7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring selenium, its metabolites, and other biomarkers of exposure and effect to selenium. 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 analytical methods used to quantify selenium in biological and environmental samples are summarized below. Table 7-1 lists the applicable analytical methods used for determining selenium and selenium compounds in biological fluids and tissues, and Table 7-2 lists the methods used for determining selenium in environmental samples.

7.1 BIOLOGICAL MATERIALS

Sampling of biological material for determination of total selenium concentrations does not usually pose a problem unless specific selenium compounds are to be identified (Bem 1981). One exception is the collection and storage of urine samples without loss of volatile selenium compounds (Bem 1981). Unless special precautions are taken, most analyses of biological materials probably underestimate the concentration of these compounds. Ideally, selenium should be measured in 24-hour urine samples that have been stored in polyethylene containers in acid medium (Sanz Alaejos and Diaz Romero 1993). Blood samples should be separated into plasma or serum and cell fractions prior to freezing if the selenium levels in these components are to be measured separately. Freezing of biological samples immediately following collection is recommended to reduce enzymatic formation of volatile selenium compounds.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (breath)	Calibrate personal sampling pump; sample at a known flow rate for a total sample size of 5–2,000 L; analyze at 190.6 nm	ICP/AES	21 ng/mL	97–105%	NIOSH 1994a (method 7300)
Blood	Mineralize using HNO_3-$HClO_4$ mixture, generate hydride, and atomize	HGAAS	$1x10^{-8}$ g/g	No data	Clinton 1977
	Digest blood sample with a nitric/perchloric acid mixture; fume mixture at 200 °C and measure 2,3-diaminonaphthalene	Fluorometric	$1.2x10^{-9}$ g/g	98%	Rongpu et al. 1986
Blood, plasma, or tissue homogenate	Digest with $Mg(NO_3)_2$ or HNO_3 at a solution temperature of 100 °C for 60–90 minutes; add HCl; and add hydroxylamine sulfate, EDTA, and urea	GC/ECD	$1x10^{-8}$ g/g	95–105%	McCarthy et al. 1981
Serum	Dilute sample with matrix modifier containing $Mg(NO_3)_2$ and $Ni(NO_3)_2$ to thermally stabilize Se; heat, dry, atomize; use Zeeman background correction	ZAAS	No data	6.2% relative standard deviation	Lewis et al. 1986b
	Dilute sample with matrix modifier containing $NiCl_2$; heat, dry, and atomize	GFAAS	No data	84–116%	Oster and Prellwitz 1982
	Nitric-perchloric acid digestion; HCl reduction; sodium borohydride reduction; measure selenium hydride	HGAAS	No data	33–73%	Oster and Prellwitz 1982
	On-line acid ashing of sample followed by hydrive generation	ICP/AES	5.5 µg/L	98–106%	Recknagel et al. 1993

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	24-hour samples analyzed to measure CT and selenium concentration in urine	Folin-Wu method for CT measurement; fluorimetric method to measure Se	No data	No data	Hojo 1981b, 1982
	Digest sample with HNO_3 and $HClO_4$	Fluorometric	No data	100±22%	Koh and Benson 1983
	Add nitric acid, platinum, and nickel	EAAS	No data	4–8% relative deviation	Saeed 1986
Human spermatozoa and protasomes	Digest with 25% tetramethylammonium hydroxide in methanol	GFAAS	$1x10^{-8}$ g/g	95.1±5.2%	Suistomaa et al. 1987
Biological samples	Decompose sample with nitric acid; use 1,2-dibromobenzene as a reagent to measure piazselenol	GC/ECD	$1x10^{-9}$ g/g	No data	Shimoishi 1977
	Spike sample with ^{82}Se; digest; acidify with HCl; react with 4-nitro-o-phenylene-diamine; measure nitro-piazselenol	IDGC/MS	$5x10^{-11}$ g/g	No data	Lewis 1988
Liver	Lyophilize sample; irradiate the sample; digest with HNO_3, $HClO_4$, and the carrier source; distill sample, and use distillate for analysis	Radiochemical NAA	$2.2x10^{-10}$ g/g	No data	Lievens et al. 1977
Protein (human liver)		INAA and gel filtration	No data	No data	Norheim and Steinnes 1975

