7. ANALYTICAL METHODS

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

A variety of analytical methods can be used to determine trace concentrations (sub-ppb to ppb) of tungsten in biological tissues. These include inductively coupled plasma-atomic emission spectroscopy (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), and neutron activation analysis (NAA), as well as other techniques, such as atomic absorption spectroscopy (AAS) and UV/Visible spectroscopy (UV/VIS). Table 7-1 lists analytical methods used for determining tungsten and tungsten compounds in biological fluids and tissues.

ICP-AES and ICP-MS have been used to determine tungsten concentrations in biological samples (Bárány et al. 2002a, 2002b; Le Lamer-Déchamps et al. 2003; Marquet et al. 1997; Paschal et al. 1998; Schramel et al. 1997). Samples are typically wet ashed with nitric acid at elevated temperatures and then diluted for analysis. Tungsten is quantified by ICP-AES using the emission line at 207.91 nm and by ICP-MS using isotope masses of ^{182}W and ^{186}W. The instrument detection limits have been determined to be 50 µg/L and 0.02–0.3 µg/L for ICP-AES and ICP-MS, respectively. Huang et al. (2002) recently developed a method using chelation ion chromatography (CIC) coupled with on-line detected by ICP-MS. The advantage of this method is the ability to analyze trace amounts of tungsten (and other metals) in complex matrices such as biological samples. Using a bis-(2-aminoethylthio) methylate (BAETM) resin column, the limit of detection was reported to be <0.05 ng/mL for this method.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human blood	Not specified	ICP-MS	0.2 µg/L	No data	Bárány et al. 2002a, 2002b
Human serum	Not specified	ICP-MS	0.04 µg/L	No data	Bárány et al. 2002a, 2002b
Human plasma	Dilute	ICP-MS	0.01 µg/L	97–102%	Le Lamar-Déchamps et al. 2003
Blood and urine	Dilute (and acidify)	ICP-AES	50 µg/L	No data	Marquet et al. 1997
Human hair and nails	Hydrolysis in nitric acid; dilute	ICP-AES	—	No data	Marquet et al. 1997
Human blood and tissue	Dry	NAA	1 µg/mg	No data	Bowen 1960
Human tissues (e.g., kidney, liver, lung)	Deep freeze (or freeze dry); grind to powder	NAA	No data	No data	Brune et al. 1980
Human urine	Dilute (and acidify)	ICP-MS	0.3 ng/mL	No data	Paschal et al. 1998
Human urine	Dilute (and acidify)	ICP-MS	0.02 µg/L	116.5%	Schramel et al. 1997
Animal tissues (e.g., liver, kidney, lung, spleen, brain, etc.)	Wet digestion using HNO_3/$HClO_4$; evaporate to dryness; dissolve in ionic buffer ($LiNO_3$/HNO_3)	DCP-AES	~0.037 µg/L	No data	Frank and Petersson 1983
Rat and dog plasma	None	ICP-AES	100 ng/mL	89–105%	Poucheret et al. 2000

AES = atomic emission spectroscopy; DCP = DC plasma; $HClO_4$ = perchloric acid; HNO_3 = nitric acid; ICP = inductively coupled plasma; $LiNO_3$= lithium nitrate; MS = mass spectrometry; NAA = neutron activation analysis

NAA techniques provide low detection limits for tungsten (0.005 µg tungsten per gram of sample), but there are few reactors at which NAA facilities and expertise are available (Dams et al. 1970). A common NAA procedure for tungsten determination is to produce the short-lived ^{187}W radionuclide (half-life of 24 hours; gamma-lines of 479.3 and 685.7 keV). Counting can be initiated after an irradiation period of 2–5 hours and a cooling period of 20–30 hours (Dams et al. 1970). Biological samples that have been analyzed for tungsten using the NAA technique include human blood and tissues (e.g., kidney, liver, and lung) (Bowen 1960; Brune et al. 1980). Because facilities at which NAA can be performed are extremely limited, NAA's most useful application is as a reference method against which other less expensive and more common methods can be compared for accuracy.

7.2 ENVIRONMENTAL SAMPLES

Many of the basic analytical methods used for determining tungsten in biological media are also used for determining tungsten levels in environmental samples (e.g., soil, water, and air). ICP-AES, ICP-MS, Flame AAS, UV/VIS spectrophotometry, and NAA are the most common techniques utilized for analysis of tungsten in environmental samples. Table 7-2 lists the methods used for determining tungsten in environmental samples.

