7. ANALYTICAL METHODS

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

7.1 BIOLOGICAL MATERIALS

The most common analytical procedures for measuring cadmium concentrations in biological samples use the methods of atomic absorption spectroscopy (AAS) and inductively coupled plasma atomic emission spectroscopy (ICP/AES). These basic methods of analysis are well defined and generally accepted for the analysis of cadmium.

Samples are prepared for AAS and ICP/AES methods in a variety of ways. Digestion with nitric acid is most common (Roberts and Clark 1986; Sharma et al. 1982). Cadmium in blood and plasma measured by graphite furnace atomic absorption spectroscopy (GFAAS) facilitated by a wet ashing pretreatment of samples resulted in good accuracy and reproducibility. The sample detection limit using this method was 0.4 μg/L (Roberts and Clark 1986). This method was also precise and highly reproducible in determining cadmium in whole blood, urine, and hair with 99–99.4% recoveries reported (Sharma et al. 1982). The matrix may also be modified with diammonium hydrogen phosphate or other agents such as palladium (Pd)-based modifiers (Moreira et al. 1995). Detection limits as low as 0.1 μg/L with recoveries ranging from 93 to 111% are reported using this technique (Subramanian and Meranger 1981; Subramanian et al. 1983). If the concentration of cadmium in the dissolved sample is below the detection limit, preconcentration techniques, such as chelation and extraction, may be employed (Gross et al. 1976; Sharma et al. 1982). Various ICP methods have been developed for measuring cadmium levels in biological materials. ICP dynamic reaction cell mass spectrometry (ICP-DRC-MS) has been shown to eliminate molybdenum-based polyatomic interferences, resulting in a reduction of observed urine cadmium concentrations, as compared to ICP-MS measurements (Jarrett et al. 2008). Since cadmium is a

ubiquitous element, the risk of contamination during sampling, processing, and analysis must be minimized by strict laboratory procedures (Elinder and Lind 1985). In procedures for micro-determination, all glass and plastic-ware should be acid-washed and subsequently rinsed with double-distilled water.

Current analytical improvements deal primarily with the methods of sample preparation and sample introduction to the analytical systems in order to lower the detection limits or decrease sample analysis time. Various improvements in the methods of extraction, preconcentration, chelation, complexation, and sample introduction have been developed for use with biological media. Detection limits as low as 0.003 µg/L were reported (Espinosa Almendro et al. 1992; Cordero et al. 1994; Jeng et al. 1994; Katskov et al. 1994; Komárek et al. 1991; Ma et al. 1994b; Welz et al. 1991).

The cadmium concentration in biological samples may also be measured by a number of other methods such as radiochemical neutron activation analysis (RNAA). One RNAA procedure involving a rapid two-step solvent extraction was used for determining cadmium in tissue samples (Tandon et al. 1994). Another method to determine cadmium in biological materials is based on the ion-exchange scheme developed by SAMSAHL where cadmium is trapped on an anion exchange resin. With this method, recovery of 98% and a detection limit of 4 µg/kg were reported. The accuracy of the method was estimated by three different approaches: analysis using radiotracers in inactive sample solutions; by analyzing standards, pipetted on filter paper, and processed as samples; and determination by RNAA (Woittiez and Tangonan 1992).

Cadmium concentration in tissue may be measured both *in vivo* (Ellis 1985; Scott and Chettle 1986) and *in vitro* (Lieberman and Kramer 1970) by neutron activation analysis (NAA). Direct *in vivo* assessment of body burden in humans focused on the measurements of cadmium in the kidney and liver by NAA. The detection limits reported are approximately 2 mg cadmium for the total kidney and 1.5 µg/g for the liver (Ellis 1985); 1.9 mg cadmium for the kidney; and 1.3 µg/g for the liver (Scott and Chettle 1986).

X-ray fluorescence is also used for *in vivo* measurement of cadmium in the kidney (Christoffersson et al. 1987; Nilsson and Skerfving 1993; Scott and Chettle 1986; Skerfving and Nilsson 1992). The *in vivo* techniques are used for clinical measurements of individuals occupationally exposed to cadmium. Additional methods applicable to the analysis of cadmium in biological media include ICP/MS (Stroh 1993; Vanhoe et al. 1994), ICP/AES (Cordero et al. 1994; Espinosa Almendro et al. 1992), and high performance liquid chromatography (HPLC) (Chang and Robinson 1993; Steenkamp and Coetzee 1994).