CT = creatinine; EAAS = electrothermal atomic absorption spectroscopy; EDTA = ethylenediaminetetraacetic acid; GC/ECD = gas chromatography/electron capture detection; GFAAS = graphite furnace atomic absorption spectroscopy; HCl = hydrochloric acid; $HClO_4$ = perchloric acid; HGAAS = hydride generation atomic absorption spectroscopy; HNO_3 = nitric acid; ICP/AES = inductively coupled plasma/atomic emission spectroscopy; IDGC/MS = isotope dilution gas chromatography/mass spectrometry; INAA = instrumental neutron activation analysis; $Mg(NO_3)_2$ = magnesium nitrate; NAA = neutron activation analysis; $NiCl_2$ = nickel chloride; Se = selenium; ZAAS = graphite furnace atomic absorption spectroscopy with Zeeman background correction

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Reduce selenium in sample from SeVI to SeIV; add zinc to the acidified sample; pass gaseous selenium hydride to AA	AA, gaseous hydride	$2x10^{-9}$ g/mL	100%	EPA 1979a (method 270.3)
	Microwave digestion; acidify with HNO_3	ICP-MS	No data	156% (fine flour); 149% milk powder)	Zhou and Liu (1997)
Water	Acidify sample with HCl, degas solution with N_2 bubbling	HGGC with photo-ionization detection	$1x10^{-12}$ g/mL (0.001 ppb)	No data	Vien and Fry 1988
	Reduce selenium to SeIV with HCl and KBr; coprecipitate with lanthanum hydroxide; centrifuge.	ICP/AES	0.06 µg/L	100% selenite; 88% selenate	Adkins et al. 1995
	Acid digestion	ICP/AES	21 ng/mL	97-105%	NIOSH (2001)
Water and waste water	Acid digestion	AA, furnace	$2x10^{-9}$ g/mL	94–112%	EPA 1979a (method 270.2)
Water and wastes	Acid digestion	AA, furnace	$5x10^{-9}$ g/mL	No data	EPA 1984b (method 200.7 CLP-M)
Solid/solid waste/sludge	Aqueous samples subject to acid digestion	AA, furnace	$2x10^{-9}$ g/mL	No data	EPA 1984c (method 7740)
	Acid digestion; measure at 196 mm	ICP and GFAAS	$7.5x10^{-8}$ g/mL	94–112%	EPA 1986c (methods 3050, 6010)
	Acid digestion with HNO_3/sulfuric acid; convert SeIV to volatile hydride	AA, gaseous hydride	$2x10^{-9}$ g/mL	100%	EPA 1997a (method 7741a)
	Acid digestion	AA, furnace	$2x10^{-9}$ g/mL	No data	EPA 1984b (method 270.2 CLP-M)

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Wastes/soil/ groundwater	Nitric acid digestion or nitric/peroxide/hydrochloric acid digestion	AA, furnace	$3x10^{-9}$ g/mL	100.5 %	EPA 1997b (method 7742)
Organic waste	Oxidize organic samples, absorb combustion products in NaOH; separate on an ion exchange column Digest aqueous sample with HNO_3 and perchloric acid	Cathodic stripping	$5x10^{-9}$ g/mL	No data	DOE 1987
Marine biological tissues	Decompose tissue sample with HNO_3 under pressure; add sulfuric and perchloric acids; heat at 310 °C to evaporate excess acid; add HCl	HGAAS	$2x10^{-7}$ g/g	No data	Welz and Melcher 1985
Marine samples	Digest sample with concentrated HNO_3 at room temperature; add HNO_3, perchloric, and sulfuric acids to complete digestion; evaporate extra acids; dissolve residue in HCl	HGAES-ICP	$5x10^{-9}$ g/mL	No data	DOE 1987
Avian eggs and liver	Digest sample with HNO_3; and hydrogen peroxide to increase solubility	GFAAS	$4x10^{-7}$ g/g	No data	Krynitsky 1987
Fat materials (butter)	Melt butter under an infrared lamp; digest with HNO_3, sulfuric, and perchloric acids	HGAAS	10 ppb	No data	Narasaski 1985

