7. ANALYTICAL METHODS

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

Blood, Urine, Serum, Cerebrospinal Fluid. Several analytical methods are available to analyze the level of lead in biological samples. The most common methods employed are flame atomic absorption spectrometry (AAS), graphite furnace atomic absorption spectrometry (GFAAS), anode stripping voltametry (ASV), inductively coupled plasma-atomic emission spectroscopy (ICP/AES), and inductively coupled plasma mass spectrometry (ICP/MS). According to Grandjean and Olsen (1984) and Flegal and Smith (1995), GFAAS and ASV are the methods of choice for the analysis of lead. In order to produce reliable results, background correction, such as Zeeman background correction that minimizes the impact of the absorbance of molecular species, must be applied. Limits of detection for lead using AAS are on the order of μg/mL (ppm) for flame AAS measurements, while flameless AAS measurements can detect blood lead levels at about 1 ng/mL (Flegal and Smith 1995). A detection limit of 0.05 ng/mL has been achieved for lead in blood samples analyzed by GFAAS (Flegal and Smith 1995). ICP/MS is also a very powerful tool for trace analysis of lead and other metals. Although ICP/MS instruments are more costly than GFAA instruments, their ability to analyze multiple metals from a single sample, low detection limits, reliability, and ease of use have increasingly made them popular for trace metal analysis. Other specialized methods for lead analysis are x-ray fluorescence spectroscopy (XRFS), neutron activation analysis (NAA), differential pulse anode stripping voltametry, and isotope dilution mass spectrometry (IDMS). The most reliable method for the determination of lead at low concentrations is IDMS (EPA 1986a; Grandjean and Olsen 1984), but due to the technical expertise required and high cost of the equipment, this method is not commonly used. It is primarily used for the development of certified standard reference materials by which other methods can determine their reliability since results of lead

analyses from numerous laboratories often do not agree (Fell 1984). Details of several methods used for the analysis of lead in biological samples are presented in Table 7-1.

Concentrations of lead in blood, urine, serum, and cerebrospinal fluid have been used as indicators of exposure to lead. Measurement of lead in blood is the most common method of assessing exposure. OSHA mandates biological monitoring of blood as a measure of workplace exposure to lead (Goyer 2001). Blood lead is also considered the most useful tool for screening and diagnostic testing (Moore 1995); the half-life of lead in blood is approximately 36 days (Todd et al. 1996). A second half-life is generally considered to be approximately 4 years (Graziano 1994) and reflects the replenishment of lead in the blood from the bone storage compartment. Sample preparation usually consists of wet ashing (digesting) the sample with strong acid and heat, and redissolving the residue in dilute acid prior to analysis so that all lead species are converted quantitatively to the same lead compound (NIOSH 1977c). Preparation methods not requiring wet ashing have also been used with good results (Aguilera de Benzo et al. 1989; Delves and Campbell 1988; Manton and Cook 1984; NIOSH 1977b; Que Hee et al. 1985a; Zhang et al. 1997). For samples analyzed by ICP/MS, ASV, AAS, and GFAAS, sensitivity is in the low- to sub-ppb (0.1–15 ppb) with good accuracy and precision (Aguilera de Benzo et al. 1989; Delves and Campbell 1988; NIOSH 1977b, 1977c; Que Hee et al. 1985a; Zhang et al. 1997). The presence of phosphate, ethylenediaminetetraacetic acid (EDTA), and oxalate can sequester lead and cause low readings in flame AAS (NIOSH 1994c). A comparison of IDMS, ASV, and GFAAS showed that all three of these methods can be used to reliably quantify lead levels in blood (Que Hee et al. 1985a). ACGIH recommends quantification of blood lead by GFAAS. ESA, Inc. has introduced a simple to use, portable device for performing blood lead measurements using a finger stick or a venous sample (ESA 1998). Results can be obtained in about 3 minutes. For analysis of urine, chelation and solvent extraction, followed by atomic absorption for quantification is the recommended method (ACGIH 1986). Estimated accuracy reported for an IDMS technique was excellent (Manton and Cook 1984). Sensitivity and precision were not reported by the authors, but they are generally considered to be excellent (EPA 1986a; Grandjean and Olsen 1984).

An indirect fluorescent method to quantify the level of Pb^{+2} in intracellular fluids has been published (Dyatlov et al. 1998). Although there are no commercially available fluorescent probes specific to Pb^{2+}, the fluorescent probe (fluo-3) frequently used to quantify levels of Ca^{2+} was employed as a means to estimate Pb^{2+} levels in calcium containing solution. The presence of Pb^{2+} depresses the fluorescent signal

