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 mercury, its metabolites, and other biomarkers of exposure to and effects of mercury. 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 analysis of metals in biological and environmental samples is complicated by the different organic and inorganic forms of the metal that may be present. For mercury, this complication is usually overcome by reducing all the mercury in the sample to its elemental state prior to analysis; this solution is not appropriate when information about the individual mercury species is desired. Mercury has an additional problem of being relatively volatile and, therefore, easily lost during sample preparation and analysis. In spite of these complications, several methods have been developed for determining trace amounts of mercury in biological and environmental samples, even in complex media. Careful attention must be paid to inadvertent contamination of the sample with mercury, especially when determining trace concentrations. Labware (glass or Teflon) should be thoroughly cleaned and acid-leached before being used for trace-level analysis. It has been shown that final soaking of laboratory ware, particularly Teflon, in hot (70 EC) 1% HCL removes any traces of oxidizing compounds (e.g., chlorine) that may subsequently destroy methylmercury in solution (Horvat 1996). Appropriate method blanks must be included.

Attention must be paid also to sample preservation to avoid perturbing the distribution of mercury compounds in the sample (Horvat 1996). The preservation of aqueous samples is often accomplished using acidification. However, suspended matter must be removed prior to acidification and dimethylmercury and Hg(0) have to be removed or else conversion of these species into methylmercury and mercury(II) can occur (Horvat 1996). For solid matrices, the preservation method of choice is freezing (Bloom 1993). Freezing preserves all major mercury species indefinitely, although coagulation will occur for sediments thus making it difficult to obtain representative subsamples of the sediment for analysis. For most metals, such storage

issues would be solved by drying the samples first, but for mercury, especially methylmercury, there is a risk of losses from volatilization. Tissue samples may be freeze-dried without loss of methylmercury. Repeated freezing and thawing of wet, biological samples can also cause loss of methylmercury (Horvat and Byrne 1992) but such degradations are dependant on the matrix.

Numerous standard or certified reference materials exist for verifying the reliability of new or modified methods, especially for total mercury; standard reference materials for individual organomercury species can be more difficult to obtain. The existing methods for determining mercury in biological and environmental matrices are described more fully in the following sections.

6.1 BIOLOGICAL MATERIALS

Many researchers have attempted to determine mercury levels in the blood, urine, tissues, and hair of humans and animals. Most methods have used atomic absorption spectrometry (AAS), atomic fluorescence spectrometry (AFS), or neutron activation analysis (NAA). In addition, methods based on mass spectrometry (MS), spectrophotometry, and anodic stripping voltametry (ASV) have also been tested. Of the available methods, cold vapor (CV) AAS is the most widely used. In most methods, mercury in the sample is reduced to the elemental state. Some methods require predigestion of the sample prior to reduction. At all phases of sample preparation and analysis, the possibility of contamination from mercury found naturally in the environment must be considered. Rigorous standards to prevent mercury contamination must be followed. Table 6-1 presents details of selected methods used to determine mercury in biological samples. Methods have been developed for the analysis of mercury in breath samples. These are based on AAS with either flameless (NIOSH 1994) or cold vapor release of the sample to the detection chamber (Rathje et al. 1974). Flameless AAS is the NIOSH-recommended method of determining levels of mercury in expired air (NIOSH 1994). No other current methods for analyzing breath were located.

In recent years, increasing attention has been paid to human exposure to mercury via dental amalgams (Skare 1995). Exposure results from elemental mercury vapor released from amalgams that is either inhaled directly or swallowed after dissolution in saliva. A Jerome 511 Gold Film Mercury Vapor Analyzer (Arizona Instrument Corp., Jerome, AZ) has been used to measure mercury vapor released from amalgam during routine dental procedures (Engle et al. 1992) or at other times to establish baseline exposure data (Halbach 1995). Accuracy and precision data were not reported. Although the detection limit for this method was not reported, mercury concentrations at μg concentrations are detectable. A similar instrument

Table 6-1. Analytical Methods for Determining Mercury in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath	Personal sampler (collection of an aliquot of air); analysis of sample at 253.7 nm.	AAS (flameless)	1 ng/sample	No data	NIOSH 1984 (method 6000)
Breath	Fasten a hopcalite sampling tube to a temple of the worker's safety glasses; draw air through the sampler.	CVAAS	No data	No data	Rathje et al. 1974
Human whole blood	Treatment of sample with dilute hydrochloric acid; addition of a pH buffer and a complexing agent (diethyldithiocarbamate); extraction of mercury species into toluene	ETAAS	2 µg/dm^3	>94%	Emteborg et al. 1992
Blood	Cleavage of both organic and inorganic mercury from blood protein thiol groups using hydrochloric acid, extraction of mercury species into toluene as their diethyldithiocarbamate (DDTC) complexes; addition of Grignard reagent to toluene phase to form butyl derivatives of the mercury species	GC/MPD	0.4 µg/L	>100%	Bulska et al. 1992
Blood and erythrocytes (inorganic, total)	Digestion of sample with H_2SO_4 (mixture of nitric and perchloric for total) overnight, reduction with $SnCl_2$, purging onto gold wire to form amalgam (preconcentration) followed by thermal release of elemental mercury.	CVAAS	0.06 ng/g (0.06 ppb) for total; 0.04 ng/g for inorganic.	75–114%	Bergdahl et al. 1995
Blood and urine	Dilution of sample in ammonia buffer; reduction with sodium borohydride	ICPAES	0.5 µg/L	100	Buneaux et al. 1992
Blood and urine	Total mercury: precipitation-extraction with 50% volume/volume hydrochloric acid containing EDTA and cysteine; centrifugation; filtration through screening column. Methyl mercury: extraction of the methyl mercury into benzene or toluene; back extract into aqueous cysteine solution	ICP-MS	0.2 µg/L	91.6–110.2	Kalamegham and Ash 1992
Blood, plasma, urine (total)	Digestion of blood and plasma samples overnight in a mixture of nitric acid and perchloric acid	CVAAS	5 nmol/L	93.4–103	Vesterberg 1991
Blood, urine, tissues (inorganic)	Dilution of blood or urine sample with water; homogenization of tissue samples with water; reduction of mercury with $SnCl_2$ followed by purging to detector	CVAAS	≈6 µg/L	77–110	Friese et al. 1990

