6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring antimony in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify antimony. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect antimony in environmental samples are the methods approved by federal agencies 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 ww - Additionally, analytical methods are included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Methods for the analytical determination of antimony in biological materials are basically the same as those used for the environmental samples that are discussed below. The most commonly used methods determine the total antimony content of the sample, not the particular antimony compound or oxidation state that is present. Methodological differences are a function of the level of antimony in the sample, digestion procedures required to solubilize the sample, and the level of potentially interfering substances in the type of sample. Antimony occurs at very low levels in biological samples. The accurate determination of trace levels of antimony in these samples may require special methods (e.g., neutron activation) that are both sensitive and selective. Atomic absorption spectroscopy and inductively coupled plasmaatomic emission spectroscopy, with or without preconcentration or separation steps, are the most commonly employed methods. Atomic absorption has three variants: direct aspiration into a flame, atomization in an electrically heated carbon rod, or generation of stibine that is then passed into a heated silica tube.

Instrumental neutron activation analysis (INAA), with or without chemical separation, has very good sensitivity and selectivity for antimony, and it has the advantage of being able to measure many elements simultaneously. However, it is slow, costly, and requires special facilities. INAA is favored for surveys where trace levels of many elements are to be determined. It is often required for measuring antimony in tissues in which the antimony level is very low. The neutron activation analysis of antimony requires an exposure to neutron fluxes for 6 hours to 2 days. After the exposure period, the samples are kept for several days before counting, This allows the activity of short half-lived isotopes to decline, and thus improves accuracy of the analysis (Iyengar et al. 1978). Nondestructive INCA can be used to measure concentrations to levels somewhat below 1 ppm. Nondestructive methods are not only advantageous because of reduced sample handling, but also

because they are independent of the sample matrix and of the efficiency of the digestion or extraction procedure. While this is generally adequate for antimony determinations in hair and lung tissues, the antimony levels in blood serum and kidney tissues are usually too low to measure without preconcentration (Iyengar et al. 1978). Detection limits may be limited by interferences from matrix elements such as sodium, potassium, phosphorus, and bromine. Lower detection limits (approximately 0.006 ppm) can be obtained by digestion and solvent extraction to eliminate these interferences (Mok and Wai 1988).

Analytical methods and detection limits for antimony in biological materials are given in Table 6-1. Antimony contained in other biological materials such as hair and nails can be determined by using the same analytical techniques as for blood and tissue, but suitable procedures for dissolving the sample matrix must be used (Takagi et al. 1986, 1988).

6.2 ENVIRONMENTAL SAMPLES

Analytical methods for antimony in environmental samples generally determine the total antimony content of the sample; determining specific antimony compounds is difficult. Some methods can be used to determine antimony in different oxidation states, but these methods are only used in special circumstances.

The most common methods used for environmental samples are atomic absorption spectrometry (AAS) (either flame or graphite furnace) and inductively coupled plasma-atomic emission spectroscopy (ICP-AES). Before the widespread use of AAS, calorimetric methods were used for the determination of antimony; the best known of these methods is the rhodamine B method (APHA 1972). The basis for the method is the formation of a pink complex when pentavalent antimony reacts with rhodamine B in the presence of an excess of chloride ions. The complex is extracted into an organic solvent and the absorbance measured at 565 nm. Trivalent antimony must be oxidized to the pentavalent state with nitric, sulfuric, and perchloric acids.