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plants		Gravimetric method	$2x10^{-6}$ g/g	No data	AOAC 1984 (method 3.101)
	Grind air-dried or fresh samples; acid digest with HNO_3 and HCl add EDTA; neutralize with NH_4OH; add HCl; shake with decalin; centrifuge decalin layer; read decalin solution with fluorometer at 525 nm within 5 minutes	Fluorometric method	$<4x10^{-6}$ g/g	No data	AOAC 1984 (method 3.102 to 3.107)
Food	Digest sample with HNO_3, perchloric, and sulfuric acids; heat; add H_2O_2; mix with EDTA, NH_4OH, and DAN; boil; add cyclohexane and shake; read cyclohexane layer at 525 nm	Titrimetric method	No data	No data	AOAC 1984 (methods 25.154 and 25.158)
Air (particulate)	Fileter particulate matter from air; irradiate and count sample	NAA, non-destructive	$1x10^{-10}$ g/m^3	No data	Dams et al. 1984
Air	Calibrate sampling pump; sample at a known flow rate for a total sample size of 13–2,000 L; analyze at 190.6 nm	ICP/AES	21 ng/mL	97–105%	NIOSH 1994a (method 7300)

AA = atomic absorption; AES = atomic emission spectrometry; CFAAS = graphite furnace atomic absorption spectroscopy; DAN = 2,3-diaminonaphthalene; EDTA = ethylenediamine tetraacetate; HCl = hydrochloric acid; HGAAS = hydride generation atomic absorption spectroscopy; HGAES = hydride generation atomic emission spectroscopy; HGGC = hydride generation gas chromatography; HNO_3 = nitric acid; ICP = inductively coupled plasma; KBr = potassium bromide; N_2 = nitrogen; NAA = neutron activation analysis; NaOH = sodium hydroxide; NH_4OH = ammonium hydroxide

7. ANALYTICAL METHODS

A variety of analytical methods can be used to determine trace concentrations (ng/g) of selenium in biological tissues. These include fluorometry, neutron activation analysis (NAA), atomic absorption spectroscopy (AAS), inductively coupled plasma-atomic emission spectroscopy (ICP-AES), inductively coupled plasma-mass spectrometry (ICP-MS), gas chromatography (GC), spectrophotometry, x-ray fluorescence analysis, and others.

Classical flame AAS techniques do not have sufficiently low detection limits for selenium to be useful for determining its presence in biological samples (Koirtyohann and Morris 1986). Hydride generation atomic absorption spectroscopy (HGAAS) has been used instead for determination of selenium in biological samples such as blood and blood constituents and meat, fruits, and vegetables (Bem 1981).

Graphite furnace atomic absorption spectroscopy (GFAAS) offers high sensitivity ($5x10^{-11}$ g selenium/g sample), but interference from the matrix can cause significant difficulties (Lewis 1988). GFAAS methods rely on the fact that numerous metal compounds react with selenium compounds to form relatively refractory metal selenides (Oster and Prellwitz 1982). Nickel, molybdenum, and platinum are commonly added to the sample to thermally stabilize the selenium. Organic materials are then destroyed by high temperature in the furnace prior to atomization of the sample at very high temperatures (e.g., 2,700 °C) (Oster and Prellwitz 1982). One advantage of GFAAS techniques is that the material in the graphite sample cell can be chemically treated *in situ* to reduce chemical interference. GFAAS techniques require correction for background absorption. Correction techniques include the deuterium continuum light source method (Hoenig and Van Hoeyweghen 1986) and the Zeeman splitting of the absorption line (Koirtyohann and Morris 1986). A Zeeman-effect system, which applies a magnetic field to the atomizer, allows the background correction to be performed at the exact analyte wavelength without the use of auxiliary light sources (Fernandez and Giddings 1982). The Zeeman-effect background correction is necessary for the determination of selenium in blood and blood products when GFAAS is used because a spectral interference from iron occurs at the selenium wavelength that cannot be corrected by a deuterium continuum source.