Table 6-1. Analytical Methods for Determining Mercury in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood, breast milk (total, inorganic)	Digestion of sample with nitric/perchloric acid overnight for total, and with H_2SO_4 overnight for inorganic; reduction and purging	CVAAS	0.1 ng/g (blood); 0.04 ng/g (milk)	97%	Oskarsson et al. 1996
Blood (total)	Irradiation of sample followed by treatment with permanganate, sulfuric acid, distilled water, ammonia, and hydroxylamine hydrochloride; treatment with ion exchange.	NAA	0.3 ng/mL	100%	Fung et al. 1995
Serum, bovine liver (total)	Digestion of sample with HNO_3 and heat in closed container in microwave oven; reduction with $SnCl_2$ and TBP; purging to gold-coated sand adsorber to preconcentrate (amalgamation); thermally desorb to detector	CVAAS	0.84 ng/g	93–111	Vermeir et al. 1989
Urine (total)	Digestion with $HNO_3/HClO_3$ and heat; evaporation; addition of NH_4Cl/ammonium solution; dilution with water	ASV	NR	100–105	Liu et al. 1990
Urine (total)	Addition of HCl to sample followed by bromate/bromide solution and equilibration for 15 minutes; decomposition of excess bromine by addition of hydroxylamine hydrochloride.	AFS	1 ng/L	95-98% (methyl mercury, phenyl mercury)	Corns et al. 1994
Urine, tissue, hair (total)	Digestion of sample with HNO_3 in closed vessel in microwave; cooling and dilution with water; reduction with $SnCl_2$; purging to detector	AFS	0.9 ng/L	94–102	Vermeir et al. 1991a, 1991b
Blood, urine, hair, fish (total, methyl Hg)	Total: digestion of sample with nitric, perchloric, and sulfuric acids; Methyl mercury in hair: digestion with HCl and extraction into benzene. Methyl mercury in blood, fish, and urine: digestion with KOH and extraction into dithizone solution, cleaned up via extractions.	Total: CVAAS, methyl mercury: GC/ECD	0.5 ng	No data	Akagi et al. 1995

Table 6-1. Analytical Methods for Determining Mercury in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Tissue, hair	Washing of hair sample with acetone and water; homogenization of hair or tissue sample in micro dismembrator; irradiation; addition of carriers; digestion with concentrated HNO_3/ H_2SO_4 solution and heat in a closed Teflon bomb; extraction of digest with $CHCl_3$ to remove bromide ion, extraction of aqueous phase with Zn-$(DDC)_2$/$CHCl_3$; counting of ^{197}Hg in organic phase	NAA	0.36 ng/g (tissue) 3.6 ng/g (hair)	85–110	Zhuang et al. 1989
Liver tissue (methyl mercury)	Extraction of sample with toluene; concentration of methylmercury in aqueous phase; mixing with bacterial cells and incubation in microreaction vessel; injection of headspace gas containing methane	GC/FID	15 ng	NR	Baldi and Filippelli 1991
Hair	Washing of samples with acetone and water; digestion with HNO_3 and heat; oxidation with permanganate solution and heat; cooling and addition of hydroxylamine hydrochloride; reduction of mercury with $SnCl_2$; purging to detector	CVAAS	NR	100–101	Pineau et al. 1990
Wrist and temporal areas	None	XRF	20 µg/g	No data	Bloch and Shapiro 1986

AAS = atomic absorption spectrometry; AFS = atomic fluorescence spectrometry; ASV = anodic stripping voltametry; $CHCl_3$ = trichloromethane; CVAAS = cold vapor atomic absorption spectrometry; ECD = electron capture detection; ETAAS = electrothermal atomic absorption spectrometry; FID = flame ionization detector; GC = gas chromatography; $HClO_3$ = perchlorous acid; Hg = mercury; HNO_3 = nitric acid; H_2SO_4 = sulfuric acid; ICPAES = inductively coupled plasma atomic emission spectroscopy; ICP-MS = inductively coupled plasma-mass spectrometry; MPD = microwave-induced plasma emission; NAA = neutron-activation analysis; NH_4Cl = ammonium chloride; NR = not reported; $SnCl_2$ = tin(II) chloride; TBP = tri-*n*-butyl-phosphate; XRF = X-ray fluorescence; Zn-$(DCC)_2$ = zinc diethyldithiocarbamate

(Jerome 431X Mercury Vapor Analyzer) was used by Chien et al. (1996) to measure elemental mercury vapor released from dental amalgams in the oral cavity and was reported to have a sensitivity of 0.003 mg/m^3. Absorbed mercury can be measured using blood and urine measurements as described below.

CVAAS is the primary method that is used to determine mercury in blood and serum (Friese et al. 1990; Ngim et al. 1988; Vermeir et al. 1988, 1989; Vesterberg 1991). Using CVAAS, concentrations in the sub- to low-ppb can be reliably measured. Both direct reduction of sample (Friese et al. 1990; Ngim et al. 1988) and predigestion followed by reduction (Oskarsson et al. 1996; Vermeir et al. 1988, 1989) produced good accuracy and precision. However, with predigestion techniques, best results were obtained on samples that were heated in a closed teflon container in a microwave oven and preconcentrated on gold-coated sand (Vermeir et al. 1989). A complimentary method to CVAAS for total mercury determination in blood is electrothermal atomic absorption (ETAAS) (Emteborg et al. 1992). Recoveries are excellent and sensitivity is 2 µg/dm^3. GC/microwave-induced plasma atomic emission detection (MPD) can also be used to measure both organic and inorganic mercury in blood samples (Bulska et al. 1992). Sensitivity is in the sub-ppb range, and recovery is excellent (100%).

