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 aluminum, its metabolites, and other biomarkers of exposure and effect to aluminum. 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 rhods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

Because of the ubiquitous nature of aluminum contamination is a major problem encountered in the analysis of aluminum by all methods except accelerator mass spectroscopy (AMS) using radioactive ^{26}Al. When using the other methods, all items used during collection, preparation, and assay should be checked for aluminum contribution to the procedure. Only by taking these stringent precautions will one be able to produce accurate results. A variety of analytical methods have been used to measure aluminum levels in biological materials, including AMS, graphite furnace atomic absorption spectrometry (GFAAS), flame atomic absorption spectrometry (FAAS), neutron activation analysis (NAA), inductively coupled plasma-atomic emission spectrometry (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), and laser ablation microprobe mass analysis (LAMMA) (Maitani et al. 1994; Owen et al. 1994; Van Landeghem et al. 1994) (see Table 6-l). Front end separation techniques such as chromatography are frequently coupled with analytical methods.

AMS is a technique that can now be used to accurately determine the atomic content in as little as a few milligrams of biological material. AMS has been used in the past for measuring long-lived radionuclides that occur naturally in our environment, but it is suitable for analyzing the concentration of radioactive ^{26}Al and stable ^{27}Al in biological samples. AMS combines a particle accelerator with ion sources, large magnets, and detectors, and is capable of a detection limit of one atom in 10^{15} (1 part per quadrillion [ppq]). This method has biomedical applications regarding the uptake and distribution of aluminum in

Table 6-1. Analytical Methods for Determining Aluminum in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Serum	Direct injection into atomizer	GFAAS	Low μg/L levels	No data	King et al. 1981
Serum	Dilution with water; addition of EDTA	GFAAS	2 μg/L	No data	Alderman and Gitelman 1980
Serum	Centrifugation and injection of supernatant	GFAAS	14.3 μg/L	97-102%	Bettinelli et al. 1985
Serum (Al-organic acid species)	Addition of sodium bicarbonate; direct injection into chromatography column	HPLC/ICP-AES	No data	No data	Maitani et al. 1994
Serum (Al-organic acid species)	Dilution with mobile phase; fractions collected for ETAAS analysis	HPLC/ETAAS	No data	98-100% in spiked and synthetic serum	Wrobel et al. 1995
Serum (Al-organic acid species)	Addition of citrate buffer; direct injection into chromatography column	HPLC/ETAAS	0.12 μg/L	99.2 ± 12.4%	Van Landeghem et al. 1994
Plasma	Dilution	GFAAS	3–39 μg/L	97-105%	Wawschinek et al. 1982
Whole blood, plasma, or serum	Dilution with water	GFAAS	24 μg/L	No data	Gardiner et al. 1981
Whole blood	Addition of sodium citrate; centrifugation; injection of supernatant	GFAAS	Low μg/L levels	No data	Gorsky and Dietz 1978

Table 6-1. Analytical Methods for Determining Aluminum in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Whole blood	Dilution with Triton X-100	GFAAS	1.9 μg/L in serum; 1.8 μg/L in plasma; 2.3 μg/L in whole blood	No data	Van der Voet et al. 1985
Urine	Digestion; ion-exchange clean-up	NAA	50 μg/L	No data	Blotcky et al. 1976
Urine and blood	Dilution with water	GFAAS or ICP-AES	Low μg/L levels	No data	Sanz-Medel et al. 1987
Urine	Direct injection	GFAAS	Low μg/L levels	No data	Gorsky and Dietz 1978
Urine	Direct injection	GFAAS	Low μg/L levels	No data	Gorsky and Dietz 1978
Urine and blood	Dilution with water	ICP-AES	1 μg/L (urine); 4 μg/L (blood)	No data	Allain and Mauras 1979
Biological tissues	Homogenization with EDTA	GFAAS	0.002–10.057 μg/g	95–106%	LeGendre and Alfrey 1976
Biological tissues	Freeze-drying; grinding for homogenization	NAA	8 μg/g	No recovery; RSD <10%	Wood et al. 1990
Biological tissues	Drying; digestion with nitric acid; dilution with water	GFAAS	0.5 μg/g	80–117%	Bouman et al. 1986
Kidney, liver, urine	Acid digestion; dilution with water	ICP-AES	No data	98.8% ± 8.6% in liver	Maitani et al. 1994