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Blood	Dilution with Triton X-100®; addition of nitric acid and diammonium phospate	GFAAS	2.4 μg/L	93–105	Aguilera et al. 1989
Blood	Dilution of sample with ammonium solution containing Triton X-100	ICP/MS	15 μg/L	96–111	Delves and Campbell 1988
Blood	Dilution of sample in 0.2% Triton X-100 and water	GFAAS	≈15 μg/L	97–150	Que Hee et al. 1985a
Blood	wet ashing, dilution	ICP/MS	0.1 ppb	94–100	Zhang et al. 1997
		GFAAS	4 ppb	90–108	
Blood and urine	Mixing of urine sample with HNO_3; filtration, chelation of lead in whole blood or filtered urine with APDC, extraction with MIBK	AAS (NIOSH Method 8003)	0.05 μg/g (blood) or 0.05 μg/mL (urine)	99 (±10.8%)	NIOSH 1994e
Blood and urine	^{206}Pb addition and sample acid digestion; lead coprecipitation by addition of $Ba(NO_3)_2$, followed by electrodeposition on platinum wire	IDMS	No data	98–99	Manton and Cook 1984
Blood and tissue	Digestion of sample with $HNO_3/HClO_4/H_2SO_4$; heat	ICP/AES (Method 8005)	0.01 μg/g (blood) 0.2 μg/g (tissue)	113	NIOSH 1994b
Blood	Addition of 50 μL of blood into reagent, mixing, and transferring to sensor strip (commercial test kit)	Gold electrode sensor	1.4 μg/dL	No data	ESA 1998
Urine	Collect 50 mL urine sample and add 5 mL concentrated HNO_3 as preservative. Extraction-filter samples through cellulose membrane, adjust pH to 8, ash filters and resins in low temperature oxygen plasma for 6 hours	ICP/AES (Method 8310)	0.1 μg/ sample (50–200 mL sample volume)	100	NIOSH 1994f
Serum blood, and urine	Filtration of sample if needed; blood requires digestion in a Parr bomb; dilution of serum or urine with acid or water	ICP/AES	10B50 μg/L	85 (serum) >80 (urine, blood)	Que Hee and Boyle 1988

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Urine (δ-amino-levulinic acid)	Dilution of sample; reaction with ethylacetoacetate and ethylacetate to form δ-amino-levulinic acid-pyrrole; reaction with Erhlich's reagent	Spectrophotometry	No data	No data	Tomokuni and Ichiba 1988
Urine (δ-amino-levulinic acid)	Acidification of sample; separate δ-aminolevulinic acid on HPLC; reaction with formaldehyde and acetylacetone	HPLC/FL	10 μg/L	No data	Tabuchi et al. 1989
Plasma, Urine (δ-amino-levulinic acid)	Derivatization of δ-aminolevulinic acid with formaldehyde and acetylacetone to form fluorescent compounds; separation using HPLC	HPLC/FL	3 μg/L	No data	Oishi et al. 1996
Serum and cerebro-spinal fluid	^{206}Pb addition and sample acid digestion; lead isolation by ion-exchange, elution, and deposition onto platinum wire	IDMS	No data	80–120	Manton and Cook 1984
Feces	Dessication and pulverization of sample; digestion with hot acid in Paar bomb	ICP/AES	10–50 μg/L	>86	Que Hee and Boyle 1988
Testes, liver, spleen, kidney	Dicing of sample and digestion in hot acid in a Paar bomb; evaporation; redissolution in HCl/HNO_3	ICP/AES	10–50 μg/L	>80	Que Hee and Boyle 1988
Spleen, liver, and kidney; Liver, kidney, muscle	Wet digestion of sample with HNO_3-$HClO_4$ mixture; Bomb digestion of sample with acid and heat or digestion with acid and dry ashing; dissolution in acid; dilution with water	GFAAS GFAAS DPASV	No data 20 μg/g (bomb); 5 μg/g (dry ashing) No data	No data 85–107 (bomb); 75–107 (dry ashing) 82–120	Blakley and Archer 1982; Ellen and Van Loon 1990
Tissues (brain, heart, lung, kidney, liver, and testes)	Dry ashing of sample; dissolution in HNO_3	AAS	No data	No data	Exon et al. 1979
Tissues	Freeze drying of samples; subjection to thermal neutron irradiation; chemical separation of elements	NAA	No data	No data	Hewitt 1988
Brain	Wet ashing of sample with mixture of acids, mixing with Metex® and analysis	ASV	No data	No data	Jason and Kellogg 1981

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Bone	Partially polarized photon directed at second phalanx of left forefinger (noninvasive technique)	K-XRF	20 μg/g	No data	Christoff-ersson et al. 1986
Bone	Partially polarized photon directed at anteromedial skin surface of mid-tibia (non-invasive technique)	L-XRF	20 μg/g	No data	Wielopolski et al. 1986
Teeth	Cleaning and sectioning of tooth; digestion with HNO_3; evaporation; redissolution in buffer solution	ASV	No data	83–114	Rabinowitz et al. 1989
Teeth	Dry ashing of sample; crushing; dry ashing again; dissolution in HNO_3	AAS	No data	90–110	Steenhout and Pourtois 1981
Hair	Cleaning of sample with acetone/ methanol; digestion with acid mixture and heat; diammonium phosphate addition as matrix modifier	GFAAS	0.16 μg/g	99	Wilhelm et al. 1989
Bone	^{109}Cd gamma-ray irradiation with source at 2.5 cm from skin of proximal tibia	K-XRF	2 μg/g	No data	Hu et al. 1989, 1990, 1991
Hair	Cleaning of sample with hexane, ethanol, and water; wet ashing with HNO_3 and H_2O_2	ICP/AES	No data	No data	Thatcher et al. 1982