Methylmercury and inorganic mercury were extracted from human whole blood samples, as their diethyldithiocarbamate complexes, into toluene and butylated them by using a Grignard Reagent (Bulska et al. 1992). The mercury species were then detected by a microwave-induced plasma atomic emission spectrometric system (GC/MPD). The absolute detection limit was calculated to be 1 pg of mercury in either the methylmercury or inorganic mercury form. This corresponds to a detection limit of about 0.4 µg/L. The method is reproducible. Methods for inorganic mercury and organic mercury (mostly methylmercury) have been reported for blood, urine, hair, and breast milk (Akagi et al. 1995; Bergdahl et al. 1995; Oskarsson et al. 1996). Total mercury is typically determined using CVAAS after complete conversion of all mercury to the volatile elemental form using harsh (nitric acid/perchloric acid, bromate/bromide) digestions followed by reduction of ionic mercury to the elemental form. Inorganic mercury can be determined after milder digestions (HCl, sulfuric acid) and reduction. The organic form is determined by the difference between total and inorganic. Sub-ng/g (ppb) detection limits are routine. Methylmercury is also determined using GC with electron capture detection (ECD) (Akagi et al. 1995).

There is evidence to suggest that urinary mercury levels are good measures of exposure to inorganic mercury in the environment (Ikingura and Akagi 1996). The primary method used to analyze urine for mercury is CVAAS (Akagi et al. 1995; Friese et al. 1990; Ngim et al. 1988; Oskarsson et al. 1996; Ping

and Dasgupta 1989, 1990; Vesterberg 1991). Methods using AFS (Corns et al. 1994; Vermeir et al. 1991a, 1991b), ASV (Liu et al. 1990), and isotope-dilution spark source (IDSS) MS have also been developed. CVAAS is sensitive (low-ppt), reliable (recovery is >76% and precision is generally <10% relative standard deviation [RSD]), and may be used on either digested or undigested samples (Friese et al. 1990; Ngim et al. 1988; Ping and Dasgupta 1989, 1990). Improved sensitivity (sub-ppt), accuracy (>90% recovery), and precision (7% RSD or better) were obtained with AFS when samples were digested in a closed container in a microwave (Vermeir et al. 1991a, 1991b). Good results have also been achieved with ASV (Liu et al. 1990) and IDSSMS (Moody and Paulsen 1988). The precision of these methods is especially high (<5% RSD), and recoveries with ASV are >90%. Both these methods require predigestion of the sample. As an alternative to CVAAS, total mercury determination in blood and urine can be performed by inductively coupled plasma-atomic emission spectroscopy (ICP-AES) or ICP-mass spectrometry (Buneaux et al. 1992; Kalamegham and Ash 1992). These methods are sensitive, with detection limits in the sub-ppb range. Recoveries (>90%) and precision (<17% coefficient of variation [CV]) are good.

AAS-based methods and NAA have been used to measure mercury in tissues. The AAS methods differ in the way the sample is released for detection. CVAAS is the best-defined of the AAS techniques. Mercury concentrations in the sub- to low-ppb have been reliably determined in tissue samples (Friese et al. 1990; Vermeir et al. 1988, 1989). Best results were obtained when the sample was digested in a closed container in a microwave oven, and the vaporized mercury was preconcentrated on gold-coated sand (Vermeir et al. 1989). Flameless AAS, which uses an electric furnace to atomize the mercury, has yielded high recoveries, but no data are available on the sensitivity or precision of the technique (Ichinose and Miyazawa 1989). Separative column atomizer AAS (SCA-AAS) introduces the mercury to the detector by running the sample through a heat-activated charcoal column (Yanagisawa et al. 1989). Little sample preparation is required, but high background interference is a problem with this method. Good results were reported for tissue samples with sub-ppm mercury concentrations (from control rats), but decreased accuracy and precision occurred in samples containing higher levels (from dosed rats). AFS offers a good alternative to CVAAS. Sensitivity was in the sub-ppt range, and recovery and precision were excellent (Vermeir et al. 1991a, 1991b). In addition, sample preparation is relatively simple and rapid. NAA permits determination of mercury in tissue samples at the sub- to low-ppb level, but erratic accuracy and precision make the method less reliable (Taskaev et al. 1988; Zhuang et al. 1989). An extraction method using zinc diethyldithiocarbamate produced good results with NAA (Zhuang et al. 1989). GC equipped with a flame ionization detector (FID) has also been used to detect methylmercury in tissues at ng levels (Baldi and Filippelli 1991). Recovery and precision data were not reported.

Studies have indicated that the mercury concentration in the hair correlates well with dietary mercury exposure (Inasmasu et al. 1986; Wilhelm and Idel 1996). Methylmercury is the primary dietary mercury contaminant and is present in large amounts in seafood (Ikingura and Akagi 1996). Most of the mercury measured in hair is methylmercury; hair is a good matrix for assessing exposure to methylmercury (Wilhelm and Idel 1996). Hair analysis has been conducted using CVAAS, AFS, and NAA (Grandjean et al. 1992; Ngim et al. 1988; Pineau et al. 1990; Suo et al. 1992; Suzuki et al. 1992; Taskaev et al. 1988; Vermeir et al. 1991a, 1991b; Zhuang et al. 1989). Segmental hair analysis is commonly used as a means of determining an historical record of exposure or uptake of mercury (Grandjean et al. 1992; Suzuki et al. 1992). The method involves cutting the hair strands into smaller segments, usually 1 cm each, and analyzing each segment separately. Detection limits for hair using CVAAS were not reported but are expected to be similar to those for tissue (sub- to low-ppb). The sensitivity of NAA is similar to that of CVAAS, but variable recoveries and precision make NAA less reliable. Good results were reported for one NAA method (Zhuang et al. 1989). Results from studies using AFS suggest this method may be the most sensitive and reliable technique (Suo et al. 1992; Vermeir et al. 1991a, 1991b). A detection limit in the sub-ppt range was obtained, and precision and accuracy were both excellent.

