7. ANALYTICAL METHODS

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

Fluorine gas is too reactive to exist in biological or environmental samples. Indeed, fluorine is too reactive to be analyzed directly by conventional methods, but rather is quantitatively converted to chlorine gas and the latter is analyzed (Shia 1994). The methods discussed below are for the analysis of the fluoride ion, or in the case of gaseous acid fluorides, hydrogen fluoride. The particular fluorine molecule is rarely identified.

7.1 BIOLOGICAL MATERIALS

Trace levels of fluoride in biological media are determined primarily by potentiometric (ion selective electrode [ISE]) and gas chromatographic (GC) methods. Colorimetric methods are available, but are more time consuming and lack the sensitivity of the other methods (Kakabadse et al. 1971; Venkateswarlu et al. 1971). Other methods that have been used include fluorometric, enzymatic, and proton activation analysis (Rudolph et al. 1973). The latter technique is sensitive to trace amounts of sample and requires minimal sample preparation. Urine and blood and other bodily fluids can be analyzed with a minimum of sample preparation. Tissue will require ashing, digestion with acid, or even fusion with alkali to free the fluoride from its matrix. The most accurate method of sample preparation is microdiffusion techniques, such as the acid-hexamethyldisiloxane (HMDS) diffusion method by Taves (1968). These methods allows for the liberation of fluoride from organic or inorganic matrices (WHO 2002). During sample preparation, the analyst must be careful to avoid sample contamination, incomplete

release from matrices, and losses due to volatilization (NRC Canada 1971). Vogel et al. (1990) reported methodologies for sample manipulation and fluoride analysis on very small sample volumes. Techniques included micropipette procedures for transferring samples, preparation of micro fluoride-selective electrodes, and methods for adapting standard electrodes for micro- and semi-micro volumes (0.005–5 μL). These techniques have been used for fluoride analysis of various biological samples, such as salvia, plaque, and tooth enamel (Vogel et al. 1990, 1992a, 1992b). Table 7-1 describes some analytical methods for determining fluorides in biological materials.

There is extensive literature on the ISE methodology because it is the most frequently used method for fluoride measurement in biological media. The fluoride ion selective membrane utilizes a membrane consisting of a slice of a single crystal of lanthanum fluoride that has been doped with europium (II) fluoride to improve its conductivity (Skoog et al. 1990). It has a theoretical response to changes in fluoride ion activity in the range of 10^{0}–10^{-6} M. It is selective to fluoride over other common anions by several orders of magnitude; only hydroxide ion causes serious interference. The pH of the solution analyzed is adjusted to approximately 5 to eliminate interference. ISE is the methodology recommended by NIOSH in Method 8308 for the determination of fluoride in urine (NIOSH 1994). Fluoride analyses using the ion selective electrode are simple, sensitive, and rapid. Recoveries are usually >90%, but this is dependent on the type of sample and the sample preparation required. Sample for ISE analysis must be prepared to solubilize the fluoride in the sample. For some samples, ashing or NaOH fusion is required. A total-ionic strength adjustment buffer (TISAB) is used to adjust samples and standards to the same ionic strength and pH; this allows the concentration, rather than the activity, to be measured directly and often read directly off a meter. The pH of the buffer is about 5, a level at which F^- is the predominant fluorine-containing species. The buffer contains cyclo-hexylene-dinitrilotetraacetic acid, which forms stable complexes with Fe(III) and Al(III), thus removing interferences by freeing fluoride ions from complexes with these ions (NIOSH 1994; Schamschula et al. 1985; Tusl 1970). Bone fluoride levels can be measured using the ISE technique after ashing of the sample (Boivin et al. 1988). Fingernail fluoride levels can be measured using the ISE technique after nail clippings were prepared by HMDS-facilitated diffusion overnight. This sample preparation was found to quantitatively remove fluoride from the nail material (Whitford et al. 1999a). Whitford et al. (1999a) also reported that soaking the fingernail samples in deionized water for 6 hours or in a solution of 1.0 ppm fluoride for 2 hours did not change the concentration of fluoride found in the nail.

