species in young or aged male and female rats	ATSDR
J.G. Penland	U.S. Department of Agriculture	Effects of dietary Mn and Ca on the human menstrual cycle	NS
O. Rabin	NIA	Mn^{+2} transport across blood brain barrier in rats	NS
R. Reams-Brown	Florida A&M University, College of Pharmacy and Pharmacuetical Sciences	Mechanism of Manganese-Induced Toxicity at the Molecular Level	ATSDR
D.J. Sanchez	Rovira and Virgili University, Spain	Maternal and developmental toxicity following subcutaneous exposure to Mn in the mouse	NS
N. Suarez	Astra Arcus Ab, Sweden,	Mn uptake and storage in cultured human neuroblastoma cells	NS
P. Sviatko	Slovak Academy of Science Czechoslovakia	Mn levels in dairy cows	NS
K.H. Thompson	University of British Columbia, Canada	Mn and vitamin E deficiencies in diabetic rats	NS
W.J. Visek	University of Illinois	Effects of Mn and arginine on protein metabolism in rats	USDA-CSRS

3. CHEMICAL AND PHYSICAL INFORMATION

3.1 CHEMICAL IDENTITY

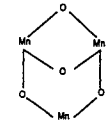
Table 3-1 lists common synonyms, trade names, and other relevant information regarding the chemical identity of manganese and several of its most important compounds.

Information regarding the chemical identity of manganese is located in Table 3-1.

3.2 PHYSICAL AND CHEMICAL PROPERTIES

Information regarding the physical and chemical properties of manganese is located in Table 3-2.

Table 3-1. Chemical Identity of Manganese and Compounds[a]

Characteristic	Manganese	Manganese (II) Chloride	Manganous Sulfate	Manganese (II, III) Oxide
Synonyms	Elemental manganese[b]; collodial manganese[b]; cutaval[b]	Manganese chloride[b]; manganese dichloride	Manganese sulfate	Trimanganese tetroxide; mangano-manganic oxide[c]
Registered trade name(s)	Cutaval[b]; Mangan[b]	No data	Sorba-Spray Manganese[b]	No data
Chemical formula	Mn	$MnCl_2$	$MnSO_4$	Mn_3O_4
Chemical structure[d]	Mn	Cl^- Mn^{+2} Cl^-		
Identification numbers:				
CAS	7439-96-5	7773-01-5	7785-87-7	1317-35-7
NIOSH RTECS	009275000[b]	009625000[b]	OP1050000[b]	OP0900000[b]
EPA hazardous waste	No data	No data	No data	No data
OHM/TADS	No data	No data	No data	No data
DOT/UN/NA/IMCO shipping	No data	No data	No data	No data
HSDB	00550[b]	02154[b]	02187[b]	No data
NCI	No data	No data	No data	No data

[a]All information obtained from Sax and Lewis 1987, except where noted.
[b]HSDB 1998
[c]Windholz 1983
[d]RTECS 1999
[e]HSDB 1999
[f] Chemfinder 1999

CAS = Chemical Abstracts Service; DOT/UN/NA/IMCO = Department of Transportation/United Nations/North America/International Maritime Dangerous Goods Code; EPA = Environmental Protection Agency; HSDB = Hazardous Substances Data Bank; NCI = National Cancer Institute; NIOSH = National Institute for Occupational Safety and Health; OHM/TADS = Oil and Hazardous Materials/Technical Assistance Data System; RTECS = Registry of Toxic Effects of Chemical Substances

Table 3-1. Chemical Identity of Manganese and Compounds[a] (continued)

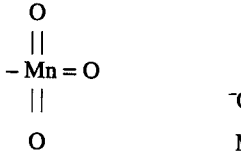
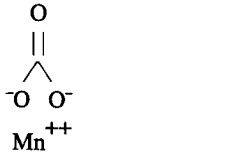
Characteristic	Manganese Dioxide	Potassium Permanganate	Manganese (II) Carbonate
Synonyms	Manganese peroxide; manganese binoxide; manganese black; battery manganese	Permanganic acid, potassium salt[c]; chameleon mineral	Carbonic acid, manganese (2+) salt[b]; manganous carbonate[b]; natural rhodochrosite[b]
Registered trade name(s)	No data	No data	No data
Chemical formula	MnO_2	$KMnO_4$	$MnCO_3$
Chemical structure	O = Mn = O		
Identification numbers:			
CAS	1313-13-9	7722-64-7	598-62-9
NIOSH RTECS	No data	SD6475000[b]	No data
EPA hazardous waste	No data	No data	No data
OHM/TADS	No data	7217279[b]	No data
DOT/UN/NA/IMCO shipping	No data	UN1490[b] IMCO 5.1[b]	No data
HSDB	No data	01218[b]	00790[b]
NCI	No data	No data	No data

Table 3-1. Chemical Identity of Manganese and Compounds[a] (continued)

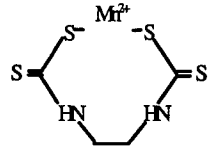
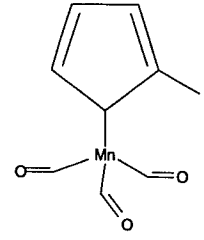
Characteristics	Mangafodipir	Maneb	Mancozeb	Methylcyclopentadienyl Manganese Tricarbonyl (MMT)
Synonyms	Mangafodipir trisodium[d]; MnDPDP[d]	Ethylenebisdithiocarbamate manganese[e]; Ethylenebis(dithiocarbamic acid) manganous salt[e]; 1,2-Ethylenediylbis(carbamodithioato) manganese[e]	Zinc manganese ethylenebisdithiocarbamate; (1,2-Ethanediyllbis(carbamodithioata))$^{-2}$ manganese zinc complex; Manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt[e]	MMT; Manganese, tricarbonyl ((1,2,3,4,5-eta)-1-methyl-2,4-cyclopentadien-1yl)-; Methylcymantrene; Tricarbonyl (2-methylcyclopentadientyl) manganese[e]
Registered trade name(s)	Teslascan[d]; Win 59010[d]	Maneb 80; Manzate Maneb; Dithane M22; Akzo Chemie Maneb; Polygram M; Sup'R Flo; Trimangol; Tubothane[e]	Dithane M-45; Dithane Ultra; Acaric M; Blecar MN; Manzin 80; FORE; Penncozeb; Manzate 200; Policar MZ; Vondozeb Plus[e]	AK-33X; Antiknock-33; CI-2; Combustion Improver-2[e]
Chemical formula	$C_{22}H_{24}MnN_4O_{14}P_2H_3Na_3$	$C_4H_6MnN_2S_4$	$C_4H_6MnN_2S_4.C_4H_6MnN_2S_4$-Zn [d]	$C_9H_7MnO_3$
Chemical structure	No data		This substance is a mixture of many individual substances and cannot be adequately represented by a single molecular structure.[f]	
ID numbers				
CAS	140678-14-4	12427-38-2	8018-01-7	12108-13-3
NIOSH RTECS	OO9163250	OP0700000	ZB3200000	48184
EPA hazardous waste	No data	U114	U114	No data
OHM/TADS	No data	No data	No data	No data
DOT/UN/NA/IMCO	No data	UN 2210; NA 2210; UN 2968; IMO 4.2; IMO 4.3	UN 2771; UN 2772; UN 3005; UN 3006; IMO 3.2; IMO 6.1	No data
HSDB	No data	4063	6792	2014
NCI	No data	No data	No data	No data

Table 3-2. Physical and Chemical Properties of Manganese and Compounds[a]

Property	Manganese	Manganese (II) Chloride	Manganous Sulfate	Manganese (II, III) Oxide
Molecular weight	54.94[c]	125.85[c]	151.00[c]	228.81[d]
Color	Gray-white[d]	Pink[d]	Pale rose-red	Black[d]
Physical state	Solid	Solid	Solid	Solid
Melting point	1,244°C[d]	650°C	700°C	1,564°C
Boiling point	1,962°C[d]	1,190°C[d]	850°C (decomposes)	No data
Density	7.21-7.44 g/cc[d]	2.977 g/cc[d]	3.25 g/cc[d]	4.856 g/cc[d]
Odor	No data	No data	Odorless	No data
Odor threshold:				
Water	No data	No data	No data	No data
Air	No data	No data	No data	No data
Solubility:				
Water	Decomposes	723 g/L (25°C)[d]	520 g/L (5°C)[d]; 700 g/L (70°C)[d]	Insoluble
Acids	Dissolves in dilute mineral acids[d]	No data	No data	Soluble in hydrochloric acid
Organic solvent(s)	No data	Soluble in alcohol, insoluble in ether	Soluble in alcohol, insoluble in ether	No data
Partition coefficients:				
Log K_{ow}	No data	No data	No data	No data
Log K_{oc}	No data	No data	No data	No data
Vapor pressure	1 mmHg at 1,292°C[b]	10 mmHg at 778°C[b]	No data	No data
Henry's law constant	No data	No data	No data	No data
Autoignition temperature	No data	Noncombustible	No data	No data
Flashpoint	No data	No data	No data	No data
Flammability limits	No data	No data	No data	No data
Conversion factors	Not applicable	Not applicable	Not applicable	Not applicable
Explosive limits	125 oz/1000 cu ft[e]	No data	No data	No data
Reactivity	Hydrogen.[e] When heated above 200°C in presence of nitrogen, forms nitrode.	No data	No data	No data

[a]All information obtained from Sax and Lewis 1987, except where noted.
[b]HSDB 1998
[c]Windholz 1983
[d]Lide 1993
[e]Sax 1988
[f]Budavari 1989
[g]NIOSH 1997 unless noted
[h]HSDB 1999
[I]US DOT 1996
[j]RTECS 1999

Table 3-2. Physical and Chemical Properties of Manganese and Compounds[a] (continued)

Property	Manganese Dioxide	Potassium Permangante	Manganese (II) Carbonate
Molecular weight	86.94[c]	158.04[c]	114.95
Color	Black	Purple	Rose[d]
Physical state	Solid	Solid	Solid
Melting point	Loses oxygen at 535°C[d]	<240° (decomposes)	Decomposes
Boiling point	No data	No data	No data
Density	5.026[d]	2.703	3.125[d]
Odor	No data	Odorless	No data
Odor threshold:			
Water	No data	No data	No data
Air	No data	No dataa	No data
Solubility:			
Water	Insoluble	63.8 g/L (20°C)[d]	Insoluble
Acids	Soluble in hydrochloric acid	Soluble in sulfuric acid[a]	Soluble in dilute acid[d], soluble in aqueous CO_2[d]
Organic solvent(s)	No data	Soluble in acetone	Insoluble in alcohol[d], insoluble in NH_3[d]
Partition coefficients:			
Log K_{ow}	No data	No data	No data
Log K_{oc}	No data	No data	No data
Vapor pressure	No data	No data	No data
Henry's law constant	No data	No data	No data
Autoignition temperature	No data	No data	No data
Flashpoint	No data	No data	No data
Flammability limits	No data	No data	No data
Conversion factors	Not applicable	Not applicable	Not applicable
Explosive limits	No data	No data	No data
Reactivity	No data	Spontaneously flammable on contact with ethylene glycol	No data

Table 3-2. Physical and Chemical Properties of Manganese and Compounds[a] (continued)

Characteristic	Mangafodipir Trisodium	Maneb[h]	Mancozeb[h]	Methylcyclopentadienyl Manganese Tricarbonyl[g]
Molecular weight	757.4[j]	265.3	541.03[j]	218.1
Color	No data	Yellow powder[f], Brown powder[c]	Greyish-yellow powder	Yellow to dark orange
Physical State	Liquid (Solution for infusion)	Solid[f]	Solid, wettable powder	Liquid, Solid below 36° F
Melting Point	No data	Decomposes before melting	Decomp. w/o melting at 192-194° C	1.5 ° C [f]
Boiling Point	No data	Decomposes at about 100 ° C	No data	449 ° F
Density	No data	1.92	No data	1.39 @ 20 ° C[h]
Odor	No data	Faint	No data	Faint, pleasant
Odor threshold	No data	No data	No data	No data
Solubility:	No data			
Water		Moderate[a], Slightly Decomposes rapidly	Insoluble[f]	Insoluble
Acids				No data
Organic solvent(s)		Soluble in chloroform, pyridine[f]; Insoluble in most common organic solvents	Practically insoluble in most organic solvents; sol. in chelating agents	Completely sol. in jet fuels and other hydrocarbon solvents[h]
Partition coefficients:	No data	No data	No data	No data
Vapor pressure	No data	Zero, negligible, <7.5 mm x10^{-8} mm Hg at 20 ° C	Practically zero, <1x10^{-5} mm Hg at 20 ° C	at 212 ° F, 7mm Hg
Henry's law constant	No data	< 4.63x10^{-9} atm-cu m/mole	No data	No data
Autoignition temperature	No data	No data	No data	No data
Flashpoint	No data	No data	137.8 ° C	230 ° F
Flammability limits	No data	No data	No data	No data
Conversion factors	No data	No data	No data	No data
Explosive limits	No data	No data	No data	No data
Reactivity	No data	Decomposes on prolonged exposure to air or moisture, Flammable/combustible, may ignite on contact with air or moist air, may decompose explosively when heated or involved in fire [i]	Slowly decomposed by heat and moisture [c], Flammable/combustible, may ignite on contact with air or moist air, may decomp. explosively when heated or involved in fire [i]	Light (decomposes)

4. PRODUCTION, IMPORT, EXPORT, USE, AND DISPOSAL

4.1 PRODUCTION

Inorganic Manganese

Table 4-1 lists the facilities in each state that manufacture or process manganese, the intended use, and the range of maximum amounts of manganese that are stored on site. There are currently 2,060 facilities that produce or process manganese in the United States. The data listed in Table 4-1 are derived from the Toxics Release Inventory (TRI96 1998). Only certain types of facilities were required to report. Therefore, this is not an exhaustive list.

Manganese is an abundant element comprising about 0.1% of the earth's crust (Graedel 1978); among the heavy metals only iron is more abundant (Cotton and Wilkinson 1972). It does not occur naturally as a base metal but is a component of over 100 minerals, including various sulfides, oxides, carbonates, silicates, phosphates, and borates (NAS 1973). The most commonly occurring manganese-bearing minerals include pyrolusite (manganese dioxide), rhodocrosite (manganese carbonate), and rhodanate (manganese silicate) (EPA 1984a; HSDB 1993; NAS 1973; Windholz 1983).

Most manganese ore is smelted in electric furnaces to produce ferromanganese, a manganese-iron alloy widely used in the production of steel (EPA 1984a; NAS 1973). Approximately 2 tons of manganese ore are required to make 1 ton of ferromanganese (NAS 1973). Production of 97–98% pure manganese metal is achieved by aluminum reduction of low iron-content manganese ore (HSDB 1998) or from the by-products of ferromanganese production. Manganese with <0.1% metallic impurities can be produced electrolytically from a manganese sulfate solution (EPA 1984a; HSDB 1998).

Manganese compounds are produced either from manganese ores or from manganese metal. For example, manganese chloride is produced by the reaction of hydrochloric acid with manganese oxide or manganese carbonate (HSDB 1993), manganese sulfate is produced as a by-product of hydroquinone production or by the action of sulfuric acid on manganese compounds (HSDB 1989), and potassium permanganate is produced by the electrolytic oxidation of manganese dioxide in a potassium hydroxide solution (HSDB 1998; Sax and Lewis 1987).

Table 4-1. Facilities That Manufacture or Process Manganese

STATE [a]	NUMBER OF FACILITIES	RANGE OF MAXIMUM AMOUNTS ON SITE IN POUNDS [b]	ACTIVITIES AND USES [c]
AL	60	100 - 49,999,999	1 ,2 ,3 ,6 ,7 ,8 ,9 ,12
AR	29	100 - 49,999,999	1 ,2 ,3 ,5 ,7,8 ,9 ,12 ,13
AZ	8	1,000 - 999,999	1 ,4 ,5 ,7 ,8 ,9, 10,12
CA	55	0 - 499,999,999	1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13
CO	14	1,000 - 9,999,999	2 ,3 ,4 ,9 ,12
CT	16	1,000 - 999,999	2 ,3 ,9 ,10
DE	1	10,000 - 99,999	1 ,5 ,8
FL	26	100 - 9,999,999	8 ,9 ,10 ,13
GA	42	0 - 9,999,999	1 ,2 ,3 ,5 ,7 ,8 ,9 ,10 ,12 ,13
HI	1	10,000 - 99,999	9
IA	51	100 - 9,999,999	1 ,2 ,3 ,5 ,7 ,8 ,9 ,10 ,12
ID	3	1,000 - 999,999	9
IL	121	0 - 49,999,999	1 ,2 ,3 ,4 ,5 ,8 ,9 ,10 ,11 ,12 ,13
IN	161	0 - 49,999,999	1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,11 ,12 ,13
KS	30	1,000 - 499,999,999	1,3 ,4 ,5 ,8 ,9 ,12 ,13
KY	63	100 - 499,999,999	1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,12 ,13
LA	17	0 - 9,999,999	1 ,2 ,3 ,5 ,7 ,8 ,9 ,10 ,12 ,13
MA	26	0 - 999,999	1 ,2 ,3 ,4 ,5 ,9 ,10
MD	17	1,000 - 49,999,999	2 ,4 ,9 ,10 ,13
ME	8	1,000 - 99,999	1 ,3 ,9
MI	128	0 - 9,999,999	1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,12 ,13
MN	28	0 - 9,999,999	8 ,9 ,10 ,12
MO	49	100 - 9,999,999	1 ,5 ,8 ,9 ,12
MS	23	100 - 49,999,999	8 ,9 ,13
MT	1	100,000,000 - 499,999,999	1 ,2 ,3 ,4 ,5 ,6 ,7
NC	57	0 - 9,999,999	1 ,2 ,3 ,5 ,8 ,9 ,10 ,11 ,12 ,13
ND	5	1,000 - 99,999	2 ,3 ,9
NE	18	0 - 9,999,999	1 ,2 ,3 ,8 ,9 ,12 ,13
NH	4	1,000 - 999,999	8 ,9
NJ	27	1,000 - 9,999,999	1 ,2 ,3 ,4 ,7 ,8 ,9 ,10
NM	1	10,000 - 99,999	9
NV	2	100,000 - 49,999,999	2 ,3 ,7
NY	63	0 - 9,999,999	1 ,2 ,3 ,4 ,5 ,7 ,8 ,9 ,10 ,12 ,13
OH	231	0 - 499,999,999	1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,12 ,13
OK	48	100 - 9,999,999	1 ,2 ,3 ,4 ,5 ,6 ,8 ,9
OR	17	1,000 - 9,999,999	2 ,3 ,9 ,12 ,13
PA	179	0 - 99,999,999	1 ,2 ,3 ,4 ,5 ,7 ,8 ,9 ,10 ,11 ,12 ,13
PR	5	0 - 999,999	9
RI	5	1,000 - 999,999	2 ,3 ,9 ,10
SC	57	0 - 9,999,999	1 ,2 ,3 ,5 ,7 ,8 ,9 ,10 ,13
SD	7	1,000 - 99,999	9 ,13
TN	54	0 - 49,999,999	1 ,2 ,3 ,4 ,5 ,6 ,7 ,8 ,9 ,10 ,12 ,13
TX	85	0 - 9,999,999	1 ,2 ,3 ,4 ,5 ,6 ,8 ,9 ,10 ,12 ,13
UT	23	1,000 - 99,999,999	2 ,3 ,7 ,9 ,12 ,13

Table 4-1. Facilities That Manufacture or Process Manganese

STATE [a]	NUMBER OF FACILITIES	RANGE OF MAXIMUM AMOUNTS ON SITE IN POUNDS [b]	ACTIVITIES AND USES [c]
VA	23	0 - 999,999	1 ,3 ,5 ,7 ,8 ,9
VT	1	10,000 - 99,999	9
WA	27	0 - 999,999	1 ,2 ,3 ,6 ,8 ,9
WI	126	0 - 49,999,999	1 ,2 ,3 ,5 ,6 ,7 ,8 ,9 ,10 ,12 ,13
WV	15	1,000 - 499,999,999	8 ,9 ,10 ,13
WY	2	0 - 999,999	1 ,5

Source: TRI96 1998

[a] Post office state abbreviations used

[b] Range represents maximum amounts on site reported by facilities in each state

[c] Activities/Uses:

1. Produce
2. Import
3. Onsite use/processing
4. Sale/Distribution
5. Byproduct
6. Impurity
7. Reactant
8. Formulation Component
9. Article Component
10. Repackaging
11. Chemical Processing Aid
12. Manufacturing Aid
13. Ancillary/Other Uses

Most manganese is mined in open pit or shallow underground mines (EPA 1984a; HSDB 1993; NAS 1973). Manganese ores were previously mined in the United States, but no appreciable quantity has been mined in the United States since 1978 (USGS 1998; U.S. Bureau of Mines 1989). The most recent data indicate that a small amount of manganiferous material (having a natural manganese content of 5–15%) was mined in Cherokee County, South Carolina, for use as a brick colorant; mining at this site comprised the only identified mining performed in 1997 (USGS 1998). No other currently operating mines in the United States were identified. Essentially all manganese ore used in manganese production in the United States is now imported (USGS 1998).

Currently, there are 1,847 facilities in the United States that indicate that they produce manganese or its compounds (TRI96 1998). These 1,847 facilities are scattered across the United States, with the largest numbers in Ohio (231), Pennsylvania (179), and Indiana (161). Over 1,894 facilities are involved in the distribution or use of manganese or manganese compounds (TRI96 1998). Table 4-1 lists the number of facilities in each state, the ranges of the maximum amounts stored at each facility, and the uses of the material (TRI96 1998).

Organic Manganese

MMT. The organomanganese compound methylcyclopentadienyl manganese tricarbonyl (MMT)is produced in either of the following ways: via the reaction of manganous chloride, cyclopentadiene, and carbon monoxide in the presence of manganese carbonyl and an element of group II or IIIA, or, via the reaction of methylcyclopentadiene with manganese carbonyl (EPA 1984a; HSDB 1999; Sax and Lewis 1987).

No production data from facilities that manufacture or process MMT were found. However, it is reported (HSDB 2000) that production in the U.S. in 1976 probably exceeded 4,966 pounds.

Maneb or mancozeb. Table 4-2 lists the facilities in the United States that manufacture or process the fungicide maneb, the intended use, and the range of maximum amounts that are stored on site. The data in Table 4-2 are derived from the Toxics Release Inventory (TRI97 1999). Only certain types of facilities were required to report. Therefore, as with the list of manganese manufacturers or processors, this is not an exhaustive list. In 1997, 3 facilities (in Arizona, New Jersey, and North Dakota) reported repackaging maneb and storing between 10,000 and 99,999 pounds of it on site. One facility in Georgia

Table 4-2. Facilities that Manufacture or Process Maneb

FACILITY	LOCATION[a]	RANGE OF MAXIMUM AMOUNTS ON SITE IN POUNDS	ACTIVITIES AND USES
BPS INC.	HELENA , AR	10,000 - 99,999	REPACKAGING
GRIFFIN LLC	VALDOSTA , GA	1,000,000 - 9,999,999	IMPORT, ON-SITE USE/PROCESSING, FORMULATION COMPONENT
AGSCO INC.	GRAND FORKS , ND	10,000 - 99,999	REPACKAGING
BARTLO PACKAGING INC.	PASSAIC , NJ	10,000 - 99,999	REPACKAGING

Source: TRI97 1999

[a] Post Office state abbreviations used

reported importing, using maneb as a formulation component, and storing between 1,000,000 and 9,999,999 pounds of maneb on site (Table 4-2).

