7. ANALYTICAL METHODS

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

The accurate and reliable determination of plutonium in biological and environmental samples is important because of the potential impact of this element on public health. Analytical methods used to measure plutonium in biological and environmental media are highly refined compared to other transuranics. Alpha spectrometry is the most widely used method for the determination of plutonium. However, this method cannot resolve the ^{239}Pu and ^{240}Pu peaks due to their similar energies (5.15 and 5.16 MeV). An independent mass spectrometric analysis is required in order to determine the individual concentrations of ^{239}Pu and ^{240}Pu (Muramatsu et al. 2001a; Wolf 2006). Other methods such as thermal ionization mass spectrometry (TIMS) and accelerator mass spectrometry (AMS) have been used for the determination of plutonium. Inductively coupled plasma-mass spectrometry (ICP-MS) has advantages of ease of operation and rapid analysis. In addition, ICP-MS can provide information about the $^{239}Pu/^{240}Pu$ ratio in a sample, which can, in turn, provide important information about the source of plutonium contamination (Muramatsu et al. 2001a; Varga et al. 2007; Wolf 2006). Interferences that may be observed with ICP-MS are caused by polyatomic ions in the plasma, such as $^{238}UH^+$ and $^{238}UH_2^+$, which can interfere with ^{239}Pu and ^{240}Pu, respectively, in samples with high concentrations of uranium (Epov et al. 2005; Figg et al. 2000).

General environmental survey instruments (e.g., alpha particle meters) are available, but they are not specific for plutonium. The predominant analytical method for measuring plutonium present at or near background concentrations in both biological and environmental media requires radiochemical separation and purification in conjunction with a quantitative measurement technique (e.g., alpha spectrometry, liquid scintillation, or mass spectrometry).

7.1 BIOLOGICAL MATERIALS

Methods for the determination of plutonium in biological materials are summarized in Table 7-1. The procedures that have been developed for the determination of small quantities of plutonium in biological samples, as well as in environmental samples, include the following steps:

- Release of plutonium from the sample's matrix into solution and the addition of plutonium tracers;
- Concentration by precipitation with a nonisotopic carrier (e.g., lanthanum or neodymium) or by solvent extraction;
- Purification by precipitation, liquid extraction, or ion exchange chromatography; and
- Determination of the plutonium content of the sample by alpha spectroscopy or other techniques.

Two common methods for releasing plutonium from the sample's matrix into solution are acid extraction and acid dissolution. Samples are wet- or dry-ashed prior to solubilization. Leaching the sample with a mixture of acids (e.g., nitric acid and hydrochloric acid) has the advantage of easily handling large sample volumes, but with the potential disadvantage of leaving plutonium compounds in the residue. The acid dissolution procedure includes the addition of excess hydrofluoric acid (HF) to the above mixture of acids and results in dissolution of much, if not all, of the sample matrix. Refractory plutonium compounds (e.g., PuO_2) are more likely to be dissolved upon addition of HF. However, dissolution of interfering elements, such as iron, phosphorous, and other rare earths (e.g., alpha-particle emitters) is also increased in acid dissolution.

A third example of a dissolution method is fusion, which is used primarily for decomposition of geological and solid environmental media and is suitable for large samples (several grams) (Wolf 2006). Fusion decomposition is performed by heating a sample with a flux reagent at atmospheric pressure in a graphite, zirconium , or platinum crucible. Common fluxes include hydroxides, peroxides, carbonates, bisulfates, hydrosulfates, pyrosulfates, tetraborates, and metaborates. Fusion with sodium hydroxide and sodium peroxide ($NaOH-Na_2O_2$) is an effective method for decomposition of silica-containing matrices. A disadvantage of fusion decomposition is that use of a large amount of flux material results in a solution with a high content of total dissolved solids, requiring chemical separation of the analyte from the dissolved flux material (Wolf 2006).