The NIOSH-recommended technique (Method 7074) for determining tungsten in air uses Flame AAS. Detection limits for tungsten are 50 µg of soluble tungsten per sample and 125 µg of insoluble tungsten per sample using an absorption line at 255.1 nm (NIOSH 1994).

Inductively coupled plasma techniques have been used to measure tungsten concentrations in water samples. Samples are typically filtered and acidified before analysis. Johannesson et al. (2000) used ICP-MS to measure the levels of total tungsten in river water samples. Detection limits for tungsten were 0.8 nmol/kg (0.15 µg/kg). For spring water, Hall et al. (1988) reported detection limits of 0.06 and 1.2 µg/L for ICP-MS and ICP-AES, respectively. In order analyze waters with high concentrations of dissolved solids (e.g., seawater), Huang et al. (2002) employed CIC coupled with ICP-MS and achieved detection limits of <0.05 ng/mL.

UV/VIS spectroscopy has been used to measure tungsten in environmental samples. Parker and Boltz (1968) used UV/VIS spectroscopy at 262 nm to determine total tungsten levels in water samples as a peroxytungstic acid complex. Quin and Brooks (1972a) measured the concentration of tungsten utilizing

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Digest with HNO_3/HF; evaporate to dryness; add NaOH/$NaSO_4$; dilute	Flame AAS	50 µg soluble W per sample; 125 µg insoluble W per sample	No data	NIOSH 1994 (Method 7074)
Workspace air	Filter air; leach soluble W using DI water; dissolve residual W HNO_3/HF after HCl extraction	Flame AAS	10 µg soluble W/L; 8 mg insoluble W per sample	90.8–103% soluble; 90.8–105% insoluble	Hull and Haartz 1980
Workspace/ urban air	Filter air for particulates	NAA	0.20±0.09 µg/m^3	No data	Haddad and Zikovsky 1985
Water	Dilute tungstate solution; add H_2SO_4 and HOOH; dilute	UV/VIS of peroxy-tungstic acid at 262 nm	No data	No data	Parker and Boltz 1968
Water	Add sodium acetate buffer; add benzoin anti-oxime then extract with MIBK; add 1-ephedrine	Flame AAS	0.1 mg/L	No data	Korrey and Goulden 1975
Water (WO_4^{2-})	Add HCl, chloropromazine HCl, and $(NH_4)_2Fe(SO_4)_2$; mix; add HOOH to initiate reaction	Spectro-photometry (λ=525 nm)	~2 µg/L	No data	Tomiyasu and Yonehara 1996
River water	Filter; acidify with HNO_3	ICP-MS	No data	No data	Konhauser et al. 1997
River water	Filter; acidify with HNO_3	ICP-MS	0.8 nmol/kg (0.15 µg/kg)	No data	Johannesson et al. 2000
River water	Filter; acidify with HNO_3	NAA	No data	No data	Tanizaki et al. 1992a, 1992b
Spring water	Acidify with HCl; add oxime dissolved in EtOH and activated charcoal; filter; ash; dissolve in HCl; dilute	ICP-AES	1.2 µg/L	No data	Hall et al. 1988
		ICP-MS	0.06 µg/L		

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Polluted waters	Microwave digestion using HF/HCl/HNO_3; column chromatography using Chelex-100 in Na resin; dilute	ICP-AES	30 mg/L	No data	Ferri et al. 1999
Seawater	Acidify, dilute	CIC-ICP-MS	<0.05 ng/mL	No data	Huang et al. 2002
Seawater	Acidify; add ammonium pyrrolidine dithiocarbamate; add activated charcoal; stir	NAA	0.05 µg/L	No data	van der Sloot et al. 1977
Soil	Dry; digest in aqua regia/perchloric acid; filter; dilute	ICP-AES	No data	No data	Sadiq et al. 1992
Soil, stream sediment, and rocks	Fuse sample with $KHSO_4$; leach with HCl; mix with $SnCl_2$; add dithiol; dissolve with petroleum spirits	UV/VIS of tungsten-dithiol complex at λ=630 nm	~1 ppm	95–105%	Quin and Brooks 1972a
Fertilizers	Digest in HNO_3, HCl, and/or $HClO_4$ acid(s); dilute	ICP-AES	0.002 mg/kg	No data	Senesi et al. 1988
Onion	Digest and redistill in HNO_3; dilute	ICP-MS	0.0180 µg/kg fresh weight	No data	Bibak et al. 1998
Vegetation	Ash; add $SnCl_2$ solution; add dithiol; dissolve with petroleum spirits	UV/VIS of tungsten-dithiol complex at λ=630 nm	0.01 ppm dry weight	95–105%	Quin and Brooks 1972a
Berries	Microwave digestion; dilute	ICP-AES	0.0001 mg/g dry weight	108%	Rodushkin et al. 1999
Wine	Dilute sample to volume.	ICP-MS	0.01 ng/mL	No data	Pérez-Jordán et al. 1998

