7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring ammonia, its metabolites, and other biomarkers of exposure and effect to ammonia. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods are included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

7.1 BIOLOGICAL MATERIALS

When ammonia is found in biological materials at physiological pH (7.2), most of it (99%) will be found as ammonium ion, due to its pK_a of 9.2. This is an important consideration for any subsequent analysis. The determination of ammonia (as dissolved NH_3 and ammonium ion) in blood, plasma, or serum is of value in detecting existing or impending hepatic coma and Reyes Syndrome (Meyerhoff and Robins 1980; Tietz 1970). The determination of ammonia in urine had historically been used as an indicator of the kidney's ability to produce ammonia; however, this procedure has been replaced by more modern and accurate tests for kidney function. Procedures for the determination of ammonia in biological samples are found in Table 7-1. Ammonia is also tested for in calculi (abnormal concretions in the body formed of mineral deposits, often found in the gall bladder, kidney, or bladder) (Tietz 1970); however, the test described therein is not quantitative and is not included in Table 7-1.

The amount of ammonia in collected blood, urine, saliva, or other biological fluid samples can be affected by several mechanisms that may lead to erroneous ammonia concentration determinations. These effects can be minimized by proper sample storage and handling. The ammonia content of freshly drawn blood rises rapidly on standing because of the deamination of labile amides such as glutamine (Henry 1964); at room temperature, the ammonia content can increase by a factor of two or three in several hours. Therefore, it is important to both keep the specimen cold (on ice) and perform the analysis as soon as possible. If the sample cannot be analyzed quickly, it may be frozen (-20 °C). The ammonia content of

Table 7-1. Analytical Methods for Determining Ammonia in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	24-Hour specimen, add HCl, refrigerate	Colorimetric (Berthelot reaction)	Not reported	Not reported	Tietz 1970
Urine	24-Hour specimen analyzed immediately, or stored up to 8 weeks at -20 °C	Indophenol reaction	Not reported	Not reported	Huizenga et al. 1994
Saliva	Freeze at -20 °C for up to 2 weeks, or store for 1 hour at 4 °C, or analyze immediately	Membrane based ammonia-selective electrode	Not reported	Not reported	Huizenga et al. 1994
Serum, plasma, whole blood	Freeze, then store at -15 °C for several days, or store on ice (4 °C) for 30–60 minutes, or analyze immediately	Colorimetric assay based on indophenol production	Not reported	Not reported	Huizenga et al. 1994
Serum, plasma, whole blood	Freeze, then store at -15 °C for several days, or store on ice (4 °C) for 30–60 minutes, or analyze immediately	Titration	Not reported	Not reported	Huizenga et al. 1994
Serum, plasma, whole blood	Freeze at -30 °C or store on ice, or analyze immediately	Membrane based ammonia-selective electrode	Not reported	-7.0–14% error, 102% average recovery	Meyerhoff and Robins 1980

iced (4 °C) blood samples remains constant for up to 60 minutes, whereas the ammonia content of frozen (-20 °C) blood samples remains constant for several days (Huizenga et al. 1994; Tietz 1970). For blood samples collected from a healthy person (and stored on ice), the ammonia content should be measured within 30–60 minutes of collection. For persons suspected of suffering from liver disease, however, the blood samples should be analyzed within 15 minutes (Huizenga et al. 1994). This more rapid assessment is necessary because some liver diseases result in high levels of γ-glutamyl transferase, an enzyme that hydrolyzes glutamine; the enzyme's activity will increase the concentration of ammonia in the sample to levels higher than was present at the time of collection (Huizenga et al. 1994). Other erroneously high measured ammonia levels can result from ammonia contamination of reagents or pick-up of ammonia from the atmosphere, including from technicians that have recently smoked a cigarette (Huizenga et al. 1994). The presence and activities of bacteria in samples can also cause changes in the concentrations of ammonia. The natural presence of bacteria present in some samples (e.g., saliva) or their presence in infected tissues (e.g., bladder infections) can lead to erroneously high ammonia concentrations, due to the presence of bacterial ureases that hydrolyze urea present in the biological samples. This reaction is the chief cause for the formation of ammonia in unacidified urine on standing (Henry 1964). Furthermore, contamination of samples by environmental bacteria following the collection of the sample may also lead to increases in ammonia concentrations. Therefore, use of sterile collection bottles for sample collection and storage is recommended if there is potential for storage of the sample prior to analysis (Huizenga et al. 1994).

