6. ANALYTICAL METHODS

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

6.1 BIOLOGICAL MATERIALS

Spectrophotometric methods, high-performance liquid chromatography (HPLC), and gas chromatography (GC) may be used to detect and measure hydrazine and dimethylhydrazines in biological materials (Alvarez de Laviada et al. 1987; Amlathe and Gupta 1988; Fiala and Kulakis 1981; Preece et al. 1992a; Reynolds and Thomas 1965; Timbrell and Hat-laud 1979). The spectrophotometer measures the absorbance of light at a particular wavelength, thereby identifying and quantifying a compound that absorbs at that wavelength. The chromatograph separates complex mixtures of organics and allows individual compounds to be identified and quantified by a detector. An electrochemical detector (ED), in the case of HPLC, and a nitrogen phosphorus detector (NPD) or flame ionization detector (FID), in the case of GC, may be used to identify hydrazine or dimethylhydrazine or their derivatives. When unequivocal identification is required, a mass spectrometer (MS) coupled to the GC column may be employed.

Prior to GC or spectrophotometric analysis, hydrazine and dimethylhydrazines must be separated from the biological sample matrix and derivatives of the compounds must be prepared. Separation is usually effected by precipitation of residual protein with acid and extraction of interfering lipids with methylene chloride (Alvarez de Laviada et al. 1987; Preece et al. 1992a; Reynolds and Thomas 1965; Timbrell and Harland 1979). Hydrazine and 1,1-dimethylhydrazine, but not 1,2-dimethylhydrazine,

may then be derivatized with an aldehyde such as pentafluorobenzaldehyde or *p*-dimethylaminobenzaldehyde. 1,2-Dimethylhydrazine, which has no free-NH_2 group, cannot be derivatized in this way but may be quantified by chromatographic methods that do not require derivatization (Fiala and Kulakis 1981). Details of selected analytical methods for hydrazine and dimethylhydrazines in biological samples are summarized in Table 6-l.

Accurate analysis of hydrazine and dimethylhydrazines in biological samples is complicated by the tendency of these chemicals to autoxidize during storage (Preece et al. 1992a). Thus, derivatization should be completed as rapidly as possible, before autoxidation can occur.

6.2 ENVIRONMENTAL SAMPLES

Determination of hydrazine and dimethylhydrazines in air, water, soil, food, and tobacco is also carried out by spectrophotometry, GC, or HPLC analysis (Amlathe and Gupta 1988; ASTM 1991b; Holtzclaw et al. 1984; Leasure and Miller 1988; Liu et al. 1974; NIOSH 1977a, 1977b, 1984; Rutschmann and Buser 1991; Schmeltz et al. 1977; Wright 1987). Several representative methods for quantifying these chemicals in each of these media are summarized in Table 6-2. EPA-validated methods are not available for analysis of hydrazine or dimethylhydrazines in any environmental medium. Two EPA methods (8250 and 8270) are recommended for analysis of 1,1-dimethylhydrazine in wastes (EPA 1990e). However, these methods do not list 1,1-dimethylhydrazine as an analyte (EPA 1990c, 1990d) and do not appear to be suitable methods for analysis of this compound since 1,1-dimethylhydrazine is likely to degrade during the GC analysis unless it has been derivatized.

Separation of hydrazine and dimethylhydrazines from environmental samples is by acid extraction when necessary. Air samples are usually collected in a bubbler with acid or on an acid-coated silica gel (NIOSH 1977a, 1977b, 1984). When GC is employed, detection may be by electron capture detector (ECD), FID, nitrogen-specific detector (NSD), thermionic ionization detector (TID), and/or MS as described above (Section 6.1).

Accurate determination of hydrazine and dimethylhydrazines in environmental samples is also complicated by the susceptibility of these chemicals to oxidization. Air samples must be analyzed immediately after collection (Cook et al. 1979). Degradation of hydrazine in aqueous samples can be prevented by acidification with sulfuric acid (WHO 1987).

TABLE 6-1. Analytical Methods for Determining Hydrazine, 1,1-Dimethylhydrazine, and 1,2-Dimethylhydrazine in Biological Samples[a]