Plutonium solutions that contain: (1) other alpha-particle emitters (e.g., americium and neptunium), or (2) large amounts of fission products (e.g., cesium), or interfering amounts of other substances such as

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Samples were spiked with a known amount of plutonium and digested with nitric acid/hydrogen peroxide Non-digested (raw) samples were also analyzed	ICP-MS	0.18 pg/L (digested samples after preconcentration) 1.9 pg/L (raw samples)	70–100% (raw samples)	Epov et al. 2005
Tissue	Wet ashed with HNO_3/H_2SO_4; collected on $Fe(OH)_2$; separation by ion exchange and electrodeposition or microprecipitation	α spectro-metry	0.0007 Bq (400 minutes)	No data	DOE 1997 Pu-04-RC
Tissue	Wet ashed with HNO_3/HF; separation by solvent extraction; electrodeposition onto platinum disc	solid state α spectrometry	0.65 Bq (400 minutes)	No data	DOE 1997 Pu-05-RC
Urine	Wet ashing with $H_2O_2/HNO_3/HCl/HF/H_2SO_4$; separation by anion exchange chromatography; electrodeposition onto platinum disc	solid state α spectrometry	0.60 Bq (400 minutes)	No data	DOE 1997 Pu-06-RC
Urine	Wet ashed with $HNO_3/H_2O_2/HCl$; purified by ion exchange chromatography	α spectro-metry	No data	No data	DOE 1997 Pu-07-RC; Pu-11-RC
Tissue	Ashed at 400 °C; dissolved in HNO_3/HCl; filtered; decomposed with HF; purified by ion exchange chromatography	α spectro-metry	No data	No data	DOE 1997 Pu-08-RC; Pu-11-RC
Tissue	Digested with HNO_3/H_2SO_4; coprecipitation of plutonium with $Fe(OH)_3$; purified by ion exchange chromatography	α spectro-metry	No data	No data	DOE 1997 Pu-09-RC; Pu-11-RC
Tissue	Ashing; electrodeposition	α spectro-metry	No data	No data	USTUR Method 600
Biological soft tissues	Wet ash; filter; extract; electrodeposition on platinum disk	α spectro-metry	No data	No data	Singh and Wrenn 1988
Urine	Evaporate; wet ash; filter; extract; electrodeposit on platinum disk	α spectro-metry	No data	No data	Singh and Wrenn 1988
Fecal matter	Wet ash; filter; extract; electrodeposition on platinum disk	α spectro-metry	No data	No data	Singh and Wrenn 1988

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Bones	Dry ash; reduce valence state; extract; electro-deposition on platinum disk	α spectro-metry	No data	No data	Singh and Wrenn 1988
Milk	Dry ashed; dissolution in HCl; extraction with triisooctylamine; coprecipitate with lanthanum fluoride; filtration	α spectro-metry	No data	No data	EPA 1984 (Method 00-09)
Plant	Dissolve starch; filter; wet ash; extract; electro-deposition on platinum disk	α spectro-metry	0.0027 pCi (0.1×10^{-4} Bq)	No data	Bunzl and Kracke 1987

ICP-MS = inductively coupled plasma-mass spectrometry

iron, calcium, uranium, and phosphorous need to undergo additional chemical separation procedures. Non-radioactive carriers, such as lanthanum fluoride (LaF_3), neodymium fluoride (NdF_3), and zirconium phenylphosphate ($ZrC_6H_6PO_4$), are used to selectively precipitate the lanthanides. Solvent extraction and ion exchange separation methods are preferred methods because of better separations. In addition, they do not involve the addition of nonvolatile substances resulting in an easier preparation of the co-precipitation source used for alpha-particle counting.

These extraction techniques can be made very efficient and selective by adjusting the oxidation state of the plutonium and other sample constituents. Common extraction methods specific for plutonium use 2-thenoyltrifluoroacetone (TTA), tetrapropylammonium trinitrate in isopropylacetone or triisooctylamine, cupferron in chloroform, tributylphoshphate, and tri-octylphosphine dioxide. Anion exchange methods with either nitric or hydrochloric acid solutions are commonly used. Cation exchange column methods are less frequently used (Brouns 1980).

Prior to measurement, the separated and purified plutonium is typically deposited as a very thin layer on a highly polished metal plancet. Two techniques that are commonly used are: (1) electrodeposition and (2) co-precipitation with a carrier. In electrodeposition, the plutonium is electrodeposited on a polished stainless steel, or platinum disk. In the co-precipitation technique, actinides can be co-precipitated from a large volume of solution using anions such as fluorides, hydroxides, and phosphate. Actinides in the tri- or tetravalent state can be removed from solution by the addition of lanthanide fluoride carriers, such as NdF_3 or LaF_3, which are used to co-precipitate the separated and purified plutonium from solution. Iron hydroxide can also be used to co-precipitate actinides from a carbonate-free solution. The precipitate is then prepared for counting by either filtration or by evaporation of a slurry of the precipitate onto a stainless steel disk (Hindman 1983; Mitchell 1960; Sill and Williams 1981; Talvitie 1972; Wolf 2006).