AAS = atomic absorption spectroscopy; APDC = ammonium pyrrolidine dithiocarbamate; ASV = anode stripping voltammetry; $Ba(NO_3)_2$ = barium nitrate; ^{109}Cd = cadmium 109 radioisotope; DPASV = differential pulse anodic stripping voltammetry; GFAAS = graphite furnace atomic absorption spectroscopy; H_2O_2 = hydrogen peroxide; HCl = hydrogen chloride; H_2SO_4 = sulfuric acid; $HClO_4$ = perchloric acid; HNO_3 = nitric acid; HPLC/FL = high performance liquid chromatography/fluorimetry; ICP/AES = inductively coupled plasma/atomic emission spectroscopy; ICP/MS = inductively coupled plasma-mass spectrometry; IDMS = isotope dilution mass spectrometry; K-XRF = K-wave X-ray fluorescence; L-XRF = L-wave X-ray fluorescence; MIBK = methyl isobutyl ketone; NAA = neutron activation analysis; NaOH = sodium hydroxide; NIOSH = National Institute for Occupational Safety and Health; ^{206}Pb = lead 206

observed in the emission spectrum of the fluo-3 Ca^{2+} complex at 530 nm, and the concentration of Pb^{2+} in solution was correlated with the observed decrease of intensity in the emission spectra.

Several biomarkers exist for monitoring exposure to lead. A number of biochemical assays are available for the assessment of lead exposure and toxicity in the human body using standard clinical laboratory techniques. Details of such assays are reported in several reviews (EPA 1986a; Grandjean and Olsen 1984; Stokinger 1981) and are also available in standard clinical laboratory methods manuals. The commonly used assays are coproporphyrin, 1,25-dihydroxyvitamin D, ALA (δ-aminolevulinic acid), and EP (erythrocyte protoporphyrin) concentrations and ALAD (ALA dehydratase) activity. All of these assays are sensitive, reliable, and well established; however, erythrocyte protoporphyrin and ALAD activity appear to be the most useful and sensitive for determining exposure to lead. A recent review (Porru and Alessio 1996) indicated that ALAD activity was proportional to blood lead concentration ranging from 10 to 40 µg/dL, and EP concentration was proportional to blood lead over the range of 30–80 µg/dL. The EP concentration was said to be useful for assessing exposure experienced over the past 3 to 4 months. Urinary ALA, however, was not proportional to blood lead until the blood concentrations reached 60–70 µg/dL, a concentration too high to be of use for early screening since other clinical symptoms should already be evident. A colorimetric method for detection of ALA in urine, in which the pyrrole from ALA is formed and reacted with Ehrlich's reagent to form a colored end product, has been used successfully (Tomokuni and Ichiba 1988). ALA has also been determined in urine using high-performance liquid chromatography (HPLC) followed by quantification of a fluorescent end product (Tabuchi et al. 1989). A similar approach to ALA determination in blood and urine was described by Oishi et al. (1996) and was more sensitive than the method of Tabuchi et al. (1989). Erythrocyte protoporphyrin bound to zinc has been quantified using hemofluorimetry (Braithwaite and Brown 1987). An HPLC/fluorescent method has been reported for determination of coproporphyrin in urine (Tomokuni et al. 1988). Other biological assays that have been used as indicators of lead exposure are serum immunoglobulins and salivary IgA (Ewers et al. 1982). While all of these biological assays are reliable and have been verified for clinical laboratory use, they are not specific for lead.

Tissues. Lead has been quantified in a variety of tissues, including liver, kidney, brain, heart, lung, muscle, and testes. Techniques for measuring lead in tissues are similar to those used for blood and urine. When AAS, GFAAS, or ASV are used for analysis, the samples may be wet ashed, digested with acid, or bomb digested (Blakley and Archer 1982; Blakley et al. 1982; Ellen and Van Loon 1990; Exon et al. 1979; Jason and Kellogg 1981; Que Hee and Boyle 1988). The information located did not allow an adequate comparison between these methods. Parr bomb digestions are recommended for estimation of

metals in biological tissues (Que Hee and Boyle 1988). Sensitivities reported for GFAAS and ICP/AES are in the low ppm range (5–20 ppm) (Ellen and Van Loon 1990) and are probably comparable for the other techniques. Differential anodic stripping pulse voltametry (DPASV) and NAA have also been used to analyze tissues for lead. Sample preparation for DPASV is the same as those for AAS, GFAAS, and ASV. Its accuracy and precision are comparable to results using GFAAS, and its sensitivity is slightly greater (Ellen and Van Loon 1990). Determination of lead in tissue samples following freeze drying, neutron irradiation, and chemical separation has been reported. An advantage of this method is that the sample does not have to be dissolved. No further information was reported for the method (Hewitt 1988).