A modification of the GFAAS method for determining selenium levels in human urine was described by Saeed (1986). In this electrothermal atomic absorption spectrometry (EAAS) method, nitric acid, nickel, and platinum are added to the graphite cell. The addition of nickel helps to mask the spectral interference from phosphates in urine. EAAS has been used to determine selenium levels in human spermatozoa (Suistomaa et al. 1987). For human blood plasma and serum, the detection limit of the EAAS method

was 0.8 µg/L (2 ng absolute), with recoveries of 87–96% for plasma and 94–104% for serum (Harrison et al. 1996).

HGAAS offers reduced chemical interference but requires larger sample volumes than GFAAS techniques (Koirtyohann and Morris 1986). HGAAS techniques have been used to measure selenium concentrations in food (Fiorino et al. 1976). These techniques use wet-sample digestion (e.g., nitric-perchloric acid) to destroy organic matter. Sample reduction to convert Se(VI) (+6 valence state) to Se(IV) (+4 valence state) is necessary prior to using sodium borohydride to reduce all selenium present to selenium hydride (Macpherson et al. 1988). The selenium hydride is thermally decomposed and atomized in the sample beam of the atomic absorption spectrophotometer. Nitric-perchloric acid is commonly used for the digestion step. Because perchloric acid is potentially explosive, use of phosphoric acid instead is also common. Following the International Union of Pure and Applied Chemists (IUPAC) interlaboratory trial for the determination of selenium in human body fluids, Welz and Verlinden (1986) reported that it was important to use a temperature of at least 200 ºC for sample decomposition when using HGAAS. They attributed the severe imprecision and systematic errors in measuring selenium in multiple samples to improper sample decomposition. Norheim and Haugen (1986) demonstrated that a combined system of a wet digestion and an automated hydride generator could analyze approximately 80 samples per day.

ICP-AES with hydride vapor generation has been used to determine total selenium in biological samples (Tracy and Moller 1990). This technique is especially suited to the analysis of small samples. Samples are wet ashed with nitric, sulfuric, and perchloric acids at temperatures up to 310 ºC. After treatment with hydrochloric acid, selenium is reduced by sodium borohydride to hydrogen selenide in a simplified continuous flow manifold. A standard pneumatic nebulizer affects the gas-liquid separation of H_2Se, which is quantified by ICP-AES at 196.090 nm. The instrument detection limit for this method has been determined to be 0.4 µg/L

Hydride generation atomic fluorescence spectrometry (HGAFS) has been used to measure selenium concentrations in urine (Sabé et al. 2001). Samples were completely mineralized using a focused microwave oven with a mixture of nitric acid and sulfuric acid for 14 minutes. Complete recovery was achieved from selenocystine (SeCys), selenomethionine (SeMet), and trimethyl selenium (TMeSe) species. The detection and limit of quantization for this method were 57 and 190 pg selenium/L.

Application of gas-liquid chromatography (GLC) to determine selenium in biological samples allows for the elimination of interference from the biological matrix. GLC requires prior decomposition of organic

matter with nitric acid. GLC techniques are based on measurement of the amount of piazselenol formed by the reaction of selenium (*IV*) with appropriate reagents in acidic media (Bem 1981). For gas chromatographic determination of selenium with an electron capture detector, 1,2-diaminoarenes can be used as reagents to produce piazselenols (McCarthy et al. 1981; Poole et al. 1977; Shimoishi 1977; Young and Christian 1973). Using 1,2-diamino-3,5-dibromobenzene as a reagent, Shimoishi (1977) obtained a detection limit of $1x10^{-9}$ g selenium per gram of sample.

Isotope dilution gas chromatography/mass spectrometry (IDGC/MS) is a highly accurate technique that is more accessible than NAA techniques. IDGC/MS has been used to determine selenium in foods, plasma and serum, red blood cells, feces, urine, and human breast milk (Lewis 1988). The minimum sample size per determination is 0.5–10 g (0.5–10 mL). In the IDGC/MS method, a stable selenium isotope is added to the sample prior to digestion. This procedure eliminates the need for quantitative sample preparation and external standardization (Lewis 1988). However, a disadvantage of this technique is that enriched isotopic standards are expensive.