In the determination of ammonia or ammonium ion in biological samples, ammonia is first liberated by distillation, aeration, ion-exchange chromatography, microdiffusion, or deproteinization (Huizenga et al. 1994). Deproteinization methods involve treatment of blood or plasma fluids with trichloroacetic acid (or perchloric acid or tungstate-sulfuric acid), followed by centrifugation. The protein-free supernatant can be assayed colorimetrically for ammonia. Traditionally, Kjeldahl distillation methods have been used to determine ammonia levels in biological tissue, but other methods (e.g., colorimetric or ion-specific electrodes) are also available. In the Kjeldahl distillation, ammonia is distilled and subsequently trapped in acid and analyzed titrametrically or colorimetrically. High values sometimes result because of the cleavage of protein amino groups and also the formation of ammonia by deamination reactions (Parris and Foglia 1983). Other techniques use the ammonia-selective electrode and enzymatic assays. Discrepancies have been reported between results using electrodes and those using more specific enzymatic procedures because the ammonia electrode responds to both ammonia and volatile amines (Parris and Foglia 1983). Chromatographic separation of ammonia and volatile amines after derivatization has also been used to obtain specificity (Huizenga et al. 1994; Parris 1984). Ammonia in

urine has been measured by Nesslerization, as well as by enzymatic assays and chromatographic approaches (Huizenga et al. 1994).

7.2 ENVIRONMENTAL SAMPLES

Water and waste water samples can be analyzed for ammonia by EPA Test Methods 1689 (EPA 2001a), 1690 (EPA 2001b), and 349.0 (EPA 1997). Analogous procedures (i.e., Method APHA 4500) have been approved and published jointly by the American Public Health Association, American Water Works Association, and Water Pollution Control Association. These methods are suitable for drinking, surface, and saline waters, and domestic and industrial effluent, and can be applied to biosolids. These and other methods for determining ammonia in environmental samples are listed in Table 7-2. Ammonia is reported as ammonia nitrogen. Two methods that are suitable for water employ colorimetric techniques, Nesslerization, and phenate methods. Nessler's reagent, an alkaline mixture of mercuric and potassium iodide, produces a yellow to brown color with ammonia, whereas the phenate reagent, alkaline phenol, and hypochlorite produce a blue color (EPA 2001a, 2001b; Greenberg et al. 1985). In the titrimetric method, the distillate is titrated with standard sulfuric acid with an appropriate indicator. The ammonia electrode employs a hydrophobic gas-permeable membrane to separate the sample solution from an internal ammonium chloride solution: ammonia diffusing through the membrane changes the pH of the internal solution and is sensed by a pH electrode. For determining NH_3-N concentrations above 5 mg/L, the titrimetric and ammonia-selective electrode methods are preferred. In contrast, gas chromatography/ mass spectrometry methods have been developed that permit NH_3 detection at concentrations near 20 μg/L for environmental waters (Mishra et al. 2001). Methods for determining ammonia in water and soil measure ammoniacal nitrogen, the sum of NH_3 and NH_4^+. In the determination of ammoniacal nitrogen in soil, exchangeable ammonium should be distinguished from nonexchangeable ammonium. The former is usually defined as that which can be extracted with KCl (or K_2SO_4) at room temperature (Bremner 1965). Nonexchangeable ammonium ion is defined as nitrogen held by clays and not displaced by 2M KCl (Bremner 1965). In the determination of nonexchangeable ammonium, organic forms of ammonia are first removed, the minerals containing the nonexchangeable ammonium are then decomposed with HF, and the ammonium ions released. In colorimetric procedures, turbidity and sample color may lead to interference. To eliminate interference, the pH of the sample may be raised and the ammonia distilled. Care should be taken to prevent losses in water samples due to volatilization and microbial transformation. To prevent such losses, samples should be acidified soon after collection and refrigerated. Care should also be taken during storage and treatment of soil samples to prevent ammonia

