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 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.

6.1 BIOLOGICAL SAMPLES

The most common analytical procedures for measuring cadmium concentrations in biological samples use the methods of atomic absorption spectroscopy (AAS) and atomic emission spectroscopy (AES). In AAS analysis, the sample is heated by a flame or in a furnace until the element atomizes. The ground-state atomic vapor absorbs monochromatic radiation from a source and a photoelectric detector measures the intensity of transmitted radiation (APHA 1989). In AES analysis, the emitted radiation resulting from the thermal energy from a flame or inductively coupled plasma discharge (ICP) is measured. Methods of AAS commonly used for cadmium measurement are flame atomic absorption spectroscopy (FAAS) and graphite furnace (or electrothermal) atomic absorption spectroscopy (GFAAS or ETAAS). The most common AES method used for cadmium analysis is 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 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 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) to solubilize cadmium (Vinas et al. 1997). 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). 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; Salmela and Vuori 1979). In one study, contamination with cadmium was found from micropipette tips; decontamination by acid washing when micro-amounts of cadmium are to be measured is recommended (Salmela and Vuori 1982).

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 (Almendro et al. 1992; Cordero et al. 1994; Jeng et al. 1994; Katskov et al. 1994; Komarek et al. 1991; Ma et al. 1994; 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 neutron activation analysis (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 inductively coupled plasma/mass spectrometry (ICP/MS) (Stroh 1993; Vanhoe et al. 1994) 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 (Gliinke 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 6-1 summarizes some of the methods used for sample preparation and analysis of cadmium in biological samples.

6.2 ENVIRONMENTAL SAMPLES

Analysis for cadmium in environmental samples is usually accomplished by AAS or AES techniques, with samples prepared by digestion with nitric acid (APHA 1989; EPA 1982b, 1983a, 1983b, 1986b, 1986d, 1986e). 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 analysis by AAS (APHA 1977; NIOSH 1984b). Adsorptive cathodic stripping voltametry (ACSV) (Nimmo and Fones 1994), differential pulse anodic stripping voltametry (DP-ASV) (Nam et al. 1994), and epithermal neutron activation analysis (NAA) (Landsberger and Wu 1993) have also been used for air analysis. Electrothermal inductively coupled plasma mass spectrometry (ETV-ICP-MS) has also been used to analyze size classified atmospheric particles for cadmium (Ltidke et al. 1997). The accuracy of the analysis of cadmium in acid digested atmospheric samples, measured by ACSV, was evaluated and compared with graphite furnace atomic absorption spectrometry (GFAAS) and inductively coupled plasma mass spectrometry (ICP-MS). The ASCV limit of detection for cadmium was 0.6 ng/mL, higher than that of GFAAS at 0.3 ng/mL but lower than that of ICP-MS for a l-minute collection period. ACSV has advantages for analysis of low concentrations of cadmium in aerosol acid digest samples (Nimmo and Fones 1994).

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

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	AAS/graphite furnace	0.1 µg/L	100.8±4.3	Subramanian and Meranger 1981
Blood/plasma	Digestion with nitric acid; wet ashed	AAS/graphite furnace	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	17 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	AAS/graphite furnace	0.09 ng/mL	92.7–111.1	Subramanian et al. 1983

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
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	Digestion with acid	GFAAS/flow injection system	0.003 µg/L	No data	Ma et al. 1994
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 enamel	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

[a]Lowest concentration found

AAS = atomic absorption spectroscopy; APDC = ammonium pyrrolidenedithiocarbamate; APTH = 1,5-bis[-(2-pyridyl)ethylidene]thiocarbonhydride; ETAAS = electrothermal atomic absorption spectroscopy; FAAS = flame atomic absorption; GFAAS = graphite furnace atomic absorption; ICP/AES = inductively coupled plasma atomic emission spectroscopy; ICP/MS = 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

Three methods standardized by EPA (1982b, 1983a, 1983b) are generally used for measuring concentrations of cadmium in water. The American Public Health Association (APHA) recommends similar methods for water: AAS/direct aspiration, AAS/graphite furnace technique, and inductively coupled plasma (ICP) (API-IA 1989). In addition, the APHA describes a calorimetric method using dithizone (APHA 1989). The graphite furnace AAS technique has greater sensitivity than the direct aspiration AAS and ICP techniques for cadmium. Techniques to compensate for chemical and matrix interferences in all three methods are described by APHA (1989) and EPA (1982b, 1983a, 1983b). Water analyzed by acid digestion and measured by the AAS/direct aspiration, AAS/furnace techniques or ICP/atomic emission method resulted in recoveries ranging from 90 to 110% (EPA 1982b, 1983a, 1983b). 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 laser-excited atomic fluorescence spectroscopy in a graphite furnace (LEAFS) (Zhau et al. 1998), GFAAS (Klemm and Bombach 1995), and ETAAS (Das and Chakraborty 1997). Preparation of the samples is generally accomplished by treatment with HCl and HNO_3.