Hair, Teeth, and Bone. Noninvasive methods using x-ray fluorescence can be used for the determination of lead concentration in bones. Lead accumulates over a lifetime in bones, so these measurements represent a metric cumulative dose, whereas measurements of lead in blood represent a more recent dose. Typical analyses encompass L x-rays of the tibia produced using an x-ray generator (Wielopolski et al. 1986); K x-rays in the second phalanx of the index finger using a cobalt source and a germanium silicon detector (Christoffersson et al. 1986); and *in vivo* bone K x-ray fluorescence (Batuman et al. 1989; Hu et al. 1989, 1990, 1991, 1998). The K x-ray fluorescence technique has been more widely used and validated than the L x-ray method, which has limitations regarding its utility for the determination of lead levels in bone (Hu et al. 1998; Preiss and Tariq 1992). The more energetic K x-rays penetrate the cortical bone deeper (2 cm) than the soft L x-rays, and are therefore more suitable for determining the average lead content over the whole bone thickness (Wedeen 1990). The better penetration also alleviates errors resulting from the measurement of overlying skin and makes the method relatively insensitive to movement of the subject during the 15-minute sampling period (Landrigan and Todd 1994). The level of lead in bone has been reported to be a good indicator of stored lead in body tissue (Ahlgren et al. 1976; Bloch et al. 1976; Rosen et al. 1987; Skerfving et al. 1993). The sensitivity of the technique is in the low ppm range and the precision is acceptable. Advantages are that no sample preparation is required and the technique can safely and easily be done on live subjects. A limitation of x-ray fluorescence measurements is that its precision is dependent upon the mass of the bone being studied (Hu et al. 1998). Therefore, thin bones of children have greater measurement errors than mature bones found in adults. Teeth have been analyzed for lead using AAS and ASV (Rabinowitz et al. 1989; Steenhout and Pourtois 1981). Samples must be dry ashed or digested with acid prior to analysis. Precision and accuracy of both AAS and ASV are good. Detection limits were not reported by the authors. A detection limit in the sub-ppm range (0.16 ppm) and high accuracy were reported for GFAAS analysis of hair samples (Wilhelm et al. 1989). ICP/AES has also been used to analyze hair for lead, but lack of data prevents a comparison with the AAS method (Thatcher et al. 1982).

The isotopic distribution of lead (IDMS) in shed teeth from children has been shown to be useful in studies of the history of exposure to lead, including the definition of the source of the exposure, e.g., mine dust vs. food (Gulson and Wilson 1994), so IDMS certainly has important applicability, if not for routine determinations. ICP/MS, however, is easier, more sensitive, allows for multi-element analysis, and provides isotopic data.

7.2 ENVIRONMENTAL SAMPLES

The primary methods of analyzing for lead in environmental samples are AAS, GFAAS, ASV, ICP/AES, and XRFS (EPA 1993). Less commonly employed techniques include ICP/MS, gas chromatography/photoionization detector (GC/PID), IDMS, DPASV, electron probe x-ray microanalysis (EPXMA), and laser microprobe mass analysis (LAMMA). The use of ICP/MS for the analysis of trace metals (including lead) has increased in recent years due to its high sensitivity and ease of sample preparation. ICP/MS is generally 3 orders of magnitude more sensitive than ICP/AES; however, it is more costly than other spectroscopic methods and is not universally available (Al-Rashdan et al. 1991; California Department of Fish and Game 2004). Chromatography (GC, HPLC) in conjunction with ICP/MS can also permit the separation and quantification of organometallic and inorganic forms of lead (Al-Rashdan et al. 1991). In analyzing lead concentrations in the atmosphere, a distinction between the levels of inorganic lead, which exists predominantly in the particulate phase, and alkyl lead, which occurs predominantly in the vapor phase, is necessary. Particulate-phase lead can be separated from the gas phase using a filter technique. The filter collects the particulate matter and allows the dissolved material to pass through for separate analysis of each form. As with the analysis of biological samples, the definitive method of analysis for lead is IDMS. Table 7-2 summarizes several methods for determining lead in a variety of environmental matrices.

Air. Various methods have been used to analyze for particulate lead in air. The primary methods, AAS, GFAAS, and ICP/AES are sensitive to levels in the low μg/m^3 range (0.1–20 μg/m^3) (Birch et al. 1980; EPA 1988b; NIOSH 1981, 1994a, 1994c, 2003; Scott et al. 1976). Accuracy and precision are generally good. GFAAS is considered to be more sensitive than AAS; however, AAS is not subject to as much interference from matrix effects as GFAAS (NIOSH 1977a, 1977d). Detection of particulate lead by generation of the lead hydride has been used to increase the sensitivity of the AAS technique (Nerin et al. 1989). Excellent accuracy and precision was reported for this method. ASV has a wide range as well as high sensitivity. It is relatively inexpensive compared to other methods (NIOSH 1977a).