Electrothemal vaporization ICP/MS has been utilized for the analysis of dentin and enamel from teeth (Grünke et al. 1996). Electrochemical methods such as adsorptive cathodic stripping voltametry (ACSV) and potentiometric stripping analysis (PSA) have been applied to hair analysis (Zhang et al. 1993), animal tissues (LaBar and Lamberts 1994), and body fluids (Ostapczuk 1993).

Table 7-1 summarizes some of the methods used for sample preparation and analysis of cadmium in biological samples.

7.2 ENVIRONMENTAL SAMPLES

Analysis for cadmium in environmental samples is usually accomplished by AAS or AES techniques, with samples prepared by digestion with acid, preconcentrated with a chelating resin, or direct aspiration with no preparation (APHA 1977a, 1977b; EPA 1983a, 1983b, 1997b; OSHA 2002a, 2004; USGS 1985). Since cadmium in air is usually associated with particulate matter, standard methods involve collection of air samples on glass fiber or membrane filters, acid extraction of the filters, and subsequent analysis (APHA 1977a, 1977b; OSHA 2002a, 2002b). Inductively-coupled plasma spectrometry (ICP) analysis in standard methods is also popular. ICP analysis for water and air samples can be run in tandem with mass spectrometry (MS) or AES (EPA 1996b, 1997b, 2003; NIOSH 2003; OSHA 2002b). ACSV (Nimmo and Fones 1994), differential pulse anodic stripping voltametry (DP-ASV) (Nam et al. 1994), and epithermal NAA (Landsberger and Wu 1993) have also been used for air analysis. The accuracy of the analysis of cadmium in acid digested atmospheric samples, measured by ACSV, was evaluated and compared with GFAAS and ICP/MS.

Several methods standardized by EPA (1983a, 1983b, 1994b, 1996a, 1996b, 1997b, 2000, 2003) are used for measuring concentrations of cadmium in water. Techniques to compensate for chemical and matrix interferences in all three methods are described by EPA (1983a, 1983b, 1994b, 1996a, 1996b, 1997b, 2000, 2003). After soils and solid wastes are extracted or solubilized by acid digestion, they may be analyzed for cadmium by the same AAS methods that are used for water (EPA 1986d, 1986e). Water can also be analyzed for cadmium by NAA methods (Saleh et al. 1993), PSA methods (Ostapczuk 1993), and anodic stripping voltametry (ASV) (Daih and Huang 1992).

Sediment and soil samples have been analyzed for cadmium using the methods of GFAAS (Klemm and Bombach 1995). Preparation of the samples is generally accomplished by treatment with HCl and HNO_3.

Table 7-1. Analytical Methods for Determining Cadmium in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Digestion with nitric acid; chelation with APDC and extraction with MIBK	AAS	<1 ng/mL[a]	99	Sharma et al. 1982
Blood	Modification of matrix with diammonium hydrogen phosphate/Triton X-100	GFAAS	0.1 µg/L	100.8±4.3	Subramanian and Meranger 1981
Blood/plasma	Digestion with nitric acid; wet ashed	GFAAS	0.4 µg/L	No data	Roberts and Clark 1986
Serum	Dilution with ammonia/Triton X-100	ICP/MS	0.01 ng/mL	No data	Stroh 1993
Tissue and blood	Microwave digestion	FAAS/flow injection system	0.15 µg/L	No data	Welz et al. 1991
Human milk	Dilution with deionized and double distilled water	AAS	<0.01 ppb[a]	No data	Schulte-Lobbert and Bohn 1977
Hair	Digestion with nitric acid	AAS	0.07 µg/g[a]	99	Sharma et al. 1982
Kidney	None (*in vivo*)	XRF	170.1 µg/g	No data	Christoffersson et al. 1987
Kidney/liver	Chelation and extraction with solvent	AAS/direct aspiration	0.01 ppm[a] (liver) 1.9 mg (kidney)	No data	Gross et al. 1976
Kidney/liver	None (*in vivo*)	NAA	1.3 µg/g (liver) 1.9 mg (kidney)	No data	Scott and Chettle 1986
Muscle	Wet ashed with concentrated sulfuric acid	NAA	50 ppb	50–65	Lieberman and Kramer 1970
Urine	Dilution with nitric acid	ETAAS	0.045 µg/L	97–101	Komárek et al. 1991
Urine	Modification of matrix with diammonium hydrogen phosphate/nitric acid	GFAAS	0.09 ng/mL	92.7–111.1	Subramanian et al. 1983
Urine	Digestion with nitric acid	AAS	5.67 ng/mL[a]	99.4	Sharma et al. 1982
Biological materials	Microwave digestion followed by extraction with APTH in MIBK	ICP/AES	0.15 ng/mL	No data	Cordero et al. 1994
Biological materials	Extraction with 1,5-bis(di-2-pyridylmethylene) thiocarbonohydrazide in MIBK	ICP/AES	0.1 ng/mL	No data	Espinosa Almendro et al. 1992

