7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring manganese, its metabolites, and other biomarkers of exposure and effect to manganese. 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.

The most common analytical procedures for measuring manganese levels in biological and environmental samples use the methods of atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES). In AAS analysis, the sample is aspirated into a flame or in a graphite furnace (GFAAS) until the element atomizes (Tsalev 1983). The ground-state atomic vapor absorbs monochromatic radiation from a source and a photoelectric detector measures the intensity of radiation absorbed at 279.5 nm (Tsalev 1983). Furnace atomic absorption analysis is often used for very low analyte levels and for the analysis of solid samples or slurries (Baruthio et al. 1988). Inductively coupled plasma-atomic emission spectrometry (ICP-AES) 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 (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. 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 are nearly always available only as total manganese present.

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 (GC) 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 (FID). These methods usually measure MMT by detecting the metallic portion of the compound and reporting detection of MMT as manganese.

X-ray absorption near edge structure (XANES) and x-ray absorption fine structure (XAFS) spectroscopy have been used for the analysis of manganese-containing particulates emitted from automobile exhaust containing MMT (Mölders et al. 2001; Ressler et al. 2000). These methods are particularly useful in determining the chemical speciation and valence state of manganese or other metal complexes attached to particulate matter.

7.1 BIOLOGICAL MATERIALS

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 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, saliva, 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). 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. Table 7-1 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, especially for samples with low levels of manganese (Tsalev 1983; Versieck et al. 1988).

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 of 93.5–102.7% (Hanzlik et al. 1979).

Table 7-1. 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 Craple 1988a
Blood	Digestion in oxidizing acid	ICP-AES	1 µg/100 g	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 Craple 1988a
Feces	Dry at 110 °C, ash at 550 °C, dissolve in nitric acid	AAS (furnace 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 longitudinal gradients	ETAAS (furnace technique)	No data	No data	Stupar and Dolinsek 1996[c]
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 (manganese compounds) 0.1–0.8 µM (zinc compounds) 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]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; HPLC = high performance liquid chromatography; ICP-AES = inductively coupled-plasma atomic emission spectroscopy; MnDPDP = mangafodipir; NIOSH = National Institute for Occupational Safety and Health

Walton et al. (1991) have described high performance liquid chromatography (HPLC) 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 to 20 pg of manganese for the various organomanganese species; the detection limit for determining manganese in MMT was 0.4 ng/mL. This limit of detection was several orders of magnitude better than those for HPLC with ultraviolet (UV) detection or HPLC-atomic fluorescence spectrometry (AFC) (Walton et al. 1991), but was worse than detection by GC-FID (DuPuis and Hill 1979). 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.

Table 7-1 summarizes some common methods for the determination of manganese in various types of biological materials.

7.2 ENVIRONMENTAL SAMPLES

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.

Several analytical methods from the EPA Office of Solid Waste publication SW-846, entitled *Test Methods for Evaluating Solid Waste, Physical/Chemical Methods* are applicable for analyzing manganese in water, soil, and wastes. In addition, the EPA Emission Measurement Center (EMS) and Office of Water (OW) have standardized methods for the measurement of manganese and other metals in environmental media. Several of these methods, including the analytical instrumentation and detection limits, are summarized in Table 7-2.

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 0.005–50 µg/L). In all cases, acid is added to the sample to prevent precipitation of manganese.

Table 7-2. Analytical Methods for Determining Manganese in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect sample on MCE or PVC filter, followed by nitric/perchloric acid ashing	Method 7300 (ICP-AES)	0.2 mg/m^3	94.7–101 (MCE) 99.3–101.9 (PVC)	NIOSH 2003a
Air	Collect sample on MCE filter, followed by hot block/HCl/HNO_3 digestion	Method 7303 (ICP-AES)	1.2 mg/m^3	No data	NIOSH 2003b
Air	Collect sample on MCE or PVC filter, followed by aqua regia ashing	Method 7301 (ICP-AES)	0.2 mg/m^3	91.2–103.5 (MCE) 77.4–93.4 (PVC)	NIOSH 2003c
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	Method 311 (AAS)	<10 µg/L	No data	APHA 1998a
Water	Filter and acidify filtrate with HNO_3 and analyze	Method 3113A (AAS furnace technique)	0.2 µg/L	No data	APHA 1998b
Water	Digest sample with HNO_3/HCl and analyze	Method 3120B (ICP-AES)	2 µg/L	No data	APHA 1998c
Water	Acidify with nitric acid	AAS (direct aspiration)	10 µg/L	100±2[a]	EPA 1983a
Water	Acid digest and analyze	Method 3125A (ICP-MS)	0.002 µg/L	91.81–110	APHA 1998d
Water	Preconcentration manganese-containing solution and 3,3'5,5'-tetramethyl-benzidine (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
Water, waste water, sludge, and soils	For dissolved constituents: filter, acidify filtrate, and analyze; for samples containing solids: digestion with HNO_3/HCl prior to analysis	Method 200.8 (ICP-MS)	0.01–0.04 µg/L (liquids); 0.05 mg/kg (solids)	95.8–96.9 (water); 95.2–103.6 (wastes)	EPA 1994b

