7. ANALYTICAL METHODS

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

Methods used for the analysis of beryllium in biological materials are reported in Table 7-1. Reviews of beryllium analysis methods in biological media have been published (Delves 1981; Tsalev and Zaprianov 1984). Although the level of beryllium in urine may be informative of the current exposure level, it is not useful for quantitative exposure analysis. In contrast, the level of beryllium in blood, serum, or plasma is predictive of the intensity of current exposure from certain beryllium compounds (Tsalev and Zaprianov 1984; see Section 3.4.2). Neither flame atomic absorption spectroscopy (AAS) nor atomic emission spectroscopy (AES) have adequate sensitivity for measuring beryllium concentrations found in body fluids and tissues. The determination of beryllium levels in these matrices using the preceding techniques requires elimination of spectral interferences. Graphite furnace (or electrothermal) atomic absorption spectroscopy with background correction (deuterium or Zeeman effect) and inductively coupled plasma atomic emission spectroscopy (ICP-AES) are common analytical methods for beryllium. These techniques have the sensitivity and accuracy to determine the content of beryllium in body fluids and tissues (Delves 1981). To avoid sample contamination, stainless steel needles should be avoided for the collection of whole blood samples. Certain polyethylene sample collection tubes with added heparin as an anticoagulant may contaminate whole blood samples (Paudyn et al. 1989). A gas chromatographic method to detect beryllium in whole blood down to a concentration level of 0.02 µg/mL is available (Taylor and Arnold 1971). Krachler et al. (1999b) measured beryllium in umbilical cord serum, colostrum, and maternal serum at concentrations to <1 µg/L using inductively coupled plasma mass

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Add EDTA; acidify	GC/EC	~20 µg/L	44–117%	Taylor and Arnold 1971
Blood (dog)	Add sodium hydroxide; dissolve by heating; chelate with tri-fluoroacetylacetone; extract with benzene	GC/EC	No data	95–117%	Frame and Ford 1974
Urine	Dilute with nitric acid	ICP-MS	0.1 µg/L	No data	Paschal et al. 1998
Urine	Acidified urine precipitated with excess ammonium hydroxide; centrifuge, dissolve in nitric acid and add lanthanum	GFAAS	0.01 µg/L	94–110%	Hurlburt 1978
Urine	Dilute with a matrix modifier	GFAAS	0.05 µg/L	107%; 94–98%	Paschal and Bailey 1986; Shan et al. 1989
Urine (human and rat)	Add EDTA to aqueous sample; adjust to pH 6; add trifluoro-acetylacetone in benzene; extract	GC/EC	1 µg/L	68–123%	Foreman et al. 1970
Feces	Digest; dry ash; dissolve residue in acid	GFAAS	1 µg/kg; ~2 µg/kg	108%; 90–110%	Delves 1981; Hurlburt 1978
Fingernails	Acid digestion; dry ash; dissolve in acid	GFAAS	~2 µg/kg	90–110%	Delves 1981
Hair-fingernails	Dissolve in nitric acid-perchloric acid (1:1)	GFAAS	<1 µg/kg	98–105%	Hurlburt 1978

Table 7-1. Analytical Methods for Determining Beryllium in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Liver (bovine)	Wet-ash tissue in a mixture of acids; chelate with acetylacteone; extract (chloroform); acidify chelate with 2-hydroxy-3-naphthoic acid reagent	Fluorescence spectroscopy	No data	No data	IARC 1980
Lung tissue	Sample subjected to dry or wet ashing	ICP-AES	0.075 mg/kg	No data	Martinsen and Thomassen 1986
Lung tissue	Dry; acid digestion; dilute in acid; standard addition	GFAAS	No data	No data	Baumgardt et al. 1986

EDTA = ethylenediaminetetraacetic acid; GC/EC = gas chromatography-electron capture; GFAAS = graphite furnace atomic absorption spectrometry; ICP-AES = inductively coupled plasma-atomic emission spectrometry; ICP-MS = inductively coupled plasma-mass spectrometry

spectroscopy (ICP-MS). Another relative method for the detection of beryllium is Laser Ion Mass Analysis (LIMA). This analysis method uses a laser beam to ionize elements (e.g., beryllium) in a small section of tissue and detects the elements by time-of-flight mass spectrometry (MS) (Williams and Kelland 1986).

Standard reference materials (SRMs) are useful to determine the accuracy of an analytical method. A standard reference urine (SRM=2,670) with a certified beryllium concentration is available from National Institute of Standards and Technology (Shan et al. 1989).