An X-ray fluorescence (XRF) technique has been used to measure mercury in the wrist and temporal areas of dentists exposed to various heavy metals in the work place (Bloch and Shapiro 1986). This technique allows simultaneous evaluation of the tissue burden of a number of different metals. Bone levels may be more closely related to long-term exposure than levels in blood, urine, and hair. The detection limit for XRF is in the low ppm.

A method for detecting methylmercury in biological samples by its enzymatic conversion to methane is an alternative biological technique for methylmercury or other organomercurial analyses (Baldi and Filippelli 1991). *Pseudomonas putida* strain FB1, a broad spectrum mercury-resistant strain, is able to enzymatically convert methylmercury to Hg^0 and methane either in whole cell or in cell-free extracts. GC/FID was used to determine methane produced by the biological derivatization of methylmercury. The detection limit was 15 ng of methylmercury extracted from 1 g of biological tissue. The coefficient of variation was 1.9%. Chemical interferences are negligible in the enzymatic determination of methyl-mercury. The specificity of this determination places the method among the most reliable ones. Recovery was not reported.

6.2 ENVIRONMENTAL SAMPLES

Mercury levels have been determined in numerous environmental matrices, including air, water (surface water, drinking water, groundwater, sea water, and industrial effluents), soils and sediments, fish and shellfish, foods, pharmaceuticals, and pesticides. The sample preparation varies with the complexity of the matrix, but most complex samples require decomposition of the matrix and reduction of the mercury to its elemental form. As described Section 6.1 for biological samples, special sample preparation methods need to be employed if inorganic and organic mercury are to be determined separately, or if the individual species of the organic mercury fraction are to be determined. More detailed information on selected methods in various environmental samples is given in Table 6-2.

Both CVAAS and CVAFS have been used to monitor air and suspended particulates in air for mercury (Baeyens and Leermakers 1989; Bloom and Fitzgerald 1988; Friese et al. 1990; NIOSH 1994; Paudyn and Van Loon 1986; Sengar et al. 1990; Stockwell et al. 1991; Temmerman et al. 1990). Both methods are sensitive, accurate, and precise, although slightly greater sensitivity was reported with AFS (low ppt) than with AAS (mid ppt); AFS is becoming a more common method of analysis (Horvat 1996). When AAS or AFS was combined with gas chromatography (GC), the different mercury species (inorganic mercury, dimethylmercury, diethylmercury, and methylmercury chloride) present in the air could be separated (Bloom and Fitzgerald 1988; Paudyn and Van Loon 1986). A colorimetric method, based on the formation of a colored complex formed in the presence of mercury, has been used as a quick and simple field test that can detect mercury present at the mid-ppb level (Cherian and Gupta 1990).

Numerous methods, including CVAAS, ASV, inductively coupled plasma (ICP) MS, ICP atomic emission spectrometry (AES), microwave-induced plasma (MIP) AES, NAA, GC/AFS, high-performance liquid chromatography (HPLC)/UV, HPLC/ECD, and spectrophotometry, have been used to determine mercury levels in aqueous media. Mercury has been measured in drinking water, surface water, groundwater, snow, waste water effluents, and sea water. Of the available methods, CVAAS is the method of choice (Baxter and Frech 1989, 1990; Birnie 1988; Eaton et al. 1995; Goto et al. 1988; Lee et al. 1989; Mateo et al. 1988; Munaf et al. 1991; Paudyn and Van Loon 1986; Ping and Dasgupta 1989; Robinson and Schuman 1989; Schintu et al. 1989; Shkinev et al. 1989) and the method recommended by EPA and AOAC (AOAC 1984; Beckert et al. 1990; EPA 1994f, 1994g). This method is very sensitive for mercury in water (sub- to low-ppt) and has been proven to be reliable. Water samples generally do not require digestion, but mercury in the samples is usually reduced to the elemental state and preconcentrated prior to analysis. When combined

Table 6-2. Analytical Methods for Determining Mercury in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (elemental)	Drawing of air through Hopcalite sorbent tube, dissolution of sorbent with HNO_3 then HCl, dilution, addition of stannous chloride	CVAAS	3 µg/m^3	100 (4.6% RSD at 0.9 µg)	NIOSH 1994 (Method 6009)
Air (methyl mercury)	Passage of air through a Tenax column, thermal desportion.	GC/AAS	0.1 ng/m^3 (methyl mercury)	No data	Paudyn and Van Loon 1986
Air (elemental mercury)	Preconcentration of mercury in sample by collection onto gold-coated sand absorber; thermal desorption and collection onto second absorber; desorb to detector	CVAFS	<1 ng/m^3	105–111	Temmerman et al. 1990
Air (methyl, dimethyl mercury)	Preconcentration of sample onto graphitized carbon substrate; separation by cryogenic gas chromatography	GC/CVAFS	0.3 pg (mercury, dimethyl mercury); 0.4 pg (diethyl mercury); 2 pg (methyl mercury chloride)	91–105	Bloom and Fitzgerald 1988
Air	Collection of sample onto gold-coated quartz wool; thermal desorption	CVAAS	0.08 ng	97–101	Friese et al. 1990
Water (total)	Addition of H_2SO_4/HNO_3 and $KMNO_4$, equilibrate, addition of $K_2S_2O_8$ and heating; addition of hydroxylamine, reduction to elemental mercury using stannous chloride, purging of sample	CVAAS	<1 µg/L (1 ppb)	79–92 (9–23% RSD)	Eaton et al. 1995 (Standard Method 3112B/3500B)
Water	Addition of permanganate and sulfuric acid and heating; addition of $K_2S_2O_8$ and hydroxylamine; extraction with dithizone	Spectro-photometry at 492 nm	2 µg/L (2 ppb)	95 at 250 µg/L	Eaton et al. 1995 (Standard Method 3500C)
Water (inorganic mercury)	Reduction of sample with $SnCl_2$ in HNO_3; purging of mercury to detector	CVAAS	0.1 ng/L	99	Lee et al. 1989
Water, sea water (inorganic)	Reduction of mercury in sample with $SnCl_2$; preconcentration onto platinum-lined graphite tube	GFAAS	<2 ng/L	94–102	Baxter and French 1989