Table 6-1. Analytical Methods for Determining Aluminum in Biological Materials *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Kidney, liver, femur	Microwave digestion with nitric acid; addition of internal standard and dilution with eluent	SEC/ICP-MS	0.04 μg/g	100 ± 14% of spiked Al in reference material	Owen et al. 1994
Brain	Freeze drying; acid digestion; dilution with $K_2Cr_2O_7$ matrix modifier	GFAAS	0.03 μ/g	No data	Xu et al. 1992a
Brain	Fixing and embedding in polymer matrix; sectioning and staining to visualize Al deposits; laser vaporization of selected sample surface into mass spectrometer	LAMMA	low μg/g range	no data	Lovell et al. 1993
Hair	Wash with isopropanol; digestion with nitric acid; dilution with water	GFAAS	0.65 μg/g	84–105%	Chappuis et al. 1988
Human blood, urine, serum, feces	Acid digestion using Parr bomb technique, microwave, or hot plate method	ICP-AES	1 μg/L	> 75%	Que Hee and Boyle 1988
All	None	AMS	1 ppq	NA	Flarend and Elmore 1997

AMS = accelerated mass spectroscopy; EDTA = ethylene diamine tetra acetic acid; GFAAS = graphite furnace atomic absorption spectrometry; ICP-AES = inductively coupled plasma - atomic emission spectroscopy; NAA = neutron activation analysis; ETAAS = electrothermal atomic absorption spectrometry; SEC/ICP-MS = size-exclusion chromatography/ICP-AES/mass spectrometry; HLPC/ICP-AES = *high-performance liquid chromatography/ICP-AES*; LAMMA = laser ablation microprobe mass analysis; NA = not applicable; ppq = parts per quadrillion

the body, but is dependent upon the availability of the radioactive ^{26}Al tracer, which is produced using a cyclotron. The first step in the analysis process is the chemical extraction of aluminum (both stable and radioactive) from the biological sample using a method which is free of aluminum contamination. The extractant is loaded into a holder and inserted through a vacuum lock into the ion source, which then employs ion bombardment to ionize the sample atoms. These are removed from the sample using magnets, and are separated by mass and charge by accelerators, bending magnets, and electron stripper screens. An electrostatic analyzer selects particles based on their energy, and a gas ionization detector counts the ions one at a time using a rate of energy loss assessment that distinguishes between any competing isobars. This method is used to assess the ^{26}Al content and the ^{26}Al/^{27}Al ratio (Elmore and Phillips 1987; Flarend and Elmore 1997).

GFAAS is the most common technique used for the determination of low-ppb (μg/L) levels of aluminum in serum plasma, whole blood, urine, and biological tissues (Alder et al. 1977; Alderman and Gitelman 1980; Bettinelli et al. 1985; Bouman et al. 1986; Chappuis et al. 1988; Couri et al. 1980; Gardiner and Stoeppler 1987; Gorsky and Dietz 1978; Guillard et al. 1984; Keirsse et al. 1987; Rahman et al. 1985; Savory and Wills 1986; Schaller and Valentin 1984; van der Voet et al. 1985; Wrobel et al. 1995; Xu et al. 1992a). This is because GFAAS offers the best combination of sensitivity, simplicity, and low cost. When used as a detector for high-performance liquid chromatography (HPLC), GFAAS can analyze for species of complexed or bound aluminum which have been separated into fractions on the chromatography columu (Van Landeghem et al. 1994).

NAA has been used to determine low levels of aluminum in biological tissues and urine (Blotch et al. 1976; Savory and Wills 1986; Wood et al. 1990; Yukawa et al. 1980). NAA involves the bombardment of a sample with neutrons, which transforms some of the stable ^{27}Al atoms into several radioactive aluminum isotopes beginning with ^{28}Al, and measurement of the induced radioactivity. Advantages of NAA include good sensitivity and relative independence from matrix (or media) effects and interferences. Moreover, this technique can be used to detect almost all elements of environmental concern in the same sample (Sheldon et al. 1986). One major problem with using NAA with aluminum is the need to correct for interfering reactions with phosphorus and silicon, which produce the same radioisotope (^{28}Al) of aluminum Other disadvantages of this technique include its high cost, the limited availability of nuclear reactors for NAA analysis, the short 2.25 minute half-life of ^{28}Al that requires prompt analysis of the sample following bombardment with neutrons, and disposal problems of radioactive waste.