NAA techniques provide lower detection limits for selenium (between 10^{-8} and 10^{-9} g selenium per gram of sample), but there are few reactors at which NAA facilities and expertise are available (Koirtyohann and Morris 1986). The most common NAA procedure for selenium determination is to produce the long-lived ^{75}Se radionuclide (half-life of 119 days) and count the samples after a 50–100-hour irradiation period and a 2–10-week cooling period. A faster NAA technique utilizes metastable ^{77m}Se, which has a much shorter half-life (17.4 seconds), so that counting can be initiated after an irradiation and cooling period of <1 minute (Koirtyohann and Morris 1986). The most common standard reference sample for NAA techniques is bovine liver tissue (Bem 1981). Biological tissues that can be analyzed for selenium using the NAA technique include bone, hair, liver, kidney, lung, serum, blood, feces, urine, brain, stomach, skin, aorta, heart, testis, pituitary gland, tooth enamel, tongue, muscle, spleen, and thyroid (Yukawa et al. 1980). For many NAA techniques, destructive sample pretreatment (involving radiochemical separation) is required to avoid interference from the biological matrix (Koirtyohann and Morris 1986). The advantages of NAA are its low detection limits and multielement capability (Molokhia et al. 1979). 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.

Spectrophotometric, fluorometric, voltammetric, and x-ray fluorescence analysis methods have also been successfully employed to determine selenium levels in blood, tissue, and human hair. Of these,

fluorometric methods are most commonly used (Koh and Benson 1983). The reaction of selenium(VI) with 2,3-diaminonaphthalene (DAN) or with 3,3-diaminobenzidine (DAB) to form a fluorescent Se-DAN or Se-DAB heterocyclic compound is the basis of the fluorometric method of selenium determination (Allaway and Cary 1964; Chen et al. 1982; Lewis 1988). The piazselenol formed with DAN as the reagent has greater fluorescence sensitivity than the piazselenol formed with DAB as the reagent and is also extractable into organic solvents from acid solution (Chen et al. 1982). Fluorometric techniques require sample digestion to destroy organic matter and sample reduction to convert the selenium to the selenium(IV) oxidation state (Macpherson et al. 1988). Loss of volatile selenium compounds is possible during sample digestion and manipulation because several steps are required. Chen et al. (1982), Hasunuma et al. (1982), and Koh and Benson (1983) developed modifications of the digestion and treatment steps for selenium determination by fluorometric methods. Their methods allow small sample sizes, can be performed in a single flask, and measure submicrogram amounts of selenium.

Some of the methods for determining selenium in biological materials have been compared within the same laboratory for accuracy and precision. Macpherson et al. (1988) compared the accuracy of three methods for the determination of selenium in biological fluid samples from biological materials with certified selenium levels. Acid decomposition fluorometry, HGAAS, and EAAS gave equally accurate results. Lewis et al. (1986) compared the graphite furnace atomic absorption spectrometry with the Zeeman-effect background correction (ZAAS) to isotope dilution mass spectrometry (IDMS) for determination of selenium in plasma and concluded that the ZAAS method compared favorably (correlation coefficient 0.987), but was half as precise as the IDMS method. Oster and Prellwitz (1982) compared HGAAS and GFAAS for the determination of selenium in serum. They concluded that the two techniques exhibited approximately equal detection limits in their laboratory.

In three studies that compared analytical methods for the detection of selenium in biological samples, all found that fluorometry gave both accurate and reliable results (Burguera et al. 1990; Heydorn and Griepink 1990; Macpherson et al. 1988). Burguera et al. (1990) indicated the acceptance of HGAAS as yielding reliable results, whereas Heydorn and Griepink (1990) reported HGAAS had a high relative standard deviation of 11.4%.