Table 6-2. Analytical Methods for Determining Mercury in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water, waste water	Oxidation of organic Hg to inorganic Hg by $KMnO_4$, $K_2S_2O_8$ and heat; reduction to elemental state with stannous ion	AAS (flameless)	No data	No data	AOAC 1984 (methods 32.095 to 33.099)
Water (total)	Digestion of sample with HNO_3/H_2SO_4 plus permanganate and persulfate solutions; reduction with hydroxylamine; purging to detector	CVAAS	0.1 mg/L	101–112	Beckert et al. 1990; EPA 1994f (method 7470a)
Water and snow	Extraction of organic mercury immediately after sampling; addition of organic mercury standards; addition of KB and a benzene/ toluene mixture; isolation and volume reduction of organic layer	GC/AAS	4 ng (dimethyl mercury); 5 ng (ethyl mercury)	No data	Paudyn and Van Loon 1986
Drinking water	Collection of sample in quartz ampoule and evaporation followed by irradiation; precipitation of Hg as sulfide; isolation of precipitate and dissolution in aqua regia; counting of ^{203}Hg	NAA	45 µg/L	95–107	Itawi et al. 1990
Water (total)	Digestion with $HNO_3/HClO_4$ and heat; volume reduction; addition of NH_4Cl/ ammonium solution; dilution with water	ASV	No data	100–105	Liu et al. 1990
Surface water	Acidification of sample with HNO_3; addition of ^{199}Hg; oxidization with potassium permanganate solution; reduction with sodium borohydride; purging to plasma	ICP/IDMS	ng/L	86–98	Haraldsson et al. 1989
Drinking water	Direct injection	DIN-ICPMS PN-ICPMS	30–40 ng/L	No data	Powell et al. 1992
Drinking water and groundwater	Separation of mercury species in sample on HPLC column using buffered methanol as eluent	HPLC/ECD	≈1.8 µg/L	77–104	Evans and McKee 1988

Table 6-2. Analytical Methods for Determining Mercury in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water, sea water	Reduction of sample with $SnCl_2$ in H_2SO_4; oxidation with $KMnO_4$; addition of Cd(IV), sodium arsenite, iodide, and H_2SO_4 to thermometric cell; inject sample	Kinetic thermometry	≈2 µg/L	No data	Mateo et al. 1988
Waste water (total)	Digestion of sample with HNO_3/H_2O_2 and heat; cooling and adjustment of pH to 6; equilibration with polyacrylamidoxime; stripping of Hg from resin by equilibration with HNO_3; filtration	ICP/AES	1.15 µg/L	96–98	Mahanti 1990
Waste water	Addition of HNO_3 to sample and evaporation; redissolution in water (pH should be ≈2); separation on ion chromatography column using $TPPS_4$/PAR/NaCl/$Na_2B_4O_7$-NaOH as post-column derivatization agent	Spectro-photometry	50 µg/L	No data	Yan et al. 1989
Industrial effluents	Concentration of sample by heating; digestion with concentrated H_2SO_4; dilution; addition of sodium salicylate to aqueous sample; adjustment of pH to 5.5–5.8; extraction with triphenylphosphine oxide solution; for high levels of Hg (mg), back-extraction with acetate buffer and analysis; for low levels of Hg (µg), addition of sodium hydrogen carbonate buffer and PAN; isolation of organic layer; dilution with toluene and analysis	Spectro-photometry	No data	99–119	Raman and Shinde 1990
Soil (total)	None	XRF	10 µg/g	No data	Grupp et al. 1989
Soil (total)	Digestion of sample with HCl/HNO_3 with heat in closed Teflon vessel in microwave; dilution; reduction with $SnCl_2$ and hydroxylammonium chloride in H_2SO_4; purging to detector	CVAAS	No data	90–110	Van Delft and Vos 1988

Table 6-2. Analytical Methods for Determining Mercury in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil, sediment, sludge (total)	Digestion of sample with aqua regia and permanganate in steam bath or with HNO_3/H_2SO_4 and permanganate in autoclave; reduction with hydroxylamine; purging to detector	CVAAS	0.1 mg/L	84–101	Beckert et al. 1990; EPA 1994g (method 7471a)
Soil, sediment (methyl Hg, phenyl Hg)	SFE of spiked sample using CO_2methanol containing diethylammonium diethyldithio-carbamate; dilution with octane, addition of pentylMgBr to form pentyl derivatives, addition of H_2SO_4, extraction of organic phase with water, treatment with anhydrous magnesium sulfate	GC/AED	2.5 ng/mL in extract	106 (methyl) 6.3% RSD; 59 (phenyl) 12% RSD)	Liu et al. 1994
Sediment, mussel (total)	Digestion of sample with concentrated acid; evaporation; redissolution in HNO_3 and dilution with water; reduction of sample with $SnCl_2$ in HNO_3; purging to detector	CVAAS	0.1 ng/L	111 (sediment); 60 (mussel)	Lee et al. 1989
Sediment (total)	Digestion of sample with HCl/HNO_3 and heat in Teflon bomb; oxidation with potassium permanganate solution; reduction with sodium borohydride; purging to plasma	ICP/MS	≈2 ng/g	96	Haraldsson et al. 1989
Solid samples (total)	Introduction of a slurry of sample in nitric acid into FIA system using on-line microwave digestion, mix with tin(II) chloride to form elemental mercury	CV-AFS	0.09 ng/g	84–108 (2.9–4% RSD)	Morales-Rubio et al. 1995
Fish (methyl mercury)	Homogenization of sample; extraction with HCl/KBr/ $CuSO_4$/toluene solution; centrifugation; mixing of organic phase with cysteine and centrifugation; mixing of aqueous phase with HCl/KBr/ $CuSO_4$/toluene solution; centrifugation; drying of organic phase over anhydrous Na_2SO_4	GC/ECD	50 ng/g (methyl mercury)	89–111	Ahmed et al. 1988