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Air (particulate lead)	Collection of particulate matter onto membrane filter; digestion with HNO_3/H_2O_2; dilution with distilled water	GFAAS (NIOSH Method 7105)	0.02 μg/ sample (1–1,500 L sample)	85–115	NIOSH 1994d
Air (particulate lead)	Collection of particulate matter onto membrane filter; wet ashing with HNO_3	AAS flame (Method 7082)	2.6 μg/sample (200–1,500 L sample)	97–100	NIOSH 1994c
Air (particulate lead)	Collection of particulate matter onto cellulose acetate membrane filter; wet ashing with $HNO_3/HClO_4$	ICP/AES (NIOSH Method 7300)	25 ng/mL	101–109	NIOSH 2003
Air (particulate lead)	Collection of particulate matter onto filter; extraction with HNO_3/HCl, heat, and sonication	ICP/AES	No data	No data	EPA 1988a
Air (particulate lead)	Collection of particulate matter onto filter; dry ashing; extraction with HNO_3/HCl; dilution with HNO_3	AAS AES	0.1 μg/m^3 0.15 μg/m^3	93 102	Scott et al. 1976
Air (particulate lead)	Collection of sample onto cellulose acetate filter; dissolution in HNO_3 with heat; addition of HCl/H_2O_2 and reaction in hydride generator with sodium borohydride to generate lead hydride	AAS	8 ng/L	100–101	Nerin et al. 1989
Air (particulate lead)	Collection of sample onto filter; addition of ^{206}Pb to filter; dissolution of filter in NaOH; acidification; separation of lead by electro-deposition; dissolution in acid	IDMS	0.1 ng/m^3	No data	Volkening et al. 1988
Air (particulate PbS)	Collection of particles onto filter, suspension in THF, recollection onto silver filter	XRD	60 μg/m^3	102.6	NIOSH 1994a
Air (particulate lead)	Collection of sample onto nucleopore polycarbonate filter; coating of filter sections with carbon	EPXMA LAMMA	No data No data	No data No data	Van Borm et al. 1990

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Air (tetramethyl and tetraethyl lead)	Adsorption of volatile compounds in filtered sample onto XAD-2 resin, desorption with pentane	GC/PID (NIOSH Method 2534 [TML] and 2533 [TEL])	0.4 µg/sample (15–100 L sample) (TML); 0.1 µg/ sample (30–200 L sample) (TEL)	97	NIOSH 1994g; 1994h
Air (particulate and organo-lead)	Collection of particulate matter collected onto glass fiber filter; passage of filtered gases through iodine monochloride bubblers; wet ashing of particulate matter; conversion of lead compounds in bubbler solution to dithiazone complex in presence of EDTA-salts and extraction with carbon tetrachloride solution followed by acid extraction	GFAAS	No data (particulate); 0.25 ng/m^3 (gaseous)	No data (particulate); 95–99 (gaseous)	Birch et al. 1980
Air (particulate and organo-lead)	Collection of particulate matter collected onto nucleopore filters; filtered gases cryogenically trapped and thermally desorbed	XRF (particulate) GC/GFAAS (gaseous)	0.3 µg/m^3 0.2 ng/m^3	46–>90 90–100	De Jonghe et al. 1981
Surface contamination (lead and its compounds)	Wiping of defined area surface using a moistened gauze pad; digestion of sample using nitric acid; dilution.	ICP/AES GFAAS	2 µg/sample 0.1 µg/sample	No data	NIOSH 1994a
Water (particulate and dissolved lead)	Filtration of water through a 0.45 µm membrane filter (dissolved lead); particulate material dissolved by wet ashing (insoluble lead)	ICP/AES (EPA Method 200.7)	42 µg/L	94–125	EPA 1983
Water (TAL)	Extraction with hexane	GC/AAS	0.5 µg/L	88–90	Chau et al. 1979
Water (TAL)	Purging of sample with gas followed by cryogenically trapping volatile species onto solid sorbent GC column	GC/AAS	0.5 ng/g	No data	Chau et al. 1980
Water (alkyl lead)	Complexation of sample with diethyldithiocarbamate; extraction with pentane; removal of water; butylation; extraction with nonane	GC/AAS	1.25 ng/L	90–108	Chakraborti et al. 1984

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Water (particulate and dissolved lead)	Filtration of water through a 0.45 µm membrane filter (dissolved lead); particulate material dissolved by wet ashing (insoluble lead)	AAS (EPA Method 239.1) GFAAS (EPA Method 239.2)	0.1 mg/L 1 µg/L	99.8–125.7 88–95	EPA 1983
Water (total lead)	Digestion of sample with acid and heat; dilution with water	AAS	1.0 ng/g	No data	Chau et al. 1979
Water (dissolved or total)	Acidification, addition of ammoniacal citrate-cyanide reducing solution; extraction with chloroform containing dithizone.	(Standard Method 3500-PbB)	No data	No data	NEMI 2005b
Water	Filtration, acidification, aspiration into a flame	AAS (Standard Method 3111B)	0.5 mg/L	No data	NEMI 2005a
Water	Digestion, analysis	GFAAS (Standard Method 3113B)	1 µg/L	101%	NEMI 2005c
Water and waste water (dissolved, total)	Filtration/acidification and analysis for dissolved; digestion followed by analysis for total	ICP/AES (Standard Method 3120B)	10 µg/L	109%	NEMI 2005d
Water, extracts or digests of waste	Filtration or digestion as appropriate (depends on matrix, dissolved or total, acid leachable, etc.)	ICP/MS (EPA Method 6020)	No data	71–137% (11–23% RSD) for aqueous solutions; 90B104% (6B28% RSD) for solid samples	EPA 1994d
Water	Filtration; addition of $Ni(NO_3)_2$ and $NH_4H_2PO_4$matrix modifiers	ETAAS	0.14 µg/L	89–101	Xu and Liang 1997
Water (total lead)	Filtration of sample followed by analysis; digestion of filter with acid	ICP/AES	10B50 µg/L	>80	Que Hee and Boyle 1988
Soil	Drying of soil sample followed by sieving; digestion with HNO_3; centrifugation	ICP/AES	0.09 µg/g	97–103	Schmitt et al. 1988

