7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring naphthalene, 1-methylnaphthalene, 2-methylnaphthalene,, its metabolites, and other biomarkers of exposure and effect to naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene. 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

Naphthalene is moderately volatile with a boiling point of 218 °C and low water solubility of 31.7 mg/L (20 °C). Its log octanol/water partition coefficient is 3.29, implying a moderate affinity for lipid tissues. It undergoes short-term bioaccumulation in tissues, but biochemical processes lead to its biodegradation and eventual elimination. Methylnaphthalenes have similar properties (see Table 4-2). All of these properties have implications for determination of naphthalene and methylnaphthalenes in biological materials.

Historically, diethyl ether has been a widely used solvent for the extraction of lipophilic organic analytes such as naphthalene from biological fluids (Zlatkis and Kim 1976). Homogenization of tissue with the extractant and lysing of cells improves extraction efficiency. When, as is often the case, multiple analytes are determined using solvent extraction, selective extraction and loss of compounds that have a low boiling point can cause errors. The commercial availability of highly purified solvents has largely eliminated problems with solvent impurities, although high costs, solvent toxicities, and restrictions on spent solvent disposal must be considered. Extraction is the first step in the overall cleanup process that places the analyte in a form and matrix suitable for introduction into the instrument used to quantitate it. Cleanup of biological samples may often be complex and involve a number of steps (Walters 1986). Directly coupled supercritical fluid extraction (SFE)-gas chromatography has been used for the

determination of polychlorinated biphenyls (Hawthorne 1988) and might also be applicable to determination of naphthalene and methylnaphthalenes in biological samples.

Naphthalene metabolites are less lipophilic than naphthalene itself. Metabolites are isolated from body fluids and tissue homogenates by extraction and separated by thin layer chromatography (TLC) and HPLC (Horning et al. 1980; Melancon et al. 1982; Stillwell et al. 1982). Final identification of metabolites, which include numerous oxygenated and sulfur-containing species, is accomplished by gas chromatography (GC) and mass spectrometry (MS).

New immunological methods are being developed for detecting selected naphthalene metabolites in urine or naphthalene protein adducts in the blood of lung lavage specimens (Cho et al. 1994b; Marco et al. 1993). Additional work in perfecting these techniques is necessary before they will be useful in research and clinical practice.

Analytical methods for the determination of naphthalene and for 1-methylnaphthalene and 2-methyl-naphthalene in biological samples are given in Table 7-1. A method for the determination of radiolabelled 2-methylnaphthalene in rat urine has been described by Melancon et al. (1982). TLC and HPLC were used to characterize 2-methylnaphthalene and its metabolites, including 2-naphthoylglycine, 2-naphthoic acid, and others.

7.2 ENVIRONMENTAL SAMPLES

Gas chromatography and HPLC are the analytical methods most commonly used for detection of naphthalene and methylnaphthalenes in environmental samples. Several variations of these methods using different collection, extraction, and/or cleanup procedures and different detection methods have been approved by EPA and NIOSH for analysis of naphthalene in ambient water, drinking water, waste water, soil, and air (EPA 1982a, 1982b, 1986a, 1986b, 1986c, 1986d, 1990a, 1990b, 1990c, 1990d, 1990e; NIOSH 1984a, 1984b). The American Public Health Association (APHA) has recommended standard methods for analysis of naphthalene in water and waste water, each of which has been accepted by EPA as equivalent to one of the EPA-approved methods (APHA 1992a, 1992b, 1992c, 1992d, 1992e, 1992f). Analytical methods for naphthalene and 2-methylnaphthalene are presented in Tables 7-2 and 7-3, respectively. Although no standard methods were located that provided information on detection

Table 7-1. Analytical Methods for Determining Naphthalene, 1-Methylnaphthalene, and 2-Methylnaphthalene in Biological Samples[a]

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Adipose tissue	Extract; bulk lipid removal; Florisil® fractionation	HRGC/MS	9 ng/g	No data	Stanley 1986
Adipose tissue (human and bovine)	Extract with hexane; Florisil® cleanup	Capillary column GC/MS	10 ng/g	90 (human) 63 (bovine)	Liao et al. 1988
Human milk	Purge with helium; desorb thermally	Capillary column GC/MS	No data	No data	Pellizzari et al. 1982
Human urine (1-naphthol analysis	No data	TLC or GS/ unspecified spectroscopy	No data	No data	Bieniek 1994
Fish tissue	Purge and trap to carbon adsorption tube; extract with carbon disulfide	HRGC/FID	<10 µg/L	43–51	Murray and Lockhart 1988
Fish tissue	Saponification with potassium hydroxide; extraction with cyclopentane-dichloro-methane; adsorption enrichment with potassium silicate/silica gel; gel permeation chromatography enrichment	Capilliary column GC/PID	20 ng/g	76–202 (naphthalene) 77–82 (1-methyl-naphthalene) 75–131 (2-methyl-naphthalene)	Lebo et al. 1991
Rat urine	Extract with ammonium carbonate/ethyl acetate; evaporate under nitrogen stream; dissolve in pyridine	GC/MS	No data	No data	Horning et al. 1980
Mouse urine	Extract with ethyl acetate; evaporate under nitrogen stream; dissolve in pyridine	GC/MS	No data	No data	Stillwell et al. 1982
Burned tobacco	Extract with methanol/water and cyclohexane; enrich in dimethyl sulfoxide; fractional distillation and evaporation under dry nitrogen	GLC/MS	No data	85–95	Schmeltz et al. 1976