The ICP-AES technique, also referred to as ICP-optical emission spectroscopy (ICP-OES), has been reported for the measurement of aluminum in biological materials and is an excellent alternative to GFAAS for those laboratories possessing the appropriate instrumentation (Allain and Mauras 1979; Lichte et al. 1980; Maitani et al. 1994; Que Hee and Boyle 1988; Que Hee et al. 1988; Sanz-Medel et al. 1987). ICP-AES is a multi-elemental technique that is relatively free of chemical interferences. The matrix problems that can exist in atomic absorption spectrometry (AAS) are minimized in ICP-AES due to the very high excitation temperature of the sample (Savory and Wills 1986). The limits of detection for the ICP-AES method have been reported to be about 1 μg and 4 μg aluminum/l of urine and blood, respectively (Allain and Mauras 1979). A major problem with using the ICP-AES technique is the intense and broad emission of calcium which increases the aluminum background and can raise the detection limit for this element (AIlain and Mauras 1979; Que Hee and Boyle 1988; Savory and Wills 1986). Titanium also interferes with aluminum analysis (Que Hee and Boyle 1988). Also the relatively high cost and complexity of this technique can limit its routine use in many laboratories. However, ICP-AES and, especially ICP-MS technologies have advanced recently largely through the efforts of the Department of Energy, and the cost of analysis has declined considerably.

Inductively coupled plasma-mass spectrometry (ICP-MS) is a powerful technique that uses an inductively coupled plasma as an ion source and a mass spectrometer as an ion analyzer. It can measure the presence of more than 75 elements in a single scan, and can achieve detection limits down to parts per trillion (ppt) levels for many elements-levels that are two or three orders of magnitude lower than those obtained by ICP-AES (Keeler 1991). It is more expensive than ICP-AES and requires more highly skilled technical operation. Aluminum levels in urine and saliva were detected down to 0.02 μg/mL and in blood serum to 0.001 μg/mL using ICP-MS (Ward 1989). Speciation studies have employed ICP-MS as a detector for aluminum in tissue fractions separated by size-exclusion chromatography (SEC) with detection limits of 0.04 μg/g in femur, kidney and brain (Owen et al. 1994).

LAMMA has been utilized for the analysis of aluminum in brain tissue affected with Alzheimer's disease (Love11 et al. 1993). This new analytical technique of nuclear microscopy can simultaneously image and analyze features in unstained and untreated tissue sections, and therefore avoids contamination problems associated with tissue prepared using conventional chemical techniques. LAMMA was used in a study that did not detect aluminum in pyramidal neurons in brain tissue from Alzheimer's disease patients (Makjanic et al. 1998). However, in tissue that had been subject to conventional procedures such as fixation and osmication, aluminum was observed in both neurons and surrounding tissue. The method,

however, requires rigorous histological sectioning and preparation prior to analysis, specialized analytical equipment and highly trained personnel.

Adequate digestion methods are important in the determination of all metals, including aluminum Que Hee and Boyle (1988) showed that Parr bomb digestions were always superior to hot plate digestions for many elements, including aluminum in feces, liver, and testes. Microwaving in closed vessels produced lower aluminum recoveries in liver than Parr bomb digestions. The Parr bomb values for citrus leaves were within 5% of the NBS certified values.

6.2 ENVIRONMENTAL SAMPLES

A number of analytical techniques have been used for measuring aluminum concentrations in environmental samples. These include GFAAS, FAAS, NAA, ICP-AES, ICP-MS, spectrophotometry using absorbance and fluorescence detection, phosphorirxtry, chromatography and gas chromatography equipped with an electron capture detector (GUECD) (Andersen 1987, 1988; Benson et al. 1990; Carrillo et al. 1992; Dean 1989; De La Campa et al. 1988; Ermolenko and Dedkov 1988; Fleming and Lindstrom 1987; Gardiner et al. 1987; Gosink 1975; Jones et al. 1988; Kopp and McKee 1978; NIOSH 1984b; Pastor et al. 1987; Tapparo and Bombi 1990; Woolfson and Gracey 1988). They are summarized in Table 6-2.