Table 6-2. Analytical Methods for Determining Mercury in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish	Drying of visceral parts of fish in oven; digestion of sample with concentrated H_2SO_4; dilution and filtration of digest; addition of sodium salicylate to aqueous sample; adjustment of pH to 5.5–5.8; extraction with triphenylphosphine oxide solution; for high levels of Hg (mg), back-extraction with acetate buffer and analysis; for low levels of Hg (µg), addition sodium hydrogen carbonate buffer and PAN; isolation of organic layer; dilution with toluene and analysis	Spectro-photometry	No data	99–119	Raman and Shinde 1990
Fish and plant materials	Preparation of sample condensate; dilution with HNO_3; addition of Ag and co-precipi-tatation of Ag and Hg with H_2S; preparation of sample electrode	IDSSMS	0.1 µg/g	95	Moody and Paulsen 1988
Fish and shell fish (methyl mercury)	Homogenization of sample; removal of organics by washing with acetone and benzene; addition of HCl to release protein-bound methyl mercury and extraction into benzene; analysis for methyl mercury chloride	GC/ECD	No data	No data	AOAC 1984 (methods 25.146–25.157)
Fish (total)	Digestion of samples with nitric acid in a microwave acid digestion bomb, reduction to elemental mercury	CVAAS	0.195 ng/mL	>95	Navarro et al. 1992
Fish muscle (total)	Digestion of sample with H_2SO_4-HNO_3	AAS (flameless)	No data	No data	AOAC 1984 (methods 25.134–25.137)
Oyster tissue, milk powder, wheat flour (total)	Digestion of sample with HNO_3 and heat in closed container in microwave over; reduction with $SnCl_2$ and TBP; purging of mercury to gold-coated sand absorber to preconcentrate; desorption to detector	CVAAS	0.84 ng/g	93–111	Vermeir et al. 1989

Table 6-2. Analytical Methods for Determining Mercury in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Wheat flour, citrus leaves, pine needles (total)	Digestion of sample using $K_2Cr_2O_7$ and H_2SO_4, heating and dilution	CVAAS	6 ng/g	91–108	Landi et al. 1990
Milk powder, oyster tissue (total)	Digestion of sample with HNO_3 in closed vessel in microwave; dilution with water; reduction with $SnCl_2$; purging to detector	AFS	0.9 ng/L	94–102	Vermeir et al. 1991a, 1991b
Food (total)	Digestion of 5 g of sample with HNO_3-$HClO_4$	AAS (flameless)	No data	No data	AOAC 1984 (methods 25.131–25.133)
Food (total)	Digestion of sample with HNO_3 and H_2SO_4 under reflux; isolation of mercury by dithizone extraction	Colorimetric dithizone method	No data	No data	AOAC 1984 (methods 25.138–25.145)
Wine (total)	Digestion of sample with concentrated HNO_3 and chromic acid; addition of hydroxylamine chlorhydrate to cold flask; transferring to mercury/hydride generator; addition of $SnCl_2$ in H_2SO_4 to reduce; purging to detector	AAS (flameless)	6 µg/L	95–107	Cacho and Castells 1989
Pharmaceuticals	Extraction of mercury with *N*-phenylcinnamohydroxamic acid; measurement of absorbance at 390 nm	Spectrophotometry	No data	No data	Agrawal and Desai 1985
Pharmaceuticals	Removal of lipids from greasy or soapy samples with diethyl ether; digestion of sample with $KMnO_4/HNO_3$; removal of excess permanganate with sodium oxalate; adjustment of pH to alkaline with ammonium chloride/ ammonia buffer and H_2O_2 ; filtration; heating and dilution; titration of mercury with 4,4'-dihydroxybenzophenone	Spectrophotometry	2 µg/L	95–111	Marquez et al. 1988

Table 6-2. Analytical Methods for Determining Mercury in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Cigarettes	Digestion by heating with concentrated H_2SO_4; dilutition and filtration of digest; addition of sodium salicylate to sample; adjustment of pH to 5.5–5.8; extraction with triphenylphosphine oxide solution; for high mercury (mg), back-extraction with acetate buffer and analysis; for low mercury (µg), addition of sodium hydrogen carbonate buffer and PAN; isolation of organic layer; dilution with toluene	Spectro-photometry	No data	99–119	Raman and Shinde 1990