7.2 ENVIRONMENTAL SAMPLES

Methods used to analyze beryllium in environmental media are presented in Table 7-2. The standard test methods approved by EPA and NIOSH for beryllium analysis in ambient and occupational samples are included in Table 7-2. Environmental samples analyzed by atomic absorption spectroscopy and gas chromatography (GC) require pretreatment to remove interfering substances and increase sensitivity (EPA 1987). At high concentrations (500 mg/kg), aluminum and silicon interfere with the analysis of beryllium by atomic absorption spectroscopy. Separation of these elements is achieved by chelation and extraction with an organic solvent. High concentrations of iron interfere with the 243.86 nm beryllium emission line used in ICP-AES (Vaessen and Szteke 2000). A method using laser spark spectroscopy has been used for the direct determination of trace quantities of airborne beryllium collected on filters (Cremers and Radziemski 1985). A recent analytical advance is laser induced breakdown spectroscopy (LIBS), a real time technique (Langner et al. 1997). This technique has been used to monitor worker exposure to beryllium in 30-second sample intervals (Langner et al. 1997).

The following SRMs for beryllium in environmental samples are available from the National Institute of Standards and Technology: San Joaquin soil, SRM=2,709; Montana soil 1, SRM=2,710; Montana soil 2, SRM=2,711; coal, SRM=1,632; fly ash, SRM=1,633; trace elements in water, SRM=1,643; orchard leaves, SRM=1,571; and filter media, SRM=2,676 (Chang et al. 1982; Epstein et al. 1978; Gladney and Owens 1976; Namiesnik and Zygmunt 1999). SRMs are available for beryllium in soils and sediments from Canadian Certified Reference Materials Project (CCRMP), National Research Council of Canada

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Wet ash collection filter with mixture of HNO_3/HCl mixture; concentrate; add HNO_3/LiCl solution	Optical emission spectroscopy	5.3 µg/L (in dissolved particles)	96–108%	Scott et al. 1976
Air	Dissolve collection filter matrix in HF; add HNO_3; water; boil; dilute	GFAAS FAAS	0.05ng/m^3 2.5 ng/m^3	No data	Zdrojewski et al. 1976
Air	None	Laser spark technique	0.45 ng/cm^{-2}	No data	Cremers and Radziemski 1985
Occupational air	Dry collection filter; add HNO_3 and sulfuric acid; solubilize with concentrated HCl	Direct current plasma AES	0.0036 µg/sample	100%	Chang et al. 1982
Occupational air	Filter collection, acid digestion	GFAAS (method 7102)	5 pg/sample	107%	NIOSH 1989b
Occupational air	Filter collection, acid digestion	GFAAS (method 7300)	1 pg/sample	No data	NIOSH 1989b
Water	Acidify with HNO_3; evaporate under heat; add HCl or HNO_3	AAS (aspiration-method 210.1)	0.005 mg/L	97–100%	EPA 1983
		AAS (furnace method 210.2)	0.2 µg/L	No data	
Water	Acidify with HNO_3	GFAES GFAAS	2 µg/L 0.06 µg/L	112% (average) 98% (average)	Epstein et al. 1978
Water	Digest; acidify; dilute	ICP-AES (method 3500-BE-C); ICP-AES (method D1976)	1 µg/L No data	No data No data	APHA 1992 ASTM 1999

Table 7-2. Analytical Methods for Determining Beryllium in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Digest; acidify; dilute	ICP-MS (method 933.14)	0.1 µg/L	No data	AOAC 1995
Seawater	Add specific volumes of EDTA, sodium acetate, benzene and Hfta to collected seawater; rinse organic phase with NaOH; UV oxidize	GC/EC	0.02 ng/L	93–104%	Measures and Edmond 1986
Sediment	Extract dry sample with HCl solution	DCP-AES	0.02 µg/g	No data	Lum and Gammon 1985
Soil and sediment	Acid digest in bomb; dilute	ICP-AES (method D1971-A)	No data	No data	ASTM 1999
Soil, sludge, sediments, and other solid wastes	Acid digestion of sample	ICP-AES (method 6010)	0.3 µg/L	97.7–100%	EPA 1988c
Solid (coal ash)	Acid digest in bomb; dilute with acid	GFAAS	0.14–4.8 µg/L	No data	Pougnet et al. 1985
Oils and waxes	Add potassium permanganate with heat; acidify; digest with heat; filter; dilute	FAAS or ICP-AES (method 3031)	No data	No data	OSW 2000

