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 hexane, its metabolites, and other biomarkers of exposure and effect to hexane. 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 SAMPLES

n-Hexane can be determined in biological fluids and tissues and breath using a variety of analytical methods. Representative methods are summarized in Table 6-1. Most methods utilize gas chromatographic (GC) techniques for determination of *n*-hexane. The three methods used for preparation of biological fluids and tissues for analysis are solvent extraction, direct aqueous injection, and headspace extraction. Breath samples are usually collected on adsorbent traps or in sampling bags or canisters prior to analysis by GC.

Solvent extraction permits concentration of analytes, thereby increasing sensitivity, but the extraction solvent can interfere with analysis, and evaporative losses make quantitation difficult. Direct aqueous injection is a very rapid method, but sensitivity is low and matrix effects can be a serious problem. The headspace extraction method involves equilibrium of volatile analytes between the liquid or solid-sample phase and the gaseous phase. The gaseous phase is then analyzed by GC. There are two main types of headspace extraction methodology: static (equilibrium) headspace extraction and dynamic headspace extraction, which is usually called the purge-and-trap method (Seto, 1994). The static headspace extraction technique is relatively simple but may be less sensitive than the purge-and-trap method. The purge-andtrap method, while providing increased sensitivity, requires more complex instrumentation and may result

Table 6-1. Analytical Methods for Determining n-Hexane in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Isotope dilution; homogenization; solvent extraction; centrifugation	GC/MS-SIM	≈100 ng/mL or ng/g	102	White et al. 1979
Blood	Headspace extraction	cap. GC/ITD	9.5 ng/L	Not reported	Schuberth 1994
Blood	Headspace; cryogenic trapping; thermal desorption	cap. GC/MSD	15 ng/L	Not reported	Brugnone et al. 1991
Urine	Headspace	cap. GC/MSD-SIM	0.5 µg/L (calculated)	98–101	Imbriani et al. 1984
Blood, urine, adipose tissue	Dynamic headspace purge	cap. GC/FID	Not reported	86–120 (blood); 48–110 (urine); 13–80 (adipose) for model compounds	Michael et al. 1980
Tissues	Isotope dilution; homogenization; solvent extraction; centrifugation	GC/MS-SIM	≈100 µg/L	104	White et al. 1979
Breath (alveolar air)	Collection in glass tube	cap. GC/MSD	Not reported	Not reported	Brugnone et al. 1991
Breath (exhaled air)	Collection using modified Haldan-Priestly tube sampler; collection on adsorbent traps; CS$_2$ desorption	GC	0.015 mg/m^3 (1 L sample)	94.6–99.4	Periago et al. 1991
Exhaled air	Collection in aluminum tube; adsorption on charcoal; CS$_2$ desorption	GC/FID	Not reported	Not reported	Cardona et al. 1996

cap. = capillary; CS$_2$ = carbon disulfide; FID = flame ionization detector; GC = gas chromatography; ITD = ion trap detector; MS = mass spectrometry; MSD = mass selective detector; SIM = selected ion monitoring

6   ANALYTICAL METHODS

in artifact formation (Seto 1994). Packed and capillary columns are used for chromatographic separation, followed by flame ionization detection (FID) or mass spectrometry (MS) techniques.

Measurement of *n*-hexane in breath has been rather widely used to evaluate environmental and occupational exposures. A variety of methods are available for monitoring *n*-hexane in breath, but most have not been tested rigorously for reliability. Breath samples may be collected in glass tubes (alveolar air) and analyzed by GC/MS (Brugnone et al.1991). No performance data or detection limits are available for this method. Exhaled air samples may be collected using modified glass tube samplers onto an adsorbent followed by solvent desorption and analysis by GC (Periago et al.1991). Good recovery (>94%) and precision (<12% relative standard deviation [RSD]) were reported; the detection limit was 0.015 mg/m^3 (4 ppb) for a 1 L sample (Periago et al.1991). Spirometers are used to collect volatile organic compounds in breath since they provide clean air for inhalation and thus prevent contamination by the environmental air. Although these systems are used extensively for monitoring environmental exposure, very little information for *n*-hexane is available. Current systems are compact and include collection of breath into chemically inert passivated canisters (Raymer et al.1994; Thomas et al.1991) and Tedlar bags.

Few well characterized, validated methods are available for the determination of *n*-hexane in blood. A purge-and-trap method for volatiles has been developed and validated by researchers at the Centers for Disease Control and Prevention (CDC) (Ashley et al.1992, 1994). Extension of the method to include *n*-hexane should be possible. Current analytical methods utilize capillary GC columns and MS detection to provide the sensitivity and selectivity required for the analysis. Detection limits are in the low ppb range (Brugnone et al.1991; Schuberth 1994). Headspace extraction followed by GC analysis has also been utilized for the determination of *n*-hexane in blood (Brugnone et al.1991; Michael et al.1980; Schuberth 1994); however, very little performance data are available.