No production data from facilities that manufacture or process mancozeb were found.

Maneb may be manufactured by any of the following methods: heating sodium ethylenebis(dithiocarbamate) with an aqueous solution of manganese(II) sulfate, reacting a water-soluble ethylenebisdithiocarbamate with manganous sulfate or chloride, neutralizing an aqueous nabam solution with acetic acid and adding manganese chloride solution, or reacting ehtylenediamine with carbon disulfide in the presence of sodium hydroxide in order to produce sodium salt, which is then treated with manganese salt to precipitate maneb (HSDB 1999). In the United States, maneb is available as a white powder that contains 80% of active ingredient.

Mancozeb is a polymeric mixture of a zinc salt and maneb containing 20% of manganese and 2.55% of zinc (HSDB 1999). Its chemical name is manganese ethylenebis(dithiocarbamate) (polymeric) complex with zinc salt. It may be in the form of a wettable or dustable powder, a suspension concentrate, or dry seed treatment.

Mangafodipir. Manganese(II) dipyridoxyl diphosphate (MnDPDP), or mangafodipir trisodium, is classified as a drug or therapeutic agent, and no production data were found for it.

4.2 IMPORT/EXPORT

Inorganic Manganese

The United States currently relies on imports to fill its need for manganese (USGS 1998). The latest data show that the United States imported a gross weight of manganese ore and concentrate (containing 20% or more manganese) of 1.05 billion pounds (478,000 metric tons) in 1996; similar imports decreased to 790 million pounds (357,000 metric tons) in 1997 (USGS 1998). Domestic steel output is the main determinant of manganese demand and is projected to be relatively stable for the remainder of the 1990s. However, steel production has grown at an annual rate of about 1.4% from 1985–96, and U.S. steel demand reached a record high in 1997 (USGS 1998). Therefore, manganese consumption could increase

by the year 2000, but the majority of the demand for manganese will continue to be met by imports (USGS 1998). In 1996 and 1997, countries from which the United States imported manganese ore and concentrates included Australia, Brazil, Gabon, Mexico, Morocco, and South Africa, with Gabon, the dominant ore source, providing approximately 60% of ore imports (USGS 1998).

The United States also exports manganese ore and concentrates, having exported 70 million pounds (31,600 metric tons) in 1996 and 186 million pounds (84,300 metric tons) in 1997. The 1997 export level of manganese ore represents the highest level since 1984 (USGS 1998). Because only one mining activity of manganiferous material was identified in 1997 (see 4.1 Production), and since this material contained a low natural manganese content, mining cannot account for the manganese ore and concentrate exports of 1996–97. Therefore, the United States' stockpile disposal program accounts for these exports; the government's inventory of manganese was lowered by about 4% due to its exports to countries including Belgium, Canada, Colombia, Israel, Italy, the Netherlands, Norway, and the United Kingdom (USGS 1998).

Ferromanganese is also imported, with import quantities of 824 million pounds (374,000 metric tons) in 1996 and 670 million pounds (304,000 metric tons) in 1997 (USGS 1998). All grades of ferromanganese exports totaled 22 million pounds (9,8000 metric tons) and 26 million pounds (11,800 metric tons) in 1996 and 1997, respectively (USGS 1998).

Organic Manganese

MMT. Import and export data were not located for MMT.

Maneb or mancozeb. No import data were located for maneb or mancozeb. Export data show that 250,000 pounds of maneb and 840,000 pounds of mancozeb were exported from the U.S. during 3 months of 1990 (Bason and Colburn 1998).

Mangafodipir. Import and export data were not located for mangafodipir.

4.3 USE

Inorganic Manganese

Metallic manganese (ferromanganese) is used principally in steel production to improve hardness, stiffness, and strength. It is used in carbon steel, stainless steel, high-temperature steel, and tool steel, along with cast iron and superalloys (EPA 1984a; HSDB 1998; NAS 1973). Currently, 85–90% of the total manganese demand is attributable to its use in steel making (USGS 1998). Manganese compounds have a variety of uses. Manganese dioxide is commonly used in production of dry-cell batteries, matches, fireworks, porcelain and glass-bonding materials, amethyst glass, and as the starting material for production of other manganese compounds (EPA 1984a; HSDB 1998; NAS 1973; Venugopal and Luckey 1978). Manganese chloride is used as a precursor for other manganese compounds, as a catalyst in the chlorination of organic compounds, in animal feed to supply essential trace minerals, and in dry-cell batteries (EPA 1984a; HSDB 1997). Manganese sulfate is used primarily as a fertilizer (60% of total consumption) and as a livestock supplement (30% of total consumption); it is also used in some glazes, varnishes, ceramics, and fungicides (EPA 1984a; HSDB 1997; Windholz 1983). Potassium permanganate's oxidizing power allows it to be used as a disinfectant; an antialgal agent; for metal cleaning, tanning, and bleaching; and as a preservative for fresh flowers and fruits. Approximately 80% of the potassium permanganate consumed in this country is used in water and waste-treatment plants for water purification purposes (HSDB 1997). Another common source of manganese is found in the street drug "Bazooka". It is a cocaine-based drug contaminated with manganese-carbonate from free-base preparation methods (Ensing, 1985).

Organic Manganese

MMT. MMT is a fuel additive developed in the 1950s to increase the octane level of gasoline and thus improve the antiknock properties of the fuel (Davis 1998; EPA 1984a; HSDB 1993; Lynam et al. 1990; NAS 1973). It can also be used as a fuel oil additive and a smoke inhibitor (HSDB 1999). MMT was introduced into Canada in 1976 and its use has increased so substantially that it completely replaced tetraethyl lead in gasoline in that country in 1990 (Zayed et al. 1999). Ethyl Corporation, the manufacturer of MMT, has been marketing its product to U.S. refineries since late 1995 (Davis 1998). There are no data concerning the extent of its use in the U.S.

Maneb or mancozeb. Maneb is used as a foliar fungicide for fruits and vegetables, in the seed treatment of vegetables and field crops (esp., small grains such as wheat), and as a fungicide for deciduous fruits and nuts. As a foliar spray, it displays a wide spectra activity, able to control blights, leafspots, blotches, and mildews on fruits, vegetables, and ornamentals. The principal diseases that it controls include early and late blight of potato and tomato, downy mildew and anthracnose on vegetables, and "rot" diseases of apricots, peaches, and grapes. In the most recent available data, 68% of maneb's use was for vegetables, 28% for seed treatment of field crops and vegetables, and 4% for deciduous fruits and nuts in 1978 (HSDB 1999).

Mancozeb is a fungicide used to control many fungal diseases such as blight, leaf spot, rust, downy mildew, and scab in field crops, fruits, nuts, vegetables, ornamentals, etc. In particular, it is used to control the following: early and late blights of potatoes and tomatoes; leaf spot diseases on celery, cucurbits, beets, berries, and currants; rusts on cereals, vegetables, roses, carnations, asparagus, beans, and plums; downy mildews on hops, vines, onions, leeks, lettuce, cucurbits, ornamentals, and tobacco; scab on apples and pears; sigatoka disease in bananas; shot-hole of stone fruit; anthracnose of beans and cucurbits; damping-off disease of vegetables; black leg of beet; needle cast in forestry; and many seed-borne diseases of cereals (HSDB 1999). Mancozeb can also be used for foliar application or as a seed treatment (HSDB 1999). Mancozeb may be applied as a foliar spray, using aerial or ground equipment, or by chemigation. Major crops treated are apples, potatoes, and tomatoes. In the United States, mancozeb is applied to approximately 80% of onion crops (HSDB 1999).

Most recent available data show, in 1989, that the combined annual use of maneb and mancozeb in the United States was 8,000–12,000 thousand pounds active ingredient (8–12 million pounds) (Bason and Colburn 1998), making maneb and mancozeb, collectively, the 16th most used pesticides. However, information regarding the quantity of specific pesticides produced or used is difficult to access because it is proprietary (Bason and Colburn 1998), and therefore, this is only an estimate. In 1989, forestry use of maneb and mancozeb, collectively, was less than 1,000 pounds active ingredient, and 1981 urban application was 32,000 pounds active ingredient.

Mangafodipir. Mangafodipir trisodium (MnDPDP) is used as both a liver- and pancreas-specific contrast agent for magnetic resonance imaging (MRI); it improves lesion detection in MRI of these

organs by selectively enhancing the normal parenchyma, but not lesions, so that the contrast between tumorous and normal tissue is increased (Wang 1998).

4.4 DISPOSAL

Manganese is listed as a toxic substance under Section 313 of the Emergency Planning and Community Right to Know Act (EPCRA) under Title III of the Superfund Amendments and Reauthorization Act (SARA) (EPA 1998). Disposal of wastes containing manganese is controlled by a number of federal regulations (see Chapter 7).

Disposal of waste manganese into water requires a discharge permit from the EPA (see Chapter 7), but disposal of solid wastes such as manganese metal or manganese compounds is not regulated under current federal law. There are incomplete federal records of this disposal because most, but not all, solid manganese wastes are disposed of by being deposited on land or by being trucked to off-site disposal facilities (TRI96 1998). The total amount of waste manganese disposed of in this way in 1996 was in excess of 230 million pounds (TRI96 1998) (See Table 5-1). No information was located regarding predicted future trends in manganese disposal.

Organic Manganese

Maneb or mancozeb. Disposal of maneb and mancozeb must comply with state and federal regulations for the management of hazardous waste. Generators of small quantities of this waste may apply for partial exemption from the hazardous waste regulations (HSDB 1999).

No information on disposal of MMT was located.

5. POTENTIAL FOR HUMAN EXPOSURE

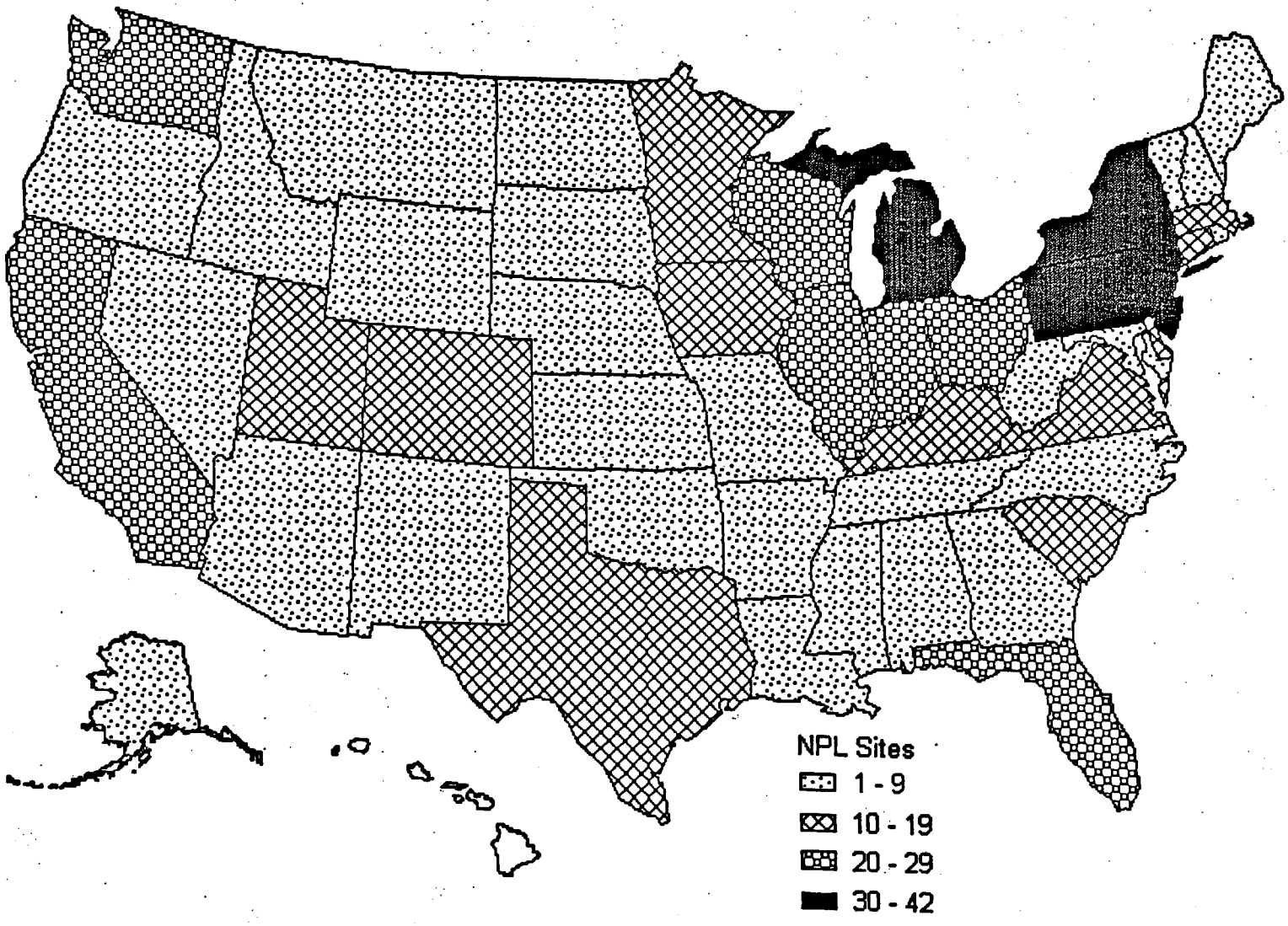
5.1 OVERVIEW

Manganese has been identified in at least 603 of the 1,467 current or former EPA National Priorities List (NPL) hazardous wastes sites (HazDat 1998). However, the number of sites evaluated for manganese is not known. The frequency of these sites within the United States can be seen in Figure 5-1. Of these sites, 598 are located in the United States, 3 are located in the Commonwealth of Puerto Rico, 1 is located in Guam, and 1 is located in the Virgin Islands. Available data indicate that manganese is detectable in soil and water at approximately 40–80% of all NPL sites (HazDat 1998) and nearly all other hazardous waste sites. In some cases, this is probably due to natural levels of manganese rather than to disposal of manganese wastes, but at some sites the levels are significantly higher than average. No data were available to indicate the presence of any organomanganese compounds (MMT, maneb, mancozeb, and mangafodipir) at any NPL hazardous waste sites.

Manganese is ubiquitous in the environment. It occurs in soil, air, water, and food. Thus, all humans are exposed to manganese, and manganese is a normal component of the human body. Food is usually the most important route of exposure for humans; typical daily intakes range from 1–5 mg/day.

Above-average exposures to manganese are most likely to occur in or near a factory or a hazardous waste site that releases significant amounts of manganese dust into air. Manganese can be released into air by combustion of unleaded gasoline that contains MMT as an antiknock ingredient. Because these releases are particulate in nature, the fate and transport of the particles are determined mainly by the wind and by the size and density of the particles. Some manganese compounds are readily soluble, so significant exposures can also occur by ingestion of contaminated drinking water. However, manganese in surface water may oxidize or adsorb to sediment particles and settle to the bottom. Manganese in soil can migrate as particulate matter in air or water, or soluble compounds may be dissolved by water and leach from the soil. The extent of leaching is determined mainly by the characteristics of the soil and is highly variable.

FIGURE 5-1. Frequency of NPL Sites with Manganese Contamination



Derived from HazDat 1998

5.2 RELEASES TO THE ENVIRONMENT

Inorganic Manganese

According to the Toxics Release Inventory (TRI), in 1996, a total of 58,360,310 pounds (26,527,414 kg) of manganese was released to the environment from 1,978 large processing facilities (TRI96 1998). Table 5-1 lists amounts released from all the facilities that manufacture or process manganese to each medium within each state in 1996 (TRI96 1998). Industrial manufacturers, processors, and users of manganese and manganese compounds are required to report the quantities of these substances released to environmental media annually (EPA 1988a). In addition, an estimated 392,340 pounds (178,336 kg) were released by manufacturing and processing facilities to publicly owned treatment works (POTWs) and an estimated 186,247,598 pounds (84,657,999 kg) were transferred offsite (TRI96 1998). The TRI data should be used with caution because only certain types of facilities are required to report. This is not an exhaustive list. Also, because these data reflect past releases, they may not be representative of current releases at these facilities.

Manganese has been identified in a variety of environmental media (air, surface water, groundwater, soil, and sediment) collected at 603 of the 1,467 NPL hazardous waste sites (HazDat 1998).

Additional releases of manganese to the environment occur from natural sources and from processes such as combustion of fossil fuel, incineration of wastes, or cement production (EPA 1985c, 1985d). Quantitative data on releases of manganese to specific environmental media are discussed below.

Organic Manganese

MMT. No data for releases of MMT to the environment from facilities that manufacture or process MMT were found.

Maneb or mancozeb. According to TRI, in 1997, a total of 23,282 pounds (10,583 kg) of maneb were released to the environment from 1 repackaging facility. Table 5-2 lists amounts of maneb released from all the facilities that manufacture or process maneb to each medium in each state in 1997 (TRI97 1998). In addition, an estimated 19,297 pounds (8,772 kg) of maneb were transferred off-site and accounted for

Table 5-1. Releases to the Environment from Facilities That Manufacture or Process Manganese

		Total of reported amounts released in pounds per year [a]						
STATE [b]	NUMBER OF FACILITIES	AIR [c]	WATER	LAND	UNDERGROUND INJECTION	POTW TRANSFER	OFF-SITE WASTE TRANSFER	TOTAL ENVIRONMENT [d]
AR	28	15,048	255	752	0	874	7,015,580	7,032,509
AZ	8	4,568	1	1	1	51	95,432	100,054
CA	55	41,870	13,005	195,109	0	9,080	1,085,258	1,344,322
CO	14	32,519	29	11,560	0	67	432,425	476,600
CT	16	846	54,000	3,800	0	758	289,429	348,833
DE	1	2,813	4	0	0	0	206,855	209,672
FL	23	7,895	0	374,683	230	878	953,881	1,337,567
GA	42	101,022	31,022	300,977	0	383	792,114	1,225,518
HI	1	0	0	0	7	0	41,616	41,623
IA	48	41,186	997	1,330,109	0	31,515	1,731,683	3,135,490
ID	3	14	0	17,491	0	0	13,478	30,983
IL	120	285,598	14,180	5,809,927	500	22,453	8,594,488	14,727,146
IN	159	297,218	25,588	2,422,228	2,900	45,032	19,377,727	22,170,693
KS	30	33,619	5	697,019	250	1,331	2,271,798	3,004,022
KY	63	155,054	11,686	153,161	0	4,413	5,458,605	5,782,919
LA	17	5,648	31,533	2,805,516	0	5	1,177,652	4,020,354
MA	26	368	19	27	0	16,310	347,007	363,731
MD	17	17,525	68,945	1,458,005	0	1,098	328,746	1,874,319
ME	8	1,189	610	0	0	10	460,914	462,723
MI	128	126,451	5,764	1,205,424	11,000	18,593	10,137,118	11,504,350
MN	27	11,716	342	50	0	56,599	1,280,310	1,349,017

Table 5-1. Releases to the Environment from Facilities That Manufacture or Process Manganese

		Total of reported amounts released in pounds per year [a]						
STATE [b]	NUMBER OF FACILITIES	AIR [c]	WATER	LAND	UNDERGROUND INJECTION	POTW TRANSFER	OFF-SITE WASTE TRANSFER	TOTAL ENVIRONMENT [d]
MO	49	42,628	755	4,661	0	41,684	1,989,279	2,079,007
MS	23	12,037	24,045	5,141,019	0	38	655,688	5,832,827
MT	1	1,503	3	2,849,437	0	2	2,153	2,853,098
NC	56	28,727	6,716	37,961	0	2,159	2,548,765	2,624,328
ND	5	553	0	0	0	0	294,710	295,263
NE	18	15,192	500	3,776	0	45	3,067,621	3,087,134
NH	4	560	0	0	0	0	36,100	36,660
NJ	27	7,555	51	250	0	2,010	467,550	477,416
NM	1	500	0	5	0	0	255	760
NV	2	13,800	0	2,330,005	0	0	0	2,343,805
NY	63	21,619	72,308	13,172	2,558	32,692	2,450,598	2,592,947
OH	227	696,933	1,055,373	13,749,832	250	56,735	26,531,656	42,090,779
OK	48	12,581	129	8,604	0	646	1,121,380	1,143,340
OR	16	6,219,388	45	281,800	0	7	1,756,325	8,257,565
PA	179	84,012	34,224	187,779	0	995	28,314,230	28,621,240
PR	5	1,000	0	19	0	33	38,222	39,274
RI	5	1,345	28	0	0	0	89,727	91,100
SC	55	31,044	175,594	231,754	0	971	10,879,559	11,318,922
SD	7	5,591	0	0	0	10	96,427	102,028
TN	54	233,442	156,151	3,856,929	0	2,621	7,554,735	11,803,878
TX	84	72,529	4,950	463,257	0	2,016	20,922,520	21,465,272

Table 5-1. Releases to the Environment from Facilities That Manufacture or Process Manganese

		Total of reported amounts released in pounds per year [a]						
STATE [b]	NUMBER OF FACILITIES	AIR [c]	WATER	LAND	UNDERGROUND INJECTION	POTW TRANSFER	OFF-SITE WASTE TRANSFER	TOTAL ENVIRONMENT [d]
UT	22	85,927	0	1,029,763	0	12,928	570,042	1,698,660
VA	22	26,221	964	267,475	0	570	684,312	979,542
VT	1	2	0	0	0	0	28,504	28,506
WA	27	6,858	52,371	500	0	45	1,193,817	1,253,591
WI	126	49,656	16,662	8,790	0	25,828	10,834,422	10,935,358
WV	15	43,042	58,629	246,842	0	850	2,025,145	2,374,508
WY	2	250	0	29,000	0	5	1,740	30,995

Source: TRI96 1998

[a] Data in TRI are maximum amounts released by each facility

[b] Post office state abbreviations used

[c] The sum of fugitive and stack releases are included in releases to air by a given facility

[d] The sum of all releases of the chemical to air, land, and water, and underground injection wells; and transfers off-site by a given facility

POTW = publicly owned treatment works

Table 5-2. Releases to the Environment from Facilities that Manufacture or Process Maneb

			Reported amounts released in pounds per year [a]						
STATE [b]	CITY	FACILITY	AIR [c]	WATER	LAND	UNDERGROUND INJECTION	POTW TRANSFER	OFF-SITE WASTE TRANSFER	TOTAL [d] ENVIRONMENT
AR	HELENA	BPS INC.	11,641	0	11,641	0	1	11,741	35,024
GA	VALDOSTA	GRIFFIN LLC	0	0	0	0	0	5,033	5,033
ND	GRAND FORKS	AGSCO INC.	0	0	0	0	0	990	990
NJ	PASSAIC	BARTLO PACKAGING INC.	0	0	0	0	0	1,533	1,533
		TOTALS	11,641	0	11,641	1	0	19,297	42,580

Source: TRIS97 1999

[a] Data in TRI are maximum amounts released by each facility

[b] Post office state abbreviations used

[c] The sum of fugitive and stack releases are included in releases to air by a given facility

[d] The sum of all releases of the chemical to air, land, and water, and underground injection wells; and transfers off-site by a given facility

POTW = publicly-owned treatment works

approximately 45% of total environmental release (TRI97 1999). No release data were found for mancozeb.

Mangafodipir. No data for releases of mangafodipir to the environment were found. Because mangofodipir is a compound used exclusively in a clinical environmental, it is not expected to be released to the environment and will not be discussed in subsequent sections concerning fate and transport.