Water and waste water samples can be analyzed for antimony by EPA Test Methods 220.1 (atomic absorption, direct aspiration), 220.2 (atomic absorption, furnace technique), or 200.7 (inductively coupled plasma-atomic emission spectroscopy) (EPA 1983b). These methods are suitable for groundwater, surface water, and domestic and industrial effluents. In open ocean water and in other water samples with a low antimony concentration, a preconcentration and/or separation procedure involving coprecipitation, chelation, selective adsorption, or hydride formation is required before analysis (Andresen and Salbu 1982; Apte and Howard 1986; Maher 1986; Sturgeon et al. 1985). The atomic absorption wavelength used for antimony is 217.6 nm. In the presence of lead concentrations of the order of 1 g/L, however, a spectral interference may occur at this resonance line, and the line at 231.1 nm should be used instead. When using direct aspiration, the spectral

TABLE 6-1. Analytical Methods for Determining Antimony in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood, tissue, or hair[a]	Acid digestion	Method 8005, ICP-AES	No data	106% at 10 μg antimony	NIOSH 1985
Urine	Treat with EDTA and refrigerate; sample stable for 1 week; wet ash	Hydride generation-AAS	10 μg/L	No data	Anonymous 1977
Feces	Digest with concentration HCl/HNO_3, extract with hexane/hydrogen peroxide, nickel matrix modifier	Graphite furnace AAS	No data	96.9%, mean	Bio/dynamics 1990

[a]Method extended to hair (Takagi et al. 1986)

AAS = atomic absorption spectrometry
EDTA = ethylenediaminetetraacetic acid
ICP-AES = inductively coupled plasma-atomic emission spectroscopy

absorption of antimony is reduced when the concentration of acid increases. Therefore, it is important to match the concentration of acid in standards and samples (EPA 1983b). Background correction of nonspecific absorption is advisable for some samples, such as those containing sulfuric acid (Ainsworth 1988). Analytical methods and detection limits for antimony in environmental media are given in Table 6-2. If the determination of dissolved antimony is required, samples should be filtered using a 0.45 μm membrane filter.

Acid digestion to assure release of antimony from the sample matrix is a crucial step in the analysis of environmental samples. Unless the particular type of sample has been well studied, it is usually important to experiment with different digestion procedures. For the release of antimony from soil, hydrogen fluoride mixed with perchloric acid or another strong acid is generally required. Aqua regia, however, has been found to be suitable. For plant and animal tissue, a combination of sulfuric and nitric acids is most satisfactory (Ainsworth 1988).

Antimony forms a volatile hydride under reducing conditions, and hydride generation has been interfaced with different analytical procedures for enhanced sensitivity and selectivity. The most popular reagent used for this reduction is sodium borohydride (Andreae 1983). It is necessary to add KI to the reaction medium to completely reduce Sb(II1). In atomic absorption, increased sensitivity can be achieved by using hydride generation because the efficiency of atomization is greater for stibine than for antimony solutions introduced into the flame. Another advantage of hydride generation is that separati.on is achieved from nonhydride-forming elements, thereby eliminating interferences. Antimony reduction is pH dependent, possibly because neutral and cationic species (but not anionic ones) are subject to reduction by negatively charged borohydride ions. By exploiting the pH dependence of the reduction, it is possible to separately determine Sb(III) and Sb(V) in natural waters (Andreae 1983; Apte and Howard 1986). Other methods that distinguish Sb(II1) from Sb(V) rely on selective extraction techniques in which Sb(II1) is extracted into an organic solvent and analyzed. After analysis, Sb(V) is reduced and extracted (Abbasi 1989; Mok and Wai 1987). When very high sensitivity is required, such as that necessary for the analysis of antimony in food, neutron activation analysis is often employed. X-ray fluorescence (XRF) and anodic stripping voltammetry (ASV) are other analytical methods that are frequently used (Costantini et al. 1985; Gillain and Brihaye 1985; Ives et al. 1984; Johnson et al. 1984).

In the determination of trace metals, major concerns include contamination and loss. Contamination can be introduced from impurities in reagents and containers and from laboratory dust. Losses may also occur due to adsorption of the analyte onto container walls. In the case of antimony, a common source of loss is volatilization during acid digestion or ashing in an AAS furnace.