Decomposition procedures have been improved and analytical methods have been modified in recent years to increase the accuracy and speed of determination of selenium concentrations in plasma, serum, and urine. Reamer and Veillon (1983) used phosphoric acid along with nitric acid and hydrogen peroxide in digestion of biological fluids instead of perchloric acid to prepare samples for fluorometry. They

concluded that phosphoric acid digestion increases the safety and convenience of the determination. Krynitsky (1987) used a modified wet digestion method for the determination of selenium in biological samples such as eggs and liver of avian species. This method uses hydrogen peroxide to enhance the solubility of the sample. Digestion with HNO_3 and $HClO_4$ is essential for accurate analysis of the total selenium in urine to ensure complete oxidation of the trimethylselenonium ion (Koh and Benson 1983).

7.2 ENVIRONMENTAL SAMPLES

Many of the basic analytical methods used for determining selenium in biological media are also used for determining selenium levels in soil, water, and air. Precautions in the collection and storage of environmental samples, however, are necessary to prevent loss of the volatile selenium compounds to the air. The destruction of organic matter before selenium measurement is also often necessary. Acidification of water samples to a pH of 1.5 is recommended to preserve selenium compounds (Muñoz Olivas et al. 1994). Nitric acid can be used, although it interferes with the hydride generation method of analysis. The best storage method for selenium compounds in water is in glass containers at 4 °C (Wiedmeyer and May 1993).

The analytic methods generally fall into two groups: (1) those that do not require the destruction of organic materials in the sample and (2) those that require the elimination of interfering matter before the selenium content can be measured. X-ray fluorescence and some of the neutron activation analysis techniques do not require sample destruction, whereas spectrophotometry, GC, atomic absorption spectrometry, polarography, titration, spark source, MS, fluorometry, and other neutron activation analysis techniques require some degree of sample destruction. Fluorometry, atomic absorption spectrometry, and neutron activation analysis are the most frequently used methods.

Inductively coupled plasma (ICP) emission techniques can be used to measure selenium concentrations. ICP techniques offer multielement capabilities, but instrumentation is costly and background interference can be a problem (Koirtyohann and Morris 1986). The NIOSH-recommended method for determining selenium in air is inductively coupled argon plasma atomic emission spectroscopy (NIOSH 1994a). Selenium may be measured in water following NIOSH Method 7300. The limit of detection for this method is 21 ng/mL using a selenium emission line at 190.6 nm (NIOSH 2001). ICP-MS has been used to determine the concentration of selenium in cloud water at detection limits of 100 and 25 pg/mL using pneumatic and ultrasonic nebulization, respectively (Richter et al. 1998).

AAS techniques are commonly used for the determination of selenium in environmental samples. Hydride generation AAS is more sensitive than flame or graphite furnace AAS for the determination of selenium in materials of variable composition. Water samples, including freshwater, river water, sea water, and surface waters, and industrial wastes, muds, sediments, and soil samples have been analyzed by AAS techniques to detect selenium at parts-per-trillion levels (Bem 1981). Selenium(VI) and selenium(IV) can be distinguished in water samples with GFAAS by selective extraction procedures. HGAAS can also be used to distinguish between selenium(VI) and selenium(IV) in environmental samples because selenium(VI) does not readily form the hydride without reduction (Koirtyohann and Morris 1986). Selenium(VI) is calculated on the basis of the total selenium minus selenium(IV) (Bem 1981).

NAA has been used to determine selenium levels in environmental samples. Dams et al. (1970) reported a detection limit of $1x10^{-10}$ g/m^3 selenium using nondestructive NAA for determining selenium in air particulate matter. For determining selenium levels in soil, radiochemical variants of NAA have been commonly employed (Bem 1981). Instrumental neutron activation analysis (INAA) is frequently used to determine selenium concentrations in water and can also be used to distinguish between selenium(IV) and selenium(VI) oxidation states (Bem 1981). INAA is also used to determine selenium concentrations in air (Bem 1981).