Recent studies have employed GC to measure fluoride concentrations in human urine and plasma (Chiba et al. 1982; Ikenishi and Kitagawa 1988; Ikenishi et al. 1988). In this method, derivatization and

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Extract with TMCS; inject organic phase (microwave induced plasma emission detector)	GC	4 µg/L	935	Chiba et al. 1982
	Add equal volume TISAB solution	ISE, NIOSH 8308	0.1 mg/L	95%	NIOSH 1994
	Add TMCS toluene solution; centrifuge; inject toluene layer	GC	>5 ng/mL	No data	Ikenishi et al. 1988
Biological fluids and tissue extracts (ionic and ionizable fluoride)	Absorb with calcium phosphate; centrifuge; analyze	ISE	10 µg/L	92–102%	Venkateswarlu et al. 1971
Saliva	Resuspend in TISAB buffer; analyze	ISE	No data	99.8%	Petersson et al. 1987; Schamschula et al. 1985
Biological fluids	Add TMCS toluene solution; centrifuge; inject toluene layer and analyze by measuring TMFS peak height	GC	5 ng/L	88.1–97.2%	Ikenishi et al. 1988
Biological tissues and fluids	Extraction from acidified sample as fluorosilane; reverse extraction as fluoride ion into alkaline solution	ISE with hanging drop assembly	>0.04 ng/sample	No data	Venkateswarlu 1974
Biological tissues	Sample pulverized to fine powder; irradiate with energetic beam of protons; detect gamma rays emitted	PAA	<10 ng/sample	No data	Rudolph et al. 1973
	Decomposition of sample at 700–1,000 °C (pyrohydrolytic technique)	Colorimetry	1 µg/sample	No data	Kakabadse et al. 1971
Tooth enamel	Soak teeth; decalcify in $HClO_4$; add TISAB; analyze	ISE	No data	No data	Schamschula et al. 1982; Shida et al. 1986
Plaque	Dried; microdiffusion; analyze	ISE	No data	97%	Schamschula et al. 1985
Bone	Ash sample; dissolve in perchloric acid; add 1,2-cyclohexylenedinitrotetraacetic acid	ISE	No data	No data	Boivin et al. 1988
Hair/fingernail	Wash in diethylether; dry; decompose in NaOH	ISE	No data	94–96%	Schamschula et al. 1985
Fingernail	HMDS-facilitated diffusion overnight	ISE	No data	No data	Whitford et al. 1999a

GC = gas chromatography; HClO4 = perchloric acid; HMDS = hexamethyldisiloxane; ISE = ion selective electrode; NaOH = sodium hydroxide; NIOSH = National Institute for Occupational Safety and Health; PAA = proton activation analysis; TISAB = total ionic strength activity buffer; TMCS = trimethylchlorosilane; TMFS = trimethylfluorosilane

extraction is achieved using trimethylchlorosilane (TMCS) in toluene to produce trimethylfluorosilane (TMFS). The organic layer is injected into the GC system and the TMFS peak height is compared with those of standard solutions. The GC method has the advantage of high sensitivity—nanogram quantities of fluoride are detectable in a milliliter of urine or plasma. This method is also useful for assessing the fluoride released from fluorine-containing drugs in biological fluids. The detection of bound fluorine provides an advantage over the ISE technique, which is not suitable for bound or organic fluoride measurements. It should also be noted that the aluminum ion may cause interference under the operating conditions of the GC, as it does with the ISE method.