λ = wavelength; AAS = atomic absorption spectroscopy; AES = atomic emission spectrometry; CIC=chelation ion chromatography; DI = deionized; EtOH = ethanol; HCl = hydrochloric acid; $HClO_4$ = perchloric acid; HF = hydrofluoric acid; HNO_3 = nitric acid; HOOH = hydrogen peroxide; H_2SO_4 = quinine sulfate; ICP = inductively coupled plasma; $KHSO_4$ = potassium hydrogen sulfate; MIBK = methyl isobutyl ketone; MS = mass spectroscopy; NAA = neutron activation analysis; NaOH = sodium hydroxide; $NaSO_4$ = sodium sulfate; $(NH_4)_2Fe(SO_4)_2$ = ammonium iron(II) sulfate; $SnCl_2$ = tin chloride; UV/VIS = ultraviolet-visible spectroscopy; WO_4^{2-} = tungstate ions

a tungsten-dithiol complex with an absorption wavelength at 630 nm. The detection limit for this technique was approximately 1 ppm in soil and 0.01 ppm dry weight in vegetation.

NAA has been used to determine tungsten levels in environmental samples. Haddad and Zikovsky (1985) reported a detection limit of 0.20±0.09 µg/m^3 tungsten using NAA for determining tungsten in workplace/urban air particulate matter. Tungsten levels in seawater have been determined by NAA after first concentrating tungsten on activated charcoal by adsorption as the ammonium pyrrolidine dithiocarbamate complex (van der Sloot et al. 1977). The detection limit is 0.05 µg tungsten/L after a simple chemical separation.

Tomiyasu and Yonehara (1996) determined the concentration of trace amounts of tungstate ions (WO_4^{2-}) using a catalytic spectrophotometric method. In the presence of iron(II), chloropromazine is oxidized by hydrogen peroxide in a hydrochloric acid solution to form a red free radical, which is further oxidized to form a colorless compound. The reaction can be followed by measuring the increase in absorbance of the red free radical at 525 nm. Tungsten(VI) inhibits the color formation, and the maximum absorbance value decreases with an increase in tungsten(VI) concentration. Tungsten(VI) has been determined by this method in the concentration range of 2–500 µg/L.

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 tungsten is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicological 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 tungsten.

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. Analytical methods with satisfactory sensitivity, precision, and reliability are available to determine the levels of tungsten in human tissues and body fluids (Bárány et al. 2002a, 2002b; Le Lamer-Déchamps et al. 2003; Marquet et al. 1997; Paschal et al. 1998; Schramel et al. 1997). Existing analytical methods are sensitive enough to measure background levels in the population and levels at which biological effects occur. For example, detection limits of <0.05 ng/mL have been reported for tungsten in biological samples (Huang et al. 2002). Standard methods of analysis for determining the levels of tungsten in human tissues and body fluids are not available and are needed for inter-laboratory comparability of results. Methods for determining levels of tungsten compounds (e.g., tungstate ions) in human tissues and body fluids are not available. Additional methods for determining tungsten compounds in human tissues and body fluids may be useful for determining exposure from different tungsten species.

Effect. There are no known sensitive and specific biomarkers of effect for tungsten. Therefore, no analytical method recommendations can be made for biomarkers of effect for tungsten at the present time.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Sensitive analytical methods are available to measure the levels of tungsten in environmental media (Hall et al. 1988; Johannesson et al. 2000; Konhauser et al. 1997; NIOSH 1994; Quin and Brooks 1972a; Sadiq et al. 1992), although very limited information is available regarding the accuracy and precision of these methods. Further studies would be useful to ascertain the accuracy and precision of methods used to determine tungsten in environmental media so that the reliability of tungsten levels may be assessed. Most analytical methods are sensitive enough to determine levels of tungsten at which health effects may occur. Some of the available methods can be used to detect tungsten at nanogram levels (Huang et al. 2002). Most of these techniques measure total tungsten and do not distinguish among various tungsten compounds. Although limited, methods are available that determine levels of tungstate ions in environmental media (Tomiyasu and Yonehara 1996). Additional methods would be useful in determining environmental levels of specific tungsten compounds such that human exposure to these compounds may be assessed.

7.3.2 Ongoing Studies

No ongoing studies investigating new methods for detection and speciation of tungsten or tungsten compounds were identified in the Federal Research in Progress database (FEDRIP 2004).