AAS = atomic absorption spectrometry; AED = atomic emission detection; AES = atomic emission spectrometry; AFS = atomic fluorescence spectrometry; Ag = silver; ASV = anodic stripping voltammetry; Cd = cadmium; $CuSO_4$ = copper sulfate; CVAAS = cold vapor atomic absorption spectrometry; CVAFS = cold-vapor atomic fluorescence spectrometry; DIN-ICPMS = direct injection nebulizer inductively coupled plasma mass spectrometry; ECD = electron capture detection or electrochemical detector; GC = gas chromatography; GFAAS = graphite furnace atomic absorption spectrometry; HCl = hydrochloric acid; $HClO_3$ = perchlorous acid; $HClO_4$ = perchloric acid; Hg = mercury; HNO_3 = nitric acid; HPLC = high-performance liquid chromatography; H_2O_2 = hydrogen peroxide; H_2S = hydrogen sulfide; H_2SO_4 = sulfuric acid; ICP = inductively coupled plasma; IDMS = isotope-dilution mass spectrometry; IDSSMS = isotope-dilution spark-source mass spectrometry; KB = potassium boride; KBr = potassium bromide; $K_2Cr_2O_7$ = potassium chromate; $KMnO_4$ = potassium permanganate; $K_2S_2O_8$ = potassium sulfhydrate; MS = mass spectrometry; NAA = neutron-activation analysis; $Na_2B_4O_7$ = sodium borohydrate; NaOH = sodium hydroxide; Na_2SO_4 = sodium sulfate; NaCl = sodium chloride; NH_4Cl = ammonium chloride; PAN = 1-(2-pyridylazo)-2-naphthol; PAR = 4-(2-pyridylazo)resorcinol; PN-ICPMS = pneumatic nebulizer inductively coupled plasma mass spectrometry; $SnCl_2$ = tin(II) chloride; TBP = tri-*n*-butyl-phosphate; $TPPS_4$ = meso-tetra(4-sulfonatophenyl)porphyrin; XRF = x-ray fluorescence

with GC, CVAAS has been used to separate and determine individual mercury species in aqueous samples (Paudyn and Van Loon 1986). Spectrophotometry has often been used to determine mercury in aqueous matrices (Abbas et al. 1989; Ajmal et al. 1989; Eaton et al. 1995; Raman and Shinde 1990; Singh et al. 1989). Sample preparation methods vary and have included separation by thin-layer chromatography (TLC) (Ajmal et al. 1989) or column chromatography (Yan et al. 1989), selective extraction (Abbas et al. 1989), and ligand formation (Raman and Shinde 1990; Singh et al. 1989). While recoveries were good, spectrophotometry is not as sensitive a technique as CVAAS. Tests of additional methods, including ASV (Liu et al. 1990), ICP/MS (Haraldsson et al. 1989), NAA (Itawi et al. 1990), AES-based techniques (Kitagawa and Nishimoto 1989; Mahanti 1990; Nakahara et al. 1988), HPLC-based techniques (Evans and McKee 1988; Shofstahl and Hardy 1990), and graphite-furnace (GF) AAS (LeBihan and Cabon 1990) indicate that these methods may also be useful for determining mercury in water samples. One of the most promising methods is GC/AFS, which has the advantages of increased sensitivity and precision compared to CVAAS and can also be used to isolate individual mercury species (Bloom 1989). A colorimetric assay has also been developed that is useful for rapid preliminary screening of field samples (Cherian and Gupta 1990).

CVAAS is the most commonly used technique for determining the mercury concentration of sediments, soils, and sludge (Bandyopadhyay and Das 1989; Beckert et al. 1990; EPA 1994g; Van Delft and Vos 1988). As with other matrices, it is sensitive, reliable, and requires little sample preparation beyond digestion of the matrix and reduction of the mercury to its elemental form. It is the method recommended by EPA for solid matrices (Beckert et al. 1990; EPA 1994g). A method based on CVAFS that uses flow injection analysis with on-line microwave digestion for the determination of total mercury has been described recently (Morales-Rubio et al. 1995). Good sensitivity (90 ppt) and precision (4% RSD) was demonstrated. Gas chromatography in conjunction with atomic emission detection (GC/AED) has been used to determine organomercury species in soils and sediments (Liu et al. 1994). Direct current ASV (DCASV) has been tested for use in determining mercury levels in river sediment (Lexa and Stulik 1989). The accuracy and sensitivity of this method are good, but it is less precise than CVAAS. A field method using XRF has been developed to monitor soil contamination (Grupp et al. 1989). This method is rapid and portable, but its high detection limit (low-ppm) makes it useful only for on-site screening.

Methods have been developed for the determination of mercury in fish, shellfish, foods, food sources, and pharmaceuticals. AAS, usually with cold vapor generation (CVAAS), is one of the primary methods used to measure mercury in these complex matrices (Carrillo et al. 1986; Friese et al. 1990; Landi et al. 1990;

Navarro et al. 1992; Odukoya 1990; Vermeir et al. 1988, 1989), because of its sensitivity and reliability. Although the sensitivity (sub- to low-ppb), accuracy, and precision are not as good as with less complex gaseous and aqueous media, it is still one of the best methods available for analysis of mercury in any matrix. Flameless AAS without cold vapor generation has also produced good results when used to determine ppb levels of mercury in wine (Cacho and Castells 1989) and fish (Filippelli 1987); it is also one of the methods recommended by AOAC for fish and food (AOAC 1984). When combined with high resolution GC (HRGC), the individual organic mercury species in fish could be determined (Jiang et al. 1989). Sub-ppt levels of mercury in powdered milk and oyster tissue were reliably determined using AFS (Vermeir et al. 1991a, 1991b). NAA was used to measure mercury levels in copepod homogenate and tomato leaves, but the sensitivity (mid- to low-ppb) and reliability were not as good as that of CVAAS or AFS (Taskaev et al. 1988; Zhuang et al. 1989). Several other methods, including IDSSMS (Moody and Paulsen 1988), HPLC/ICP/MS (Bushee 1988), square-wave voltametry (ASV) (Mannino et al. 1990), ASV (Golimowski and Gustavsson 1983), MIP/AES (Natajaran 1988), GC/ECD (Ahmed et al. 1988; AOAC 1984), and spectrophotometry (Agrawal and Desai 1985; Marquez et al. 1988) have also been used to analyze fish, plant material, and pharmaceuticals for mercury. HPLC/ICP/MS has the additional advantage of permitting separation and quantitation of individual mercury species (Bushee 1988). An AOAC-recommended colorimetric method is available for screening food samples (AOAC 1984).