7.2 ENVIRONMENTAL SAMPLES

The ISE method is the most widely used method for determining fluoride levels in the environmental media. Table 7-2 describes this and other methods for determining fluoride in environmental samples. Table 7-3 describes methods for determining hydrogen fluoride in air. ISE methods are simple to perform and have good precision and sensitivity. Fluoride-specific electrodes are commercially available. The method detects only free fluoride ions in solution. Because of the inherent restriction of this technique, several approaches have been recommended to prepare the sample for analysis. Lopez and Navia (1988) assayed total fluoride (bound and free) in food and beverages by initially acid hydrolyzing samples at 100 °C in borosilicate vials. This closed-system approach decreases contamination, eliminates dry ashing, and yields high recoveries. Dabeka and McKenzie (1981) employed microdiffusion with 40% perchloric acid to food samples in Petri dishes at 60 °C for 24–48 hours. Difficulties arose in controlling contamination and fluoride loss in the Petri dishes, and low recoveries were reported. Preparation of total fluoride in dry plant material (i.e., hay, barley, straw, corn, grass) was described by Eyde (1982); samples were fused in nickel crucibles with sodium hydroxide at 350–475 °C. The ash was diluted and filtered for analysis. This method is more tedious than the others, and fluoride loss is expected from the high fusion temperatures. All of these preparatory techniques can liberate bound fluoride from the sample matrices. It is important to prevent interference of other ions and to avoid fluoride loss at high decomposition temperatures before potentiometric analyses. Kakabadse et al. (1971) described a pyrohydrolytic technique for tea, coca, or tobacco samples that could be employed prior to colorimetric or ISE analysis. Decomposition of the sample at 700–1,000 °C is mediated by a current of air or pure oxygen to evolve hydrogen fluoride. An advantage of this approach is that fluoride is collected from inorganic and organic fluorides in one operation. Ashing, which may produce loss of organic fluorine, is eliminated.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Ambient air collected using teflon tubing; detect with continuous flow analyzer	ISE	0.1 µg/L	No data	Danchik et al. 1980
	Sample at 1–2 L/minute using cellulose ester membrane filter and alkaline-impregnated backup pad to collect particulate and gaseous fluorides. Extract hydrogen fluoride and soluble fluorides with water; insoluble fluorides require NaOH fusion	IC/conductivity detector; NIOSH 7906	3 µg/sample (gas); 120 µg/sample (particulate)	No data	NIOSH 1994
	Sample at 1–2 L/minute using cellulose ester membrane filter and alkaline-impregnated backup pad to collect particulate and gaseous fluorides; extract hydrogen fluoride and soluble fluorides with 50 mL 1:1 TISAB: water; insoluble fluorides require NaOH fusion	ISE, NIOSH 7902	3 µg/sample	No data	NIOSH 1994
	Syringe-sampling; dilute with 50% (v/v) 1,2-dioxane containing Amadec-F	Colorimetry	0.3 ppm	No data	Bethea 1974
Water	Dilute sample; add barium chloride; complex with zirconium-xylenol orange for color development	Colorimetry	2,000 µg/L	No data	Macejunas 1969
	Sample added to sulfuric acid and distilled to remove interferences; distilled sample treated with SPADNS reagent; color loss resulting from reaction of reagent with fluoride is determined at 570 nm and concentration read off standard curve	Colorimetry; EMSLC Method 340.1	0.10 mg/L	No data	EPA 1998c
	Mix sample and standard 1:1 with TISAB (for soluble fluorides)	ISE, OSW Method 9214	0.500 mg/L	No data	EPA 1996
	No sample treatment required	ISE, EMSLC Method 340.2	0.100 mg/L	No data	EPA 1998c
	Bellack distillation[a], after which fluoride ion reacts with the red cerous chelate of alizarin complexone in an autoanalyzer	Colorimetry, EMSLC Method 340.3	0.050 mg/L	No data	EPA 1998c