5.2.1 Air

Inorganic Manganese

According to the Toxics Release Inventory, in 1996, the estimated releases of manganese of 8,896,662 pounds (4,043,937 kg) to air from 1,978 large processing facilities accounted for about 15% of total environmental releases (TRI93 1995). Table 5-1 lists amounts released from these facilities. The TRI data should be used with caution, however, since only certain types of facilities are required to report. This is not an exhaustive list. Also, because these data reflect past releases, they may not be representative of current releases at these facilities.

Manganese has been identified in air samples collected at 18 of the 603 NPL hazardous waste sites where it was detected in some environmental media (HazDat 1998).

The main sources of manganese release to the air are industrial emissions, combustion of fossil fuels, and reentrainment of manganese-containing soils (EPA 1983c, 1984a, 1985c, 1985d, 1987a; Lioy 1983). The principal sources of industrial emissions are ferroalloy production and iron and steel foundries, and the principal sources of combustion emissions are power plants and coke ovens (EPA 1983c, 1985c, 1985d). Total emissions to air from anthropogenic sources in the United States were estimated to be 36 million pounds in 1978, with about 80% (29 million pounds) from industrial facilities and 20% (7 million pounds) from fossil fuel combustion (EPA 1983c). Air emissions reported by industrial sources for 1996 total 8.9 million pounds (TRI96 1998). In 1996, air emissions from 227 facilities in Ohio, the state with the widest range of releases, ranged from 0 to 449,000 pounds/year; only one state (Hawaii) reported no emissions (TRI96 1998).

Air erosion of dusts and soils is also an important atmospheric source of manganese, but no quantitative estimates of manganese release to air from this source were located (EPA 1984a). Volcanic eruptions may also release manganese to the atmosphere (Schroeder et al. 1987).

Organic Manganese

MMT. MMT was banned as an additive in unleaded gasoline by EPA in 1977 (EPA 1978, 1979, 1981); however, combustion of gasoline containing MMT (before the ban) may have contributed to urban air manganese levels. In 1995 the ban was lifted, and a court decision ordered EPA to register the product for use as a fuel additive, although testing for health effects continues (EPA 1995a). Analysis of manganese levels in the air indicates that vehicular emissions contributed an average of 13 nanograms(ng) manganese/m^3 in southern California, while vehicular emissions were only about 3 ng/m^3 in central and northern California (Davis et al. 1988). A survey of ambient air concentrations of fine ($PM_{2.5}$) manganese in rural sites in U.S. national parks and in urban sites in California indicated that from 1988 to 1993, ambient concentrations of manganese ranged from 1 ng/m^3 in rural sites to 3 ng/m^3 in urban sites (Wallace and Slonecker 1997). Part of the increase in fine manganese during this period was considered to be the result of the use of MMT in leaded gasoline. It was estimated that automobiles were responsible for 37% of the fine manganese levels in California in 1992. In 1994, the automobile was estimated to contribute to 12% of the fine manganese levels. Wallace and Slonecker (1997) estimated that the background contribution of windblown soil to fine manganese concentration was 1–2 ng/m^3. It has been estimated that if MMT were used in all gasoline, urban air manganese levels would be increased by about 50 ng/m^3 (Cooper 1984; Ter Haar et al. 1975). The U.S. EPA (EPA 1994) estimated that a "substantial number of people" could be exposed to manganese particulate levels above 0.1 µg/m^3 if there were 100% usage of gasoline containing MMT.

Utilizing various environmental modeling approaches, it was estimated in 1994 that air levels of manganese in most USA urban areas would increase less than 0.02 µg/m^3 if MMT were used in all unleaded gasoline (Lynam et al. 1994). Slightly higher levels were predicted for Los Angeles because of extremely high traffic density and unique atmospheric and geographic features. The estimates were based on the assumption that - 30% of the manganese combusted is emitted from the tailpipe. However, emission tunnel experiments revealed that only 10–15% of the manganese in the combusted gasoline was emitted from the tailpipe.

There is concern in Canada that combustion of MMT may be one of the main sources of manganese contamination in the urban environment, particularly in areas of high traffic density (Loranger and Zayed 1997a; Loranger et al 1994a). In this country, a 10% per year increase in manganese emission rates from MMT in gasoline since 1981 has been estimated (Loranger and Zayed 1994). A positive relationship between atmospheric manganese concentration and traffic density has been reported (Loranger and Zayed 1997a; Loranger et al 1994a). The principal emission product of MMT combustion is a fine particulate matter (0.1–0.4 μm diameter) consisting of manganese oxide (Egyed and Wood 1996; Ter Haar et al. 1975), manganese phosphate, and some manganese sulfate (Lynam et al. 1999). The finding of soluble manganese (<0.4 μm) in snow samples obtained close to a highway in Montreal, Canada suggested a possible contamination from mobile sources (Loranger and Zayed 1997; Loranger et al. 1995). However, it has been difficult to assess the exact contribution of mobile sources to overall contamination from natural and industrial sources because of the physico-chemical characteristics of manganese particulate, environmental factors affecting its dispersion, and the difficulties in distinguishing between mobile sources of manganese and background manganese levels (Loranger and Zayed 1997a; Veysseyre et al. 1998).

Based on dispersion modeling estimates using theoretical emission rates, actual traffic volume, and meteorological conditions near a major highway in Canada, the contribution of mobile sources of manganese to atmospheric background manganese was predicted to be approximately 50% at a distance of 25 m (manganese concentration of 0.026 $\mu g/m^3$) and less than 8% at a distance of 250 m (manganese concentration of 0.003 $\mu g/m^3$) from the major highway (Loranger et al. 1995b; Loranger and Zayed 1997a). The total uncertainty in the model predictions was estimated to be 50% (Loranger et al. 1995b).

Despite the estimated 10% per year increase in manganese emission rates from the use of MMT in gasoline in Canada, atmospheric manganese concentrations in Montreal have remained fairly constant between 1981 and 1990, and have decreased markedly in 1991 and 1992 (Loranger and Zayed 1994). The decline in manganese concentration after 1990 may have been due to a shutdown in 1991 of a ferromanganese plant located near Montreal. Air concentrations are in general below the U.S.EPA reference concentration (RfC) of 0.05 $\mu g/m^3$ for respirable manganese. However, in 1998, it was observed that some atmospheric concentrations in specific microenvironments with important traffic density were higher than the RfC (Zayed et al. 1999).

Maneb or mancozeb. According to TRI, in 1997, the estimated releases of 11,641 pounds (5,291 kg) of maneb to air from 1 repackaging facility accounted for about 27% of total environmental release (TRI97 1999). Table 5-2 lists amounts of maneb released from these facilities.

5.2.2 Water

Inorganic Manganese

According to the Toxics Release Inventory, in 1996, the estimated releases of manganese of 1,917,483 pounds (871,583 kg) to water from 1,978 large processing facilities accounted for about 3% of total environmental releases (TRI96 1998). Table 5-1 lists amounts released from these facilities. The TRI data should be used with caution, however, since only certain types of facilities are required to report. This is not an exhaustive list. Also, because these data reflect past releases, they may not be representative of current releases at these facilities.

Manganese has been identified in surface water and groundwater samples collected at 270 and 486, respectively, of the 603 NPL hazardous waste sites where it was detected in some environmental media (HazDat 1998).

Manganese may be released to water by discharge from industrial facilities or as leachate from landfills and soil (EPA 1979b, 1984a; Francis and White 1987; TRI91 1993). Reported industrial discharges to surface waters, transfers to public sewage, and underground injection (releases to groundwater) for 1996 totaled 1.9 million, 0.4 million, and 0.02 million pounds, respectively (TRI96 1998). In 1991, reported industrial discharges were much less and ranged from 0 to 380 thousand pounds/year for surface water, 0 to 126 thousand pounds/year for transfers to public sewage, and 0 to 250 pounds/year for underground injection per state (TRI91 1993).

Based on comparison to typical background levels of manganese in surface water or groundwater (see Section 5.4.2), it seems likely that some waste sites where manganese is detected contain only natural levels. Although ambient manganese levels are about 200 µg/L in a number of cases, high levels (in excess of 1,000 µg/L) have been detected indicating that manganese wastes may lead to significant contamination of water at some sites. For example, at one site in Ohio where "heavy metals" had been disposed, manganese concentrations up to 1,900 µg/L were found in on-site wells (Cooper and Istok

1988). Levels in water at two NPL sites in Missouri ranged from 0.009 to 3.7 µg/L (MDNR 1990). No information is available on the method used to determine these values, so it is not clear whether the data refer to total or dissolved manganese.

Organic Manganese

Maneb or mancozeb. According to TRI, in 1997, there was an estimated release of 0 pounds of maneb to water from facilities that manufacture or process maneb (TRI97 1999). In recent investigations of MMT occurrence in rain water and storm runoff collected along highways, MMT was found in most of the samples (Yang and Chau 1999). It is not understood why a readily photodegraded compound still exists in rain and water.

5.2.3 Soil

Inorganic Manganese

According to the Toxics Release Inventory, in 1996, the estimated releases of manganese of 47,528,469 pounds (21,603,850 kg) to soil from 1,978 large processing facilities accounted for about 81% of total environmental releases (TRI96 1998). Table 5-1 lists amounts released from these facilities. The TRI data should be used with caution, however, since only certain types of facilities are required to report. This is not an exhaustive list. Also, because these data reflect past releases, they may not be representative of current releases at these facilities.

Manganese has been identified in soil, sediment, and leachate samples collected at 237, 172, and 82, respectively, of the 603 NPL hazardous waste sites where it was detected in some environmental media (HazDat 1998).

Land disposal of manganese-containing wastes is the principal source of manganese releases to soil. Reported industrial releases to land in 1987 totaled 44 million pounds (TRI87 1989). In 1996, reported industrial releases to land ranged from 0 to 13.8 million pounds/year per state (TRI96 1998). An estimated 81% (47,500,000 pounds) of the total environmental release (58,400,000 pounds) of manganese was to land (TRI96 1998). No other data were located on releases of manganese to soils or sediments.

Organic Manganese

Maneb or mancozeb. According to TRI, in 1997, the estimated releases of 11,641 pounds (5,291 kg) of maneb to land from 1 repackaging facility accounted for about 27% of total environmental release (TRI97 1999).

5.3 ENVIRONMENTAL FATE

5.3.1 Transport and Partitioning

Elemental manganese and inorganic manganese compounds have negligible vapor pressures (see Table 3-2) but may exist in air as suspended particulate matter derived from industrial emissions or the erosion of soils. Manganese-containing particles are mainly removed from the atmosphere by gravitational settling, with large particles tending to fall out faster than small particles (EPA 1984a). The half-life of airborne particles is usually on the order of days, depending on the size of the particle and atmospheric conditions (Nriagu 1979). Some removal by washout mechanisms such as rain may also occur, although it is of minor significance in comparison to dry deposition (EPA 1984a; Turner et al. 1985).

In a study completed by Evans (1989), there were two mechanisms involved in explaining the retention of manganese and other metals in the environment by soil. First, through cation exchange reactions, manganese ions and the charged surface of soil particles form manganese oxides, hydroxides, and oxyhydroxides which in turn form absorption sites for other metals. Secondly, manganese can be adsorbed to other oxides, hydroxides, and oxyhydroxides through ligand exchange reactions. When the soil solution becomes saturated, these manganese oxides, hydroxides, and oxyhydroxides can precipitate into a new mineral phase and act as a new surface to which other substances can absorb (Evans 1989).

The behavior of heavy metals in the combustion gases of urban waste incinerators was studied by Fernandez et al. (1992). Manganese was detected inside gaseous fly ash particles in the form of oxides and chlorides. When these soluble oxides and chlorides reach environmental media they can leach out and become mobile (Fernandez et al. 1992).

The transport of manganese in air is largely determined by its particle size. About 80% of the manganese in suspended particulate matter is associated with particles having a mass median equivalent of <5 μm

(WHO 1981). The compound's small particle size (approximately 80% with a mass median equivalent diameter (MMAD) <5 μm and approximately 50% with an MMAD <2 μm) favors widespread airborne distribution and is within the respirable range (WHO 1981).

The transport and partitioning of manganese in water is controlled by the solubility of the specific chemical form present, which in turn is determined by pH, Eh (oxidation-reduction potential), and the characteristics of the available anions. The metal may exist in water in any of four oxidation states. Manganese(II) predominates in most waters (pH 4–7) but may become oxidized at a pH >8 or 9 (EPA 1984a). The principal anion associated with Mn(II) in water is usually carbonate (CO_3^{-2}), and the concentration of manganese is limited by the relatively low solubility (65 mg/L) of $MnCO_3$ (Schaanning et al. 1988). In relatively oxidized water, the solubility of Mn(II) may be controlled by manganese oxide equilibria (Ponnamperuma et al. 1969), with manganese being converted to the Mn(II) or Mn(IV) oxidation states (Rai et al. 1986). In extremely reduced water, the fate of manganese tends to be controlled by formation of a poorly soluble sulfide (EPA 1984a).

Manganese is often transported in rivers as suspended sediments. It has been reported that most of the manganese in a South American river came from industrial sources and was bound to suspended particles in the water (Malm et al. 1988).

In an aquifer studied in France, manganese was shown to originate from within the aquifer itself (Jaudon et al. 1989). In the presence of decreased dissolved oxygen in the groundwater, Mn(IV) has been shown to be reduced both chemically and bacterially into the Mn(II) form (Jaudon et al. 1989). This oxidation state is water soluble and easily released into the groundwater.

Manganese in water may be significantly bioconcentrated at lower trophic levels. A bioconcentration factor (BCF) relates the concentration of a chemical in plant and animal tissues to the concentration of the chemical in the water in which they live. Folsom et al. (1963) estimated that the BCF of manganese was 2,500–6,300 for phytoplankton, 300–5,500 for marine algae, 800–830 for intertidal mussels, and 35–930 for coastal fish. Similarly, Thompson et al. (1972) estimated that the BCF of manganese was 10,000–20,000 for marine and freshwater plants, 10,000–40,000 for invertebrates, and 100–600 for fish. In general, these data indicate that lower organisms such as algae have larger BCFs than higher organisms. In order to protect consumers from the risk of manganese bioaccumulation in marine mollusks, EPA has set a criterion for manganese at 0.1 mg/L for marine waters (EPA 1993).

The tendency of soluble manganese compounds to adsorb to soils and sediments depends mainly on the cation exchange capacity and the organic composition of the soil (Curtin et al. 1980; Hemstock and Low 1953; Kabata-Pendias and Pendias 1984; McBride 1979; Schnitzer 1969). Baes and Sharp (1983) noted that soil adsorption constants (the ratio of the concentration in soil to the concentration in water) for Mn(II) span five orders of magnitude, ranging from 0.2 to 10,000 mL/g, increasing as a function of the organic content and the ion exchange capacity of the soil; thus, adsorption may be highly variable. In some cases, adsorption of manganese to soils may not be a readily reversible process. At low concentrations, manganese may be "fixed" by clays and will not be released into solution readily (Reddy and Perkins 1976). At higher concentrations, manganese may be desorbed by ion exchange mechanisms with other ions in solution (Rai et al. 1986). For example, the discharge of waste water effluent into estuarine environments resulted in the mobilization of manganese from the bottom sediments (Helz et al. 1975; Paulson et al. 1984). The metals in the effluent may have been preferentially adsorbed resulting in the release of manganese.

Organic Manganese

MMT. MMT is generally unstable in light and is expected to degrade quickly in air. Although recent information indicates that MMT levels in the environment can increase from automobile emissions (Zayed et al. 1999), no information on the transport and partioning of MMT in the environment was located. Transport and partitioning of inorganic manganese compounds derived from the combustion of gasoline containing MMT are discussed above.

Maneb or Mancozeb. Few data on the transport and partitioning of these maneb and mancozeb were located. Calumpang et al. (1993) reported a half-life of 2.9 days for mancozeb determined in a silty clay loam soil placed in experimental columns and maintained under field conditions. In other studies, the half-life for maneb in soil was estimated to be 3 weeks and 4-8 weeks (Nash and Beall 1980; Rhodes 1977). Using chemical and physical properties, Beach et al. (1995) estimated that the half-life in soils for maneb and mancozeb to 70 days. The high K_{oc} value estimated for these compounds (>2000) suggested to these authors that the potential for leaching into ground water would be low. However, the potential for solubility in surface water was considered to be moderate, despite the relatively low solubility of maneb and reportedly insolubility of mancozeb.

5.3.2 Transformation and Degradation

5.3.2.1 Air

Inorganic Manganese

Very little information is available on atmospheric reactions of manganese (EPA 1984a). Manganese can react with sulfur dioxide and nitrogen dioxide, but the occurrence of such reactions in the atmosphere has not been demonstrated.

Organic Manganese

MMT. MMT photolyses rapidly by sunlight in the atmosphere with a very short half-life, i.e., less than 2 minutes (Ter Haar et al. 1975; Garrison et al. 1995). MMT is converted to a mixture of solid manganese oxides and carbonates. The organic portion of the solid consists of a complex mixture of acids, esters, and hydrocarbon polymers that results from the partial oxidation of the cyclopentadienyl ring, carbon monoxide insertion reactions and polymerization of multifunctional compounds (Ter Haar et al. 1975).

Maneb or mancozeb. Maneb, once released to the atmosphere, will exist primarily in the particulate-phase in the ambient atmosphere, where it can be removed by wet and dry deposition (Eisenreich et al. 1981; HSDB 1999). Maneb may undergo some photodegradation in sunlit air (Freitag et al. 1985; HSDB 1999). Mancozeb will also exist as particulate matter in the atmosphere (HSDB 1999).

5.3.2.2 Water

Inorganic Manganese

Manganese in water may undergo oxidation at high pH or Eh (see Section 5.3.1.2) and is also subject to microbial activity. For example, Mn(II) in a lake was oxidized during the summer months, but this was inhibited by a microbial poison, indicating that the oxidation was mediated by bacteria (Johnston and Kipphut 1988). The microbial metabolism of manganese is presumed to be a function of pH, temperature, and other factors, but no data were located on this.

Organic Manganese

MMT. The rate of MMT degradation in natural aquifer and sediment systems was determined to be very slow under anaerobic conditions (Garrison et al. 1995). Calculated half-lives ranged from approximately 0.2 to 1.5 years at 25°C. However, MMT photolyzed rapidly in purified, distilled water exposed to sunlight. The disappearance of MMT followed first-order kinetics, with a calculated half-life of

0.93 min. Reaction products included methylcyclopentadiene, cyclopentadiene, carbon monoxide, and a manganese carbonyl that readily oxidized to trimanganese tetroxide.

Maneb or mancozeb. Maneb released to water may be subject to abiotic degradation to ethylene thiuram disulfide, ethylene thiourea (ETU), ethylenediamine (EDA), and ethylene thiuram monosulfide (ETM) (Hylin 1973; HSDB 1999). The rate of degradation is influenced by the aeration of water and pH. In addition, maneb may undergo some photodegradation in sunlit water (Freitag et al. 1985; HSDB 1999). Maneb is not expected to undergo significant volatilization from water. Mancozeb hydrolyzes, with a half-life less than 1–2 days at pH 5–9, in water rapidly (HSDB 1999).

5.3.2.3 Sediment and Soil

Inorganic Manganese

The oxidation state of manganese in soils and sediments may be altered by microbial activity. Geering et al. (1969) observed that Mn(II) in suspensions of silt or clay loams from several areas of the United States was oxidized by microorganisms, leading to the precipitation of manganese minerals. Other studies (Francis 1985) have shown that bacteria and microflora can increase the mobility of manganese in coal-waste solids by increasing dissolution of manganese in subsurface environments.

Organic Manganese

MMT. The hydrophobicity of MMT (log K_{ow} = 3.7) suggests that it can sorb to soil or sediment particles (Garrison et al. 1995). MMT was found to be stable in a stream bottom sediment under anaerobic conditions. Photodegradation of MMT is not likely to occur in sediments, and it may equilibrate between the sediment, sediment porewater, and water column manganese (Garrison et al. 1995).

Maneb or mancozeb. In a laboratory experiment, maneb was shown to degrade readily (within 2 days) to ETU in soil (Nash and Beall 1980). A half-life of maneb in soil was determined to be 36 days. Maneb was found not to move below 1 cm depth of soil. However, soluble ^{14}C-degradation products (<40 µg/L) of maneb (not measurable as EDA or ETU) did move through the soil with leachate water. The mobility of maneb in soil is influenced by the level of organic matter in the soil, such that mobility decreases with

increasing organic matter (Helling et al. 1974). A half-life of maneb in soil was reported to be 4–8 weeks in fine sand with 2.4% organic matter content (Rhodes 1977). ^{14}C-residues did not leach much below a 5-inch soil depth.

Mancozeb degrades rapidly in soil (Calumpang et al. 1993). Under field conditions, the half-lives of mancozeb, ethylenethiourea (ETU), and ethyleneurea (EU) in soil were 2.9, 2.5, and 4.8 days, respectively. Mancozeb is immobile in soil. Ethylene leached to a maximum of 8 cm of soil 14 days after application of mancozeb.

5.4 LEVELS MONITORED OR ESTIMATED IN THE ENVIRONMENT

Reliable evaluation of the potential for human exposure to manganese depends in part on the reliability of supporting analytical data from environmental samples and biological specimens. In reviewing data on manganese levels monitored or estimated in the environment, it should also be noted that the amount of chemical identified analytically is not necessarily equivalent to the amount that is bioavailable.

5.4.1 Air

Inorganic Manganese

Table 5-3 summarizes data collected over a period of nearly 30 years from numerous urban, nonurban, and source-dominated areas of the United States. Direct comparisons of data from different time periods are complicated because of changes in sample collection and analytical methodology. However, it is clear that manganese levels tend to be higher in source-dominated and urban areas than in nonurban areas. These data also indicate that concentrations in all areas have tended to decrease over the past three decades (EPA 1984a; Kleinman et al. 1980). This is probably due primarily to the installation of emission controls in the metals industry (EPA 1984a, 1985d). A concurrent decrease in total suspended particulates (TSP) was observed in most areas. Annual averages of manganese in urban and rural areas without significant manganese pollution are in the range of 0.01–0.07 $\mu g/m^3$ (WHO 1997). The daily intake of manganese in the air by the general population in areas without manganese emitting industries was estimated to be below 2 µg/day (WHO 1981). In areas with major foundry facilities, intake may rise to 4–6 µg/day, and in areas associated with ferro- or silicomanganese industries, it may be as high as 10 µg, with 24-hour peak values exceeding 200 µg/day (WHO 1981).

Table 5-3. Average Levels of Manganese in Ambient Air[a]

Sampling location	Concentration (ng/m^3)		
	1953–1957	1965–1967	1982
Nonurban	60	12	5
Urban	110	73	33
Source dominated	No data	250–8,300	130–140

[a]Adapted from EPA 1984a

During 1988–1993, ambient concentration of fine ($PM_{2.5}$) manganese ranged from 1 ng/m^3 in rural sites in U.S. National Parks to 3 ng/m^3 in urban sites in California (Wallace and Slonecker 1997). There is concern in Canada regarding the combustion of MMT as an important source of manganese contamination in the urban environment, especially in areas of high traffic density. For instance, Loranger and Zayed (1997a) reported significantly higher levels of both respirable and total manganese levels at a high traffic density site (0.024 µg/m^3 and 0.050 µg/m^3, respectively) in Montreal in contrast to a low traffic density site (0.015 µg/m^3 and 0.027 µg/m^3, respectively). Temporal variation of respirable and total manganese was similar for both sites, and atmospheric manganese concentrations reflected a positive relationship with the traffic density. However, as discussed in Section 5.2.1, it has been difficult to assess the exact contribution of the combustion of MMT by vehicles to manganese levels in the environment.