Several other environmental matrices have been analyzed for mercury content. These include coal fly ash (Horvat and Lupsina 1991; Lexa and Stulik 1989), coal dust (Wankhade and Garg 1989), minerals (Bichler 1991), pesticides (Sharma and Singh 1989), gasoline (Costanzo and Barry 1988), and oily waste (Campbell and Kanert 1992). The methods used include CVAAS, DCASV, NAA, spectrophotometry, and GC/alternating current plasma detection (ACPD). The data on each method for each matrix were insufficient for making comparisons.

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 mercury 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 mercury.

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.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. There are reliable methods for detecting and quantifying elemental mercury in human breath, blood, urine, milk, tissues, hair, and bones. The method of choice is CVAAS (Akagi et al. 1995; Friese et al. 1990; Pineau et al. 1990; Ping and Dasgupta 1989, 1990; Rathje et al. 1974; Vermeir et al. 1988, 1989; Vesterberg 1991). Other methods that have produced good results include ETAAS (Emteborg et al. 1992), AFS (Corns et al. 1994; Vermeir et al. 1991a, 1991b; Suo et al. 1992), flameless AAS (NIOSH 1994), IDSSMS (Moody and Paulsen 1988), XRF (Bloch and Shapiro 1986), NAA (Fung et al. 1995; Zhuang et al. 1989), GC/MPD (Bulska et al. 1992), ICP-AES (Buneaux et al. 1992), and ICP-MS (Kalamegham and Ash 1992). Using these methods, mercury levels at μg to pg concentrations are detectable. This makes them useful for measuring background and higher levels (Ikingura and Agaki 1996). Many of the methods can also distinguish between organic and inorganic mercury. No further methods for analysis of elemental mercury in biological fluids and tissues are needed. Additional research will be needed to validate the determination of individual mercury species (i.e., methylmercury, phenyl mercury, mercury acetate, etc.) in matrices determined to be important. Methods exist for the separation and detection of these species, but few standard reference materials exist for comparative studies.

Biochemical indicators of possible renal dysfunction (increased urinary NAG levels, and elevated porphyrins) have been associated with increased urinary levels of mercury (Rosenman et al. 1986; Wada et al. 1969; Woods 1996). Functional indicators of adverse neurological effects (reduced nerve conduction velocity, prolonged nerve latency, increased tremor frequency, increased reaction time, reduced hand-eye coordination, and performance on memory and verbal intelligence tests) have also been correlated with increased urinary levels of mercury (Levine et al. 1982; Piikivi et al. 1984; Smith et al. 1970, 1983; Verberk et al. 1986; Vroom and Greer 1972; Williamson et al. 1982). Decreased nerve conduction velocity has been correlated with increased tissue levels of mercury (Shapiro et al. 1982). These biomarkers are not specific for mercury and may be induced by exposure to other metals and chemicals or to disease conditions. Other

nonspecific indicators of possible mercury exposure (insomnia, emotional instability, paresthesia, and abnormal EEG) that have been observed in exposed individuals cannot be quantified, but an increased incidence in specific populations may be correlated with increased urinary levels of mercury in the population (Davis et al. 1974; Jaffe et al. 1983; McFarland and Reigel 1978). The existing analytical methods that have been discussed for exposure can reliably measure the levels in blood, urine, and tissue at which these effects occur. Standard methods exist to measure the effects that can be quantified. No further methods need to be developed.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. There are analytical methods to detect and measure elemental and organic mercury in air, water, sediment, soil, sludge, foods, plant materials, and other environmental matrices. The methods used include CVAAS (the most commonly used and recommended method) (AOAC 1984; Baxter and Frech 1989; Eaton et al. 1995; EPA 1994f, 1994g; Munaf et al. 1991; Navarro et al. 1992; Paudyn and Van Loon 1986; Ping and Dasgupta 1989), AFS (Bloom 1989; Bloom and Fitzgerald 1988; Morales-Rubio et al. 1995; Vermeir et al. 1991a, 1991b), IDSSMS (Moody and Paulsen 1988), flameless AAS (Cacho and Castells 1989; Filippelli 1987; NIOSH 1994), and several other methods. Several of the methods have been proven reliable and are sensitive enough to measure background levels. Methods also exist to determine individual mercury species (Bloom and Fitzgerald 1988; Liu et al. 1994; Paudyn and Van Loon 1986). No further methods are needed for mercury analysis in environmental samples. Additional work would be required to validate methods for individual organomercury species in particular matrices.

6.3.2 Ongoing Studies

Ongoing studies concerning the detection and measurement of mercury in biological or environmental samples identified through a search of Federal Research in Progress (FEDRIP 1998) are shown in Table 6-3.

Table 6-3. Research on New Methods for the Detection of Mercury

Investigator	Sponsor	Research
RJ Schlager ADA Technologies Englewood, CA	USDoE, Energy Research	Developing a continuous emission monitor for total and organic mercury in stack gases
PC Efthimion EEI Pluckemin, NJ	EPA	Developing a continuous emission monitor for flue gas based on plasma emission using a microwave-powered source
CW Brown Brooks Rand, Ltd. Seattle, WA	DoE	Developing a monitor for methyl mercury based on luminescence
D Mcallister Biode, Inc. Cape Elizabeth, ME	DoE	Developing a simple sensor for use in waste, surface, and groundwater using a shear horizontal acoustic plate mode (SHAPM) sensor, a form of piezoelectric sensor
LG Piper Physical Sciences, Inc., Andover, MA	DoE	Developing a sensor for mercury in exhaust stack effluents from coal burning power plants based on the fluoerescence of merucry excited by active nitrogen
JCMay	FDA	Developing methods for the determination of mercury and trace metals in injectable products based on high performance liquid chromatography in conjunction with ICP-MS

Source: FEDRIP 1998