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Passive collection using 0.01 N H_2SO_4 in liquid sorbent badge	Method 6701, ion chromatography, conductivity detection	1 µg NH_3/sample	No bias between 6.9 and 48 ppm; +19% at 148 ppm	NIOSH 1987
Air	Air samples from stack emissions collected through an in-stack filter to remove particulates and ammonium salts, and then bubbled through 0.1 N H_2SO_4	EPA Method 30; ion chromatography	1 µg NH_3/sample	98.5±1.3%; Bias of 0.996 ppm for a spiked sample of 6.43 ppm. Correction factor of 0.87 needs to be applied	Eaton et al. 1996
Air	0.8 µm prefilter may be used; ammonia trapped on sulfuric acid silica gel	NIOSH method 6015, Colorimetric determination of indophenol by visible light spectrophotometry	0.5 µg NH_3/sample	Not determined	NIOSH 1994
Air	0.8 µm prefilter may be used; ammonia trapped on sulfuric acid silica gel	NIOSH method 6016. Ion chromatography	2 µg NH_3/sample	102±3.8%	NIOSH 1996
Air	Chromatomembrane cells preextract and preconcentrate sample	Ion chromatography with conductivity detection	6 µg NH_3/sample	Not reported	Erxleben et al. 2000
Air	Collection in H_2SO_4-coated activated carbon beads in sampling tube	Ion chromatography	2 µg NH_3/sample	95–110% recovery	Bishop et al. 1986
Air	Known volume of air drawn through prefilter and H_2SO_4-treated silica gel	NIOSH S347, ammonia-specific electrode	Not reported	97.6% mean recovery	SRI 1988
Water	Sample mixed with borate buffer	Method 1689, ion selective probe	0.1 mg/L	Not reported	EPA 2001a

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Sample collected, preserved with H_2SO_4, and chilled to 4 °C. Samples should not be stored for >28 days	Method 1690, colorimetric determination of indophenol blue, following reaction of any ammonia with alkaline phenol and hypo-chlorite	0.2 mg/L	Not reported	EPA 2001b
Water	None	Method 350.1 colorimetric, automated phenate	0.1 mg/L	107 and 99% recoveries at 0.16 and 1.44 mg NH_3-N/L, respectively	EPA 1983
Estuarine and coastal water	Samples filtered through 0.45 μm membrane filter, refrigerated and analyzed within 3 hours	Method 349.0, automated colorimetric determination by reactions that form indophenol blue	0.3 μg/L	92.2–109.1% recovery, n=14	EPA 1997a
Water	Removal of residual chlorine with sodium thiosulfate, distillation	Method 350.2 Nessier reagent, colorimetric, titrimetric; or ammonia specific electrode	0.05 mg N/L for colorimetric and potentiometric; 1.0 mg N/L for titrametric	28.12 to -0.46R bias between 0.21 and 1.92 mg N/L	EPA 1983
Water	None	Method 350.3 ion selective electrode	0.03 mg N/L	96 and 91% recoveries at 0.19 and 0.13 mg N/L, respectively	EPA 1983
Soil, exchange-able ammonium	Extract soil with 2N KCl	Method 84-3, steam distillation with MgO, titration	Not reported	Not reported	Bremner 1965
Soil, non-exchangeable (fixed) ammonium	Pretreat soil with KOBr-KOH, shake with 5 N HF-1N HCl for 24 hours	Method 84-7, steam distillation with KOH, titration	Not reported	Not reported	Bremner 1965

loss or gain. It has been demonstrated that dry soil can rapidly adsorb trace amounts of ammonia from the atmosphere and that extensive amounts of ammonia can be lost during air drying (Bremner 1965). Additionally, in samples containing both ammonium and nitrite, losses during air drying may occur due to the reaction between these ions and the resulting formation and release of nitrogen gas (Bremner 1965).

The detection limit of analytical methods for determining ammonia in air depends on the amount of air collected in a liquid or solid adsorbent. Sampling is performed with passive samplers or by drawing a volume of air through the adsorbent using a pump. Particulate contaminants such as ammonium salts may be removed by a prefilter. For determination of ammonia in the ambient atmosphere, larger volumes of air must be sampled than those appropriate for determinations of ammonia in occupational settings (e.g., industrial, agricultural) where ammonia levels are higher. Improvements in methodologies have led to development of techniques that permit continuous monitoring of atmospheric ammonia down to 0.1 $\mu g/m^3$ (Pranitis and Meyerhoff 1987). Several passive monitoring systems report detection limits of 0.05–1.0 $\mu g/m^3$ and have collection rates ranging from 2.7 to 2,000 mL/minute (Kirchner et al. 1999). One method used for ambient atmospheric sampling employs a specially designed flow-through, ammonia-selective electrode with a sniffer tube, whereas the methods used for occupational settings often use passive collectors with media (usually acids impregnated onto filters) housed within protective cases. Ammonia concentrations on these passive collectors are then determined by a wide range of methods, including colorimetric assays (e.g., indophenol determination), the Berthelot reaction, or ion chromatography (Kirchner et al. 1999).