Organic Manganese

MMT. In Montreal, Canada, atmospheric concentrations of MMT, and respirable and total manganese, were measured in 5 microenvironments including a gas station, an underground car park, downtown Montreal, near an expressway, and near an oil refinery (Zayed et al. 1999). The overall mean concentrations of respirable manganese, total manganese, and MMT measured for all the microenvironments were 0.036 µg/m^3, 0.103 µg/m^3, and 0.005 µg/m^3, respectively. It was noted by the authors that respirable manganese (0.053 µg/m^3) measured near the expressway was equal to the U.S. EPA Reference Concentration (RfC) of 0.05 µg/m^3. Moreover, by comparing the RfC to the 95th percentile of atmospheric manganese in Montreal, it appears that possibly 5% of a "theoretical population" generated by Monte-Carlo simulations would be exposed to a higher concentration and could be at risk (Loranger and Zayed 1997b).

Maneb or mancozeb. Ambient levels of maneb (calculated from manganese) were <0.02, 0.03, and 0.77 mg/m^3 in a tractor cabin, breathing zone, and during weighing of the pesticide, respectively (Savolainen et al. 1989).

5.4.2 Water

Inorganic Manganese

Many 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, and alcohol consumption) can significantly influence the uptake of manganese by an individual (EPA 1993b). 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 (EPA 1993b).

Concentrations of manganese in surface water are usually reported as dissolved manganese. Although total manganese may be a better indicator, since manganese adsorbed to suspended solids may exceed dissolved manganese in many systems, the bioavailability of manganese in this form has not been established (EPA 1984a; NAS 1977). In a 1962–1967 survey of U.S. surface waters, dissolved manganese was detected in 51% of 1,577 samples, at a mean concentration of 59 µg/L. Individual values ranged from 0.3 to 3,230 µg/L. Mean concentrations for 15 different drainage basins in the United States ranged from 2.3 µg/L in the western Great Lakes to 232 µg/L in the Ohio River drainage basin (Kopp and Kroner 1967). A later (1974–1981) survey of United States river waters reported a median dissolved manganese concentration of 24 µg/L in samples from 286 locations, with values ranging from <11 µg/L (25th percentile) to >51 µg/L (75th percentile) (Smith et al. 1987). Natural concentrations of manganese in seawater reportedly range from 0.4 to 10 µg/L (EPA 1984a).

Mean manganese concentrations in groundwater are similar to those in surface water, although some individual samples may be considerably higher. Reported mean groundwater concentrations were 20 and 90 µg/L in an analysis of California shallow groundwater from two geologic zones (Deverel and Millard 1988). Values up to 1,300 µg/L and 9,600 µg/L have been reported in neutral and acidic groundwater, respectively (EPA 1984a). Concentrations of 9,500–18,600 µg/L have been reported in four private wells in Connecticut (CDHS 1990). It is not known whether these measurements were total or dissolved manganese.

A 1962 survey of public drinking water supplies in 100 large United States cities reported 97% contained <100 µg/L of manganese (Durfor and Becker 1964). Similarly, a 1969 survey of 969 systems reported 91% contained <50 µg/L, with a mean concentration of 22 µg/L (U.S. DHEW 1970). Several other

studies reported similar manganese concentrations, with mean values ranging from 4 to 32 µg/L (EPA 1984a; NAS 1980a; WHO 1981).

Organic Manganese

MMT. No studies were located reporting surface or ground water concentrations of MMT.

Maneb or mancozeb. No studies were located regarding environmental concentrations of maneb or mancozeb in groundwater, surface water, or public water supplies. Beach et al. (1995) estimated the likelihood of ground water and surface water contamination of maneb and mancozeb in different agricultural environments and for different crops. They reported that maneb and mancozeb are not susceptible to leaching, but would be highly susceptible to sediment runoff in Arizona (lettuce production), soil adsorbed runoff in Florida (tomato production) and Michigan (asparagus production). In addition, mancozeb would be highly susceptible to soil-adsorbed runoff in Texas (watermelon production). No studies to date have investigated potential increases in manganese concentrations of surface or ground water bodies as a result of maneb or mancozeb use.

5.4.3 Sediment and Soil

Inorganic Manganese

Manganese comprises about 0.1% of the earth's crust (Graedel 1978; NAS 1973), and manganese occurs naturally in virtually all soils. Average natural ("background") levels of manganese in soils range from around 40 to 900 mg/kg, with an estimated mean background concentration of 330 mg/kg (Cooper 1984; Eckel and Langley 1988; EPA 1985c; Rope et al. 1988; Schroeder et al. 1987). The maximum value reported was 7,000 mg/kg (Eckel and Langley 1988).

Accumulation of manganese in soil usually occurs in the subsoil and not on the soil surface; 60–90% of manganese is found in the sand fraction of the soil (WHO 1981). A preliminary survey was conducted in Utah to provide an initial field measurement of the contamination by manganese oxides from exhaust in roadside soil and plant species due to the addition of MMT to motor vehicle fuels. Soil (0–5 cm) manganese concentrations were strongly correlated with distance from roadways with moderate and moderately high traffic volumes (Lytle et al. 1994). In addition, exchangeable manganese was found to

be significantly higher in an organic soil located at stations with a high traffic density comparing to another one with a low traffic density (Brault et al. 1994). The average soil manganese concentration measured at 1 meter from a moderate to moderately-high traffic volume roadside was 3,046 µg/g dry weight. At 15m, the average soil manganese concentration decreased to 254 µg/g dry weight.

Organic Manganese

MMT. No studies were located which measured the concentration of MMT in sediment or soil; all studies have focused on increased concentrations of manganese in these media as a result of combustion of the fuel additive.

Maneb or mancozeb. No studies were located that measured the concentration of these compounds after application of the compounds in the field on existing crops. All studies to date have involved localized application or laboratory measurements intended to report half-lives of the compounds in particular environments (Helling et al. 1974; Nash and Beall 1980; Rhodes 1977). None of the studies has reported increased manganese concentrations in either soil or sediment as a result of application of these fungicides.

5.4.4 Other Environmental Media

Inorganic Manganese

Manganese is a natural component of most foods. A summary of mean manganese concentrations in 234 foods analyzed by the FDA is included in Table 5-4. The highest concentrations (up to 50 ppm) are found in nuts, tea, legumes, pineapples, and whole grains, with lower levels (up to 5 ppm) found in milk products, meats, fish, and eggs (Davis et al. 1992a; Pennington et al. 1986). Tea and leafy green vegetables were the major dietary sources of manganese for young women taking part in a dietary study in Wisconsin (Davis et al. 1992a). Bioaccumulation of manganese by plants was examined using oats (*Avena nova*) and beans (*Phaseolus vularis*) (Brault et al. 1994). These plants were grown in sandy and organic soil at a control site (greenhouse) and at two outdoor sites weakly near <20,000 vehicles/day, and 132,000 vehicles/day respectively. The highest manganese accumulation was found in the fruits and stems of oats grown in the organic and sandy soils at the station with the highest traffic density. Lönnerdal (1997) reported that infant formulas currently contain 30–75 ppb (0.03–0.075 ppm) manganese, as

Table 5-4. Manganese Concentrations in Selected Foods[a]

Type of food	Range of mean concentrations (ppm)
Nuts and nut products	18.21–46.83
Grains and grain products	0.42–40.70
Legumes	2.24–6.73
Fruits	0.20–10.38
Fruit juices and drinks	0.05–11.47
Vegetables and vegetable products	0.42–6.64
Desserts	0.04–7.98
Infant foods	0.17–4.83
Infant formulae (soy) and enteral products	0.31–2.87
Infant formulae (cow-milk based)	0.03-0.075
Meat, poultry, fish, and eggs	0.10–3.99
Mixed dishes	0.69–2.98
Condiments, fats, and sweeteners	0.04–1.45
Beverages (including tea)	0.00–2.09
Soups	0.19–0.65
Milk and milk products	0.02–0.49

[a]Adapted from Cook 1997, Lönnerdal 1997, Pennington et al. 1986

compared to concentrations of 3–10 ppb (0.003–0.01 ppm) in breast milk and 30 ppb (0.03 ppm) in cow's milk.

During a 1992 survey conducted by Canada's Department of Fisheries and Oceans, concentrations of manganese were detected in the muscle samples of bluefin tuna (*Thunnus thynnus*) (Hellou et al. 1992). Concentrations of manganese in 14 samples of fish muscle ranged from 0.16 to 0.31 µg manganese/g dry weight, with a mean of 0.22 µg/g. Although the analysis was administered with a high accuracy of 94% using inductively coupled plasma mass spectrometry (ICP-MS), the sample population was small.

In the field survey conducted by Lytle et al. (1994), terrestrial and aquatic plant samples were collected along motorways and local urban roadways throughout Utah during 1992 and 1993. Manganese was detected in the plant samples, with manganese concentrations ranging from 30.2 to 13,680 µg/g dry weight. Manganese was detected in plants found nearest to the motorway. Loranger et al. (1994b) evaluated the use of the pigeon as a monitor for manganese contamination from motor vehicles in urban and rural areas of Canada, a country in which MMT has been used to replace lead in gasoline. Manganese concentrations were similar in the 2 groups of pigeons for all tissues except the liver and feces; urban pigeons had about 35% more manganese than rural ones. Loranger et al. (1994b) suggested that although pigeon feces and liver may be good biomarkers of manganese contamination, it is premature to associate the excess manganese with the combustion of MMT.

Organic Manganese

MMT. No studies were located concerning concentrations of MMT on food stuffs.

Maneb or mancozeb. Maneb and mancozeb residues in various food and crop samples have been evaluated. According to the FOODCAM database, a national database of information generated by state agencies involved in the regulation of agricultural, public health, and the environment, maneb residues were detected (concentrations not reported) in only 2 of 13,085 food samples analyzed during 1989 (Minyard and Roberts 1991). No maneb residues were found in 39 fruit and vegetable samples in California during 1989 (Okumara et al. 1991). Residues of maneb (<0.1–4.0 ppm) and ETU (<0.05 ppm) were detected in various crop samples obtained from an area where maneb was used as a fungicide (Pease and Holt 1977).

Mancozeb residues were not detected in treated crop samples (apples, dried onion, and grapes) that were analyzed as a part of the comprehensive California Priority Pesticide Program for 1989 (Okumura et al. 1991). Other samples of grapes had residues that were within tolerance limits for mancozeb. Tomato fruits sprayed with mancozeb up to four times in 45 days had residue levels below detection limits except for green fruits harvested 30 days after the first spray and green or ripe fruits harvested 30-45 days after the fourth spray (residue levels ranged from 0.96 mg/kg to 3.27 mg/kg) (Patil et al. 1995). Residue levels decreased to below detection limits in green or ripe tomatos when the fruit were harvested 10 days after spraying or later. These data indicate that mancozeb residue levels on tomatos are dependent on the number of mancozeb applications and time of harvest. Following an accidental aerial spray of a grain field, residues of mancozeb (expressed as maneb) and ETU were detected in barley, oats, and wheat (Rosenberg and Siltanen 1979). The highest concentrations of residues were detected 1 meter from a potato field, the intended target area for aerial spraying. At this distance, samples of barley, oats, and wheat contained 21, 5.5, and 15 ppm maneb, and 0.08, 0.01, and 0.09 ppm ETU, respectively.

5.5 GENERAL POPULATION AND OCCUPATIONAL EXPOSURE

The EPA has reported that 7,550 cases of manganism have been recorded in the literature since the first report in 1937 (EPA 1993b). Typical daily human exposure levels to manganese from water, air, and food are summarized in Table 5-4 (EPA 1984a). As the table illustrates, the most significant exposure for the general population is from food, with an average ingestion rate of 3,800 µg/day (EPA 1984a). Other estimates of daily intake for adults range from 2,000 to 8,800 µg (EPA 1984a; NAS 1977; Patterson et al. 1984; Pennington et al. 1986; WHO 1984a). Even though gastrointestinal absorption of manganese is low (3–5%), oral exposure is the primary source of absorbed manganese (Table 5-5).

Manganese intake among individuals varies greatly, depending upon dietary habits. For example, an average cup of tea may contain 0.4–1.3 mg of manganese (Pennington et al. 1986; Schroeder et al. 1966). Thus, an individual consuming three cups of tea per day might receive up to 4 mg/day from this source alone, increasing the average intake from all dietary sources.

The EPA Reference Dose (RfD)/Reference Concentration (RfC) workgroup in June 1990 set an RfD for manganese in food of 0.14 mg manganese/kg/day, equivalent to 10 mg/day for a 70-kg man based on chronic manganese uptake (EPA 1993b). The Food and Nutrition Board of the NRC estimated the adequate and safe intake of manganese for adults at 2–5 mg/day (NAS 1980b). This level was chosen

Table 5-5. Summary of Typical Human Exposure to Manganese[a]

Parameter	Exposure medium		
	Water	Air	Food
Typical concentration in medium	4 µg/L	0.023 µg/m^3	1.28 µg/calorie
Assumed daily intake of medium by 70-kg adult	2 L	20 m^3	3,000 calories
Estimated average daily intake by 70-kg adult	8 µg	0.46 µg[b]	3,800 µg
Assumed absorption fraction	0.03[c]	1[c]	0.03[d]
Approximate absorbed dose	0.24 µg	0.46 µg	114 µg

[a]Adapted from EPA 1984a
[b]Assumes 100% deposition in the lungs
[c]No data; assumed value
[d]See Section 2.3.1.2

because it includes an "extra margin of safety" of 5 mg/day below the level of 10 mg/day, which the NRC considered to be safe for occasional intake (IRIS 1993). It is possible that a significant proportion of Americans, especially females, are not consuming sufficient manganese (Davis and Greger 1992; NAS 1980a; Pennington et al. 1986). However, infants may be ingesting more than the estimated safe and adequate dose of 0.3–1.0 mg/day for their age group (Pennington et al. 1986) because of the relatively high manganese levels in prepared infant foods (Table 5-3) and formulas (Lönnerdal 1997).

In the workplace, exposure to manganese is most likely to occur by inhalation of manganese fumes or manganese-containing dusts. This is a concern mainly in the ferromanganese, iron and steel, dry-cell battery, nd welding industries (WHO 1986). Exposure may also occur during manganese mining and ore processing. Ordinarily, manganese is mined in an open pit or shallow underground mine; however, manganese carbonate used to be mined from deep mines in Butte, Montana (HSDB 1993). The most recent data indicate that only a very small amount of manganese is still mined in the United States; in 1997, manganiferous material having a natural manganese content between 5 and 15% for use in coloring brick was mined in Cherokee County, South Carolina (USGS 1998). Excluding insignificant quantities of similar low-grade manganiferous ore, the United States has not mined significant amounts of manganese since 1978 and now relies on imports to fill its needs (U.S. Bureau of Mines 1989). Therefore, mining is no longer a source of manganese exposure for many workers in the United States. In 1980, it was estimated that in the United States about 300 workers were exposed to pure manganese and about 630,000 workers were exposed to other forms of manganese (NOES 1989). Concentrations of 1.5–450 mg manganese/m^3 have been reported in U.S. manganese mines (EPA 1984a), 0.30–20 mg manganese/m^3 in ferroalloy production facilities (Saric et al. 1977), and 3–18 mg manganese/m^3 in a dry-cell battery facility (Emara et al. 1971). Steel-manufacturing facilities are significant employers in the U.S. There is a potential for manganese exposure to workers in these facilities. Current occupational exposures in a metal-producing plant in the United States were reported as a mean of 0.066 mg/m^3, median of 0.051 mg/m^3 as respirable dust, and 0.18 mg/m^3 in total dust (Gibbs et al. 1999). Exposure levels should not exceed the OSHA time-weighted average Permissible Exposure Limit (PEL) of 1 mg total manganese/m^3 (see Table 7-1). Assuming inhalation of 10 m^3 of air during an average workday, maximum occupational exposure would be 10 mg manganese/day. This exceeds the average exposure from ambient air by a factor of more than 10^4 and is about 2.5 times the average exposure from the diet. Thus, for workers in industries using manganese, the major route of exposure may be inhalation from workplace air rather than from ingestion of food.

Occupational exposure to manganese resulting from the combustion of MMT in Montreal, Canada has been studied. Sierra et al. (1995) conducted a study of Montreal automotive workers (garage mechanics) and nonautomotive workers (control group). Exposure to manganese was measured for 5 consecutive working days. In addition, their environmental exposure (at home) was measured on 2 days of the same week. Air sampling was performed by portable pumps; for sampling at homes, workers were asked to wear the pumps as much as possible. At the workplace, the mechanics were exposed to manganese concentrations ranging from 0.010–6.673 μg/m^3 (mean of 0.45 μg/m^3), while nonautomotive workers were exposed to concentrations manganese ranging from 0.011–1.862 μg/m^3 (mean of 0.04 μg/m^3). The average environmental concentrations for the mechanics (0.012 μg/m^3) and for the nonautomotive workers (0.008 μg/m^3) were similar to manganese concentrations measured in Montreal in 1992. Based on measurements of manganese particle size distributions, Sierra et al. (1995) estimated that less than 10% of the manganese exposure of the garage mechanics was due to MMT; however, the exact contribution of MMT could not be determined.

A similar study conducted in Montreal by these investigators, but involving taxi drivers and garage mechanics, revealed that garage mechanics at work were exposed to an average of 0.250 μg/m^3 and taxi drivers to 0.024 μg/m^3 (Zayed et al. 1994). In another study, exposure of office workers and taxi drivers to both respirable and total manganese was evaluated (Zayed et al. 1996). Manganese concentrations measured for the office workers ranged from 0.001–0.034 μg/m^3 (respirable manganese) and from 0.002–0.044 μg/m^3 (total manganese). For the taxi drivers, the manganese concentrations ranged from 0.007–0.032 μg/m^3 (respirable manganese) and from 0.008–0.073 μg/m^3 (total manganese). Zayed et al. (1996) concluded that the higher exposure to atmospheric manganese in the outdoor urban environment may be at least partly due to the use of MMT in cars. Nevertheless, these investigators indicated that the exposures of taxi drivers to manganese were well below existing exposure and health guidelines.

In order to assess the potential health risks from MMT combustion, Loranger and Zayed (1995) conducted a multi-media assessment (i.e., food, water, and ambient air) of manganese exposure in two groups of workers (garage mechanics and blue-collar workers) potentially exposed to different levels of manganese from MMT. Garage mechanics were exposed to higher air manganese concentrations (0.42 μg/m^3) than blue-collar workers (0.04 μg/m^3). However, for the garage workers, exposure to atmospheric manganese represented only approximately 4% of the total absorbed dose, while ingestion of food represented 95.7% of the total multi-media dose. For the blue collar workers, atmospheric

manganese contributed only 0.3% to the total absorbed dose, whereas ingestion of food represented 99.2% of the total multi-media dose. These results were consistent with values of multi-media doses predicted by GADUS, an environmental fate/exposure model (Loranger and Zayed 1997b). Based on governmental standards or criteria for occupational and environmental exposures, Loranger and Zayed (1995) concluded that the manganese levels in food and air may not cause any problems for these workers.

Based on an analysis of data obtained from a large, continuous personal exposure study in Toronto, Canada, a city with widespread use of MMT, it was determined that the general population was exposed to low concentrations (median concentration was 0.008 $\mu g/m^3$) of airborne $PM_{2.5}$ manganese (Lynam et al. 1999). Ambient levels of manganese in Toronto were approximately the same as cities where MMT is not used. Traffic densities were not take into consideration. Also, high levels of airborne manganese were measured in the Toronto subway.

Occupational exposure to maneb and mancozeb can occur by the inhalation or dermal routes during the formulation and spray application of these pesticides (HSDB 1999). The general population is unlikely to be exposed to excess levels of pesticide residues on food, since crops analyzed for these compounds were found to have either non-detectable levels of the compounds or levels within allowable limits (Okumura et al. 1991) (see Section 5.4.4).

5.6 EXPOSURES OF CHILDREN

This section focuses on exposures from conception to maturity at 18 years in humans and briefly considers potential pre-conception exposure to germ cells. Differences from adults in susceptibility to hazardous substances are discussed in 2.6 Children's Susceptibility.

Children are not small adults. A child's exposure may differ from an adult's exposure in many ways. Children drink more fluids, eat more food, and breathe more air per kilogram of body weight, and have a larger skin surface in proportion to their body volume. A child's diet often differs from that of adults. The developing human's source of nutrition changes with age: from placental nourishment to breast milk or formula to the diet of older children who eat more of certain types of foods than adults. A child's behavior and lifestyle also influence exposure. Children crawl on the floor, they put things in their

mouths, they may ingest inappropriate things such as dirt or paint chips, they spend more time outdoors. Children also are closer to the ground, and they do not have the judgement of adults in avoiding hazards (NRC 1993). Children and children in strollers may also have increased exposure to exhaust fumes due to their height above ground.

Children would be exposed to manganese in the same manner as adults. The main source of exposure of children to manganese is through food. Infants and young toddlers may be exposed to concentrations higher than the estimated safe and adequate dose for their age group because of the increased levels of the element in infant formulas as compared to breast milk (Collipp et al. 1983; Cook 1997; Dorner et al. 1989; Keen et al. 1986; Lönnerdal et al. 1983, 1994). Manganese concentrations in blood serum of children of different ages are provided in Table 2-7. The data indicate that manganese concentrations decrease slightly from the time the infant is 5 days of age until he or she is 12 months of age (Alarcón et al. 1996; Rukgauer et al. 1997). Manganese concentrations increase after this time, and they have been measured as an average of 1.4±1.25 µg/L in children aged 1 month to 18 years (Rükgauer et al. 1997).

Children are exposed *in utero* because manganese in maternal blood crosses the placenta to satisfy the fetus's need for manganese. The compound has been measured in cord blood plasma of premature and full-term infants and their mothers (Wilson et al. 1991). Full-term babies have higher (but not statistically significantly different) blood concentrations of manganese than premature babies, and pregnant women have higher blood concentrations than nonpregnant women. However, no correlations were observed between maternal and infant concentrations of manganese. Manganese in breast milk has been found to range from 3.4 to 10 µg/L (Arnaud and Favier 1995; Collipp et al. 1983) depending on the maturity of the milk. The Food and Nutrition Board of the NRC based the recommended manganese intake of infants on the analyses of pooled human milk samples. Accordingly, manganese intakes of infants fed some formulas appear high, but no signs of toxicity have been observed (Lönnerdal et al. 1983). It is unknown whether nursing mothers exposed to higher-than-average concentrations of manganese would excrete increased concentrations of the metal in their breast milk.

Young children often eat dirt (exhibiting what is called soil pica, the ingestion of a material unfit for food) and exhibit frequent hand-to-mouth activity; they can be exposed to manganese through this unique pathway if the soils contain the metal. Current estimates indicate that soil pica may be more prevalent in the general population than previously thought and that most children periodically ingest soil to varying degrees; this may be a potential health concern (EPA 1986d; Stanek and Calabrese 1995). However, no

information was found concerning the bioavailability of manganese from soil and, therefore, determining the actual risk posed to children from this exposure pathway is difficult. This behavior should not pose an increased risk of exposure to manganese in most residential situations where the manganese levels are in the normal or background range. If the soils are from a hazardous waste site that contains high concentrations of manganese, then increased exposure to the compound may occur. However, until bioavailability of manganese from soil is determined, a child's ingestion of a given amount of soil containing manganese cannot be translated into actual levels of exposure.