Gas liquid chromatography allows for elimination of interference from the matrix when analyzing environmental samples. When analyzing biological samples, a variety of reagents can be used to convert selenium to piazselenols for measurement with an electron capture detector. Spectrophotometric determinations of selenium are performed using organic reagents, whereas fluorometric analysis relies on piazselenol fluorescence to measure submicrogram levels of the element.

The hydride generation GC with photoionization detection (HGGC-PD) method for selenium determination was developed by Vien and Fry (1988). The combined usage of a photoionization detector and a cold trap provided at least two orders of magnitude improvement in detectability over the existing GC systems. The detection limit for the HGGC-PD method was $1x10^{-12}$ g selenium/mL (0.001 ppb) for 28 mL samples. An advantage of the HGGC-PD technique is the ability to perform simultaneous determinations of at least four different hydride-forming elements (Vien and Fry 1988).

EPA's Contract Laboratory Program (EPA 1984b) requires the participating laboratories to meet the Contract Required Detection Level (CRDL) for selenium of $5x10^{-9}$ g selenium/mL (5 µg selenium/L) using proven instruments and approved analytical techniques, including ICP and atomic absorption methods.

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

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. Methods that distinguish among the various selenium compounds are not commonly used to estimate human exposure to selenium, but have been used in specialized metabolic studies. Analytical methods currently used to measure concentrations of selenium in biological fluids or human tissue samples as an indication of human exposure are described in Table 7-1. Attempts to use measures of whole blood GPX activity levels as indicators of human exposure to selenium have not been successful. Errors can result if the selenium-dependent GPX activity is not distinguished from the nonselenium-dependent GPX activity (Edwards and Blackburn 1986). In addition, whole blood selenium concentrations and GPX activity appear to correlate with one another only at low blood selenium levels (<0.100 mg selenium/L) (Allaway et al. 1968; Valentine et al. 1980). GPX activity levels measured in

platelets have provided an indication of selenium exposure levels at low blood selenium levels (Nève et al. 1988). Whether platelet GPX activity levels would provide an indication of selenium status in populations with plasma selenium levels above 0.012 mg selenium/L is not known. There is great variability in the exposure data available for humans. Therefore, until larger databases of selenium concentrations in biological materials from affected and unaffected populations are available, no recommendations for analytical methods can be made.

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

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Numerous analytical methods are available for the determination of selenium levels in environmental media (AOAC 1984; Bem 1981; Dams et al. 1970; DOE 1987; EPA 1984b, 1986c; Koirtyohann and Morris 1986; NIOSH 1994a; Vien and Fry 1988). However, most of these do not distinguish among the various selenium compounds. Many of the available methods can be used to detect selenium at subnanogram levels. For the determination of selenium only, fluorometry, chromatography, or spectrometry are the preferred techniques. When conducting a multielemental analysis or when analyzing a complex matrix, more sophisticated methods are required.

It is possible to detect selenium levels as low as 1 ng/m^3 of air using neutron activation analysis. Standardized methods for selenium determination in different environmental samples such as water, soil, sludge, and industrial waste are available in the above-mentioned literature.

There are fewer methods available for distinguishing among the inorganic forms of selenium in the environment. HGAAS, INAA, and GFAAS with selective extraction procedures can be used to distinguish between selenium(VI) and selenium(IV) in samples of soil and water. Methods for determining selenium sulfide levels in the environment are lacking, but would be useful for the identification and measurement of this potentially carcinogenic selenium compound.

Very limited information is available regarding the sensitivity, reliability, and specificity of the existing methods. Further studies to determine these factors would be useful.

7.3.2 Ongoing Studies

N.J. Miller-Ihli and coworkers at the Agricultural Research Service (Beltsville, Maryland) are conducting studies to develop single and multielement methods for the determination of trace elements of nutritional and health concern (e.g., selenium). Some techniques proposed in their studies include: GFAAS and electrothermal vaporization inductively coupled plasma-mass spectrometry (ICP-MS); inductively coupled plasma-atomic emission spectrometry (ICP-AES); electrothermal vaporization ICP-MS (USS-ETV-ICP-MS) and USS-GFAAS; and capillary zone electrophoresis (CZE) coupled with ICP-MS (FEDRIP 2002).