TABLE 6-2. Analytical Methods for Determining Antimony in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Filter collection and acid digestion	Method 301, colorimetric (Rhodamine B)	1.0 μg	95%-102% between 2 and 10 μg of antimony	APHA 1972
	Filter collection and acid digestion and reduction of Sb(V) with NaI	Hydride generation-AAS	4 ng	10% accuracy at 40 ng antimony	DeDoncker et al. 1983
Air (stilbine)	Filter collection (20 L sample) on $HgCl_2$-coated silica gel; desorption and treatment with concentrated HCl, ceric sulfate, and liquid extraction	NIOSH 6008, colorimetric (Rhodamine B)	No data	98.6% between 0.12 and 1.0 μg antimony	NIOSH 1987
Water, waste water	Acid digestion	Method 204.1, AAS/direct aspiration	0.2 mg/L	96% and 97% at 5 and 15 mg antimony/L	EPA 1983a
	Acid digestion, sample solutions should contain 2% HNO_3	Method 204.2, AAS/furnace technique	3 μg/L	Not applicable	EPA 1983a
	Filter and acidify sample	Method 200.7, ICP-AES	32 μg/L	Not applicable	EPA 1983a
Soil, sediment sludge, solid waste	Digestion with 4:1 HNO_3 and HCl[a]	Method 3050 (modified)[a] ICP-AES	No data	3% accuracy at 33 ppm antimony	EPA 1986
Food	Acid digestion and resin separation following irradiation	INAA	0.1-0.3 ppb	No data	Cunningham 1987

[a]The digestion procedure in Method 3050 is not suitable for antimony. A satisfactory digestion procedure has been proposed by Kimbrough and Wakakuwa (1989).

AAS = atomic absorption spectrometry; HCl = hydrochloric acid; $HgCl_2$ = mercuric chloride; HNO_3 = nitric acid; ICP-AES = inductively coupled plasma-atomic emission spectroscopy; INAA = instrumental neutron activation analysis; NaI = sodium iodide; NIOSH = National Institute for Occupational Safety and Health; Sb(V) = antimony (+5)

6. ANALYTICAL METHODS

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA as amended directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of antimony 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 antimony.

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 or eliminate the uncertainties of human health assessment. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

6.3.1 Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods for determining antimony in biological materials are well developed, and there are methods available to most laboratories that are satisfactory for testing biological samples that naturally contain high concentrations of antimony or for occupational exposure testing (Anonymous 1977; NIOSH 1985). Since antimony occurs at very low levels in many biological materials, methods such as INAA that require special facilities must often be used to achieve adequate sensitivity (Iyengar et al. 1978). Standardized methods are available from NIOSH and other sources to measure antimony in blood, urine, and tissue (NIOSH 1985). Several authors have reported that antimony concentrations in hair, nails, blood, or urine are elevated in exposed individuals; therefore, antimony levels in these samples can be used as a biomarker for exposure to antimony (Katayama and Ishidi 1987). Available analytical methods are capable of determining the levels of antimony in these media in both normal and occupationally exposed persons (Bakagi et al. 1986, 1988; Iyenger et al. 1988). Methods with sufficient sensitivity (e.g., INAA), however, are not available in most laboratories.

No biomarkers that could be used to characterize effects of antimony have been identified, Should subtle biochemical or physiological changes unique to antimony be identified, methods to analyze for these changes could possibly be developed if they don't already exist.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining antimony in environmental media are well developed and adequate. Standardized methods are available from EPA, NIOSH, and other sources (APHA 1972; Cunningham 1987; DeDonker et al. 1983;

EPA 1983a, 1986; NIOSH 1987). Since most analytical methods measure total antimony, the methods for analyzing for the parent compound and degradation product are identical.

6.3.2 On-going Studies

Analytical methods for antimony and antimony compounds are currently being developed at EPA's Environmental Monitoring Systems Laboratory in Cincinnati, Ohio (EPA 1989b). No on-going studies regarding new analytical methods for measuring antimony in biological materials were located In the available literature.