Children who suffer from cholestatic liver disease or who have gastrointestinal disorders that mandate they be given parenteral nutrition may be at increased risk from overexposure to manganese. Increased manganese concentrations in blood and brain, and symptoms of neuromotor dysfunction were observed in an 8-year-old girl with cholestatic liver failure (Devenyi et al. 1994). Children with or without chronic liver disease and a 5-year-old boy who had gastrointestinal disorders, all of whom were administered parenteral nutrition, had abnormal MRI scans indicative of manganese accumulation (Fell et al. 1996; Ono et al. 1995) accompanied by motor disorders (Fell et al. 1994).

Because manganese is a trace element that is essential for normal human health and is predominantly obtained from food, it is unlikely that toxic amounts of manganese will be absorbed from food. However, diets vary and some are higher in manganese than others (diets high in grains and tea, for instance). One case study suggested that a 59-year-old man developed manganism-like symptoms from abusing vitamins and minerals. This man had very high manganese concentrations in blood, urine, feces, hair, and brain (Banta and Markesbery 1977). Both manganese and iron are bound by transferrin and these elements compete for the binding protein in the body. Therefore, diets that are low in iron allow transferrin to bind more manganese. For this reason, it is important to provide children with a balanced diet to maintain optimal iron and manganese stores in the body. Studies show that adults absorb only 3–5% of manganese ingested from the diet (Davidsson et al. 1988, 1989; Mena et al. 1969); infants have increased absorption relative to adults (Dorner et al. 1989). Neonatal animals also exhibit increased absorption relative to older animals (Ballatori et al. 1987; Miller et al. 1975; Rehnberg et al. 1981).

Children may be exposed to organic manganese compounds through a variety of routes. They may be exposed to MMT combustion products via inhalation of these products in air, or ingestion of them after deposition on the soil. Children may be exposed to maneb and mancozeb by eating fruits and vegetables that have residues of these pesticides on them

5.7 POPULATIONS WITH POTENTIALLY HIGH EXPOSURES

As discussed in Section 5.5, workers in industries using or producing manganese are mostly likely to have high exposure to manganese, primarily by inhalation of manganese dusts in workplace air. Populations living in the vicinity of ferromanganese or iron and steel manufacturing facilities, coal-fired power plants, or hazardous waste sites may also be exposed to elevated manganese particulate matter in air or water, although this exposure is likely to be much lower than in the workplace. Populations living in regions of natural manganese ore deposits may be exposed to above-average levels in soil, water, or air. Children are especially likely to receive above-average doses from manganese-containing soils since they have a higher intake of soil (mainly through hand-to-mouth contact) than adults (Calabrese et al. 1989).

People ingesting large amounts of foods high in manganese also have a potential for higher-than-usual exposure. Included in this group would be vegetarians, who ingest a larger proportion of grains, legumes, and nuts in their diets than the average U. S. population, and heavy tea drinkers. While the intake of manganese from vegetarians may exceed the estimates of daily dietary intake, the bioavailability of manganese from vegetable sources is substantially decreased by dietary components such as fiber and phytates (EPA 1993b). In addition to the population with these dietary habits, individuals with iron deficiency show increased rates of manganese absorption (Mena et al. 1969, 1974); iron deficiency leads to increased brain manganese concentrations in experimental animals (Aschner and Aschner 1990).

Manganese is eliminated from the body primarily through the bile. Interruption of the manufacture or flow of bile can impair the body's ability to clear manganese. Several studies have shown that adults and children (Devenyi et al. 1994; Fell et al. 1996; Hauser et al. 1994, 1996; Pomier-Layrargues et al. 1998; Rose et al. 1999; Spahr et al. 1996), as well as experimental animals (Rose et al. 1999), with cholestatic liver disorders have increased manganese levels in their blood and brain and are at risk from potentially increased exposure to manganese due to their decreased homeostatic control of the compound.

In addition to oral diets, people on partial and total parenteral nutrition may be exposed to increased amounts of manganese. Forbes and Forbes (1997) found that of 32 patients receiving home parenteral nutrition due to digestive problems, 31 had elevated serum manganese levels (0.5–2.4 mg/L compared to normal range of 0.275–0.825 mg/L). It is unclear whether these levels reflected steady-state conditions due to the time the samples were taken. However, these levels are much higher than other studies involving patients on TPN; thus, it is unlikely that these levels represent steady-state conditions. Further,

the normal range reported by authors is elevated compared to other studies, suggesting the possibility that the blood samples were contaminated with exogenous manganese. The authors observed no clinical evidence of toxicity in the patients. Fourteen of the patients suffered iron deficiency anemia; because low iron concentrations are associated with increased manganese uptake, the anemia may have exacerbated the increased blood manganese concentrations. Increased blood manganese levels and MRI scans indicative of increased manganese in brains have been reported in children fed entirely on parenteral nutrition (Fell et al. 1996; Kofritsa et al. 1998; Ono et al. 1995). Interestingly, only in the Fell et al. (1996) study were neurotoxic effects reported. Whole-blood manganese in the children from this study ranged from 9.9–110 µg/L. Devenyi et al. (1994) found hyperintense signals in the brain of an 8-year-old child who had cholestatic liver disease and exhibited dystonia and other motor dysfunctions. Nagatomo et al. (1999) reported that two elderly patients who had been administered TPN for 3–4 months exhibited clinical signs of manganism (including masked facies, marked rigidity, hypokinesia) with associated elevated blood manganese levels and hyperintense signals on MRI, localized to the basal ganglia, especially the globus pallidus. Signs of manganism abated upon levodopa treatment and the administration of Ca-EDTA; the high intensity signals on MRI abated when manganese supplementation ceased. In addition to patients on parenteral nutrition, uremic patients on hemodialysis have been found to have increased manganese levels due to increased concentrations of manganese in the dialysis solution (Lin et al. 1996). These studies indicate that while increased levels of manganese in blood and brain are often associated with TPN administration, adverse neurological effects are not always reported. Nagatoma et al. (1999) found increased serum concentrations of manganese and brain abnormalities in two patients who showed parkinsonism with psychiatric symptoms after 3–4 months of total parenteral nutrition. Discontinuation of manganese supplementation in the parenteral diet, coupled with levodopa treatment gradually improved both the symptoms and brain abnormalities in the patients.

In comparison to other groups within the general population, persons living close to high density traffic areas, automotive workers, and taxi drivers may be exposed to higher concentrations of manganese arising from the combustion of MMT. Farmers, people employed as pesticide sprayers, home gardeners, and those involved in the manufacture and distribution of maneb and mancozeb may be exposed to higher concentrations of these pesticides than the general public. People who ingest fruits and vegetables that have been treated with these pesticides and that contain higher-than-usual residues of the compounds (due to incomplete washing or over-application) may be exposed to increased concentrations of the pesticides. It is possible that medical workers may be exposed to higher concentrations of mangafodipir than the general population, although exposure routes other than i.v. are not expected to pose a significant risk.

5.8 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of manganese is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of manganese.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce or eliminate the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

5.8.1 Identification of Data Needs

Physical and Chemical Properties. The fundamental physical and chemical properties of manganese and manganese compounds are known (see Table 3-2), and additional research does not appear necessary.

Production, Import/Export, Use, Release and Disposal. Information is available on U.S. import of manganese ore and production of ferromanganese (HSDB 1989; TRI87 1989; TRI91 1993; U.S. Bureau of Mines 1989), but more recent data would be valuable. It is clear that most manganese is used in steel production, but detailed information on the amount and type of manganese compounds used in various other products was not located and would be helpful in evaluating possible consumer exposures. Information on the import, export, and use of MMT in U.S. fuels would be very helpful in targeting potentially exposed populations for the identification of adverse health effects.

According to the Emergency Planning and Community Right-to-Know Act of 1986, 42 U.S.C. Section 11023, industries are required to submit chemical release and off-site transfer information to the EPA. The Toxics Release Inventory (TRI), which contains this information for 1991, became available in May of

1993. This database will be updated yearly and should provide a list of industrial production facilities and emissions.

Data from the TRI database provide valuable information on the amounts of manganese released to different environmental media (e.g., air, soil, and water) each year, although details on the chemical form and physical state of the waste materials are not included. These disposal practices are not regulated under current federal law. TRI data may not be complete estimates of total release. Also, because these data reflect past releases, they may not be representative of current releases at these facilities.

Environmental Fate. The partitioning of manganese between water and soil can be fairly well predicted using thermodynamic equilibrium concepts, if soil-specific information is available (Baes and Sharp 1983; Rai et al. 1986). However, the kinetics of these reactions have not been studied in detail. Kinetic studies could help determine the residence time of manganese released into water or soil. Information on the conversion of manganese to different forms, especially the combustion products of MMT, in the air, water, and soil is also needed. The fate of manganese particles released into the air is determined by the particle size, and the direction and distance of particle transport at a site can be predicted from meteorological data and particle size data (EPA 1984a; Nriagu 1979). Transport of manganese in water is determined mainly by the solubility of the manganese compounds present, although suspended particles may also be transported in flowing waters (EPA 1984a; Schaanning et al. 1988).

The primary transformations which manganese undergoes in the environment are oxidation/reduction reactions (EPA 1984a; Rai et al. 1986). Reactions of manganese with airborne oxidants have not been studied. Information on the rate and extent of such reactions would be helpful in understanding the fate of atmospheric releases. The transformation of manganese in water or soil is dependent mainly on Eh, pH, and available counter ions (EPA 1984a). In some soils, manganese may also be oxidized by bacteria (Geering et al. 1969; Johnston and Kipphut 1988). More work is needed on the environmental factors, such as soil composition and pH, that may determine the form in which manganese will appear and thus impact manganese availability and absorption.

Modeling has also provided interesting insight into the contribution of the combustion of MMT to atmospheric manganese (Loranger et al. 1995b). According to the model estimations, the contribution of

direct emissions from motor vehicles to the atmospheric background manganese (as measured from sampling stations) would be about 50% at <25m and less than 8% at 250m. These results are confirmed with an *in situ* study using snow as the environmental indicator where the average deposition rates of manganese for the top and bottom layers ranged from 0.01 to 0.21 mg/m^2day (Loranger et al. 1996). The average concentrations of manganese decreased with distance from the road. However, it was impossible to distinguish between directly-emitted manganese from automobiles, manganese enriched road dust, and the naturally-occurring manganese in crustal materials. No study to date has provided the complete answer to this question and this constitutes one of the major remaining data needs regarding the environmental significance of manganese from MMT and the resulting potential for exposure.

Bioavailability from Environmental Media. Manganese is known to be absorbed following inhalation or oral exposure (Mena et al. 1969; Pollack et al. 1965), but dermal exposure is not considered to be significant. The uptake of manganese from air, food, milk, and water has been studied (Davidsson et al. 1988, 1989a). However, absorption from soil has not been investigated. In view of the potential for tight binding of manganese to some soil types, studies on this subject would be valuable in evaluating risk to humans, especially children who may ingest contaminated soils near hazardous waste sites. Additional information would also be valuable on the relative bioavailability of different manganese compounds across various environmental media.

Food Chain Bioaccumulation. It has been established that while lower organisms (e.g., plankton, aquatic plants, and some fish) can significantly bioconcentrate manganese, higher organisms (including humans) tend to maintain manganese homeostasis (EPA 1984a; Folsom et al. 1963; Thompson et al. 1972). This indicates that the potential for biomagnification of manganese from lower trophic levels to higher ones is low, and it does not appear that additional research in this area is essential at this time.

Exposure Levels in Environmental Media. Reliable monitoring data for the levels of manganese in contaminated media at hazardous waste sites are needed so that the information obtained on levels of manganese in the environment can be used in combination with the known body burden of manganese to assess the potential risk of adverse health effects in populations living in the vicinity of hazardous waste sites.

Manganese levels have been monitored in all environmental media, including air, water, soil, and food (EPA 1984a; NAS 1980a; Pennington et al. 1986). The EPA has estimated average human intake levels of manganese from water, air, and food (EPA 1984a). These estimates were based mainly on monitoring data from the 1960s and 1970s. More recent data on airborne MMT and manganese would be valuable in identifying any trends in environmental contamination and potential populations that may suffer higher manganese body burdens following increased use of MMT (Davis 1999; Weiss 1999).

More specific data on levels in the environment around those particular sites where manganese is believed to have been dumped would be helpful in determining the extent of exposure levels around such waste sites. In particular, data on the concentration of manganese in the air around hazardous waste sites would be valuable in assessing the potential significance of this exposure pathway.

Exposure Levels in Humans. This information is necessary for assessing the need to conduct health studies on these populations. Manganese is a normal component of human tissues and fluids (Sumino et al. 1975; Tipton and Cook 1963). Increased average levels of manganese have been detected in blood and urine of populations exposed to high concentrations of manganese in the workplace (Roels et al. 1987b), but no similar data are available for populations surrounding hazardous waste sites. Surveys of manganese levels in the blood or urine of populations living near waste sites could be useful in identifying groups with above-average levels of manganese exposure. However, because of the variability in these values, it is not likely that such data would be helpful in identifying individuals with above-average exposures. More information is also needed to determine whether iron-deficient populations have a higher manganese body burden. manganese and iron have many physico/chemical similarities and there is a possibility of competition between these elements. Increased manganese concentrations have been shown to inhibit the metabolic function of the iron-dependent enzyme, aconitase (Zheng et al. 1998). Iron deficiency is the single most prevalent nutritional deficiency in the world, and so the potential health risk associated with iron deficiencies exacerbating the brain manganese burden may represent a crucial issue of exposure and susceptibility, and has yet to be evaluated. Air concentrations in areas with high traffic density are sometimes higher than the guide level (Zayed et al. 1999), therefore, some individuals could be at risk. Research focusing on the environmental level of exposure of certain groups of the population, such as those living near a major highway, is needed.

Exposures of Children. Children are exposed daily to manganese. The compound is an essential trace element vital for the body to function properly. However, exposure and body burden studies are lacking for children, especially infants and toddlers. These data are needed because studies in infants indicate that absorption of manganese is high. Studies in animals indicate that neonates do not excrete manganese as readily as more mature animals. Two studies link ingestion exposure of excess manganese to neurological deficits (poorer performance in school and on neurobehavioral tests; He et al 1994; Zhang et al. 1995.). Intake values for the subjects in these studies were not reported. It would be useful to know if effects such as these are associated with accumulation of manganese in the brain. Although the primary pathway for exposure is the diet, studies involving exposures to airborne manganese (e.g., in dust that may be present at a nearby hazardous waste site or manganese-processing plant) would aid in understanding other pathways that may contribute significantly to children's total body burden of manganese. In addition, since most manganese is provided from plant foods, which are often limited in children's diets, dietary exposure/body burden studies would aid in understanding manganese requirements for maintaining good health.

Soil ingestion is likely the only unique exposure pathway for children. Additional studies concerning bioavailability of manganese from soil would provide important information concerning the proportion of the total daily manganese intake that could originate from ingested soils.

Although infants differ in their weight-adjusted intake of manganese, it is unknown whether older children differ in this parameter. Studies concerning this endpoint would be very valuable.

Studies involving inhalation or ingestion exposure to MMT in the young are very few (Komura and Sakamoto 1992b, 1994). Although these studies indicate that MMT had very little measurable effect on development, only one dose level was used. Although analytical data indicate that environmental MMT is unlikely to persist (Lynam et al. 1999), it is unknown what typical body burdens of manganese might be in children following long-term exposure to MMT combustion products. Additional studies measuring these endpoints in the young would be helpful.

Aside from limited developmental studies involving gestational exposure, there are no data on the effects of exposure of the young to maneb and mancozeb. Studies evaluating acute, intermediate, and chronic exposures to these pesticides via inhalation and oral exposures are needed.

Child health data needs relating to susceptibility are discussed in 2.12.2 Identification of Data Needs.

Exposures Registries. No exposure registries for inorganic or organic manganese were located. This substance is not currently one of the compounds for which a subregistry has been established in the National Exposure Registry. The substance will be considered in the future when chemical selection is made for the establishment of subregistries. The information that is amassed in the National Exposure Registry facilitates the epidemiological research needed to assess adverse health outcomes that may be related to exposure to this substance.

5.8.2 Ongoing Studies

On-going remedial investigations and feasibility studies at NPL sites contaminated with manganese will add to the available database on exposure levels in the environment and exposure levels in humans.

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring manganese, its metabolites, and other biomarkers of exposure and effect to inorganic and organic manganese compounds. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

Inorganic Manganese

Flame atomic absorption analysis is the most straightforward and widely used method for determining manganese (Tsalev 1983). In this method, a solution containing manganese is introduced into a flame, and the concentration of manganese is determined from the intensity of the color at 279.5 nm. Furnace atomic absorption analysis is often used for very low analyte levels (Baruthio et al. 1988), and inductively coupled plasma atomic emission analysis is frequently employed for multianalyte analyses that include manganese. Neutron activation analysis is also a very effective method for determining manganese concentrations in different samples (Kennedy et al. 1990; Rose et al. 1999). This technique uses no reagents and a minimum of sample handling; thus potential contamination with exogenous sources of manganese can be avoided. In addition, the technique has a low detection limit in biological tissues (4 ng/g) and high precision (Kennedy 1990). Further, the technique can be used for environmental samples as well as biological samples. Other methods for measuring manganese include spectrophotometry, mass spectrometry, neutron activation analysis, and X-ray fluorimetry.

It is important to note that none of these methods distinguish between different oxidation states of manganese or between different manganese compounds. Thus monitoring data on manganese is nearly always available only as total manganese present.

Organic Manganese

MMT. Levels of organometallic species in environmental and toxicological samples are typically in ppb concentrations, ng/mL in solution, or ng/g in solids (Walton et al. 1991). Therefore, methods of determination must be both selective and sensitive, achieved usually by coupling liquid or gas chromatography with detection via electrochemical, mass spectrometry, and atomic spectrometry detectors. A number of analytical methods for quantifying MMT in gasoline have been described including simple determination of total elemental manganese by atomic absorption and gas chromatography followed by flame-ionization detection. These methods usually measure MMT by detecting the metallic portion of the compound and reporting detection of MMT as manganese.

Maneb or mancozeb. The analysis of alkylenebis(dithiocarbamates) of some bivalent metal ions is hampered by their low solubility, low stability, and polymeric structure (Bardarov and Zaikov 1989). Furthermore, the methods developed for their determination have low selectivity. Indirect methods include spectrophotometric, gas chromatographic (GC), or thin-layer chromatographic (TLC) determinations of the reaction products, liberated after reduction (in an acidic medium) by carbon disulfide (Bardarov and Zaikov 1989). It is important to note that these methods are typically unable to distinguish among various dithiocarbamates since most can be degraded to CS_2. Other methods for determination of maneb or mancozeb, which are ethylenebisdithiocarbamates, or EBDCs, rely on the measurement of the metallic portion of the compounds, and therefore, many of these methods are similar to those for detection of inorganic manganese. Some newer methods are presented that have greater selectivity or use novel approaches different from carbon disulfide evolution.

6.1 BIOLOGICAL MATERIALS

Inorganic Manganese

Normally, determination of manganese in biological materials requires digestion of the organic matrix prior to analysis. For tissue samples or feces (detection limits ranging from 0.2 µg/g to <1 µg/g)), this is usually done by treatment with an oxidizing acid mixture such as 3:1:1 (v/v/v) nitric:perchloric:sulfuric acid mixture (Kneip and Crable 1988a). Fluid samples such as blood or urine may be digested in the same way (blood, detection limits = 1 µg/100 g, 10 µg/L), or manganese can be extracted by an ion exchange

resin (urine, detection limit = 0.5–2 µg/L) or by chelating agents such as cupferon in methylisobutylketone (urine, detection limit = <1 µg/L) . Recently, a method for directly measuring concentrations of trace elements in hair that does not require digestion prior to analysis has been developed (Stupar and Dolinsek 1996). While the authors used their technique to determine chromium, lead, and cadmium levels in hair, it is assumed that their slurry sampling or direct solid sampling technique might also work for manganese determination. Neutron activation analysis is also an effective analysis tool for measuring manganese in biological tissues (Rose et al. 1999, Vitarella et al. 2000). Table 6-1 summarizes some of the methods used for sample preparation and analysis of manganese in biological materials. It is important to note that special care is needed to avoid contamination of biological materials with exogenous manganese (Tsalev 1983; Versieck et al. 1988).

Organic Manganese

MMT. GC-FID may be used to determine levels of MMT in biological tissues and fluids with a detection limit of 1–2 ppm and percent recovery from 93.5 to 102.7% (Hanzlik et al. 1979).

More recently, Walton et al. (1991) have described high performance liquid chromatography coupled with laser-excited atomic fluorescence spectrometry (LEAFS) to detect various species of MMT. The detection limit for this GC-LEAFS method ranged from 8–20 pg of manganese for the various organomanganese species; the detection limit for determining manganese in MMT was 8 pg (0.4 ng/mL). This limit of detection was several orders of magnitude better than those for HPLC-UV or HPLC-AFC detection (Walton et al. 1991), but was worse than detection by GC-FID (DuPuis and Hill 1959). Walton et al. (1991) used their method to determine manganese species present in rat urine after rats had been administered MMT prepared in propylene glycol via subcutaneous injection.

Maneb or mancozeb. Headley (1996) used an inductively coupled plasma-atomic emission spectrometry (ICP-AES) method for occupational exposure estimations that measures mancozeb by determining the elemental manganese portion of the pesticide in a sample. The method was successful in analyzing urine (0.02 mg/L), wash water (0.02 mg/L), tank mixes (0.02 mg/L), cellulose acetate filters (0.5 µg), and fabric from patches and clothing (0.5 µg), with detection limits in parentheses. However, ICP-AES methods cannot differentiate among various forms of manganese, so it is important that background

Table 6-1. Analytical Methods for Determining Manganese in Biological Materials[a]

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Extraction into methylisobutyl-ketone as the cupferon chelate	AAS (furnace technique)	<1 µg/L[b]	No data	Baselt 1988
Urine	Extract with resin, ash resin	ICP/AES	<1 µg/L[b]	100±10%	NIOSH 1984d
Blood	Acid digestion	ICP/AES	1 µg/dL	98±2.1%	Kneip and Crable 1988a
Blood	Digestion in oxidizing acid	ICP/AES	1 µg/100g	98±2.1%	NIOSH 1984c
Tissue	Digestion in oxidizing acid	ICP/AES	0.2 µg/g	98±2.1%	NIOSH 1984c
Tissue	Acid digestion	ICP/AES	0.2 µg/g	104±5.6%	Kneip and Crable 1988a
Feces	Dry at 110°, ash at 550°, dissolve in nitric acid	AAS (flame technique)	<1 µg/g	102±7%	Friedman et al. 1987
Hair	Digestion in concentrated nitric:perchloric acid (3:1) mixture	Flameless AAS	<0.2 µg/g	No data	Collipp et al. 1983
Hair	(a) slurry sample introduction technique (hair powder added to twice distilled water to measure bulk hair trace elements, or (b) direct introduction of hair segments to measure lontigudinal gradients	ETAAS (furnace technique)	No data	No data	Stupar and Dolinsek 1996[d]

Table 6-1. Analytical Methods for Determining Manganese in Biological Materials[a] *(continued)*

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Methods for determination of Maneb or Mancozeb					
Urine	Acid digestion in nitric acid or nitric acid-H_2O_2 mixture	Acid digestion in nitric acid or nitric acid-H_2O_2 mixture	0.020mg/L (based on 0.004 mg Mn/L) in urine	No data	Headley et al. 1996
Biological fluids and tissues	Extract into hexane containing biphenyl solutions containing 5-250 ppm MMT in 50 ppm biphenyl	Extract into hexane containing biphenyl solutions containing 5-250 ppm MMT in 50 ppm biphenyl	1-2 ppm	93.5-102.7%	Hanzlik et al. 1979
Tissues	Macerate autopsy tissues into fine slurry; Add anhydrous sodium sulphate, acetonitrile extraction with chloroform; Solution concentrated and residue dissolved in acetone	Macerate autopsy tissues into fine slurry; Add anhydrous sodium sulphate, acetonitrile extraction with chloroform; Solution concentrated and residue dissolved in acetone	approx. 0.5μg	90-95%	Tewari and Singh 1979

Table 6-1. Analytical Methods for Determining Manganese in Biological Materials[a] *(continued)*

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Methods for determination of MnDPDP					
Human plasma	Mix heparinized blood samples of patients receiving MnDPDP via injection with solid trisodium phosphate dodecahydrate pH 10.0±0.2; ultrafiltrate	Mixed-bed resin HPLC-Anion exchange and reverse-phase	0.8-2.3 µM (Mn cmpds) 0.1-0.8 µM (Zn cmpds) of 50 µL injection volume	85-115%	Toft et al. 1997a

[a]Magnetic resonance imaging (MRI) has been useful in determining brain accumulation of manganese but is not a quantitative method; therefore, it is not listed as an entry in this table.
[b]Estimated from sensitivity and linearity data
[c]Per sample size of 50 to 200 mL
[d]Methods were used to determine levels of chromium, lead, and cadmium in hair. Manganese concentrations in hair were evaluated for some, but not all, of the samples and tested one, but not both, new methods. However, it is assumed that both techniques will work for the trace element manganese.