NIOSH has recommended Methods 7013 (FAAS) and 7300 (ICP-AES) for detecting aluminum and other elements in filter samples of workplace air particulates. The applicable working ranges are 0.5-10 mg/m^3 for a 100-L air sample by Method 7013 and 0.005-2.0 mg/m^3 for a 500-L air sample by Method 7300 (NIOSH 1984b).

GFAAS and FAAS are the techniques (Methods 202.1 and 202.2) recommended by EPA for measuring low levels of aluminum in water and waste water (Kopp and McKee 1978). Detection limits of 100 μg of aluminum/L of sample and 3 μg of aluminum/L of sample were obtained using the FAAS and GFAAS techniques, respectively (Kopp and McKee 1978). Spectrophotometry and GUECD have also been employed to measure low-ppb (11μg/L) levels of aluminum in water (Dean 1989; Ermolenko and Dedkov 1988; Gosink 1975). Flow-injection systems using absorbance (Benson et al. 1990) and fluorescence detection (Carrillo et al. 1992) have been used to monitor aqueous aluminum levels in the field and in the

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect sample on cellulose filter and digest with nitric acid	Method 7013 (FAAS)	2 μg/sample	NA	NIOSH 1984b
Air	Collect sample on cellulose filter and digest with nitric acid	Method 7300 (ICP-AES)	1 μg/sample	NA	NIOSH 1984b
Water	Add o,o'- and o,p-dihydroxyazo compounds to sample and analyze at 545 nm	Spectrophotometer	4 μg/L	NA	Ermolenko and Dedkov 1988
Water	Add acetate and trifluoroacetylacetone in benzene to sample and shake; add sodium hydroxide, shake, and analyze extract	GC/EDC	Low μg/L levels	No data	Gosink 1975
Water and waste water	Digest sample with nitric acid and analyze	FAAS or GFAAS	100 μg/L (FAAS); 3 μg/L (GFAAS)	NA	Kopp and McKee 1978 (Methods 202.1 and 202.1)
Soil	Filter sample and clean-up on chromatography column	GFAAS	No data	No data	Gardiner et al. 1987
Fly ash	Dry sample in vacuum and irradiate	NAA	No data	NA	Fleming and Lindstrom 1987
Plants	Digest sample with nitric acid and analyze	Spectrophotometer	7 μg/L	NA	Dean 1989
Rock, magma, soil, paint, citrus leaves	Acid digest sample using Parr bomb or microwave	ICP-AES	0.001 μg/L	90%	Que Hee and Boyle 1988
Dialysis fluids	Dilute sample with acidic Triton X-100	Phosphorimetry	3 μg/L	No data	Andersen 1987

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Dialysis fluids (continued)	Add Ferron and cetyltrimethyl-ammonium bromide solution to sample and measure phosphorescence at 586 nm	Phosphorimetry	5.4 μg/L	No data	De La Campa et al. 1988
Rock, soil	Digest with acid	AMS	10^{-15} g/g sample	NA	Flarend and Elmore 1997

AMS = accelerated mass spectroscopy; FAAS = flame atomic absorption spectrometry; GC/ED = gas chromatography/electron capture detector; GFAAS : graphite furnace atomic absorption spectrometry; ICP-AES = inductively couples plasma-atomic absorption spectrometry; NA = not applicable; NAA = neutron activation analysis

laboratory setting, with detection limits as low as 0.3 µg/L. Ion chromatography using spectrophotometric detection and on-line preconcentration gives an effective detection limit <1 µg/L in aqueous samples. GFAAS is the method of choice for measuring low-ppb levels of aluminum in dialysis fluids (Andersen 1987, 1988; Woolfson and Gracey 1988).

The GFAAS and NAA techniques have been employed for measuring aluminum levels in soil and fly ash, respectively (Fleming and Lindstrom 1987; Gardiner et al. 1987). Que Hee and Boyle (1988) employed ICP/AES to measure aluminum in rocks, soils, volcano magma, and print. Aluminum silicate matrices require disruption by hydrofluoric acid/nitric acid digestion in Parr bombs to achieve >90% recoveries of aluminum and other elements in preparation for ICP-AES analysis using wet ashing (Que Hee and Boyle 1988). Aluminum in air particulates and filters has been determined by pressurized digestion and ICP-AES detection (Dreetz and Lund 1992). Microwave digestions in closed polypropylene bottles gave the same concentrations of aluminum for rocks and soils.