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Dust	Wiping of hard surface of known dimension; acid digestion	ICP/AES AAS GFAAS	Varies	No data	ASTM 1998f (ASTM E 1728); ASTM 1998b (ASTM E 1644); ASTM 1998a (ASTM E 1613)
Soil	Drying of soil followed by homogenization, digestion with nitric acid and hydrogen peroxide, dilution	ICP/AES AAS GFAAS	Varies	No data	ASTM 1998e (ASTM E 1727) ; ASTM 1998d (ASTM E 1726) ; ASTM 1998a (ASTM E 1613)
Soil	Drying of soil sample followed by sieving, digestion with HNO_3, filtration	AAS	No data	No data	Mielke et al. 1983
Soil	Drying of sample and sieving for XRF; digestion of sieved sample with HNO_3 and heat for AAS	XRF AAS	No data No data	65–98 63–68	Krueger and Duguay 1989
Soil	Drying of sample, dry ashing, digestion with acid, and dilution with water	AAS	2 μg/g	79–103	Beyer and Cromartie 1987
Soil	Digestion with HNO_3 and H_2O_2; evaporation; redissolution with HNO_3; filtration	FI-HG-AAS	2 μg/L	98–101	Samanta and Chakraborti 1996.
Soil, wastes, and ground-water	Acid digestion of sample, dilution with water, and filtration	AAS (EPA method 7420) GFAAS (EPA method 7421)	0.1 mg/L 1 μg/L	No data No data	EPA 1986c
Soil, dust, and paint	Digestion of sample with hot acid; evaporation of water; redissolution in HNO_3	AAS	12 ng/g	>80	Que Hee et al. 1985b

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Sediment	Digestion of sample with hot HNO_3/H_2SO_4	GFAAS	No data	92–95	Bloom and Crecelius 1987
Sediment, fish (TAL)	Homogenization of fish; addition of EDTA to sample; extraction with hexane; centrifugation; isolation off organic layer for analysis	GC/AAS	0.01 μg/g (sediment) 0.025 μg/g	81–85 72–76	Chau et al. 1979
Sediment, (fish), vegetation (TAL)	Purging of sample with gas followed by cryogenically trapping volatile species onto solid sorbent GC column	GC/AAS	0.1 ng/g (solid)	No data	Chau et al. 1980
Sediment, fish, vegetation (total lead)	Digestion of sample with acid and heat; dilution with water	AAS	50 ng/g (sediment) 10 ng/g (fish and vegetation)	No data No data	Chau et al. 1980
Dried paint	Sample collection using heat gun, cold scraping, or coring methods; microwave digestion with nitric acid and hydrochloric acid	ICP/AES AAS GFAAS	Varies	No data	ASTM 1998g (ASTM E 1729); ASTM 1998c (ASTM E 1645); ASTM 1998a (ASTM E 1613)
Milk	Addition of 50 μL $(C_2H_5)_4NOH$ in ethanol to 25 μL milk followed by heating and dilution with water to 125 μL	GFAAS	No data	No data	Michaelson and Sauerhoff 1974
Evaporated milk	Dry ashing of sample; dissolution in HNO_3	ASV	0.005 μg/g	99	Capar and Rigsby 1989
Mussel, tomato	Digestion of sample with acid or acid plus catalyst; generation of lead hydride	GFAAS	4 ng/g	94–95	Aroza et al. 1989
Agricultural crops	Dry ashing of sample with H_2SO_4 and HNO_3; dilution with water	DPASV	0.4 ng/g	85–106	Satzger et al. 1982

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Grains, milk mussel, fish	Bomb digestion of sample with acid and heat or digestion with acid and dry ashing; dissolution in acid; dilution with water	GFAAS	20 μg/g (bomb); 5 μg/g (dry ash)	85–107	Ellen and Van Loon 1990
			No data	75–107	
		DPASV		82–120	
Edible oils	Microwave digestion with acid mixture;	ICP/AES	50 ng/g	75–107	Allen et al. 1998
	$(NH_4)_2PO_4$ added as matrix modifier	GFAAS	30 ng/g	78–117	
Citrus leaves and paint	Chopping or pulverization of sample; digestion with hot acid; evaporation of water; redissolution in acid	ICP/AES	10–50 μg/L	75–82 (citrus leaves); 89–96 (paint)	Que Hee and Boyle 1988
Feathers	Clean feathers with non ionic detergent; rinse with deionized water for 2–3 minutes.	ICP/MS	10 ppb	No data	California Department of Fish and Game 2004