AAS = atomic absorption spectroscopy; ICP/AES = inductively coupled plasma atomic emission spectroscopy; NIOSH = National Institute for Occupational Safety and Health; TLC = thin-layer chromatography

levels of manganese are known and low enough to not influence the levels of manganese attributable to mancozeb (Headley 1996).

Table 6-1 summarizes some common methods for the determination of manganese in various types of biological materials.

6.2 ENVIRONMENTAL SAMPLES

Inorganic Manganese

Manganese in air exists as particulate matter, so sampling is done by drawing air through a filter in order to collect the suspended particles. A variety of filter types (e.g., glass fibers and cellulose acetate) and sampling devices (e.g., low volume, high volume, and dichotomous) are available, depending on the particle sizes of concern and the concentration range of interest. In some cases, material on the filter may be analyzed directly (e.g., by X-ray fluorescence), or the filter may be digested by ashing in acid prior to analysis. In general, sensitivity is dependent on the volume of air drawn through the filter prior to analysis, and typically, detection limits are 1–2 µg/sample.

Water may either be analyzed directly, or, if the concentration of manganese is low, a concentration step (e.g., evaporation, extraction, and binding to a resin) may be employed (detection limits ranging from 0.005 µg/L–50 µg/L) . In all cases, acid is added to the sample to prevent precipitation of manganese. Using a new method, the catalytic kinetic method of analysis, Beklemishev et al. (1997) measured the concentrations of manganese in tap and river water. Their analytical method relies on an indicator reaction that is catalyzed by Mn(II) (the oxidation of 3,3',5,5'-tetramethylbenzidine [TMB] by potassium periodate [KIO_4]) and is carried out on the surface of a paper-based sorbent. The advantages of this new technique are that it has a much lower detection limit (0.005 µg/L) than do established methods and is transportable, allowing it to be used for rapid tests in the field (i.e., spot tests and similar procedures).

Determination of manganese levels in soils, sludges, or other solid wastes requires an acid extraction/digestion step prior to analysis. The details vary with the specific characteristics of the sample, but usually treatment will involve heating in nitric acid, oxidation with hydrogen peroxide, and filtration and/or centrifugation to remove insoluble matter.

Recently, manganese levels in foods have been determined in order to define more clearly human dietary requirements or levels of absorption of manganese from the diet (Tinggi et al. 1997). Atomic absorption spectrometry has been the most widely used analytical technique to determine manganese levels in a broad range of foods, as well as other environmental and biological samples (Tinggi et al. 1997). Tinggi et al. (1997) contributed a wet digestion technique using a 12:2 (v/v) nitric:sulfuric acid mixture for their determination, and, for food samples with low levels of manganese, they found that the more sensitive graphite furnace atomic absorption analysis was required. Because manganese is often found at very low levels in many foods, its measurement requires methods with similarly low detection limits; these researchers identified detection limits of 0.15 mg/kg (ppm) and 1.10 µg/kg (ppb) for flame and graphite furnace atomic absorption spectrometry, respectively (Tinggi et al. 1997). Neutron activation analysis is an effective technique for measuring manganese in environmental samples; it provides a low detection limit and high precision (Kennedy 1990).

Organic Manganese

MMT. A number of analytical methods for quantifying MMT in gasoline have been described including simple determination of total elemental manganese by atomic absorption (Smith and Palmby 1959) and gas chromatography followed by flame-ionization detection (FID) (DuPuis and Hill 1979). The former has measured manganese concentrations from 0.1 to 4 grams per gallon of gasoline after dilution of the sample with isooctane to minimize the effects of differences in base stock composition and is accurate to about 3% of the amount of manganese present. The latter has a detection limit of 1.7×10^{-14} g/s (0.017 pg/s) and could easily measure 6 mg/gallon of manganese in a gasoline sample; it is one of the most sensitive approaches. Aue et al. (1990) described a method in which MMT is detected in gasolines by gas chromatography coupled with flame photometric detection (FPD); the chemiluminescence of manganese is measured to determine MMT levels in a method that uses simple, inexpensive, and commercially available instrumentation. MMT levels can be determined down to 0.6 ppm (w/w) in gasoline (Aue et al. 1990). In another method showing excellent performance, Quimby et al. (1978) used gas chromatography followed by atmospheric pressure helium microwave detection system (or, microwave emission detector, MED); this method has a high degree of selectivity (1.9×10^{6}) and a detection limit of 0.25 pg/s at a wavelength of 257.6 nm.

Gas chromatography followed by electron-capture detection (ECD) (Gaind et al. 1992) or alternating current plasma (ACP) emission detection (Ombaba and Barry 1994) (detection limit: 62 pg as manganese) has also been described for determination of MMT in gasoline.

Gas chromatography followed by alternating current plasma (ACP) emission detection has been described for detecting MMT in air samples; airborne MMT concentrations as low as 0.001 mg/m^3 can be measured (Ombaba and Barry 1994).

Maneb or mancozeb. Like with many other dithiocarbamate pesticides, the most commonly used methods of detecting maneb or mancozeb involve degrading the active ingredient in the pesticide to carbon disulfide, CS_2. The CS_2 is then detected by spectrophotometry of a colored complex (Keppel et al. 1971) or by gas chromatography of the gas either in the headspace (McCleod and McCully 1969) or absorbed in a solvent layer (Headley 1996). The CS_2 evolution method is the AOAC method used widely to identify amounts of dithiocarbamates in pesticide formulations (HSDB 1999).

Examples of application of the carbon disulfide method include the determination of residues in or on food. McLeod and McCully (1969) developed a head space gas procedure for screening food samples (plants) for pesticide residues; while the method can quickly determine the presence of ferbam, maneb, nabam, thiram, zineb, and ziram in samples, it cannot differentiate among the dithiocarbamates or quantitate amounts of maneb. The CS_2 produced upon hydrolysis with hydrochloric acid-stannous chloride reagent is determined by gas chromatography; maneb recoveries ranged from 75–95% in samples of lettuce, cucumber, carrot, apple, cabbage, and strawberries. Alternatively, residues may be determined after reaction with acid to form carbon disulfide by measurement with gas liquid chromatography (Zielinski and Fishbein 1966) or standard colorimetric methods (HSDB 1999). Ahmad et al. (1996) describe an improved headspace gas-liquid chromatography (GLC) procedure used to measure dithiocarbamate residues in fruits and vegetables by detection of CS_2, followed by verification of EBDCs by conversion to ETU (ethylenethiourea), a degradation product. Although this method reduces false-positive results, it cannot differentiate among various dithiocarbamate pesticides and also may overestimate the apparent CS_2 content (Ahmad et al. 1996).

Rao et al. (1993) present a modification of the usual CS_2 evolution method (which measures CS_2 by spectrophotometry) with a method that converts maneb to a manganese-PAN complex that is extracted in

isobutyl methyl ketone (MIBK); the complex then absorbs at 550 nm and can be measured from 0.37 to 3.75 micrograms/mL. Their method determines micro-quantities of maneb in commercial formulations, synthetic mixtures, grain, and in the presence of various other dithiocarbamates. The authors note that this method is particularly selective since other pesticides like ziram, zineb, and ferbam which usually interfere in other methods did not interfere under their experimental conditions.

Hylin et al. (1978) present an ultraviolet absorption method for analysis of maneb formulations. The maneb is converted to nabam, a water soluble ethylenebisdithiocarbamate, by treatment with Na_4EDTA. The converted maneb is measured at 284 nm, and a conversion formula is given to calculate the % maneb.

Newsome (1974) presents a method in which, following hydrolysis with hydrochloric acid containing stannous chloride, maneb is converted to an ethylenediamine that is recovered on an ion-exchange column and determined by gas chromatography as the bis-trifluoroacetate (IARC 1976). The limit of detection was 0.1 mg/kg.

Walash et al. (1993) developed a spectrophotometric method for determination of maneb and its decomposition product, ETU, in some vegetables by using EDTA as a solvent which causes release of the EBDC moiety from maneb followed by reaction of EBDC with either 2,6-dibromoquinone chlorimide (DBQ) or 2,6-dichloroquinone chlorimide (DCQ). The result is a red solution that absorbs at 495 nm. Amounts as low as 2 ppm were detected in cucumber and tomato fruits.

Noguer and Marty (1997) propose a new high sensitivity biosensor method that allows the detection of dithiocarbamate fungicides at concentrations less than 10 ppb. By using the fact that these compounds strongly inhibit yeast aldehyde dehydrogenase (AIDH), they are trying to develop an amperometric bienzymic sensor for dithiocarbamate detection based on AIDH inhibition that determines the concentration of dithiocarbamates by measuring changes in current; while the method's operational stability is still in need of improvement, the method was able to measure 1.48 ppb maneb.

Rangaswamy and Vijayashankar (1975) describe a method that relies upon the periodate oxidation of the manganese in mancozeb to permanganic acid in the presence of phosphoric acid; this method can determine the active ingredient in formulations and can identify residues.

Afsar et al. (1987) have developed a method to differentiate mancozeb from a mixture of maneb and zinc salts or from a mixture of maneb and zineb. Compounds are distinguished on the basis of color differences after treatment of the saturated solutions of fungicides in *n*-propanol-acetone mixture first with dithizone and then with monosodium dihydrogen phosphate. Stevenson (1972) presented a similar earlier method that distinguished maneb, zineb, mancozeb, and selected fungicidal mixtures by successive application of acid dithizone, sodium hydroxide, and acid dithizone to the spot.

Spot tests for the in-field detection of mancozeb in water use copper (II) chloride-acetic acid as a coloring reagent. A cupric salt is formed which is soluble in chloroform extractant to produce a red-brown solution; this method has a detection limit of 15 µg (Rathore et al. 1996).

Because maneb and mancozeb have vapor pressures of virtually zero, they may be present in air only as dust (Maini and Boni 1986). Workroom air sampling has been performed by collection of maneb or mancozeb on filters followed by hydrochloric acid hydrolysis, with stannous chloride reduction, and analysis of the resulting carbon disulfide using gas chromatography (Maini and Boni 1986; Woodrow et al. 1995).

Table 6-2 summarizes some common methods for the determination of manganese in various types of environmental media.

6.3 ADEQUACY OF THE DATABASE

Section 104(I)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of manganese is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of manganese.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on filter, direct analysis	XRF	2 µg/sample	No data	NIOSH 1984a
Air	Collection on filter, acid digestion	ICP/AES	1 µg/sample (5 µg/m^3)	84-93%	NIOSH 1984b
Water	Acidify with nitric acid	AAS (furnace technique)	0.2 µg/L	No data	EPA 1983b
Water	Acidify with nitric acid	AAS (flame) AAS (furnace) ICP/AES	2 µg/L 0.01 µg/L 1 µg/L	No data No data No data	Taylor 1982
Water	Acidify with nitric acid	AAS (direct aspiration)	10 µg/L	100±6%	APHA 1985a
Water	Adjust pH to 2-4, extract with APDC into MIBK	AAS (direct aspiration)	<10 µg/L	No data	APHA 1985b
Water	Acidify with nitric acid	AAS (furnace technique)	0.2 µg/L	No data	APHA 1985c
Water	Acidify with nitric acid	ICP/AES	2µg/L	No data	APHA 1985d
Water	Acidify with nitric acid	AAS (direct aspiration)	10 µg/L	100±2%[a]	EPA 1983a
Water	Acidify, oxidize	Colormetric	50 µg/L	100±26%	APHA 1985e
Water	Preconcentrate manganese-containing solution and 3,3',5,5'-tetramethylbenzidine (TMB) onto filter paper; add oxidant KIO_4 to catalyze oxidation; measure absorbance	Catalytic kinetic method of analysis	0.005 µg/L	No data	Beklemishev et al. 1997

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water and wastes	Acid digestion	ICP/AES	2 µg/L	100±6%	EPA 1982
Water and wastes	Acid digestion	AAS	10 µg/L	100±2%	EPA 1986c
Water and wastes	Acid digestion	ICP/AES	2 µg/L	93±6%	EPA 1986b
Sediments, sludges, soils	Acid digestion, oxidation, filtration/centrifugation	AAS, ICP/AES	Variable, depending on matrix	93±6%	EPA 1986a, 1986b
Foods	Digest wet or dry foods with HNO_3–H_2SO_4 mixture (12:2 mL)	AAS [flame(F) or graphite furnace(GF)]	F-AAS: 0.15 mg/kg GF-AAS: 1.10 μg/kg	No data	Tinggi et al. 1997
Methods for MMT Determination					
Air	Draw known volume of air through XAD-2 sampling tubes for 10-60 minutes	GC-ECD	0.001 mg/m^3 (in 10 L sample); 0.02 ng from a 2.0 µL injection of a 0.01 µg/mL MMT solution	No data	Gaind et al. 1992

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Gasoline	Dilute gasoline in acetone (1:10)	Capillary GC-ACP detector	62 pg/s	No data	Ombaba and Barry 1994
Gasoline	Dilute with hexane (1:99); direct injection	GC-ECD	No data	No data	Gaind et al. 1992
Gasoline	Inject sample	GC-MED	0.25 pg/s	No data	Quimby et al. 1978
Gasoline	Inject sample	GC-FPD	0.6 ppm	No data	Aue et al. 1990
Methods for Determination of Maneb or Mancozeb					
Air	Trap mancozeb on glass fiber filters at 14-16 L/min; hydrochloric acid hydrolysis with stannous chloride reduction	Sulfur-mode flame photometric gas chromatography	0.5 μg/filter = 23 ng/m^3	No data	Woodrow et al. 1995
Water	Heat water sample in presence of tin(II) chloride and 2,2,4-trimethylpentane (isooctane); CS_2 dissolves in isooctane Note: result corresponds to total of dithiocarbamates that undergo this reaction	GC-FPD	0.84 μg/L as maneb	No data	Barceló 1993

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Solution prepared in NaOH, copper (II) chloride-acetic acid used as coloring agent	Visual spot test	15 μg (may be lowered by preconcentration via column chromatographyo r liquid-liquid extraction	No data	Rathore et al. 1996
Maneb formulations	Treat with Na_4EDTA to convert maneb to nabam, a water soluble ethylenebisdithiocarbamate	Ultraviolet absorption: Measure converted maneb at 284 nm	No data	No data	Hylin et al. 1978
Commercial formulations, Grain, Dithiocarbamate mixture	Combine maneb with PAN at pH 9.2; Extract Mn-PAN complex in isobutyl methyl ketone (MIBK)	Spectrophotometry	0.37 µg/mL-3.75 µg/mL	97.5-98.5% (grain); 99.0-100.6% (synthetic mixtures)	Rao et al. 1993
Maneb	Hydrolysis with hydrochloric acid containing stannous chloride	Ion-exchange column, GC	0.1 mg/kg	No data	Newsome 1974 in IARC 1996

Table 6-2. Analytical Methods for Determining Manganese in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Wash water, tank mixes, cellulose acetate filters, gauze patches, clothing	Acid digestion in nitric acid or nitric acid-H_2O_2 mixture	ICP-AES (measured as Mn)	0.020mg/L (based on 0.004 mg Mn/L) in wash water and tank mixes; 0.5 µg (based on 0.1 µg Mn) in cellulose acetate filters, gauze patches, clothing	No data	Headley et al. 1996
Cucumber, tomato	Crush cucumber or tomato; extract by sonication with EDTA and methanol; 0.2% solution 2,6-dibromoquinone 4-chlorimide (DBQ) or 2,6-dichloroquinone 4-chlorimide (DCQ) added	Spectrophotometry	≤2ppm	Percent extraction: 95.5% (cucumber), 89.2% (tomato)	Walash et al. 1993
Formulations; Grains: sorghum, paddy, and wheat;	For formulations, dissolve Dithane M-45 in HNO_3; dilute with 3N HNO_3; add potassium periodate and phosphoric acid	AAS	0.6 µg to 6 µg Dithane M-45 (at 20g sample level);	95-100% (formulations)93-99% (paddy samples)	Rangaswamy and Vijayashankar 1975

[a]Percent recovery at manganese concentration greater than 80 µg/L; at lower concentrations (10–20 µg/L) percent recoveries were greater than 120%.

AAS = atomic absorption spectrometry; APDC = ammonium pyrrolidine dithiocarbamate; APHA = American Public Health Association; EPA = Environmental Protection Agency; FPD = flame photometric detection; ICP/AES = inductivity coupled plasma atomic emission spectroscopy; MED = microwave emission detector; MIBK = methyl isobutyl ketone; NIOSH = National Institute for Occupational Safety and Health; XRF = x-ray fluorescence

that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect

Exposure. Sensitive and selective methods are available for the detection and quantitative measurement of manganese in blood, urine, hair, feces, and tissues (Baselt 1988; Collipp et al. 1983; Friedman et al. 1987; Kneip and Crable 1988a; NIOSH 1984c, 1984d). Since levels in biological samples are generally rather low, sample contamination with exogenous manganese can sometimes occur (Tsalev 1983; Versieck et al. 1988). Development of standard methods for limiting this problem would be useful. As discussed in Section 2.5.1, measurement of average manganese concentrations in these materials has proved useful in comparing groups of occupationally exposed people to nonexposed people (Roels et al. 1987b) but has not been especially valuable in evaluating human exposure in individuals (Rehnberg et al. 1982). This is due to the inherent variability in intake levels and toxicokinetics of manganese in humans, rather than a limitation in the analytical methods for manganese. Development of noninvasive methods for measuring whole-body or tissue-specific manganese burdens would be valuable in estimating human exposure levels but would be limited by the same considerations of individual variability that limit existing methods.

Effect. No reliable biomarkers of manganese effect are known. Biochemical changes such as altered blood or urinary levels of steroids, neurotransmitters, or their metabolites are plausible biomarkers of exposure, but this possibility has not been thoroughly investigated. Although methods exist for the analysis of these biochemicals, further work to improve the analyses does not seem warranted unless the utility of this approach is established.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. All humans are exposed to manganese, primarily through food (EPA 1984a). Near a hazardous waste site that contains manganese or a factory that uses manganese, humans could receive above-average exposure by inhalation of air or ingestion of water, soil, or food. Methods exist for the analysis of manganese in all of these media, and the sensitivity of these methods is sufficient to detect levels of potential human health concern (APHA 1985a, 1985b, 1985c, 1985d, 1985e; EPA 1982, 1986b, 1986c; NIOSH 1984a, 1984b). However, there is a data need for analytical methods that can differentiate

between the differing manganese species in various environmental media. This information will add to our knowledge of whether a specific manganese species present at a waste site is cause for concern.

6.3.2 Ongoing Studies

No information was located regarding ongoing research on methods for analysis of manganese in biological materials or environmental samples.

7. REGULATIONS AND ADVISORIES

Because of its potential to cause adverse health effects in exposed people, a number of regulations and guidelines have been established for manganese. These are summarized in Table 7-1.

An MRL of 0.00004 mg manganese/m^3 (0.04 µg manganese/m^3) in respirable dust has been derived for chronic inhalation exposure to manganese. The MRL is based on a surrogate NOAEL value of 0.074 mg manganese/m^3 (74 µg manganese/m^3) that was determined using benchmark dose analysis (BMD) of the raw data from the study by Roels et al. (1992) which reported neurological effects among battery factory workers exposed to manganese. A NOAEL of 0.071 mg manganese/m^3 (71 µg manganese/m^3) derived from BMD analysis of the individual exposure and response data in foundry workers from the study by Iregren (1990) is comparable. These two $BMDL_{10}$ NOAEL estimates are consistent with a NOAEL of 0.051 mg/m^3, median respirable dust, for exposures in a metal producing plant reported by Gibbs et al. (1999). The sensitive endpoint used as the basis for this MRL, neurological effects among manganese exposed workers, was also reported in studies by Roels et al. (1987a), Mergler et al. (1994), and Lucchini et al. (1995, 1999).

Based on the $BMDL_{10}$ value of 0.074 mg manganese/m^3 , a chronic inhalation MRL of 0.00004 mg manganese/m^3 (0.04 µg manganese/m^3) was derived using (1) an uncertainty factor of 10 for human variability, (2) factors of 5/7 and 8/24 to account for intermittent exposure (5 days/week, 8 hours/day), (3) an uncertainty factor of 10 to account for limitations in the inhalation database, including the lack of data on developmental effects and data on the potential for reproductive effects in women, and the potential for differences in toxicity from different forms of manganese and, (4) a modifying factor of 5 for the potential for increased susceptibility in children based upon differences in the pharmacokinetic handling of manganese in the young.

The upper range of the estimated safe and adequate daily dietary intake of 5 mg/day (NRC 1989) has been adopted as a provisional guidance value (0.07 mg/kg/day) for oral exposure to manganese. This guidance is necessary because, although manganese is an essential nutrient, its prevalence at hazardous waste sites puts some individuals at risk for exposure to toxic levels.

The EPA has derived a chronic inhalation RfC of 5×10^{-5} mg/m^3 for respirable manganese (IRIS 1998). This value is based on the LOAEL of 0.15 mg/m^3 from a study of people exposed to manganese dioxide (Roels et al. 1992). The LOAEL was calculated by dividing the geometric mean concentration of the lifetime-

integrated respirable dust concentration by the average duration of employment in the facility. EPA calculated the RfC by adjusting for continuous exposure and dividing by an uncertainty factor of 1,000 (a factor of 10 for use of a LOAEL, a factor of 10 to protect sensitive individuals, and a factor or 10 for database limitations reflecting both the less-than-chronic periods of exposure and the lack of developmental data, as well as potential but unquantified differences in the toxicity of different forms of manganese). The estimated breathing rate in the exposed workers was assumed to be 10 m^3/workday.