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	Extract with TMCS; analyze organic phase (microwave induced plasma emission detector)	GC	4 µg/L	93–100%	Chiba et al. 1982
Waste water	Centrifuge sample to settle solids; filter and dilute	Anion exclusion chromatography	200 µg/L	No data	Hannah 1986
Water, rain	Dilute sample with TISAB buffer; analyze in flow injection system	ISE	2 µg/L	No data	Fucsko et al. 1987
Food, beverage	Homogenize sample; acid hydrolysis in a closed system	ISE	0.1 µg/g	97%	Lopez and Navia 1988
	Sample pulverized to powder	PAA	1 µg/g dry weight	No data	Shroy et al. 1982
Tea, cocoa, tobacco	Decomposition at 700–1,000 °C in moist current of oxygen or air; collect hydrogen fluoride; react to form Ce(III)alizarin-complexan	Colorimetry	>1 µg	No data	Kakabadse et al. 1971
Milk, peas, pears	Sample is dried and ground to powder; microdiffusion in Petri dish; analyze	ISE	0.2–5 µg/g	54–109%	Dabeka and McKenzie 1981
Vegetation	Fluorine-19 sample activation	INAA	14 µg/sample	No data	Knight et al. 1988
	Extraction of sample	ISE	>0.05 µg/g	>95%	Jacobson and Heller 1971
	Fusion with NaOH; dissolve in tiron buffer	ISE	10 µg/g	No data	Sager 1987
Feed	Sample is dried and acidified	ISE	15 µg/g	90–108%	Melton et al. 1974
Household products	Dilute sample, add buffer; addition procedure	ISE	No data	98–104%	Schick 1973
Plants	Sample dried and fused in nickel crucibles; filter	ISE	>0.3 µg/g	87–102%	Eyde 1982

[a]Bellack distillation uses $HClO_4/AgClO_4$ to remove chloride.

Ce III = cesium ion (+3 oxidation state); EMSLC = EPA Environmental Monitoring Systems Laboratory in Cincinnati; GC = gas chromatography; HPLC = high pressure liquid chromatography; IC = ion chromatography; INAA = instrumental neutron activation analysis; ISE = ion selective electrode; NaOH = sodium hydroxide; NIOSH = National Institute for Occupational Safety and Health; OSW = Office of Soild Waste; PAA = proton activation analysis; SPADNS = sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate; TISAB = total ionic strength activity buffer; TMCS = trimethylchlorosilane; (v/v) = volume/volume

Table 7-3. Analytical Methods for Determining Hydrogen Fluoride in Environmental Samplesa

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Personal air sampled at 1–2 L/minute for total sample of 12–800 L onto treated pad; soak pad in 25 mL water and 25 mL TISAB; collect using teflon tubing and analyze with continuous flow analyzer.	ISE, NIOSH 7902	0.7 µg fluoride/ sample	No data	NIOSH 1994
	Personal air sampled at 0.2–0.3 L/min for total sample size of 3–100 L using silica gel sample tube; boil sorbent from sample tube in bicarbonate/carbonate buffer for 10 minutes.	IC/conductivity detector, NIOSH 7903	0.7 µg/sample	No data	NIOSH 1994
	Personal air sampled at 1–2 L/minute using cellulose ester membrane filter and alkaline-impregnated backup pad to collect particulate and gaseous fluorides; extract hydrogen fluoride and soluble fluorides with water; insoluble fluorides requires NaOH fusion.	IC/conductivity detector, NIOSH 7906	3 µg/sample (gas); 120 µg/ sample (particulate)	No data	NIOSH 1994
	Hydrogen fluoride vapor collected with dosimeter containing poly-propylene element.	ISE	100 µg/L	No data	Young and Monat 1982
	Dual cellulose filter to separate particulate and gaseous fluoride; heat filters at 75 °C; extract; dilute with TISAB buffer.	ISE	1.2 µg/filter	No data	Einfeld and Horstman 1979

[a]Some methods measure both gaseous (HF) and particulate fluorides.