[a]Data are for naphthalene only unless otherwise specified.

FID = flame ionization detector; GC = gas chromatography; GLC = gas-liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; PID = photoionization detector; TLC = thin layer chromatography

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect in charcoal tube; elute with carbon disulfide	GC/FID	15 mg/m^3	No data	NIOSH 1977
Air	Collect in charcoal tube; elute with carbon disulfide	GC/FID	10 µg/sample	No data	NIOSH 1984a
Air	Collect in charcoal tube; elute with organic solvent	GC/FID	0.5 µg/sample	No data	NIOSH 1984b
Air	Collection filter or tube; extract with acetonitrile	HPLC/FD	0.080 µg/filter or 0.070 µg/tube	No data	Hansen et al. 1991
Indoor air	Medium flow rate samples; extract with methylene chloride; exchange to cyclohexane; clean up; exchange to acetonitrile	HPLC/UV	250 pg/µL	No data	EPA 1990a
Indoor air	Medium flow rate samples; extract with methylene chloride	GC/MS	No data	No data	EPA 1990a
Water	Purge and trap	HRGC/PID	0.06 µg/L	102±6.3	Ho 1989
Water	Extract with methylene chloride; exchange to cyclohexane; clean up; exchange to acetonitrile	HPLC/UV	1.8 µg/L	78±8.3	EPA 1982a
Water	Extract with methylene chloride at pH 11 and 2; concentrate	GC/MS	1.6 µg/L	75±35	EPA 1982b
Water	Adsorb on small bed volume Tenax® cartridges; thermally desorb	GC/MS	No data	No data	Pankow et al. 1988
Drinking water	Liquid-liquid extraction with methylene chloride; exchange to acetonitrile	HPLC/UV	3.3 µg/L	76–96	EPA 1990d
Drinking water	Liquid-solid extraction with methylene chloride; exchange to acetonitrile	HPLC/UV	2.2 µg/L	49.6–75.2	EPA 1990e
Drinking water	Purge and trap	Packed column GC/PID	0.01–0.05 µg/L	92	APHA1992e
Drinking water	Purge and trap	Capillary column GC/MS	0.02–0.2 µg/L	98–104	APHA 1992d
Drinking water	Purge and trap	Capillary column GC/PID	No data	102	APHA 1992f

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Wastewater	Extract with methylene chloride	Isotope dilution, capillary column GC/MS	10 µg/L	75–149	EPA 1990c
Wastewater	Extract with methylene chloride; exchange to cyclohexane; clean up; exchange to acetonitrile	HPLC/UV	1.8 µg/L	21.5–100	APHA 1992b
Water	Extract with methylene chloride	Capillary column GC/MS	10 µg/L[a]	No data	EPA 1986c
Wastes, non-water miscible	Extract with methylene chloride	Packed column GC/MS	160 mg/kg	No data	EPA 1986b
Soil	Extract with methylene chloride	Packed column GC/MS	1 mg/kg	No data	EPA 1986b
Soil, sediment	Extract with methylene chloride	Capillary column GC/MS	660 µg/kg	No data	EPA 1986c
Wastes, soil	Extract with methylene chloride	GC/FTIR	20 µg/L[a, b]	No data	EPA 1986d

[a]Identification limit in water. Detection limits for actual samples are several orders of magnitude higher, depending upon the sample matrix and extraction procedure employed.
[b]Based on a 2 µL injection of a 1 L sample that was extracted and concentrated to a volume of 1 mL.

FD = fluorescence detection; FID = flame ionization detector; FTIR = Fourier transform infrared spectrometry; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectroscopy; PID = photoionization detection; UV = ultraviolet spectrometry

Table 7-3. Analytical Methods for Determining 2-Methylnaphthalene in Environmental Samples[a]

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery
Soil, sediment	Extract with methylene chloride	Capillary column GC/MS	660 µg/kg	No data
Water	Extract with methylene chloride	Capillary column GC/MS	10 µg/kg	No data

[a]EPA 1986c

GC = gas chromatography; MS = mass spectroscopy

limits or accuracy for 1-methylnaphthalene, this compound may be analyzed in environmental media by GC and HPLC methods (HSDB 1995).