Sample matrix	Preparation method	Analytical metod	Sample detection limit	Percent recovery	Reference
Urine	Precipitate residual protein with hydrochloric acid and ammonium sulfate; extract interfering lipids with methylene chloride; derivatize aqueous fraction with pentafluoro-benzaldehyde; extract with ethyl acetate.	GC/NPD	8 μmol[b]	79±14	Preece et al. 1992
Urine	Extract with methylene chloride; discard extract; derivatize aqueous fraction with p-chlorobenzaldehyde; extract with methylene chloride; dry and dissolve in ethyl acetate.	GC/NPD	0.05 μg/mL	No data	Timbrell and Harland 1979
Urine	Deproteinate with trichloroacetic acid; derivatize with vanillin in ethanol; acidify with sulfuric acid.	Spectrophotometry	0.065 μg/mL	99.4–100	Amlathe and Gupta 1988
Urine[c]	Dilute with deionized water.	Ion-exchange HPLC/ECD	8 ng[b]/sample	No data	Fiala and Kulakis 1981
Plasma Liver Tissue	Precipitate residual protein with hydrochloric acid and ammonium sulfate; extract interfering lipids with methylene chloride; derivatize aqueous fraction with pentafluoro-benzaldehyde; extract with chloroform.	GC/MS	≈20 nmol/mL[b]	103±9	Preece et al. 1992

TABLE 6-1. Analytical Methods for Determining Hydrazine, 1,1-Dimethylhydrazine, and 1,2-Dimethylhydrazine in Biological Samples *(continued)*

Sample matrix	Preparation method	Analytical metod	Sample detection limit	Percent recovery	Reference
Plasma[c]	None	Ion-exchange HPLC/ED	8 ng[b]/sample	No data	Fiala and Kulakis 1981
Serum Liver/brain tissue	Acidify; derivatize with p-dimethyl-aminobenzaldehyde in ethanol.	Spectrophotometry	0.025 µg[b]/sample	No data	Alvarez de Laviada et al. 1987
Serum	Treat with trichloroacetic acid; centrifuge; derivatize supernatant with p-dimethylaminobenzaldehyde in ethanol.	Spectrophotometry	0.05 µg/mL[b]	No data	Reynolds and Thomas 1965

[a] Applicable to hydrazine only unless otherwise noted.
[b] Lowest detected amount.
[c] Method applicable to 1,1-dimethylhydrazine and 1,2-dimethylhydrazine as well as hydrazine.

ED = electrochemical detector; GC = gas chromatography; HPLC = high performance liquid chromatography; MS = mass spectroscopy; NPD = nitrogen-phosphorus detector.

TABLE 6-2. Analytical Methods for Determining Hydrazine, 1,1-Dimethylhydrazine, and 1,2-Dimethylhydrazine in Environmental Samples[a]

Sample matrix	Preparation method	Analytical metod	Sample detection limit	Percent recovery	Reference
Air	Collect in bubbler with hydrochloric acid; neutralize with sodium hydroxide; derivatize with p-dimethylaminobenz-aldehyde; dilute with glacial acetic acid.	Spectrophotometry	0.9 µg/sample	No data	NIOSH 1984
Air[b]	Adsorb on sulfuric acid-coated silica gel; elute with water; derivatize with 2-furaldehyde; extract with ethyl acetate	GC/FID	0.002 mg/m^3 [c] (hydrazine) 0.04 mg/m^3 [c] (1,1-dimethyl-hydrazine)	No data	NIOSH 1977b
Air[d]	Collect in bubbler with hydrochloric acid; derivatize with phosphomolybdic acid	Spectrophotometry	0.02 mg/m^3	No data	NIOSH 1977a
Air[b]	Collect in a microimpinger containing acetone and glacial acetic acid to trap and derivatize in one step	GC/NSD	4 ppb (5 µg/m^3)	97–104	Holtzclaw et al. 1984
Water	Acidify with hydrochloric acid; derivatize with p-dimethylamino-benzaldehyde	Spectrophotometry	5 µg/L	97.5–100.3	ASTM 1991b
Water	Derivatize with vanillin in ethanol; acidify with sulfuric acid	Spectrophotometry	0.065 ppm	99.2–100.4	Amlathe and Gupta 1988

TABLE 6-2. Analytical Methods for Determining Hydrazine, 1,1-Dimethylhydrazine, and 1,2-Dimethylhydrazine in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical metod	Sample detection limit	Percent recovery	Reference
Soil[b]	Extract with sulfuric acid; derivatize with 2,4-pentanedione	GC/TID	0.1 ppm (hydrazine) 0.5 ppm (1,1-dimethyl-hydrazine)	98–100 (hydrazine) 94–101 (1,1-dimethyl-hydrazine)	Leasure and Miller 1988
Food[d]	Extract with L-ascorbic acid; derivatize with 2-nitrobenzaldehyde; cleanup on alumina column	GC/ECD	10 ppb	72–122	Wright 1987
Food[d]	Derivatize with pentafluorobenzoyl chloride; extract with methylene chloride	GC/MS	0.01 ppm	24–100	Rutschmann and Buser 1991
Tobacco/ tobacco smoke	Derivatize with pentafluorobenzaldehyde; enrich the resulting decafluorobenz-aldehyde azine by thin layer chromatrography; extract with ether	GC/ECD	0.1 ng/cigarette	No data	Liu et al. 1974

[a] Applicable to hydrazine only unless otherwise noted.
[b] Applicable to hydrazine and 1,1-dimethylhydrazine.
[c] Lower limit of range.
[d] Applicable to 1,1-dimethylhydrazine only.