The EPA has derived a chronic oral RfD of 0.14 mg/kg/day for manganese (IRIS 1998). This value is equal to the average daily intake of manganese in the diet (10 mg/day) that is considered adequate and safe. The RfD was derived assuming an average body weight of 70 kg, and was based on a composite of data including chronic humans' NOAELs (WHO 1973), "safe and adequate levels" from the National Academy of Sciences (NRC 1989), and research by Schroeder et al. (1966). An uncertainty factor was not employed because (a) the information used to determine the RfD for manganese was taken from many large populations, (b) humans exert an efficient homeostatic control over manganese such that body burdens are kept constant with variations in diet, (c) there are no subpopulations that are believed to be more sensitive to manganese at this level, and (d) manganese is an essential element that is required for normal human growth and maintenance of health. However, the EPA has recommended that a "modifying factor of 3 be applied when assessing risk from manganese in drinking water or soil because the study by Kondakis et al. (1989) raises significant concerns about possible adverse neurological effects at doses not far from the range of essentiality.

Table 7-1. Regulations and Guidelines Applicable to Manganese

Agency	Description	Information	References
INTERNATIONAL			
WHO	Guideline value in drinking water for aesthetic quality	0.1 mg/L	WHO 1984a
	Recommended exposure limit in workplace air - respirable manganese particles	0.3 mg/m^3	WHO 1986
	Recommended air quality guideline for Europe (annual average)	0.15 µg/m^3	WHO 1997
NATIONAL			
Regulations:			
a. Air:			
EPA OAQPS	Ban on use of methylcyclopentadienyl manganese tricarbonyl (MMT) as a fuel additive in unleaded gasoline	Yes	EPA 1978, 1979, 1981
	Ban on use of MMT as fuel additive in unleaded gasoline overturned	Yes	EPA 1995
OSHA	PEL TWA		OSHA 1998 (29 CFR 1910.1000) (Table Z-1)
	Manganese fume, as manganese	5 mg/m^3(C)*	
	Manganese cyclopentadienyl tricarbonyl, as manganese (skin)	0.1 mg/m^3	
	Manganese tetroxide	1 mg/m^3	
	Manganese, elemental and inorganic compounds (proposed)	0.2 mg/m^3	
	STEL		
	Manganese fume	3 mg/m^3	
	Ceiling		
	Manganese compounds, as manganese	5 mg/m^3	
b. Water:			
EPA OWRS	General permits under NPDES for total manganese	Yes	40 CFR 122.21, April 2, 1992, Appendix D, Table IV
c. Food:			
FDA	Concentration in bottled water	0.05 mg/L	FDA 1993 (21 CFR 103.35)
d. Other:			
EPA OERR	Reportable quantity		EPA 1998a (40 CFR 302.4)
	Manganese, tricarbonyl methyl-cyclopentadienyl	1 lb	
	Potassium permanganate	100 lbs	EPA 1998a (40 CFR 302.4)
	Reportable quantity		EPA 1998b
	Manganese, tricarbonyl methyl-cyclopentadienyl	100 lbs	(40 CFR 355)
	Extremely Hazardous Substance TPQ		EPA 1998b
	Manganese, tricarbonyl methyl-cyclopentadienyl	100 lbs	(40 CFR 355)
EPA OSW	Monitor at hazardous waste facilities to establish groundwater quality	Yes	40 CFR 265.92 January 31, 1985

Table 7-1. Regulations and Guidelines Applicable to Manganese (continued)

Agency	Description	Information	References
NATIONAL (cont.)			
EPA OTS	Toxic chemical release reporting manganese; manganese compounds	Yes	EPA 1998c (40 CFR 372.65)
DOJ DEA	Potassium permangate ranked as essential chemical in illegal drug production. Records of sales and uses required for amounts over 500 kg.	Yes	DOJ 1990
Guidelines:			
a. Air:			
ACGIH	TLV TWA		ACGIH 1998
	Manganese dust and compounds, as manganese	5 mg/m^3	
	Manganese tetroxide, compound and manganese fume, as manganese	1 mg/m^3	
	Manganese cyclopentadienyl tricarbonyl, as manganese (skin)	0.1 mg/m^3	
	2-Methylcyclopentadienyl manganese tricarbonyl, as manganese	0.2 mg/m^3	
	STEL		
	Manganese fume	3 mg/m^3	
b. Water:			
EPA ODW	Secondary MCL for aesthetic quality	0.05 mg/L	40 CFR 143.3 January 30, 1991 (EPA 1998d)
c. Other:			
EPA	Carcinogenic Classification	Group D[e]	IRIS 1998
	RfD (oral)	0.14 mg/kg/day[f]	
	RfC (inhalation)	5x10^{-5} mg/m^3	
STATE			
Regulations and Guidelines:[b]			
a. Air:	Acceptable ambient air concentrations (unless otherwise specified)		NATICH 1992 (unless otherwise specified)
Manganese			
Arizona		8.00 µg/m^3 (24 hr)	
Connecticut		20 µg/m^3 (8 hr) 100 µg/m^3 (30 min)	
Florida-Pinella		12.0 µg/m^3 (24 hr)	
Louisiana		27.6 µg/m^3 (8 hr)	
Nevada		1.19E-1 mg/m^3 (8 hr)	
North Carolina		3.10E-2 mg/m^3 (24 hr)	
North Carolina-Forco		.031 mg/m^3 (24 hr)	
North Dakota		3.0E-2 mg/m^3 (1 hr)	
Oklahoma		100.0 µ/m^3 (24 hr)	
Oregon	de minimus Emission rates	0.8 tons/yr	OR DEQ 1999
Pennsylvania		2.4E-01 µg/m^3 (annual)	
Rhode Island		2.0 µg/m^3 (1 hr)	
South Carolina	Maximum allowable concentration	25 µg/m^3	SC DHEC 1999
South Dakota		20 µg/m^3 (8 hr)	
Texas		30.0 µ/m^3 (annual)	
Vermont		119 µg/m^3 (annual)	VT DEC 1999a
Virginia		17.0 µg/m^3 (24 hr)	
Washington	Manganese dust and compounds, threshold level	0.5 tons/yr	WA DE 1999a
Washington-Southwest		16.7 µ/m^3 (24 hr)	
Manganese cyclopentadienyl tricarbonyl (MMT)			
California	Ban on MMT in unleaded gasoline	Yes	CA ARB 1999

Table 7-1. Regulations and Guidelines Applicable to Manganese (continued)

Agency	Description	Information	References
Connecticut		2.0 µg/m³ (8 hr) 10 µg/m³ (30 min)	CT DEP 1999
Florida-Pinella		.24 µg/m³ (24 hr)	
Nevada	Ban on MMT in unleaded gasoline	Yes	NV DCNR 1999a
Nevada		2.00 µg/m³ (8 hr)	
New Hampshire	Ambient air limit	0.357 µg/m³ (24 hr)	NH DES 1999
North Carolina-Forco		.6 µg/m³ (24 hr)	
North Dakota		1.0 µg/m³ (8 hr)	
Texas		.1 µg/m³ (annual)	
Virginia		1.70 µg/m³ (24 hr)	
Washington	Threshold level	0.1 tons/yr	WA DE 1999a
Washington-Southwest		.3 µg/m³ (24 hr)	
Manganese fume, as manganese			
Connecticut	Hazardous limiting values for hazardous air pollutants	20 µg/m³ (8 hr) 100 µg/m³ (30 min)	CT DEP 1999
Idaho		0.05 mg/m³ (24 hr)	ID DHW 1999a
Manganese tetroxide			
Connecticut	Hazardous limiting values for hazardous air pollutants	20 µg/m³ (8 hr) 100 µg/m³ (30 min)	CT DEP 1999
Nevada		0.24 mg/m³ (8 hr)	
New Hampshire	Ambient air limit	1.006 µg/m³ (24 hr)	NH DES 1999
North Carolina		6.20 µg/m³ (24 hr)	
North Carolina-Forco	6.2 µg/m³ (24 hr)		
North Dakota		10 µg/m³ (8 hr)	
Texas		1.0 µg/m³ (annual)	
Virginia		16 µg/m³ (24 hr)	
Potassium permanganate, as manganese			
New Hampshire	Ambient air limit	1.006 µg/m³ (24 hr)	NH DES 1999
b. Water:			
Drinking water	Drinking water quality standards		FSTRAC 1990 (unless otherwise specified)
Illinois		150 µg/L[e]	
Kansas		50 µg/L	
Nevada	Secondary standard (Standard above which must notify public)	0.1 mg/L (100 µg/L) 0.05 mg/L (50 µg/L)	NV DCNR 1996b
New Hampshire	Secondary MCL	0.05 mg/L (50 µg/L)	NH DES 1999
New Mexico		200 µg/L	NMHED 1990
New York	MCL If both Mn and Fe present	300 µg/L [total] ≤ 0.5 mg/L (500 µg/L)	NY DEC 1999
Wisconsin		50 µg/L[4]	WDHSS 1990
Groundwater			
Idaho	Secondary standard	0.05 mg/L (50 µg/L)	ID DHW 1999b
New Hampshire	For potable drinking supply	50 µg/L	NH DES 1999
New Jersey	Ground water quality criteria for potable drinking water supply	50 µg/L	NJ DEP 1999
Vermont	Enforcement standard Preventative action limit	840 µg/L 420 µg/L	VT DEC 1999
Washington		0.05 mg/L (50 µg/L)	WA DE 1999b

Table 7-1. Regulations and Guidelines Applicable to Manganese (continued)

Agency	Description	Information	References
Water for irrigation Nevada		200 µg/L	NV DCNR 1999c

[a]Group D = not classifiable as to human carcinogenicity
[b]All data on state regulations and guidelines from NATICH 1992 unless noted otherwise.
[c]Only for communities serving less than or 1,000 persons or less than or 300 service connections
[d]Groundwater standard
[e]C = ceiling limit
[f]This reference dose is for the total oral intake of manganese. When assessing exposure to manganese from food, the modifying factor is 1; however, when assessing exposure to manganese from drinking water or soil, a modifying factor of 3 is recommended.

ACGIH = American Conference of Governmental Industrial Hygienists; DEA = Drug Enforcement Agency; DOJ = Department of Justice; EPA = Environmental Protection Agency; FDA = Food and Drug Administration; MCL = Maximum Contaminant Level; NIOSH = National Institute for Occupational Safety and Health; NPDES = National Pollutant Discharge Elimination System; OAQPS = Office of Air Quality Planning and Standards; ODW = Office of Drinking Water; OERR = Office of Emergency and Remedial Response; OSHA = Occupational Safety and Health Administration; OSW = Office of Solid Wastes; OTS = Office of Toxic Substances; OWRS = Office of Water Regulations and Standards; PEL = Permissible Exposure Limit; RfC = reference concentration; RfD = reference dose; STEL = Short Term Exposure Limit; TLV = Threshold Limit Value; TPQ = Threshold Planning Quantity; TWA = Time-Weighted Average; WHO = World Health Organization

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9. GLOSSARY

Acute Exposure—Exposure to a chemical for a duration of 14 days or less, as specified in the Toxicological Profiles.

Adsorption Coefficient (K_{oc})—The ratio of the amount of a chemical adsorbed per unit weight of organic carbon in the soil or sediment to the concentration of the chemical in solution at equilibrium.

Adsorption Ratio (Kd)—The amount of a chemical adsorbed by a sediment or soil (i.e., the solid phase) divided by the amount of chemical in the solution phase, which is in equilibrium with the solid phase, at a fixed solid/solution ratio. It is generally expressed in micrograms of chemical sorbed per gram of soil or sediment.

Bioconcentration Factor (BCF)—The quotient of the concentration of a chemical in aquatic organisms at a specific time or during a discrete time period of exposure divided by the concentration in the surrounding water at the same time or during the same period.

Cancer Effect Level (CEL)—The lowest dose of chemical in a study, or group of studies, that produces significant increases in the incidence of cancer (or tumors) between the exposed population and its appropriate control.

Carcinogen—A chemical capable of inducing cancer.

Ceiling Value—A concentration of a substance that should not be exceeded, even instantaneously.

Chronic Exposure—Exposure to a chemical for 365 days or more, as specified in the Toxicological Profiles.

Developmental Toxicity—The occurrence of adverse effects on the developing organism that may result from exposure to a chemical prior to conception (either parent), during prenatal development, or postnatally to the time of sexual maturation. Adverse developmental effects may be detected at any point in the life span of the organism.

Embryotoxicity and Fetotoxicity—Any toxic effect on the conceptus as a result of prenatal exposure to a chemical; the distinguishing feature between the two terms is the stage of development during which the insult occurred. The terms, as used here, include malformations and variations, altered growth, and in utero death.

EPA Health Advisory—An estimate of acceptable drinking water levels for a chemical substance based on health effects information. A health advisory is not a legally enforceable federal standard, but serves as technical guidance to assist federal, state, and local officials.

Immediately Dangerous to Life or Health (IDLH)—The maximum environmental concentration of a contaminant from which one could escape within 30 min without any escape-impairing symptoms or irreversible health effects.

Intermediate Exposure—Exposure to a chemical for a duration of 15–364 days, as specified in the Toxicological Profiles.

Immunologic Toxicity—The occurrence of adverse effects on the immune system that may result from exposure to environmental agents such as chemicals.

***In Vitro*—**Isolated from the living organism and artificially maintained, as in a test tube.

***In Vivo*—**Occurring within the living organism.

Lethal Concentration$_{(LO)}$ (LC_{LO})—The lowest concentration of a chemical in air which has been reported to have caused death in humans or animals.

Lethal Concentration$_{(50)}$ (LC_{50})—A calculated concentration of a chemical in air to which exposure for a specific length of time is expected to cause death in 50% of a defined experimental animal population.

Lethal Dose$_{(LO)}$ (LD_{LO})—The lowest dose of a chemical introduced by a route other than inhalation that is expected to have caused death in humans or animals.

Lethal Dose$_{(50)}$ (LD_{50})—The dose of a chemical which has been calculated to cause death in 50% of a defined experimental animal population.

Lethal Time$_{(50)}$ (LT_{50})—A calculated period of time within which a specific concentration of a chemical is expected to cause death in 50% of a defined experimental animal population.

Lowest-Observed-Adverse-Effect Level (LOAEL)—The lowest dose of chemical in a study, or group of studies, that produces statistically or biologically significant increases in frequency or severity of adverse effects between the exposed population and its appropriate control.

Malformations—Permanent structural changes that may adversely affect survival, development, or function.

Minimal Risk Level—An estimate of daily human exposure to a dose of a chemical that is likely to be without an appreciable risk of adverse noncancerous effects over a specified duration of exposure.

Mutagen—A substance that causes mutations. A mutation is a change in the genetic material in a body cell. Mutations can lead to birth defects, miscarriages, or cancer.

Neurotoxicity—The occurrence of adverse effects on the nervous system following exposure to chemical.

No-Observed-Adverse-Effect Level (NOAEL)—The dose of chemical at which there were no statistically or biologically significant increases in frequency or severity of adverse effects seen between the exposed population and its appropriate control. Effects may be produced at this dose, but they are not considered to be adverse.

Octanol-Water Partition Coefficient (K_{ow})—The equilibrium ratio of the concentrations of a chemical in n-octanol and water, in dilute solution.

Permissible Exposure Limit (PEL)—An allowable exposure level in workplace air averaged over an 8-hour shift.

q_1*—The upper-bound estimate of the low-dose slope of the dose-response curve as determined by the multistage procedure. The q_1* can be used to calculate an estimate of carcinogenic potency, the incremental excess cancer risk per unit of exposure (usually µg/L for water, mg/kg/day for food, and µg/m^3 for air).

Reference Dose (RfD)—An estimate (with uncertainty spanning perhaps an order of magnitude) of the daily exposure of the human population to a potential hazard that is likely to be without risk of deleterious effects during a lifetime. The RfD is operationally derived from the NOAEL (from animal and human studies) by a consistent application of uncertainty factors that reflect various types of data used to estimate RfDs and an additional modifying factor, which is based on a professional judgment of the entire database on the chemical. The RfDs are not applicable to nonthreshold effects such as cancer.

Reportable Quantity (RQ)—The quantity of a hazardous substance that is considered reportable under CERCLA. Reportable quantities are (1) 1 pound or greater or (2) for selected substances, an amount established by regulation either under CERCLA or under Sect. 311 of the Clean Water Act. Quantities are measured over a 24-hour period.

Reproductive Toxicity—The occurrence of adverse effects on the reproductive system that may result from exposure to a chemical. The toxicity may be directed to the reproductive organs and/or the related endocrine system. The manifestation of such toxicity may be noted as alterations in sexual behavior, fertility, pregnancy outcomes, or modifications in other functions that are dependent on the integrity of this system.

Short-Term Exposure Limit (STEL)—The maximum concentration to which workers can be exposed for up to 15 min continually. No more than four excursions are allowed per day, and there must be at least 60 min between exposure periods. The daily TLV-TWA may not be exceeded.

Target Organ Toxicity—This term covers a broad range of adverse effects on target organs or physiological systems (e.g., renal, cardiovascular) extending from those arising through a single limited exposure to those assumed over a lifetime of exposure to a chemical.

Teratogen—A chemical that causes structural defects that affect the development of an organism.

Threshold Limit Value (TLV)—A concentration of a substance to which most workers can be exposed without adverse effect. The TLV may be expressed as a TWA, as a STEL, or as a CL.

Time-Weighted Average (TWA)—An allowable exposure concentration averaged over a normal 8-hour workday or 40-hour workweek.

Toxic Dose (TD_{50})—A calculated dose of a chemical, introduced by a route other than inhalation, which is expected to cause a specific toxic effect in 50% of a defined experimental animal population.

Uncertainty Factor (UF)—A factor used in operationally deriving the RfD from experimental data. UFs are intended to account for (1) the variation in sensitivity among the members of the human population, (2) the uncertainty in extrapolating animal data to the case of human, (3) the uncertainty in extrapolating from

data obtained in a study that is of less than lifetime exposure, and (4) the uncertainty in using LOAEL data rather than NOAEL data. Usually each of these factors is set equal to 10.

APPENDIX A

ATSDR MINIMAL RISK LEVELS AND WORKSHEETS

The Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) [42 U.S.C. 9601 et seq.], as amended by the Superfund Amendments and Reauthorization Act (SARA) [Pub. L. 99–499], requires that the Agency for Toxic Substances and Disease Registry (ATSDR) develop jointly with the U.S. Environmental Protection Agency (EPA), in order of priority, a list of hazardous substances most commonly found at facilities on the CERCLA National Priorities List (NPL); prepare toxicological profiles for each substance included on the priority list of hazardous substances; and assure the initiation of a research program to fill identified data needs associated with the substances.

The toxicological profiles include an examination, summary, and interpretation of available toxicological information and epidemiologic evaluations of a hazardous substance. During the development of toxicological profiles, Minimal Risk Levels (MRLs) are derived when reliable and sufficient data exist to identify the target organ(s) of effect or the most sensitive health effect(s) for a specific duration for a given route of exposure. An MRL is an estimate of the daily human exposure to a hazardous substance that is likely to be without appreciable risk of adverse noncancer health effects over a specified duration of exposure. MRLs are based on noncancer health effects only and are not based on a consideration of cancer effects. These substance-specific estimates, which are intended to serve as screening levels, are used by ATSDR health assessors to identify contaminants and potential health effects that may be of concern at hazardous waste sites. It is important to note that MRLs are not intended to define clean-up or action levels.

MRLs are derived for hazardous substances using the no-observed-adverse-effect level/uncertainty factor approach. They are below levels that might cause adverse health effects in the people most sensitive to such chemical-induced effects. MRLs are derived for acute (1–14 days), intermediate (15–364 days), and chronic (365 days and longer) durations and for the oral and inhalation routes of exposure. Currently, MRLs for the dermal route of exposure are not derived because ATSDR has not yet identified a method suitable for this route of exposure. MRLs are generally based on the most sensitive chemical-induced end point considered to be of relevance to humans. Serious health effects (such as irreparable damage to the liver or kidneys, or birth defects) are not used as a basis for establishing MRLs. Exposure to a level above the MRL does not mean that adverse health effects will occur.

MRLs are intended only to serve as a screening tool to help public health professionals decide where to look more closely. They may also be viewed as a mechanism to identify those hazardous waste sites that are not expected to cause adverse health effects. Most MRLs contain a degree of uncertainty because of the lack of precise toxicological information on the people who might be most sensitive (e.g., infants, elderly, nutritionally or immunologically compromised) to the effects of hazardous substances. ATSDR uses a conservative (i.e., protective) approach to address this uncertainty consistent with the public health principle of prevention. Although human data are preferred, MRLs often must be based on animal studies because relevant human studies are lacking. In the absence of evidence to the contrary, ATSDR assumes that humans are more sensitive to the effects of hazardous substance than animals and that certain persons may be particularly sensitive. Thus, the resulting MRL may be as much as a hundredfold below levels that have been shown to be nontoxic in laboratory animals.

Proposed MRLs undergo a rigorous review process: Health Effects/MRL Workgroup reviews within the Division of Toxicology, expert panel peer reviews, and agencywide MRL Workgroup reviews, with participation from other federal agencies and comments from the public. They are subject to change as new information becomes available concomitant with updating the toxicological profiles. Thus, MRLs in the most recent toxicological profiles supersede previously published levels. For additional information regarding MRLs, please contact the Division of Toxicology, Agency for Toxic Substances and Disease Registry, 1600 Clifton Road, Mailstop E-29, Atlanta, Georgia 30333.

APPENDIX A

MINIMAL RISK LEVEL (MRL) WORKSHEET

Chemical Name: Manganese
CAS Number: 7439-96-5
Profile Status: Peer Review Draft 2
Route: [X] Inhalation [ ] Oral
Duration: [ ] Acute [ ] Intermediate [X] Chronic
Graph Key:
Species: Human

Minimal Risk Level: 0.00004 mg manganese/m^3

Reference: Roels et al. 1992

Experimental design: Neurological effects of manganese exposure were evaluated in 92 male workers in a dry alkaline battery factory. The control group was 101 age- and area-matched workers not occupationally exposed to manganese but with similar work schedules and workloads. Workers were exposed an average duration of 5.3 years (range 0.2-17.7 years) to average (geometric mean) concentrations of 215 µg manganese/m^3 and 948 µg manganese/m^3 in respirable and total dust, respectively. The authors noted that the work processes had not changed significantly in the last 15 years, indicating that past exposures should be comparable to those measured in the study. Neurological function was measured using an audioverbal short term memory test, a simple visual reaction time test using a chronoscope, and three manual tests of hand steadiness, coordination, and dexterity. This report provided good documentation of individual exposure data and characterization of the population studied.

Effects noted in study and corresponding doses: Manganese-exposed workers performed significantly worse than the controls on the neurobehavioral tests, with particular differences in simple reaction time, eye-hand coordination, and hand steadiness. The authors provided their data on the manganese-exposed group evaluated in this study. These data included individual exposure levels and whether the individual had an abnormal performance in the neurobehavioral tests. A dose-response curve was constructed using benchmark dose analysis (BMD) of these data. From this plot a lower 95% confidence limit was estimated around the level of manganese exposure expected to result in a 10% response rate, the $BMDL_{10}$, which was considered an acceptable surrogate for a NOAEL. The authors note the data do not provide a clear-cut dose-effect relationship.