Table 7-1. Analytical Methods for Determining Cadmium in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Biological materials	Digestion with acid	GFAAS/flow injection system	0.003 µg/L	No data	Ma et al. 1994a
Biological fluids (blood, urine)	Acidification	PSA	0.001 µg/kg	No data	Ostapczuk 1993
Biological materials	Dry tissues; irradiation followed by acid digestion	RNAA	4 µg/kg	98	Woittiez et al. 1992
Teeth, dentin, and enalmel	Digested in nitric acid, diluted with water	ETV-ICP-MS PN-ICP-MS	No data	No data	Grünke et al. 1996
Whole blood, urine	Modified with palladium based modifier	ETAAS	0.22 µg/L	No data	Moreira et al. 1995
Biological materials	Digested with nitric acid and hydrogen peroxide	B-9001-95; ICP-AES	No data	93	USGS 1996

[a]Lowest concentration found

AAS = atomic absorption spectroscopy; APDC = ammonium pyrrolidenedithiocarbamate; APTH = 1,3-bis-[1-(2-pyridyl)ethylidene] thiocarbon-hydride; ETAAS = electrothermal atomic absorption spectroscopy; FAAS = flame atomic absorption; GFAAS = graphite furnace atomic absorption; ICP/AES = inductively coupled plasma atomic emission spectroscopy; ICPIMS = inductively coupled plasma mass spectrometry; MIBK = methyl isobutyl ketone; NAA = neutron activation analysis; PSA = potentiometric stripping analysis; RNAA = radio chemical neutron activation analysis; XRF = x-ray fluorescence

The most common method for analysis of cadmium in foods is AAS (Bruhn and Franke 1976; Dabeka 1979; Muys 1984), with GFAAS being one of the most common AAS methods used (Cabrera et al. 1995). The FDA's Total Diet Study 1991–1996 analyzed cadmium and other element concentrations in food by dry ash mineralization and GFAAS (Capar and Cunningham 2000). RNAA (Greenberg et al. 1979), differential pulse ASV (Satzger et al. 1982, 1984), and the calorimetric dithizone method (AOAC 1984) may also be employed. The AAS techniques appear to be most sensitive, with recoveries ranging from 94 to 109% (Bruhn and Franke 1976; Muys 1984). A method used to isolate cadmium by first extracting with bismuth diethyldithiocarbamate ($Bi[DDC]_3$) and then with zinc diethyldithiocarbamate ($Zn[DDC]_2$) in chloroform and then measuring by RNAA showed 94–106% recovery (Greenberg et al. 1979).

Table 7-2 summarizes some of the methods used for sample preparation and analysis of cadmium in environmental samples.

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 cadmium 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 cadmium.