AA = atomic absorption; AAS = atomic absorption spectroscopy; AES = atomic emissions spectroscopy; ASV = anode stripping voltammetry; $(C_2H_5)_4NOH$ = tetraethylammonium hydroxide; DPASV = differential pulse anodic stripping voltammetry; EDTA = ethylenediamine tetraacetic acid; EPA = Environmental Protection Agency; EPXMA = electron probe X-ray micro-analysis; ETAAS = electrothermal atomic absorption spectroscopy; GC = gas chromatography; GFAAS = graphite furnace atomic absorption spectrometry; HCl = hydrochloric acid; $HClO_4$ = perchloric acid; HNO_3 = nitric acid; H_2O_2 = hydrogen peroxide; H_2SO_4 = sulfuric acid; ICP/AES = inductively coupled plasma-atomic emission spectroscopy; ICP/MS = inductively coupled plasma-mass spectrometry; IDMS = isotope dilution mass spectrometry; LAMMA = laser microprobe mass analysis; MS = mass spectrometry; NaOH = sodium hydroxide; NG = nanogram; NIOSH = National Institute for Occupational Safety and Health; ^{206}Pb = lead 206; PID = photoionization detector; TAL = tetraalkyl leads; TEL = tetraethyl lead; THF = tetrahydrofuran; TML = tetramethyl lead; XRD = X-ray diffraction; XRF = X-ray fluorescence

Advantages of ICP/AES are that it has a wide range and allows analysis of several elements at once. However, the technique is expensive in terms of equipment and supplies (NIOSH 1981). XRFS has been used to analyze for particulate lead in air (DeJonghe et al. 1981). While sensitivity was good, recovery was highly variable and relatively low compared to other methods. The highest sensitivity was obtained with IDMS, as expected (Volkening et al. 1988). As previously stated, this is the definitive method for determining lead in environmental, as well as biological samples. Two sophisticated methods, EPXMA and LAMMA, have been used to determine the inorganic lead species present in particulate matter in air (Van Borm et al. 1990).

Determination of lead vapor in air requires prior filtering of the air to exclude particulate lead, and trapping of the gaseous components. Gaseous lead is also referred to as organic lead or alkyl lead, the most common being the tetraalkyl species. Organic lead species may be trapped by liquid or solid sorbents, or cryogenically (Birch et al. 1980; DeJonghe et al. 1981; NIOSH 1978b). Gas chromatography (GC) is used to separate the different alkyl species. Detection by GFAAS and PID has been reported (DeJonghe et al. 1981; NIOSH 1978b). GFAAS detection is more sensitive than PID, but both have good accuracy.

Water. As with air, water can be analyzed for both particulate and dissolved (organic) lead. Particulate lead collected on a filter is usually wet ashed prior to analysis. Comparison of the GFAAS and AAS methods for particulate lead showed the former technique to be about 100 times more sensitive than the latter, although both offer relatively good accuracy and precision (EPA 1983). ICP/MS has been used to determine lead in water (EPA 1994d). Chelation/extraction can also be used to recover lead from aqueous matrices (APHA 1998). GC/AAS has been used to determine organic lead, present as various alkyl lead species, in water (Chakraborti et al. 1984; Chau et al. 1979, 1980). Sample preparation for organic lead analysis was either by organic solvent extraction (Chakraborti et al. 1984; Chau et al. 1979) or purge-and-trap (Chau et al. 1980). Sensitivity was in the ppb to ppt range and reliability was similar for all three methods. Total lead can be determined by digesting samples with acid and analyzing by either AAS or the more sensitive GFAAS (EPA 1986c).

Dusts, Sediments, and Soil. Both total and organic lead have been determined in dusts, sediments, and soils. In most cases, the sample must be digested with acid to break down the organic matrix prior to analysis (ASTM 1998b, 1998d; Beyer and Cromartie 1987; Bloom and Crecelius 1987; EPA 1986c; Krueger and Duguay 1989; Mielke et al. 1983; Que Hee and Boyle 1988; Que Hee et al. 1985b; Samanta and Chakraborti 1996; Schmitt et al. 1988); however, organic extraction (Chau et al. 1979) and purge-

and-trap (Chau et al. 1980) have also been used. The primary detection methods are ICP/AES, AAS, or GFAAS (GFAAS being more sensitive, but also more susceptible to interference). When quantification of organic lead is desired, GC is employed to separate the alkyl lead species (Chau et al. 1979, 1980). Precision and accuracy are acceptable for these atomic absorption-based methods (Beyer and Cromartie 1987; Bloom and Crecelius 1987; Chau et al. 1979; EPA 1986c; Krueger and Duguay 1989; Que Hee et al. 1985b). ICP/AES is reported to be more sensitive and reliable than atomic absorption techniques (Schmitt et al. 1988), but sample collection and preparation methods have been shown to strongly influence the reliability of the overall method (Que Hee et al. 1985b). Sampling of house dust and hand dust of children requires special procedures (Que Hee et al. 1985b). XRFS appears to provide a simpler method of measuring lead in soil matrices; however, the available data do not permit an assessment of the techniques sensitivity and reliability for soil analysis (Krueger and Duguay 1989). XRFS has been shown to permit speciation of inorganic and organic forms of lead in soil for source elucidation (Manceau et al. 1996).

Other Matrices. Lead has been determined in several other environmental matrices, including paint, fish, vegetation, agricultural crops, and various foods. As with soil, the methods of choice are ICP/AES, AAS, or GFAAS. Samples may be prepared using one of the methods described for sediment and soil or by wet or dry ashing (Aroza et al. 1989; ASTM 1998d; Capar and Rigsby 1989; Que Hee and Boyle 1988; Que Hee et al. 1985b; Satzger et al. 1982). ASV and DPASV have also been used with good sensitivity (ppb) and reliability to analyze for lead in other environmental media (Capar and Rigsby 1989; Ellen and Van Loon 1990; Satzger et al. 1982).