Air samples for analysis may be collected on filters or charcoal tubes. Since naphthalene may exist in both the vapor phase and the particle phase in air (Harkov 1986), collection on a charcoal tube is the preferred method for sampling naphthalene from air for analysis (NIOSH 1977, 1984a, 1984b).

Naphthalene is usually extracted from the matrix with organic solvents (liquid-liquid or liquid-solid extraction) or by purge and trap with an inert gas. SFE techniques for extraction of organic compounds from environmental matrices are currently being studied by EPA. A protocol for SFE with carbon dioxide for many organic compounds, including naphthalene, from soils and sediments has been developed (EPA 1991f).

A technique for the detection of naphthalene in PAH-contaminated media has been developed (Heitzer et al. 1994). The technique measures bioluminescence in the genetically engineered microorganism *Pseudomonas fluorescens* HK44, which carries a transcriptional gene for naphthalene and salicylate metabolism. After the addition of the bacteria to sterile water, naphthalene was detected down to 1.55 µg/L, the lowest concentration studied. In an experiment using JP-4 jet fuel, naphthalene was detected down to 0.55 µg/L in the effluent of the biosensor (Heitzer et al. 1994).

Detectors used for identification and quantification of naphthalene and methylnaphthalenes include the flame ionization detector (FID), photoionization detector (PID), ultraviolet detection (UV), Fourier transform infrared detection (FTIR), and fluorescence detection (FD). Mass spectrometry is used for confirmation.

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 naphthalene, 1-methylnaphthalene, and 2-methyl-naphthalene is available. Where adequate information is not available, ATSDR, in conjunction with 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 naphthalene, 1-methyl-naphthalene, and 2-methylnaphthalene.

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 and selective methods are available for the qualitative and/or quantitative measurement of naphthalene and many of its metabolites present in biological materials such as adipose tissue and urine (EPA 1986g; Horning et al. 1980; Liao et al. 1988). In contrast to the relative ease of measuring naphthalene once it has been isolated from its sample matrix, the development of improved techniques for sample preparation would be beneficial.

Metabolites of naphthalene in biological materials are not readily determined in routine practice because of the lack of standard methods for their quantification. Furthermore, there is a need for modern validated standard methods for analysis of naphthalene itself in biological materials. It would also be helpful to have a method that can be used to associate levels of naphthalene or its metabolites in biological media with levels of naphthalene exposure in the environment.

A method for the determination of 2-methylnaphthalene and its degradation products in rat urine has been reported (Melancon et al. 1982). It would be useful to determine if this method could also be applied to human urine and other biological samples.

Effect. There are currently no methods that can be used to correlate levels of naphthalene, 2-methyl-naphthalene, or their metabolites in biological tissues or fluid with the probable onset of adverse health effects. The development of such methods would be useful insofar as they estimate the doses required to produce cataracts and hemolytic effects.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining naphthalene in water, air, and waste samples with excellent selectivity and sensitivity have been developed and are undergoing constant improvement (EPA 1982a, 1982b, 1986a, 1986b, 1986c, 1986d, 1990a, 1990b, 1990c, 1990d, 1990e; NIOSH 1984a, 1984b). For each medium, the existing methods are adequate to measure background levels in the environment and levels at which health effects occur. Standard methods for 1-methylnaphthalene and 2-methylnaphthalene would be helpful in assessing data comparability.

It would be useful to have the means to rapidly and directly measure organic compounds such as naphthalene, 1-methylnaphthalene, and 2-methylnaphthalene in water and other environmental media without the necessity for tedious sample processing. The recently developed bioluminescent probe for naphthalene (Heitzer et al. 1994) may help satisfy this data need.

Degradation products of naphthalene in environmental media are difficult to determine. This difficulty is not so much an analytical problem as it is a problem of knowing the fundamental environmental chemistry of these compounds in water, soil, air, and biological systems.

There are some difficulties associated with sampling naphthalene from the atmosphere, where it is partially associated with particulate matter. High-volume sampling with glass fiber filters provides conditions conducive to artifact formation (Harkov 1986), thus introducing errors into the analysis of atmospheric naphthalene. This is an area in which further improvements would be useful.

7.3.2 Ongoing Studies

No ongoing studies involving analytical techniques of naphthalene, 1-methylnaphthalene, or 2-methyl-naphthalene were found in a search of the Federal Research in Progress database (FEDRIP 2003).