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. Measurements of cadmium in liver and kidney are all useful biological indices for human exposure to cadmium (Roels et al. 1981b). Human milk, human placentas, and maternal and neonatal

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on glass fiber filter; ashed with hydrochloric and nitric acids	Method 311; AAS	0.005 µg/m^3	90	APHA 1977b
Air	Collection on membrane filter; ashed with hydrochloric and nitric acids	Method 7048; AAS	0.05 µg per sample	No data	NIOSH 1994
Air	Collection on membrane filter; digestion with nitric acid and perchloric acid	Method 7300; ICP	0.3 ng/mL	99.8–105.2	NIOSH 2003
Air	Collection using filters, wipes, or bulk materials; desorbed with water extractions and mineral acid digestions	Method 121; AAS/AES	0.004 µg/mL	99.5	OSHA 2002a
Air	Collection on membrane filter; digested in nitric acid, sulfuric acid, and hydrogen peroxide	Method 125G; ICP-AES	0.14 µg[a] 0.47 µg[b]	No data	OSHA 2002b
Air	Collection on membrane filter; digested with nitric acid and small amounts of hydrochloric acid	Method 189; AAS/ AAS-HGA	0.2 µg/m^3 (AAS)[a] 0.70 µg/m^3 (AAS)[b] 0.007µg/m^3 (AAS-HGA)[a] 0.025 µg/m^3 (AAS-HGA)[b]	No data	OSHA 2004
Air	Collection on membrane filter, wipe, or bulk material; digest with nitric and hydrochloric acids	Method 206; ICP-AES	0.0062 µg/mL[a] 0.0205 µg/mL[b]	No data	OSHA 1991
Air	Irradiation UF filters	Epithermal NAA	8 ng	No data	Landsberger et al. 1993
Air (aerosols)	Acid digestion with filters	ACSV	0.6 ng/mL	100	Nimmo and Fones 1994
Atmospheric particles	Direct analysis	ETV-ICP-MS	pg/m^3 range	No data	Lüdke et al. 1997
Water	Digestion with nitric acid	Method 213.1; AAS/direct aspiration	5 µg/L	94±24	EPA 1983a
Water	Digestion with nitric acid	Method 213.2; AAS/ GFAAS	0.1 µg/L	96–99	EPA 1983b

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	On-line preconcentration with ion exchange or sorbent extraction columns	GFAAS/ flow injection system	0.8 ng/L	No data	Welz et al. 1992
Water	Digestion with nitric acid	Method 1637; chelation and GFAAS	0.0075 µg/L	No data	EPA 1996a
Water	Digestion with nitric acid	Method 1638; ICP-MS	0.025 µg/L	No data	EPA 1996b
Water	Preconcentrated with chelating resin	Method 1640; Online Chelation/ ICP-MS	0.0024 µg/L	No data	EPA 1997b
Water	Digested with hydrochloric and nitric acids	Method 200.5; AVICP-AES	0.1 µg/L	98±1.1	EPA 2003
Water and Wastes	Digestion with acids	Method 200.7; ICP-AES	1 µg/L (aqueous); 0.2 mg/kg (solids)	82–98	EPA 1994a
Various	Digestion with nitric and hydrochloric acids	Method 6010C; ICP-AES	No data	97	EPA 2000
Water and sediments	No preconcentration or pretreatment	I-1135; AAS	10 µg/L	No data	USGS 1985
Water	Digested with whole water	I-4471-97; ICP-OES	5 µg/L	No data	USGS 1998a
Various	Direct aspiration with no preconcentration or pretreatment	I-5135; AAS	10 µg/L	No data	USGS 1985
Soil	Digestion with nitric acid	Method 7130; AAS/direct aspiration	0.005 mg/L	No data	EPA 1986e
Soil	Digestion with nitric acid	Method 7131; GFAAS	0.1 µg/L	No data	EPA 1986d
Soil and sediment	Ultrasonic slurry in dilute nitric acid	GFAAS	No data	100±10	Klemm and Bombach 1995

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediment	Digestion with hydrochloric and nitric acid	LEAFS	500 fg	No data	Zhou et al. 1998
Soil and sediment	Digestion with hydrofluoric acid and nitric acid; complexation with DDPA using on-line sorbent extraction system	GFAAS/ flow injection system	0.8 µg/L	No data	Ma et al. 1994b
Food	Dry ashed; oxidization with nitric acid	ASV/ differential pulse	1 ng/g	99–108	Satzger et al. 1984
Food	Dry ashed; complexation with APCD; extraction with isoamyl acetate	AAS	0.1 ng/g	97.5±2.5	Bruhn and Franke 1976
Food	Extraction with $Bi(DDC)_3$ then with $Zn(DDC)_2$ in chloroform	RNAA	0.029 µg/g[c]	94–106	Greenberg et al. 1979
Food (24 hour diet)	Microwave digestion with nitric acid and hydrogen peroxide	GFAAS	0.004 µg/g	94–101	Yang et al. 1995
Food	Dry ashed; complexation with NaDDTC; extraction with IBMK	GFAAS	0.1 ppb[c]	94–109	Muys 1984
Food	Homogenization followed by wet ashing	GFAAS	0.01 ppb	94–108	Zhang et al. 1997
Fruit	Homogenized fruit slurried with zirconia	ETAAS	0.3 ng/g	97.7±0.3	Cabrera et al. 1995