IC = ion chromatography; ISE = ion selective electrode; NIOSH = National Institute for Occupational Safety and Health; TISAB = total ionic strength activity buffer, v/v = volume/volume

Fluoride ions form stable, colorless complexes with certain multivalent ions, such as $(AlF_6)^{3-}$, $(FeF_6)^{3-}$, and $(ZrF_6)^{3-}$. Most colorimetric methods for the determination of fluoride are based on the bleaching of colored complexes of these metals with organic dyes when fluoride is added (WHO 1984). The degree of bleaching is determined with a spectrophotometer, and the concentration of fluoride ions is assessed by comparison with standard solutions. In EPA Method 340.1, the sodium 2-(parasulfophenylazo)-1,8-di-hydroxy-3,6-naphthalenedisulfonate (SPADNS) reagent is used, and the color loss is measured at 570 nm (EPA 1998c). In EPA Method 340.3, the red cerium complex with alizarin complex one turns blue on the addition of fluoride (EPA 1998c).

Ion chromatography (IC) utilizes anion exchange resins as a stationary phase to separate fluoride ions from other species. In most cases, conductivity detectors are used to detect the ions in the eluent. Both the stationary phase and the eluent must be chosen to separate fluoride from overlapping ions. Hannah (1986) used a variant of ion exchange chromatography, namely anion exclusion chromatography, to analyze fluoride in waste water. This method is generally applied to the separation of weak organic acids and its use for fluoride determinations is based on the fact that fluoride is an anion of a weak acid, hydrogen fluoride, with a pK_a of 3.19, similar to that of weak organic acids. The acids elute in order of increasing pK_a. At low pH, anions of strong acids remain disassociated and are excluded from the resin and are rapidly eluted. Hydrogen fluoride exists primarily in the molecular form, and interacts with the resin, delaying its elution. In this way, fluoride is sufficiently separated from ionic interferences to be reliably quantified. Interfering anions, such as chloride, emerge as one peak before the fluoride elutes. Resolution can be controlled by adjusting the pH.

Fluorides in air may be present in the gas phase (generally hydrogen fluoride) or in the particulate phase. Sampling may involve trapping the particulate phase on a membrane filter and the hydrogen fluoride on an alkaline impregnated backup pad as in NIOSH Method 7906 (NIOSH 1994). Several modifications have been suggested for the air sample collection. Einfeld and Horstman (1979) found that gaseous fluoride, to some extent, may get trapped in the filter for particulate fluoride. They suggest that postsampling heat treatment promotes desorption of the gaseous fluoride from the particulate phase. The use of Teflon® tubing and materials in the analyzer is indicated for controlling loss of sample ions (Candreva and Dams 1981; Danchik et al. 1980).

For the analysis of pollutants in the environment, EPA has approved the ISE (Method 340.2) and colorimetric methods (Methods 340.1 and 340.3) for determining inorganic fluoride in water (EPA 1998).

NIOSH recommends the use of ISE (Method 7902) and IC methods (Methods 7903 and 7906) for the determination of fluoride and hydrogen fluoride in air (NIOSH 1994).

Fluoride gas or vapors in ambient air are measured primarily with the ISE method. NIOSH Method 7902 uses this technique for the determination of hydrogen fluoride and particulate fluorides in air (NIOSH 1994). The hydrogen fluoride gas and particulate fluorides are collected on separate filters before determination. Several modifications have been suggested for the air sample collection. Einfeld and Horstman (1979) found that gaseous fluoride may get trapped in the filter for particulate fluoride to some extent. They utilized postsampling heat treatment to desorb hydrogen fluoride from particulates. The use of Teflon® tubing and materials in the analyzer is indicated for controlling fluoride loss (Candreva and Dams 1981; Danchik et al. 1980).