The U.S. Department of Energy Environmental Measurement Laboratory Procedures Manual and the U.S. Transuranium and Uranium Registries Radio Analysis Procedures Manual provide techniques for the determination of plutonium in biological samples using alpha spectroscopy (DOE 1997; USTUR 2001).

The other two alpha-particle emitting plutonium isotopes, ^{236}Pu and ^{242}Pu, are normally not found in environmentally significant quantities, and are not common constituents of nuclear fuels or waste waters. Therefore, they can be used as tracers to aid in the analysis of other isotopes. In this calibration procedure, a known quantity of a tracer is added to the sample being analyzed in order to determine the yield. This is the percentage of the total amount of plutonium in the sample that is actually measured in

the electrodeposited amount after the separation, purification, and preparation of the source (Brouns 1980).

The most critical step in the analysis of biological samples is complete dissolution of the sample to assure solubilization of all plutonium compounds. Biological samples are generally dissolved by wet ashing or a combination of wet and dry ashing. High temperatures (700–1,000 °C) during ashing should be avoided in order to prevent the formation of an insoluble form of plutonium dioxide (Nielson and Beasley 1980; Sill 1975). Plutonium that has been distributed to urine, blood, or soft tissue as a result of metabolic processes is usually in a readily soluble form. Lung tissue, feces, and excised tissue from wound sites will likely contain insoluble forms of plutonium and will require treatment with HF and repeated ashings to effect solubilization. Tissues, feces, and vegetation require repeated treatment with a mixture of concentrated nitric acid (HNO_3), perchloric acid ($HClO_4$), and sulfuric acid (H_2SO_4) in order to oxidize the large amount of organic materials in these samples. If an insoluble residue remains after repeated ashings, then fusion of the residue with gram quantities of an inorganic flux (e.g., sodium carbonate, sodium pyrosulfate) can be used to effect solution. Known amounts of a plutonium isotope are commonly added subsequent to the dissolution step so that the percentage of plutonium recovered after separation and purification (i.e., the yield) may be determined. This added plutonium must be in the same chemical form as the plutonium in the sample or the yield estimates will not reflect the percentage of plutonium recovered from the dissolved sample (EPA 1976a; Nielson and Beasley 1980).

Methods used for concentrating plutonium in a sample by a carrier are often specific to one oxidation state of the plutonium. For example, the classical bismuth phosphate-lanthanum fluoride method of concentrating plutonium from urine samples is specific to plutonium in the tri- and tetravalent states and will leave plutonium(VI) in solution. The fate of the various oxidation states of plutonium in humans is not well understood and analysis procedures must insure reduction or oxidation of plutonium into appropriate oxidation states. Liver and kidney samples may contain metals (e.g., iron) that may greatly reduce chemical yields during the final electrodeposition step (EPA 1976a).

Sensitive methods for analysis of plutonium in urine are particularly important for estimating occupational plutonium body burdens. Routinely available instrumentation, such as the alpha spectrometer, can readily detect these low concentrations. More sensitive methods are commonly required for urine samples in order to assess chronic exposures to plutonium. These low detection limits were first achieved in the past by nuclear emulsion track counting. In this method, the electrodeposited sample is exposed to nuclear track film, subsequent to the isolation of plutonium. The alpha-particle

emitting isotopes of plutonium will leave tracks on the film, which are counted to quantify the amount of plutonium. Nuclear emulsion track counting has been used in the past to measure plutonium concentrations in the urine of workers at a nuclear reactor plant (Nielson and Beasley 1980). A type of scintillation counting has been used to measure ^{239}Pu and americium-241 (^{241}Am) in animal tissues (NCRP 1985).