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 lead 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 lead.

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. Methods are available for measuring inorganic lead in blood, serum, urine, cerebrospinal fluid, tissues, bone, teeth, and hair (Aguilera de Benzo et al. 1989; Batuman et al. 1989; Blakley and Archer 1982; Blakley et al. 1982; Christoffersson et al. 1986; Delves and Campbell 1988; Ellen and Van Loon 1990; Exon et al. 1979; Hu et al. 1989, 1990, 1991; Jason and Kellogg 1981; Manton and Cook 1984; NIOSH 1977b, 1977c, 1994c, 2003; Que Hee and Boyle 1988; Que Hee et al. 1985a; Wielopolski et al. 1986; Zhang et al. 1997). Available methods for determining lead in body fluids are sensitive and reliable for measuring background exposure levels, as well as exposure levels at which health effects have been observed to occur. Blood lead levels have been found to correlate best with exposure concentrations (Moore 1995; Rabinowitz et al. 1985). Methods of quantifying lead in tissues, bone, teeth, and hair are generally reliable, but are only sensitive at relatively high exposure concentrations. Since the elimination half-time of lead in blood is approximately 30 days, PbBs generally reflect relatively recent exposures. Lead in bone is considered a biomarker of cumulative exposure to lead because lead accumulates in bone over the lifetime and most of the lead body burden resides in bone. There is a need for more sensitive methods of detection for matrices so that correlations between lead levels in these media and exposure concentrations can be more reliably determined. Several nonspecific biomarkers are used to assess exposure to lead. These include ALAD activity and ALA, EP, coproporphyrin, and 1,25-dihydroxyvitamin D concentrations (Braithwaite and Brown 1987; EPA 1986a; Grandjean and Olsen 1984; Oishi et al. 1996; Porru and Alessio 1996; Stokinger 1981; Tabuchi et al. 1989; Tomokuni and Ichiba 1988; Tomokuni et al. 1988). Lead interferes with the conversion of zinc protoporphyrin (ZPP) to heme by the enzyme ferrochelatase and a correlation has been observed between lead blood levels and ZPP; therefore, levels of ZPP can also be used as a biomarker of lead exposure (Goyer 2001). The methods for determining these biomarkers are generally sensitive and reliable. No additional research for these biomarkers appears to be needed. There is a need to identify and quantify those molecules responsible for lead transport within the body; the measurement of lead associated with these compounds could provide additional information about exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Numerous analytical methods are available for measuring inorganic and organic lead

compounds in air, water, sediments, dust, paint, soil, fish, agricultural products, and foodstuffs (NEMI 2005a, 2005b, 2005c, 2005d; Eckel and Jacob 1988; EPA 1982a, 1986a, 1988b, 1989d, 1989e, 1990, 1994d; Lee et al. 1989; Maenhaut et al. 1979; Mielke 1993; Mielke et al. 1983, 1984/1985, 1989). Most of these are sensitive and reliable for determining background concentrations of lead compounds in the environment and levels at which health effects might occur. The most frequently used methods are AAS, GFAAS, ASV, and ICP/AES, the methods recommended by EPA and NIOSH (ASTM 1998a; Birch et al. 1980; EPA 1988b; NIOSH 1981, 1994c, 2003; Scott et al. 1976). The definitive method is IDMS, which is used to produce reference standards by which laboratories can determine the reliability of their analyses (Volkening et al. 1988). No additional analytical methods for determining low levels of lead compounds in environmental media are needed. Additional method development work is needed if individual lead species in environmental media are to be accurately determined. ICP/MS based methods should be critically examined.

7.3.2 Ongoing Studies

Ongoing studies regarding analytical methods for lead were reported in the Federal Research in Progress database (FEDRIP 2005), and are summarized in Table 7-3.

Table 7-3. Ongoing Research Regarding the Analytical Methods for Lead in Environmental and Biological Samples

Investigator	Affiliation	Research description	Sponsor
Chillrud S	Columbia University, New York, New York	Core-Geochemistry Laboratory: A laboratory is being developed to support several ongoing research projects, including projects involving the analytical measurement of lead in environmental samples and human tissue. The instrumentation that will be used includes a VG sector 54-30 Thermal Ionization Mass Spectrometer (TIMS), a Hitachi Z8200 Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS), a VG High-Resolution inductively coupled plasma-mass spectrometry (ICP-MS).	National Institute of Environmental Health Sciences
Mutti A	University of Parma, Parma Italy	Metals in exhaled breath condensate as chronic obstructive pulmonary disease (COPD) biomarkers: Develop biomarkers for COPD involving the analysis of exhaled breath condensate for the presence of lead by electro-thermal atomic absorption spectroscopy (ETAAS) and ICP-MS.	National Heart, Blood, and Lung Institute
Parsons PJ	New York State Department of Health, Human Toxicology and Molecular Epidemiology	Bone Lead Standardization Program: The aim of this proposal is to create a Standardization Program for Bone Lead measurements (BLSP) obtained via reference methods and via *in vivo* x-ray fluorescence (XRF).	National Institute of Environmental Health Sciences

Source: FEDRIP 2005