[a]Qualitative detection limit
[b]Quantitative detection limit
[c]Lowest concentration found

AAS = atomic absorption spectroscopy; ACSV = adsorptive cathodic stripping voltametry; APCD = ammonium pyrrolidino carbodithioate; ASV = anodic stripping voltametry; AVICP-AES = axially viewed inductively coupled plasma-atomic emission spectrometry; $Bi(DDC)_3$ = bismuth diethyldithiocarbamate; DDPA = ammonium diethyldithiophosphate; ETV-ICP-MS = electrothermal vaporization inductively coupled plasma mass spectrometry; GFAAS = graphite furnace atomic absorption; HGA = heated graphite atomizer; IBMK = isobutyl methyl ketone; ICP = inductively coupled plasma; LEAFS = laser-excited atomic fluorescence spectrometry; MS = mass spectrometry; NAA = neutron activation analysis; NaDDTC = sodiumdiethyl-dithiocarbomate; OES = optical emission spectroscopy; RNAA = radiochemical neutron activation analysis; $Zn(DDC)_2$ = zinc diethyldithiocarbamate

blood have been investigated as means to determine exposures of women and infants to cadmium (Baranowska 1995; Abadin et al. 1997). Sensitive and selective methods are available for the detection and quantitation of cadmium in these biological materials (Elinder and Lind 1985; Sharma et al. 1982). Improved methods for sample preparation and *in vivo* analysis of liver and kidney content are needed to assist in monitoring environmentally exposed populations.

Effect. Sensitive methods are also available for measuring biological markers of cadmium effect, particularly urine or serum concentration of β2-microglobulin, retinol-binding protein, metallothionein, and creatinine (Kawada et al. 1990; Roels et al. 1989; Topping et al. 1986).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Cadmium is ubiquitous in the environment and does not degrade. It is found in air, water, soil, sediments, and food. Analytical methods exist for the analysis of cadmium in all of these environmental media, and these methods have the sensitivity to measure background levels and detect elevated concentrations due to anthropogenic sources such as hazardous waste sites (EPA 1983a, 1983b, 1994b, 1996a, 1996b, 1997b, 2000, 2003). Additional research to reduce chemical and matrix interferences are needed to improve the speed and accuracy of the analyses.

7.3.2 Ongoing Studies

The National Report on Human Health Exposure to Environmental Chemicals is an ongoing biomonitoring assessment conducted by CDC. This survey measures over 200 chemicals in blood and urine from random samples collected from participants in the National Health and Nutrition Examination Survey (NHANES). The National Exposure Report was last published in 2009 and an update of biomonitoring levels for some of the chemicals, including cadmium, was published in 2012 (CDC 2012).

The information in Table 7-3 was found as a result of a search of the Federal Research in Progress database (FEDRIP 2008).

Table 7-3. Ongoing Analytical Methods Studies on Cadmium

Investigator	Affiliation	Research description	Sponsor
Parker D	University of California	Isotopic dilution methods for probing the bioavailability of trace elements in soils and sediments	U.S. Department of Agriculture
Pierzynski G	Kansas State University	Chemistry, bioavailability, and toxicity of constituents in residuals and residual treated soils	U.S. Department of Agriculture
Schwab AP; Joern B; Johnston C	Purdue University	Chemistry and bioavailability of waste constituents in soils	U.S. Department of Agriculture
Santra S	University of Central Florida	Selective detection of toxic heavy metal ions using highly sensitive quantum dot probes	National Science Foundation
Swain G	Michigan State University	Diamond microelectrode arrays: New materials for the electrochemical detection of aqueous analytes	U.S. Department of Agriculture

Source: FEDRIP 2008