Young and Monat (1982) developed a dosimeter to be worn on the lapel in the workplace for monitoring airborne fluoride vapor. A replaceable collection element adsorbs the fluoride vapors. Samples are desorbed with TISAB solution and analyzed on the ISE. The study authors noted its convenience, stability, retentivity, and insensitivity to moisture at 5–88% humidity and competing sulfur dioxide vapors. Interference may occur from reactive volatile fluorine compounds. Wind, temperature, and atmospheric pressure can affect results. The dosimeter yields a sample detection range of 0.1–387 ppm fluoride in air.

Two analytical methods for fluorine determination have been developed based on neutron or proton activation of fluorine-9 (Knight et al. 1988; Shroy et al. 1982). Instruments measure the emitted gamma rays or x-rays using lithium-drifted germanium detectors. This approach has wide application, since it does not depend on a specific sample matrix or chemical form. However, the need for a special facility with a source of neutrons or protons limits its use.

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 fluorides, hydrogen fluoride, and fluorine 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 fluorides, hydrogen fluoride, and fluorine.

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. Sensitive, reproducible analytical methods are available for detecting fluorides in biological materials following short-term exposure (such as plasma and urine) and long-term exposure (i.e., bone). The most common technique is the ISE method because it is reliable, simple, and sensitive, and has good recoveries (NIOSH 1994; Venkateswarlu et al. 1971). GC is also useful for detection of trace levels of fluoride in plasma and urine (Chiba et al. 1982; Ikenishi and Kitagawa 1988; Ikenishi et al. 1988). Both methods can measure samples at concentrations at which health effects may occur.

Urinary fluoride is a widely accepted biomarker of recent fluoride exposure and has frequently been used as an indicator of fluoride exposure in occupational studies (Chan-Yeung et al. 1983a; Kaltreider et al. 1972) and to determine exposure from drinking water (Spak et al. 1985). A minimum fluoride level of 4 mg/L in the urine using the ISE technique has been recommended as an indicator of recent fluoride exposure in workers (Derryberry et al. 1963). Other possible biomarkers of fluoride exposure include fluoride concentrations in tooth enamel (Shida et al. 1986), hair (Schamschula et al. 1982), nails (Schamschula et al. 1982; Whitford et al. 1999a), saliva (Petersson et al. 1987), blood (Jackson and Hammersley 1981), and bone (Baud et al. 1978; Bruns and Tytle 1988; Fisher 1981; Sauerbrunn et al. 1965) for which analytical methods are available.

Effect. For biomarkers of effect following chronic exposure, investigators have looked for skeletal fluorosis using radiographs. Bone density is a common index used for evaluation (Kaltreider et al. 1972). Guminska and Sterkowicz (1975) found an increase in erythrocyte enzyme activity (i.e., enolase, pyruvate kinase, ATPase) that may reflect altered glucose metabolism during prolonged fluoride exposure. These

biochemical alterations are suggested for possible diagnostic purposes, but they represent a response that may be induced in the body by a physiological change or other chemical agents. Therefore, more specific analytical methods are needed for measuring biomarkers of effect.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available for determining fluoride levels in environmental samples. Methods determine the fluoride concentration and not the particular fluorine-containing compound. Therefore, analytical methods do not distinguish between parent compound and degradation product. The ISE method is the most common method for measuring fluoride in environmental samples. It is a convenient, sensitive, and reliable method, but fluoride ions must first be released from any matrix and rendered free in solution. Methods are available for preparing various types of environmental samples for analysis (Dabeka and McKenzie 1981; EPA 1998c; Eyde 1982; Kakabadse et al. 1971; Lopez and Navia 1988; NIOSH 1994; NRC Canada 1971; WHO 1984).

7.3.2 Ongoing Studies

One ongoing study regarding techniques for measuring and determining fluoride in biological and environmental samples was located. Noah Seixas of the University of Washington proposes to adapt real-time instruments for monitoring HF and SO_2 and a nonspecific particulate, integrating currently available, electrochemical sensor, and light scattering technology and, using these instruments, to monitor exposure in four aluminum smelting operations (FEDRIP 2002).