Table 7-2. Analytical Methods for Determining Beryllium in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Dissolve in HNO_3; dry, then treat with HCl-$HClO_4$ and heat; filter	ICP-AES	No data	No data	Awadallah et al. 1986
Food	Freeze-dry or blender-grind food composites; solubilize with	ICP-AES	2.5 µg/kg	No data	Wolnick et al. 1984
	HNO_3, $HClO_4$, H_2SO_4, or HCl		2 µg/kg	98%	Capar and Yess 1996

AAS = atomic absorption spectrometry; AES = atomic emission spectrometry; AOAC = Association of Official Analytical Chemists; APHA = American Public Health Association; ASTM = American Society for Testing and Materials; DCP-AES = direct current plasma-atomic absorption spectroscopy; EDTA = ethylenediamine tetraacetic acid; FAAS = flame atomic absorption spectrometry; GC/EC = gas chromatography-electron capture; GFAAS = graphite furnace atomic absorption spectrometry; GFAES = graphite furnace atomic emission spectrometry; HCl = hydrochloric acid; $HClO_4$ = perchloric acid; HF = hydrogen fluoride; Hfta = 1,1,1-trifluoro-2,4-pentanedione; HNO_3 = nitric acid; H_2SO_4 = sulfuric acid; ICP-AES = inductively coupled plasma-atomic emission spectrometry; ICP-MS = inductively coupled plasma-mass spectrometry; LiCl = lithium chloride; NaOH = sodium hydroxide; NIOSH = National Institute for Occupational Safety and Health; OSW = Office of Solid Waste of the U.S. Environmental Protection Agency; UV = ultraviolet

NRCC and the U.S. Geological Survey (USGS): soil, SO 1; soil-sandy, SO 2; soil-limestone til, SO 3; soil-silty, SO 4; arable soil, TILL 2; Lake sediment, IKSD 4; sediment MESS-1; sediment BCSS-1; and marine sediment, MAG 1 (Namiesnik and Zygmunt 1999; Waldichuk et al. 1987).

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 beryllium is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (NTP), is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of beryllium.

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. As discussed in Section 3.8.1, the beryllium level in blood/serum/plasma is an accurate biomarker of exposure to certain forms of beryllium (James and Williams 1985; Stokes and Rossman 1991; Zorn et al. 1986). The level of beryllium in normal blood is 1 µg/kg (Zorn et al. 1986). No analytical method capable of determining beryllium in blood at or below this level has been reported in the literature. The routine analytical methods presently available are useful for detecting beryllium levels in the blood of occupationally exposed persons.

Effect. There are several methods for measuring effects due to beryllium exposure (see Section 3.8.2). An antigen-specific lymphocyte proliferation test confirms exposure and may be useful in early diagnosis of individuals with chronic beryllium disease; several methods for the lymphocyte proliferation test have been reported (Bobka et al. 1997; Kreiss et al. 1989; Mroz et al. 1991; Rossman et al. 1988; Stokes and

Rossman 1991). Another method that can be used for the positive diagnosis of chronic beryllium disease when other symptoms are evident is LIMA of histological sections of lung or skin granulomas. LIMA can detect parts per million (or mg/kg) levels of beryllium in these tissues (Williams and Kelland 1986).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The concentration of beryllium in approximately 95% of drinking waters in the United States is <0.01 µg/L (EPA 1980; Iwan 1987). Although a few methods are available (see Table 7-2) to detect beryllium at such low concentrations, no routine methods are available to quantify beryllium concentrations in most U.S. drinking waters. Similarly, the detection limit for beryllium in fresh vegetables by the commonly used analytical method (see Table 7-2) is 2.5 µg/kg. At this detection limit, beryllium was not found in two foods tested (Wolnik et al. 1984). Developing a routine analytical method to detect low levels of beryllium in foods would be useful. The data on the levels of beryllium in drinking water and total diet samples from ambient sources are significant in determining background levels of daily intake from these routes.

7.3.2 Ongoing Studies

The FEDRIP database lists an ongoing National Institute of Environmental Health and Sciences study by Jolly et al. at ELS Technology, Inc. (Lakewood, Colorado) that is investigating a portable low-cost analyzer for the detection of beryllium in toxic metal aerosols (FEDRIP 2001).