Table 7-2. Analytical Methods for Determining Manganese in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water and wastes	Acid digestion	AAS	10 µg/L	100±2	EPA 1986c
Water, solids, sediment,	For dissolved constituents: filter, acidify filtrate, and analyze; for samples containing solids: digestion with HNO_3/HCl prior to analysis	Method 6010C (ICP-AES)	0.93 µg/L	No data	EPA 2007a
Foods	Digest wet or dry foods with HNO_3–H_2SO_4 mixture (12:2 mL)	AAS (flame) AAS (furnace)	AAS (flame): 0.15 mg/kg AAS (furnace): 1.10 µg/kg	No data	Tinggi et al. 1997
Foods	Digestion with nitric, sulfuric, perchloric acid solution	ICP-AES	0.2 mg/kg	96.2–97	Capar and Cunningham 2000
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
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

[a]Percent recovery at manganese concentration >80 µg/L; at lower concentrations (10–20 µg/L), percent recoveries were >120%.

AAS = atomic absorption spectrometry; ACP = alternating current plasma; AES = atomic emission spectroscopy; APDC = ammonium pyrrolidine dithiocarbamate; APHA = American Public Health Association; ECD = electron-capture detection; EPA = Environmental Protection Agency; FPD = flame photometric detection; GC = gas chromatography; ICP = inductivity coupled plasma; MCE = mixed cellulose ester; MED = microwave emission detector; MS = mass spectrometry; NIOSH = National Institute for Occupational Safety and Health; PVC = polyvinyl chloride; XRF = x-ray fluorescence

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 novel 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.

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).

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 FID (DuPuis and Hill 1979). The former has measured manganese concentrations from 0.1 to 4 g/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 an absolute detection limit of 1.7×10^{-14} g/sample (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 GC followed by atmospheric pressure helium microwave detection system (or microwave emission detector [MED]); this method has a high degree of selectivity ($1.9x10^6$) and a detection limit of 0.25 pg/s at a wavelength of 257.6 nm.

GC 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. GC followed by 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).

Table 7-2 summarizes some common methods for the determination of manganese in various types of environmental media.

7.3 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 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.

7.3.1 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. 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. Smith et al. (2007) have discussed the limitations of using blood and urine levels of manganese as biomarkers of exposure and have suggested further investigation of using manganese levels in teeth and hair as exposure biomarkers. The use of tooth enamel as a potential biomarker has been explored by Ericson et al. (2007). Josephs et al. (2005) have also discussed the limitations of using manganese levels in serum or urine as a direct measure of exposure since free manganese does not accumulate in the circulatory system. Magnetic resonance imaging (MRI) in conjunction with analysis of manganese in whole blood (MnB), plasma (MnP), or red blood cells has been used in the diagnosis of manganism in humans (Jiang et al. 2007). Abdelouahab et al. (2010) have shown that MAO activity is a potentially useful biomarker for manganese exposure. Montes et al. (2008) conducted a study supporting prolactin as a potential biomarker for manganese exposure. Development of additional 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.

There is a need to evaluate the accuracy and reproducibility of analytical measures of manganese in biological media, so that analytical variability is not inappropriately incorporated into natural biological variability in reported data, as may now be the case.

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 1984). 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 air (NIOSH 2003a, 2003b, 2003c), water (APHA 1998a, 1998b, 1998c, 1998d; EPA 1994b, 2007a), and soils and sediment (EPA 2007a). Methods are also available to analyze manganese in food (Capar and Cunningham 2000; Tinggi et al. 1997).

7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2008) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs pertinent to the analysis of manganese in biological or environmental samples. Donald Smith and co-workers at the University of California, Santa Cruz are studying the role of manganese in neurodegenrative disease using particle induced x-ray emission (PIXE) analyses of *in situ* brain regional manganese levels of rodents (FEDRIP 2008). Carmen Enid Martinez and co-workers at Pennsylvania State University are studying the elemental distribution in soil particles using novel techniques that include synchrotron-based microprobe x-ray fluorescence (XRF) and x-ray diffraction (XRD) in addition to scanning electron microscopy coupled to energy or wavelength dispersive x-ray analysis (SEM/E-W-DS). Metal solubility measurements are to be studied by inductively coupled plasma emission spectroscopy (ICP), anodic/cathodic stripping voltammetry (A/C-SV), and ion-selective electrodes (ISE).