Epov et al. (2005) reported a method where nondigested urine samples could be analyzed with a detection limit of 1.9 pg/L. The authors noted that in the case of an emergency, urine analysis without digestion could provide a rapid determination (about 1 hour) of plutonium levels in urine. However, sample digestion would be needed if more precise and sensitive analysis is required. Four hours are required to analyze urine samples with digestion (Epov et al. 2005).

7.2 ENVIRONMENTAL SAMPLES

Methods for the determination of plutonium in environmental samples are summarized in Table 7-2. The separation and extraction methods used to prepare biological samples for plutonium analysis are commonly used for environmental samples. Large volumes of air samples (e.g., 10,000 m^3) should be collected in order to obtain detectable amounts of plutonium in particulate in air (EPA 1976a).

Field survey instruments for measuring photons of ^{241}Am in surface soils and on airborne particulates are available (e.g., Field Instrument for Detecting Low Energy Radiation or FIDLER) with a minimum detection limit of approximately twice the magnitude of a background level of ^{239}Pu ($1x10^3$–$2x10^3$ pCi/m^2; 37–74 Bq/m^2). The FIDLER uses a sodium iodide or calcium fluoride crystal and photon-height discrimination in order to detect the 17 keV x-rays emitted from the progeny of plutonium, or the 60 keV gamma photons of ^{241}Am. These instruments are useful for identifying areas of contamination, but cannot be used to accurately predict the concentration of plutonium in surface soils (EPA 1976a). This instrument has been used in aerial surveys of large area sources, such as the Nevada Test Site.

Since soil-adsorbed plutonium contamination exists as discrete particles of various sizes, analysis of larger soil volumes (25–100 g) is recommended (EPA 1976a). Commonly, soil samples with high amounts of carbonate are difficult to analyze. More rapid, efficient, and economical procedures have been developed to sequentially analyze a number of radioactive actinides (Hindman 1986).

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Recovery	Reference
Air filter, soil, water, vegetation	Fusion with KF and pyrosulfate; dissolution in HCl; coprecipitation on $BaSO_4$; dissolution of $BaSO_4$ and reprecipitation with DPTA	α spectrometry	No data	85–95%	DOE 1999c CHEM-TP-A.20
Water	Separation of radionuclides by Eichrom resins	α spectrometry	0.6 mBq (400 minutes)	93%	DOE 1997 Se-03
Soil	Plutonium isotopes are leached from soil using HNO_3/HCl	α spectrometry	1 mBq (400 minutes)	No data	DOE 1997 Pu-02-RC
Air filter	Digestion with HNO_3 followed by treatment with HF; decompose filters composed of organic polymer overnithe at 450 ºC prior to digestion	α spectrometry	No data	No data	DOE 1997 Pu-01-RC; Pu-11-RC; G-03
Soil, sediment	Plutonium isotopes are leached with HNO_3/HCl; purification by ion exchange chromatography; microprecipitation	α spectrometry	1 mBq (400 minutes)	No data	DOE 1997 Pu-12-RC
Water	Heated in HNO_3/HCl; evaporation and dissolution in HNO_3; purified by ion exchange; microprecipitation	α spectrometry	No data	No data	DOE 1997 Pu-07-RC; Pu-11-RC; G-03
Vegetation	Ashed at 400 ºC; dissolved in HNO_3/HCl; filtered; decomposed with HF; purified by ion exchange chromatography	α spectrometry	No data	No data	DOE 1997 Pu-08-RC; Pu-11-RC
Vegetation	Digested with HNO_3/H_2SO_4; coprecipitation of plutonium with $Fe(OH)_3$; purified by ion exchange chromatography	α spectrometry	No data	No data	DOE 1997 Pu-09-RC; Pu-11-RC

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Recovery	Reference
Food	HNO_3, closed-vessel microwave digestion	ICP-MS	0.020 pg/g	100±20%	Evans et al. 2003
Air	Dry ash; filter; extract; reduce valence; coprecipitate with lanthanum fluoride	α spectrometry	No data	No data	EPA 1984 (EPA Method 00-04)
Soil, coal, fly ash, ores, vegetation, biotia, and water	Ashed or evaporated; dissolved with HF, $HClO_4$, and HCl; extraction with triisooctylamine/p-xylene; stripped with HNO_3; wet ashed; co-precipitated with lanthanum fluoride; filtration	α spectrometry	No data	No data	EPA 1984 (EPA Method Pu-01)
Water, soil, air, vegetation, and animal tissue	Ashing; ion exchange separation; electrodeposition	α spectrometry	0.02 pCi/sample	No data	EPA 1979
Water	Filter; extract; coprecipitate with lanthanum fluoride	α particle counter (either proportional or scintillation detectors)	No data	No data	EPA 1980 (EPA method 907.0)
Drinking water	Acidify; oxidation with sodium nitrite; precipitation; extraction with tri-isooctylamine; co-precipitated with lanthanum fluoride	α particle counter	No data	93%	EPA 1982 (EPA Method 911)