Dose and endpoint used for MRL derivation:

[ ] NOAEL [ ] LOAEL [X] Other $BMDL_{10}$ of 74 µg manganese/m^3

Conversion to Continuous Exposure:

[X] 5/7 to account for intermittent exposure (5 days per week)
[X] 8/24 to account for intermittent exposure (8 hours per day)

Uncertainty and Modifying Factors used in MRL derivation:

[X] 10 for human variability
[ ] 10 for the use of a LOAEL
[X] 10 for the potential for differences in toxicity from different manganese forms and other limitations in the database for inhalation exposures, including lack of data on developmental effects and reproductive effects in females
[X] 5 for modifying factor for potentially increased susceptibility in children based on differential pharmacokinetics in the young

Was a conversion used from ppm in food or water to a mg/body weight dose?
If so, explain: No

If an inhalation study in animals, list the conversion factors used in determining human equivalent dose: NA

Other additional studies or pertinent information which lend support to this MRL:

Dr. Anders Iregren also provided individual data on total manganese exposure and performance on neurobehavioral tests for the occupational cohort that participated in his study (Iregren 1990; Wennberg et al. 1991). A benchmark dose analysis was also performed with these data under contract with ATSDR (Clewell and Crump 1999) and the $BMDL_{10}$ value derived from this evaluation was 0.071 mg manganese/m^3 based upon the reported observation that the respirable fraction ranged upwards to 80% of the total dust measured. This $BMDL_{10}$ value is essentially the same as that estimated for the Roels et al. (1992) study, thus giving support to the value obtained for the current MRL study. More recently, Gibbs et al. (1999) reported that exposure to 0.051 mg manganese/m^3 (median, respirable manganese) was a NOAEL among workers at a metal producing plant when evaluated using both novel and older neurobehavioral test methods. However, individual exposure and test performance data from this study were not available to ATSDR for conducting a benchmark dose analysis. Nonetheless, the NOAEL reported by Gibbs et al. (1999) is consistent with the $BMDL_{10}$ values derived from raw data provided by Drs. Roels and Iregren. Several epidemiological studies also report preclinical neurological effects and support the Roels et al. (1992) and Iregren (1990) studies. An older study by Roels et al. (1987a) involved workers chronically exposed to manganese dusts at a concentration of 1 mg/m^3 in a factory using manganese oxides and salts. The authors noted a slight increase in frequency of weakness and tremor, and decreased scores on psychomotor tests (eye-hand coordination, hand steadiness, short-term memory, simple reaction time). In this study the exposure concentration did not distinguish between respirable dust and nonrespirable dust. In a recent study by Mergler et al. (1994), 145 workers from a ferromanganese and silicomanganese alloy factory were observed for adverse effects from exposure to manganese dusts. Environmental levels of total manganese in dust were measured at 0.014-11.48 mg/m^3 (median 0.151 mg/m^3, mean 1.186 mg/m^3). Mean duration of exposure was 16.7 years. Manganese workers showed decreased performance on tests of motor function, had difficulty in set shifting, and exhibited significantly lower levels of cognitive flexibility. Lucchini et al. (1995) reported neurobehavioral effects in 58 workers exposed to manganese dusts for 1–28 years (mean, 13 years). These workers were observed during a period of forced cessation from work and exhibited decreased neurobehavioral performance (finger tapping, symbol digit, digit span, and additions tests). Environmental levels of total dust ranged from 0.027–0.27 mg/m^3 (geometric means), with respirable dust given as 50–60% of the total dust. A recent study in occupational workers in a ferroalloy plant reported

decreased performance in neurobehavioral tests (e.g., symbol digit; finger tapping, dominant and non-dominant hand; digit span) was correlated with the CEI of workers who were employed in high, medium, or low-exposure areas. While airborne manganese concentrations were correlated with blood and urine manganese levels, CEI values were not (Lucchini et al. 1999). In a longitudinal follow-up study, Roels and colleagues observed that manganese concentrations were directly, and inversely, correlated with performance on a test measuring eye-hand coordination in a population of exposed workers at a dry-alkaline battery plant Roels et al. (1999). These workers were employed during a time of decreasing concentrations of airborne manganese as described in the following subgroups: low (~0.31–0.16 mg/m^3), medium (~0.90–0.250 mg/m^3), and high-exposure (~3.00–1.20 mg/m^3). Visual reaction time and hand steadiness tests did not reveal improved performance when manganese concentrations decreased. Further, increased performance by the medium- and high-exposure groups on the eye-hand coordination test during the period of lowest manganese exposure still did not reach the level of the control population, thus suggesting a permanent neurological effect from manganese exposures at these levels.

APPENDIX B

USER'S GUIDE

Chapter 1

Public Health Statement

This chapter of the profile is a health effects summary written in non-technical language. Its intended audience is the general public especially people living in the vicinity of a hazardous waste site or chemical release. If the Public Health Statement were removed from the rest of the document, it would still communicate to the lay public essential information about the chemical.

The major headings in the Public Health Statement are useful to find specific topics of concern. The topics are written in a question and answer format. The answer to each question includes a sentence that will direct the reader to chapters in the profile that will provide more information on the given topic.

Chapter 2

Tables and Figures for Levels of Significant Exposure (LSE)

Tables (2-1, 2-2, and 2-3) and figures (2-1 and 2-2) are used to summarize health effects and illustrate graphically levels of exposure associated with those effects. These levels cover health effects observed at increasing dose concentrations and durations, differences in response by species, minimal risk levels (MRLs) to humans for noncancer end points, and EPA's estimated range associated with an upper-bound individual lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. Use the LSE tables and figures for a quick review of the health effects and to locate data for a specific exposure scenario. The LSE tables and figures should always be used in conjunction with the text. All entries in these tables and figures represent studies that provide reliable, quantitative estimates of No-Observed-Adverse-Effect Levels (NOAELs), Lowest-Observed-Adverse-Effect Levels (LOAELs), or Cancer Effect Levels (CELs).

The legends presented below demonstrate the application of these tables and figures. Representative examples of LSE Table 2-1 and Figure 2-1 are shown. The numbers in the left column of the legends correspond to the numbers in the example table and figure.

LEGEND

See LSE Table 2-1

(1) Route of Exposure One of the first considerations when reviewing the toxicity of a substance using these tables and figures should be the relevant and appropriate route of exposure. When sufficient data

exists, three LSE tables and two LSE figures are presented in the document. The three LSE tables present data on the three principal routes of exposure, i.e., inhalation, oral, and dermal (LSE Table 2-1, 2-2, and 2-3, respectively). LSE figures are limited to the inhalation (LSE Figure 2-1) and oral (LSE Figure 2-2) routes. Not all substances will have data on each route of exposure and will not therefore have all five of the tables and figures.

(2) Exposure Period Three exposure periods - acute (less than 15 days), intermediate (15–364 days), and chronic (365 days or more) are presented within each relevant route of exposure. In this example, an inhalation study of intermediate exposure duration is reported. For quick reference to health effects occurring from a known length of exposure, locate the applicable exposure period within the LSE table and figure.

(3) Health Effect The major categories of health effects included in LSE tables and figures are death, systemic, immunological, neurological, developmental, reproductive, and cancer. NOAELs and LOAELs can be reported in the tables and figures for all effects but cancer. Systemic effects are further defined in the "System" column of the LSE table (see key number 18).

(4) Key to Figure Each key number in the LSE table links study information to one or more data points using the same key number in the corresponding LSE figure. In this example, the study represented by key number 18 has been used to derive a NOAEL and a Less Serious LOAEL (also see the 2 "18r" data points in Figure 2-1).

(5) Species The test species, whether animal or human, are identified in this column. Section 2.5, "Relevance to Public Health," covers the relevance of animal data to human toxicity and Section 2.3, "Toxicokinetics," contains any available information on comparative toxicokinetics. Although NOAELs and LOAELs are species specific, the levels are extrapolated to equivalent human doses to derive an MRL.

(6) Exposure Frequency/Duration The duration of the study and the weekly and daily exposure regimen are provided in this column. This permits comparison of NOAELs and LOAELs from different studies. In this case (key number 18), rats were exposed to 1,1,2,2-tetrachloroethane via inhalation for 6 hours per day, 5 days per week, for 3 weeks. For a more complete review of the dosing regimen refer to the appropriate sections of the text or the original reference paper, i.e., Nitschke et al. 1981.

(7) System This column further defines the systemic effects. These systems include: respiratory, cardiovascular, gastrointestinal, hematological, musculoskeletal, hepatic, renal, and dermal/ocular. "Other" refers to any systemic effect (e.g., a decrease in body weight) not covered in these systems. In the example of key number 18, 1 systemic effect (respiratory) was investigated.

(8) NOAEL A No-Observed-Adverse-Effect Level (NOAEL) is the highest exposure level at which no harmful effects were seen in the organ system studied. Key number 18 reports a NOAEL of 3 ppm for

the respiratory system which was used to derive an intermediate exposure, inhalation MRL of 0.005 ppm (see footnote "b").

(9) LOAEL A Lowest-Observed-Adverse-Effect Level (LOAEL) is the lowest dose used in the study that caused a harmful health effect. LOAELs have been classified into "Less Serious" and "Serious" effects. These distinctions help readers identify the levels of exposure at which adverse health effects first appear and the gradation of effects with increasing dose. A brief description of the specific endpoint used to quantify the adverse effect accompanies the LOAEL. The respiratory effect reported in key number 18 (hyperplasia) is a Less serious LOAEL of 10 ppm. MRLs are not derived from Serious LOAELs.

(10) Reference The complete reference citation is given in chapter 8 of the profile.

(11) CEL A Cancer Effect Level (CEL) is the lowest exposure level associated with the onset of carcinogenesis in experimental or epidemiologic studies. CELs are always considered serious effects. The LSE tables and figures do not contain NOAELs for cancer, but the text may report doses not causing measurable cancer increases.

(12) Footnotes Explanations of abbreviations or reference notes for data in the LSE tables are found in the footnotes. Footnote "b" indicates the NOAEL of 3 ppm in key number 18 was used to derive an MRL of 0.005 ppm.

LEGEND

See Figure 2-1

LSE figures graphically illustrate the data presented in the corresponding LSE tables. Figures help the reader quickly compare health effects according to exposure concentrations for particular exposure periods.

(13) Exposure Period The same exposure periods appear as in the LSE table. In this example, health effects observed within the intermediate and chronic exposure periods are illustrated.

(14) Health Effect These are the categories of health effects for which reliable quantitative data exists. The same health effects appear in the LSE table.

(15) Levels of Exposure concentrations or doses for each health effect in the LSE tables are graphically displayed in the LSE figures. Exposure concentration or dose is measured on the log scale "y" axis. Inhalation exposure is reported in mg/m^3 or ppm and oral exposure is reported in mg/kg/day.

(16) NOAEL In this example, 18r NOAEL is the critical endpoint for which an intermediate inhalation exposure MRL is based. As you can see from the LSE figure key, the open-circle symbol indicates to a

NOAEL for the test species-rat. The key number 18 corresponds to the entry in the LSE table. The dashed descending arrow indicates the extrapolation from the exposure level of 3 ppm (see entry 18 in the Table) to the MRL of 0.005 ppm (see footnote "b" in the LSE table).

(17) CEL Key number 38r is 1 of 3 studies for which Cancer Effect Levels were derived. The diamond symbol refers to a Cancer Effect Level for the test species-mouse. The number 38 corresponds to the entry in the LSE table.

(18) Estimated Upper-Bound Human Cancer Risk Levels This is the range associated with the upper-bound for lifetime cancer risk of 1 in 10,000 to 1 in 10,000,000. These risk levels are derived from the EPA's Human Health Assessment Group's upper-bound estimates of the slope of the cancer dose response curve at low dose levels (q_1*).

(19) Key to LSE Figure The Key explains the abbreviations and symbols used in the figure.

The Relevance to Public Health section provides a health effects summary based on evaluations of existing toxicologic, epidemiologic, and toxicokinetic information. This summary is designed to present interpretive, weight-of-evidence discussions for human health end points by addressing the following questions.

1. What effects are known to occur in humans?

2. What effects observed in animals are likely to be of concern to humans?

3. What exposure conditions are likely to be of concern to humans, especially around hazardous waste sites?

The section covers end points in the same order they appear within the Discussion of Health Effects by Route of Exposure section, by route (inhalation, oral, dermal) and within route by effect. Human data are presented first, then animal data. Both are organized by duration (acute, intermediate, chronic). *In vitro* data and data from parenteral routes (intramuscular, intravenous, subcutaneous, etc.) are also considered in this section. If data are located in the scientific literature, a table of genotoxicity information is included.

The carcinogenic potential of the profiled substance is qualitatively evaluated, when appropriate, using existing toxicokinetic, genotoxic, and carcinogenic data. ATSDR does not currently assess cancer potency or perform cancer risk assessments. Minimal risk levels (MRLs) for noncancer end points (if derived) and the end points from which they were derived are indicated and discussed.

Limitations to existing scientific literature that prevent a satisfactory evaluation of the relevance to public health are identified in the Data Needs section.

SAMPLE

1 6

TABLE 2-1. Levels of Significant Exposure to [Chemical x] – Inhalation

Key to figure[a]	Species	Exposure frequency/ duration	System	NOAEL (ppm)	LOAEL (effect)		Reference
					Less serious (ppm)	Serious (ppm)	
2 6 INTERMEDIATE EXPOSURE							
	5	6	7	8	9		10
3 6 Systemic	9	9	9	9	9		9
4 6 18	Rat	13 wk 5d/wk 6hr/d	Resp	3[b]	10 (hyperplasia)		Nitschke et al. 1981
CHRONIC EXPOSURE							
					11		
Cancer					9		
38	Rat	18 mo 5d/wk 7hr/d			20	(CEL, multiple organs)	Wong et al. 1982
39	Rat	89–104 wk 5d/wk 6hr/d			10	(CEL, lung tumors, nasal tumors)	NTP 1982
40	Mouse	79–103 wk 5d/wk 6hr/d			10	(CEL, lung tumors, hemangiosarcomas)	NTP 1982

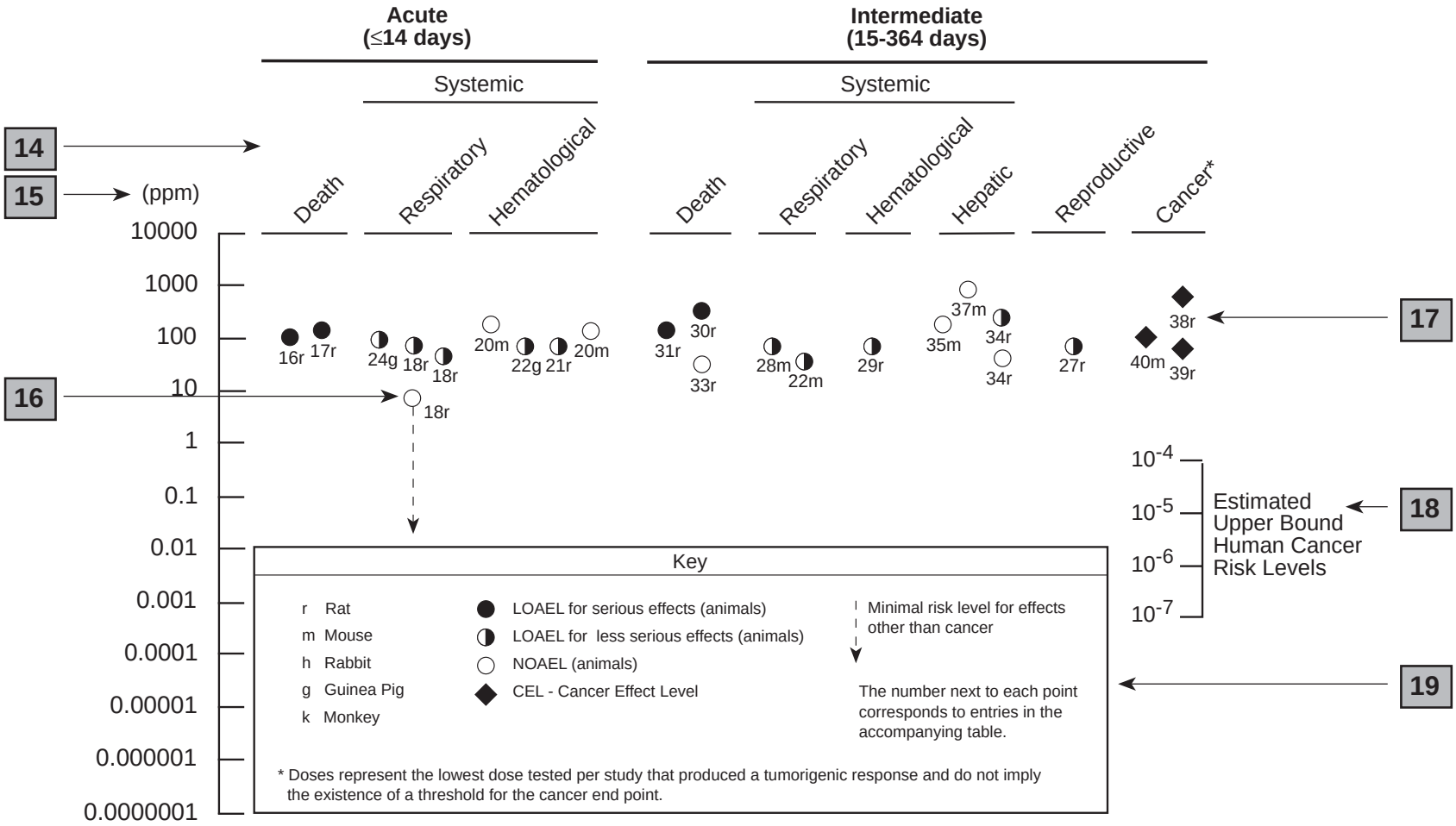
[a] The number corresponds to entries in Figure 2-1.

12 6 [b] Used to derive an intermediate inhalation Minimal Risk Level (MRL) of 5 x 10^{-3} ppm; dose adjusted for intermittent exposure and divided by an uncertainty factor of 100 (10 for extrapolation from animal to humans, 10 for human variability).

CEL = cancer effect level; d = days(s); hr = hour(s); LOAEL = lowest-observed-adverse-effect level; mo = month(s); NOAEL = no-observed-adverse-effect level; Resp = respiratory; wk = week(s)

SAMPLE

13 → **Figure 2-1. Levels of Significant Exposure to [Chemical X] – Inhalation**



Chapter 2 (Section 2.5)

Relevance to Public Health

Interpretation of Minimal Risk Levels

Where sufficient toxicologic information is available, we have derived minimal risk levels (MRLs) for inhalation and oral routes of entry at each duration of exposure (acute, intermediate, and chronic). These MRLs are not meant to support regulatory action; but to acquaint health professionals with exposure levels at which adverse health effects are not expected to occur in humans. They should help physicians and public health officials determine the safety of a community living near a chemical emission, given the concentration of a contaminant in air or the estimated daily dose in water. MRLs are based largely on toxicological studies in animals and on reports of human occupational exposure.

MRL users should be familiar with the toxicologic information on which the number is based. Chapter 2.5, "Relevance to Public Health," contains basic information known about the substance. Other sections such as 2.8, "Interactions with Other Substances," and 2.9, "Populations that are Unusually Susceptible" provide important supplemental information.

MRL users should also understand the MRL derivation methodology. MRLs are derived using a modified version of the risk assessment methodology the Environmental Protection Agency (EPA) provides (Barnes and Dourson 1988) to determine reference doses for lifetime exposure (RfDs).

To derive an MRL, ATSDR generally selects the most sensitive endpoint which, in its best judgement, represents the most sensitive human health effect for a given exposure route and duration. ATSDR cannot make this judgement or derive an MRL unless information (quantitative or qualitative) is available for all potential systemic, neurological, and developmental effects. If this information and reliable quantitative data on the chosen endpoint are available, ATSDR derives an MRL using the most sensitive species (when information from multiple species is available) with the highest NOAEL that does not exceed any adverse effect levels. When a NOAEL is not available, a lowest-observed-adverse-effect level (LOAEL) can be used to derive an MRL, and an uncertainty factor (UF) of 10 must be employed. Additional uncertainty factors of 10 must be used both for human variability to protect sensitive subpopulations (people who are most susceptible to the health effects caused by the substance) and for interspecies variability (extrapolation from animals to humans). In deriving an MRL, these individual uncertainty factors are multiplied together. The product is then divided into the inhalation concentration or oral dosage selected from the study. Uncertainty factors used in developing a substance-specific MRL are provided in the footnotes of the LSE Tables.

APPENDIX C

ACRONYMS, ABBREVIATIONS, AND SYMBOLS

ACGIH	American Conference of Governmental Industrial Hygienists
ADME	Absorption, Distribution, Metabolism, and Excretion
atm	atmosphere
ATSDR	Agency for Toxic Substances and Disease Registry
BCF	bioconcentration factor
BSC	Board of Scientific Counselors
C	Centigrade
CDC	Centers for Disease Control
CEL	Cancer Effect Level
CERCLA	Comprehensive Environmental Response, Compensation, and Liability Act
CFR	Code of Federal Regulations
CLP	Contract Laboratory Program
cm	centimeter
CNS	central nervous system
d	day
DHEW	Department of Health, Education, and Welfare
DHHS	Department of Health and Human Services
DOL	Department of Labor
ECG	electrocardiogram
EEG	electroencephalogram
EPA	Environmental Protection Agency
EKG	see ECG
F	Fahrenheit
F_1	first filial generation
FAO	Food and Agricultural Organization of the United Nations
FEMA	Federal Emergency Management Agency
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
fpm	feet per minute
ft	foot
FR	*Federal Register*
g	gram
GC	gas chromatography
gen	generation
HPLC	high-performance liquid chromatography
hr	hour
IDLH	Immediately Dangerous to Life and Health

IARC	International Agency for Research on Cancer
ILO	International Labor Organization
in	inch
i.p.	intraperitoneal
i.u.	international unit
i.v.	intravenous
Kd	adsorption ratio
kg	kilogram
kkg	metric ton
K_{oc}	organic carbon partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC	liquid chromatography
LC_{Lo}	lethal concentration, low
LC_{50}	lethal concentration, 50% kill
LD_{Lo}	lethal dose, low
LD_{50}	lethal dose, 50% kill
LOAEL	lowest-observed-adverse-effect level
LSE	Levels of Significant Exposure
m	meter
meq	milliequivalent
mg	milligram
min	minute
mL	milliliter
mm	millimeter
mmHg	millimeters of mercury
mmol	millimole
mo	month
mppcf	millions of particles per cubic foot
MRL	Minimal Risk Level
MS	mass spectrometry
NIEHS	National Institute of Environmental Health Sciences
NIOSH	National Institute for Occupational Safety and Health
NIOSHTIC	NIOSH's Computerized Information Retrieval System
ng	nanogram
nm	nanometer
NHANES	National Health and Nutrition Examination Survey
nmol	nanomole
NOAEL	no-observed-adverse-effect level
NOES	National Occupational Exposure Survey
NOHS	National Occupational Hazard Survey
NPL	National Priorities List
NRC	National Research Council
NTIS	National Technical Information Service
NTP	National Toxicology Program
OSHA	Occupational Safety and Health Administration

PEL	permissible exposure limit
pg	picogram
pmol	picomole
PHS	Public Health Service
PMR	proportionate mortality ratio
ppb	parts per billion
ppm	parts per million
ppt	parts per trillion
REL	recommended exposure limit
RfD	Reference Dose
RTECS	Registry of Toxic Effects of Chemical Substances
s.c.	subcutaneous
sec	second
SCE	sister chromatid exchange
SIC	Standard Industrial Classification
SMR	standard mortality ratio
STEL	short term exposure limit
STORET	STORAGE and RETRIEVAL
T_4	thyroxine
TPN	total parenteral nutrition
TLV	threshold limit value
TSCA	Toxic Substances Control Act
TRI	Toxics Release Inventory
TWA	time-weighted average
U.S.	United States
UF	uncertainty factor
yr	year
WHO	World Health Organization
wk	week

>	greater than
≥	greater than or equal to
=	equal to
<	less than
≤	less than or equal to
%	percent
α	alpha
β	beta
δ	delta
γ	gamma
µm	micrometer
µg	microgram