The most common method for analysis of cadmium in foods is AAS (Bruhn and Franke 1976; Dabeka 1979; Muys 1984), with GFAAS (ETAAS) being one of the most common AAS methods used (Cabrera et al. 1995; Yang et al. 1995; Zhang et al. 1997). Electrothermal vaporization isotope dilution inductively coupled plasma mass spectrometry (ETV-ID-ICP-MS) has been utilized for the analysis of fish samples (Li and Jiang 1998). Radiochemical neutron activation analysis (RNAA) (Greenberg et al. 1979; Dermelj et al. 1996), differential pulse anodic stripping voltametry (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],) and then with zinc diethyldithiocarbamate ($Zn[DDC]_2$) in chloroform and then measuring by RNAA showed 94-106% recovery (Greenberg et al. 1979).

Table 6-2 summarizes some of the methods used for sample preparation and analysis of cadmium in environmental samples.

Table 6-2. 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 1977
Air	Collection on membrane filter; ashed with hydrochloric and nitric acids	Method 7048; AAS	0.05 µg per sample	No data	NIOSH 1984b
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	0.005 mg/L	97.8 at 0.071 mg/L	EPA 1983a
Water	Digestion with nitric acid	Method 213.2; AAS/furnance technique	0.01 µg/L	96–99	EPA 1983b
Water	Digestion with nitric acid	Method 200.7; ICP/atomic emission	4 µg/L	90–110	EPA 1982b
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
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; AAS/furnance technique	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
Sediment	Digestion with hydrochloric and nitric acid	LEAFS	500 fg	No data	Zhou et al. 1998

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
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. 1994
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 µg/kg	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 [a]	94–106	Greenberg et al. 1979
Food (24 hr 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	AAS/graphite furnace	0.1 ppb [a]	94–109	Muys 1984
Food	Homogenization followed by wet ashing	GFAAS	0.01 ppb	94–108	Zhang et al. 1997
Food	Homogenization and placement in vials	NAA	No data	No data	Dermelj et al. 1996
Fruit	Homogenized fruit slurried with zirconia spheres	ETAAS	0.3 ng/g	97.7± 0.3	Cabrera et al. 1995

[a]Lowest concentration found

AAS = atomic absorption spectroscopy; ACSV = adsorptive cathodic stripping voltametry; APCD = ammonium pyrrolidino carbodithioate;ASV = anodic stripping voltametry; $Bi(DDC)_3$ = bismuth diethyldithiocarbamate; DDPA = ammonium diethyldithiophosphate; GFAAS = graphite furnace atomic absorption; IBMK = isobutyl methyl ketone; ICP = inductively coupled plasma; NAA = neutron activation analysis; NaDDTC = sodium-diethyl-dithiocarbomate; RNAA = radiochemical neutron activation analysis; $Zn(DDC)_2$ = zinc diethyldithiocarbamate; LEAFS = Laser-excited atomic fluorescence spectrometry; ETV-ICP-MS = electrothermal vaporization inductively coupled plasma mass spectrometry

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 cadmium 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 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.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Measurements of cadmium in blood, urine, liver, hair, and kidney are all useful biological indices for human exposure to cadmium (Roels et al. 1981b; Granereo et a1.1998; Wasiak and Ciszewska 1995). Human milk, human placentas, and maternal and neonatal blood have been investigated as means to determine exposures of women and infants to cadmium (Baranowska 1995; Abadin et al. 1997). Dentin and enamel from children's teeth have also been analyzed to assess exposure (Grüke et al. 1996). 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.

Sensitive methods are also available for measuring biological markers of cadmium effect, particularly urine or serum concentration of P,-microglobulin, retinol-binding protein, metallothionein, and creatinine (Kawada et al. 1990; Roels et al. 1989; Topping et al. 1986). Additional studies to establish background levels of these indicators in unexposed populations are needed to evaluate the sensitivity of these biomarkers of effect.

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 (APHA 1989; EPA 1982b, 1983a, 1983b, 1986b, 1986d, 1986e). Additional research to reduce chemical and matrix interferences are needed to improve the speed and accuracy of the analyses.

6.3.2 Ongoing Studies

The EPA is conducting a pilot program for comprehensive monitoring of human exposure.

The National Human Exposure Assessment Study (NHEXAS) is being conducted in three regions of the United States in order to establish relationships between environmental concentrations, exposure, dose, and health response and to determine the incidence and causes of high exposures, especially for biologically susceptible persons. One of the aims of the pilot study is to test measurement methodology for a variety of pollutants, including cadmium, in food, air, and water. As an adjunct to this pilot study, the EPA and the State of Minnesota are conducting a study of children's exposure to toxic chemicals, including cadmium.