DPTA = diethylenetriamine-pentaacetic acid; ICP-MS = inductively coupled plasma-mass spectrometry

The U.S. Department of Energy Environmental Measurement Laboratory provides techniques for the determination of plutonium in various biological and environmental samples using alpha spectroscopy. EPA methods are available for the determination of plutonium in air, soil, coal fly ash, ores, vegetation, biotia, and water. APHA has standard methods for determination of gross alpha and beta radioactivity and gamma-emitting radionuclides in water, Methods 7110 and 7120, respectively; however, no methods that are specific to plutonium isotopes are reported (APHA 1998a, 1998b). No methods were reported by the AOAC for the determination of plutonium (AOAC 1990).

Alpha spectrometry is the most common analytical method for measuring plutonium concentrations in environmental samples. Other measurement techniques available are liquid scintillation, mass spectrometry (MS), and gamma spectrometry. ICP-MS has been used increasingly for the determination of plutonium in environmental samples (Muramatsu et al. 2001a). Low concentrations of plutonium in environmental samples with high salt and organic matter content cause signal suppression and make it difficult to obtain an accurate plutonium determination. Preconcentration and matrix separation are typically required in these analyses (Epov et al, 2005; Figg et al. 2000).

MS is used by some research laboratories to determine the concentration of each plutonium isotope, including the naturally-occurring ^{244}Pu. MS determines the number of atoms of a given mass number and, therefore, can measure the concentration of all of the plutonium isotopes, not only the alpha-particle emitters as in alpha spectrometry. MS is several orders of magnitude more sensitive than alpha spectrometry in determining the quantities of plutonium isotopes with long half-lives, which also tend to be the heavier isotopes.

Quantities of ^{241}Pu, a beta-particle emitter, can be quantified from assumed isotopic abundance ratios; estimated in-growth of its progeny ^{241}Am by gamma spectrometry; or MS (EPA 1976a). ^{241}Am is produced from the beta decay of ^{241}Pu and, therefore, can be used to indirectly measure the concentration of ^{241}Pu (Metz and Waterbury 1962). Direct determination of ^{241}Pu by measurement of its low energy beta-particle decay has been reported using liquid scintillation analysis (Martin 1986).

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 plutonium 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 plutonium.

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

Analytical methods are available and are adequately sensitive to detect plutonium isotopes in biological materials (e.g., blood, urine, and bone) and in environmental samples (e.g., water, soil, air, and food). No data needs are identified at this time.

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. There are methods available for measuring the isotopes of plutonium in biological samples. The measurement of plutonium in the urine is considered a biomarker of exposure to plutonium. Methods are available to detect plutonium in the urine. However, no information was available concerning the reliability of these methods for determining plutonium levels in the urine. Plutonium can be determined sensitively and selectively by alpha spectrometry and ICP-MS in urine and tissues (DOE 1997; Epov et al. 2005). Analytical methods with satisfactory sensitivity and precision are available to determine levels of plutonium in human tissues and body fluids.

Effect. Existing methods are sensitive enough to measure background levels for plutonium in the population and levels at which biological effects occur.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Environmental media are analyzed to identify contaminated areas and to determine if contaminant levels constitute a concern for human health. The detection of plutonium in air, water, and soil is of concern due to the potential for human exposure. There are many steps involved in the analysis

of plutonium in environmental media. Alpha spectrometry a satisfactory method available for the determination of plutonium in water, air, and solid waste samples (DOE 1997, 1999c).

7.3.2 Ongoing Studies

No ongoing studies pertaining to analytical methods for plutonium were identified in a search of the Federal Research in Progress database (FEDRIP 2007).