ECD = electron capture detection; FID = flame ionization detector; GC = gas chromatography; MS = mass spectroscopy; NSD = nitrogen specific detector; TID = thermionic ionization detector

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 hydrazines is available. Where adequate information is not available, ATSDR, in conjunction with the 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 hydrazines.

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.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods are available for determining the levels of hydrazine, 1,1-dimethylhydrazine, and 1,2-dimethylhydrazine in biological samples, including urine, plasma, serum, liver tissue, and brain tissue (Alvarez de Laviada et al. 1987; Amlathe and Gupta 1988; Fiala and Kulakis 1981; Preece et al. 1992a; Reynolds and Thomas 1965; Timbrell and Harland 1979). These methods generally employ standard chromatographic and spectrophotometric procedures with detection limits ranging from 0.02 to 0.065 μg/rnL, and therefore, most likely are sufficiently sensitive to measure levels at which biological effects occur following recent exposures. The limited data available indicate that these methods are accurate and reliable if analyses are performed rapidly, before autoxidation can occur. The background levels of hydrazines in biological samples in the general population have not been determined; if hydrazines are-present at all, they are most likely present at levels below current detection limits. The detection limits for current methods are sufficiently sensitive to detect levels at which effects occur. Since hydrazines can occur in the body following exposure to drugs such as isoniazid and hydralazine (Timbre11 and Harland 1979), and many of the metabolites of hydrazines are ubiquitous or may occur following exposure to other chemicals, measures should be taken to ensure exposure to these confounding chemicals has not

occurred. Other metabolites such as azomethane, azoxymethane, and methylazoxymethanol are unique to exposure to 1 ,2 dimethylhydrazine. Studies which identify specific biomarkers for past exposure to hydrazines, in conjunction with the development of accurate and reliable methods for detecting such biomarkers, would be useful in estimating exposure to hydrazines at hazardous waste sites.

The effects of hydrazines have been fairly well characterized in humans and animals, and include neurological, hepatic, and carcinogenic effects (Chlebowski et al. 1984; Gershanovich et al. 1976; Haun and Kinkead 1973; Rinehart et al. 1960; Thorup et al. 1992; Wilson 1976). Methods exist for measuring serum transaminase levels, vitamin B_6 status, and occult blood in stool samples, all of which may serve as biomarkers of effect for hydrazines. Although these methods are fairly accurate and reliable, none of them are specific for effects of hydrazines. Studies which identify biomarkers of effect that are specific to hydrazines, in conjunction with the development of accurate and reliable methods for detecting such biomarkers, would be useful in determining if individuals have been exposed to predicting recent exposures to hydrazines at hazardous waste sites.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods are available to detect and quantify hydrazine and dimethylhydrazines in air, water, soil, food, and tobacco (Amlathe and Gupta 1988; ASTM 1991b; Holtzclaw et al. 1984; Leasure and Miller 1988; Liu et al. 1974; NIOSH 1977a, 1977b, 1984; Rutschmann and Buser 1991; Wright 1987). Air is the medium of most concern for human exposure to this chemical. Exposure may also occur from water, especially in the vicinity of hazardous waste sites or industrial sources. The existing analytical methods can provide determinations for these chemicals at levels sufficiently low to meet regulatory requirements (NIOSH 1977a, 1977b, 1984). Assuming that an adequate quantity of air is passed through the collector (for example: a volume of at least 41 m^3 is required to detect a level equivalent to the intermediate inhalation MRL of $2x10^{-4}$ ppm for 1,1-dimethylhydrazine, assuming a detection limit of 0.9 μg/sample), current methods are sufficiently sensitive to measure levels near the MRL value for 1,1-dimethylhydrazine. However, their tendency to degrade and their chemical reactivity limit the accuracy of analyses of thesechemicals in all media. Improved methods of extraction and analysis that minimize these reactions would enhance recovery of these chemicals from environmental samples and provide a better estimate of environmental levels, especially in drinking water and soil at hazardous waste sites.

In addition, methods are available to measure degradation products of hydrazine and dimethylhydrazines (see Section 5.3.2) in environmental samples and can be used to determine the environmental impact of these chemicals.

6.3.2 On-going Studies

On-going studies to improve analytical methods for hydrazine and dimethylhydrazines includes continuing research to improve HPLC columns and EDs. In addition, the Naval Research Laboratory has been investigating pattern recognition techniques using microsensors capable of measuring hydrazine in air at ppb concentrations (Anon 1987). These improvements are designed to overcome sampling problems and increase sensitivity and reliability of the analyses.