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 aluminum 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 aluminum.

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. GFAAS is the method of choice for measuring low-ppb levels of aluminum in whole blood, serum plasma, urine, and various

biological tissues (Alder et al. 1977; Alderman and Gitelman 1980; Bettinelli et al. 1985; Bouman et al. 1986; Chappuis et al. 1988; Couri et al. 1980; Gardiner and Stoeppler 1987; Gorsky and Dietz 1978; Guillard et al. 1984; Keirsse et al. 1987; Rahman et al. 1985; Savory and Wills 1986; Schaller and Valentin 1984; van der Voet et al. 1985). Chromatographic techniques coupled with GFAAS detection have been used to separate various metal species and determine aluminum content in serum (Maitani et al. 1994; Van Landeghem et al. 1994). The NAA and ICP-AES methods have also been used to measure ppb levels of aluminum in biological tissues and fluids (Blotcky et al. 1976; Savory and Wills 1986; Yukawa et al. 1980). ICP-MS has the requisite sensitivity to detect low-ppb levels of aluminum (Ward 1989) in biological and environmental media though it is more expensive than GFAAS. However, the cost of ICP-MS, as well as ICP-AES, analyses has decreased significantly over the last few years. LAMMA can detect aluminum deposits in specific structures of the brain and might be used to correlate the effects of aluminum accumulation (Lovell et al. 1993). These techniques are sensitive for measuring background levels of aluminum in the population and levels of aluminum at which health effects might begin to occur.

Although sensitive analytical methods are available for measuring the presence of aluminum in biological tissues and fluids, it is not known whether data collected using these techniques have been used to correlate the levels of aluminum in biological materials to exposure and effect levels. The problem of contamination during tissue preparation (Makjanic et al. 1998) makes this task more challenging.

At present, no biomarkers of exposure and effect other than the parent compounds are available for aluminum There are no data to indicate whether other biomarkers, if available, would be preferred over chemical analysis for monitoring exposure to aluminum.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. FAAS and ICP-AES have been used to measure aluminum in air (Dreetz and Lund 1992; NIOSH 1984b). For measuring aluminum in water and waste water, spectrophotometry (Benson et al. 1990; Carrillo et al. 1992; Ermolenko and Dedkov 1988), GUECD (Gosink 1975), and FAAS and GFAAS (Kopp and McKee 1978) have been employed. GFAAS has been used to analyze aluminum in the soil (Gardiner et al. 1987), and GFAAS (Andersen 1987) as well as phosphorimetry (De La Campa et al. 1988) have been useful in determining aluminum levels in dialysis fluids. The method used to measure aluminum levels in flyash is NAA (Fleming and Lindstrom 1987). The media of most concern for potential exposure to aluminum are water and dialysis fluids. GFAAS technique is sensitive

for measuring background levels of aluminum in water (Kopp and McKee 1978) and dialysis fluids (Andersen 1987; Woolfson and Gracey 1988) and levels of aluminum at which health effects might begin to occur. GFAAS and FAAS are the techniques (Methods 202.1 and 202.2) recommended by EPA for detecting aluminumlevels in water and waste water (Kopp and McKee 1978). GFAAS is the method of choice for measuring low-ppb levels of aluminum in dialysis fluids (Andersen 1987; Woolfson and Gracey 1988). ICP-AES has been utilized to detect aluminum in biological media (leaves, feces, serum blood, liver, spleen, kidney, urine, and testes) and environmental matrices (rocks, soils, water, volcano magma, paint) in addition to other elements (Que Hee and Boyle 1988) and, more recently, ICP-MS has been shown to be useful for even more sensitive analyses of such media. No additional methods for detecting elemental aluminum in environmental media appear to be necessary at this time. A need exists for developing a range of NIST analytical standards for calibrating instruments and assessing the accuracy and precision of the various analytical methods.

6.3.2 Ongoing Studies

No ongoing studies have been identified.