Ammonia may be present in air in both the vapor phase as ammonia gas and in the particulate phase as ammonium salts. While some analytical methods may distinguish between these phases, most standard methods do not. Methods have been developed that determine gaseous ammonia alone or gaseous and particulate forms of ammoniacal nitrogen separately. These methods use filter packs or sampling tubes coated with a selective adsorbent (denuder tube) to separate the phases (Dimmock and Marshall 1986; Knapp et al. 1986; Rapsomanikis et al. 1988). In these methods, gaseous ammonia is trapped by acids that act as adsorbents (e.g., citric acid, oxalic acid, phosphoric acid) on a coated filter or denuder tube (Kirchner et al. 1999). In filter methods, errors may arise due to ammonia interactions occurring on the filter and volatilization of retained ammonium salt (Dimmock and Marshall 1986; Rapsomanikis et al. 1988). There is evidence that ammonium nitrate in particulate matter is in equilibrium with ammonia. The presence of ammonium nitrate may lead to overestimation of the actual concentration of ammonia, but underestimation of the concentration of ammonium (Doyle et al. 1979). For additional review of the

methodologies for determining ammonia in water and air, see MacCarthy et al. (1987) and Fox (1987), respectively.

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 ammonia 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 ammonia.

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. No known unique biomarkers for exposure or effects exist for ammonia. Until one has been identified, methodology for the determination of biomarkers must be preceded by an experimental determination of a unique biomarker of human exposure or effect.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining ammoniacal nitrogen in environmental media are well developed and adequate. Standardized methods are available from EPA, NIOSH, and other sources. Analytical methods are also well developed for oxidation products of ammonia. Since there are multiple sources of these compounds in the environment, their analysis is not generally used to study the disappearance of ammonia.

7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2003) database provides additional information obtainable from a few ongoing studies that may fill some of the data needs identified in Section 7.3.1. These studies are summarized below and in Table 7-3. By and large, most of the studies that were reported were related to the detection and measurement of atmospheric ammonia concentrations. Many of these focused on the development of novel sensor devices, which would provide better data for estimating ammonia emission and deposition rates. A company in Atlanta, Georgia is developing an optical ammonia sensor to measure agricultural emissions. This sensor is capable of detecting ammonia concentrations at the 100 ppb range and above. Similarly, the Georgia Institute of Technology is developing an optical sensor that will permit measurements of ammonia in the atmosphere at the 100 ppb range. Another company in Burlington, Massachusetts is developing a diode laser absorption remote sensor for measuring ammonia at trace concentrations, but no detection ranges have been specified. Another company in Massachusetts is developing a solid-state electrochemical sensor that is based on ionomer (i.e., an ion-containing polymer) membrane technology. This technology, however, is not intended for atmospheric ammonia sampling, but for instrument monitoring where ammonia gas may have negative impacts. This particular application seeks to produce these monitors for use in fuel cell systems, where free ammonia can negatively impact performance.

Table 7-3. Ongoing Studies on the Development of Analytical Approaches to the Study of Ammonia

Investigator	Affiliation	Research description	Sponsor
Attar AJ	Perfect View, Inc., Raleigh, North Carolina	Development of a low-cost ammonia detector (a small detector panel, about the size of a credit card, that changes color in response to ammonia in the atmosphere) for use in animal production facilities.	SBIR
Edwards J	Photonic Sensors, Atlanta, Georgia	Development of an optical ammonia sensor to measure agricultural emissions. This sensor is capable of detecting ammonia concentrations at the 100 ppb range.	Cooperative Agreement
Goldstein N	Spectral Sciences, Burlington, Massachusetts	Development of a diode laser absorption sensor for measurement of trace concentrations of ammonia, for potential applications in atmospheric chemistry and pollution monitoring.	U.S. DOE
Laconti AB	Giner, Inc., Waltham, Massachusetts	Development of a solid-state electrochemical sensor that is based on ionomer membrane technology for instrument monitoring where ammonia gas may have negative impacts.	SBIR
Ozanich RM	Northwest Instrument Systems, Richland, Washington	Development of an online system for analyzing ammonia (and other nitrogen containing chemicals) in water systems.	SBIR
Walsh JL Jr.	Georgia Institute of Technology, Atlanta, Georgia	Development of an optical ammonia sensor to measure agricultural emissions. This sensor is capable of detecting ammonia concentrations at the 100 ppb range, and would be useful for real-time monitoring of the injection of anhydrous ammonia fertilizer onto crops.	U.S. DOE

Source: FEDRIP 2002, 2003

SBIR = Small Business Innovative Research; U.S. DOE = U.S. Department of Energy