Some methods are available for determining *n*-hexane in urine and tissues. A modified dynamic headspace extraction method for urine, mother's milk, and adipose tissue has been reported (Michael et al.1980). Volatiles swept from the sample are analyzed by capillary GC/FID. Acceptable recovery was reported for model compounds; detection limits were not reported (Michael et al.1980). A solvent extraction procedure utilizing isotope dilution followed by GUMS analysis has been reported for tissues (White et al.1979). Recovery was good (104%) and detection limits are approximately 100 ng/mL (White et al.1979).

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Exposure to *n*-hexane is evaluated by measuring the levels of this compound in blood, urine, and exhaled breath and by measuring the levels of 2,5-hexanedione, a neurotoxic metabolite of *n*-hexane, in urine. A summary of the methods used for determining biomarkers for *n*-hexane is presented in Table 6-2.

Urine is often analyzed for the neurotoxic metabolite 2,5-hexanedione to evaluate *n*-hexane exposure. The major metabolites of *n*-hexane present in urine are 2,5-hexanedione and 4,5-dihydroxy-2-hexanone. Acid treatment (a routine step in chemical analysis of urine) converts 4,5-dihydroxy-2-hexanone to 2,5-hexanedione (Fedtke and Bolt 1986a, 1986b). Thus, 2,5-hexanedione may be expressed as *"free"* compound (without acid treatment) or *"total"* compound (with acid treatment) (Cardona et al.1996). No reports were located describing conjugated metabolites of *n*-hexane in urine (e.g. glucuronides, sulfates). Urinalysis for metabolites provides adequate sensitivity when exposure is relatively high but may not provide adequate sensitivity for evaluating low exposures (Kawai et al.1992). As analytical methods have improved it has become clear that 2,5-hexanedione can also be detected in the acid-treated urine of individuals without specific exposure to *n*-hexane (Fedtke and Bolt 1986a; Perbellini et al.1993). It is possible that small amounts of *n*-hexane are produced normally in the body as the result of fatty acid metabolism. A reference value for an Italian population is available (Bavazzano et al.1998). Specific methods also exist for measuring another *n*-hexane metabolite in urine, 2-hexanone (Table 6-2).

6.2 ENVIRONMENTAL SAMPLES

Methods are available for determining *n*-hexane in a variety of environmental matrices. A summary of representative methods is shown in Table 6-3. Validated methods, approved by agencies and organizations such as EPA, the American Society for Testing and Materials (ASTM), APHA, and NIOSH, are available for air matrices. GC/FID and GC/MS are the most widely used analytical techniques for quantitating concentrations of *n*-hexane in environmental matrices. Because of the complexity of the sample matrix and the usually low concentrations of volatile organic compounds in environmental media, sample preconcentration is generally required prior to GC analysis. Air samples may be collected and concentrated on adsorbent prior to analysis. Methods suitable for determining trace amounts of *n*-hexane in aqueous and other environmental media include three basic approaches to the pretreatment of the sample: gas purge-andtrap, headspace extraction gas analysis, and extraction with organic solvents.

n-Hexane may be determined in occupational air using collection on a charcoal adsorbent, followed by solvent desorption (Hakkola and Saarinen 1996; Rosenberg et al.1992), or thermal desorption (Tang et al.

Table 6-2. Analytical Methods for Determining Biomarkers of n-Hexane in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Tissues and blood (2-hexanone)	Isotope dilution; homogenization; solvent extraction; centrifugation	GC/MS-SIM	≈0.05 µg/g	105 (tissues), 98 (blood)	White et al. 1979
Tissues and blood (2,5-HD)	Isotope dilution; homogenization; solvent extraction; centrifugation	GC/MS-SIM	≈0.08 µg/g	104 (tissues), 108 (blood)	White et al. 1979
Urine (2,5-HD)	Acid hydrolysis; derivatization	HPLC/UV	30 ng/L (calculated)	≈94	Gori et al. 1995
Urine (2,5-HD)	Acid hydrolysis; SPE extraction; cleanup by SPE	HPLC/UV; confirmation by cap. GC/FTIR	0.05 mg/L	85	Columbini et al. 1992
Urine (2,5-HD)	Derivatization; SPE extraction	cap. GC/ECD	4.7 ng/mL (calculated)	Not reported	van Engelen et al. 1995
Urine (2,5-HD)	Derivatization; solvent extraction	cap. GC/ECD	2.8 ng/mL (calculated)	Not reported	van Engelen et al. 1995
Urine (2,5-HD)	Solvent extraction	cap. GC/FID	10 ng/mL (calculated)	98.6–106	van Engelen et al. 1995
Urine (total and free 2,5-HD)	Acid hydrolysis (total 2,5-HD); fractionation on sorbent minicolumns; solvent extraction	cap. GC/FID	Not reported	Not reported	Cardona et al. 1996

cap. = capillary; GC = gas chromatography; ECD = electron capture detector; FID = flame ionization detection; FTIR = Fourier transform infrared spectrometry; 2,5-HD = 2,5-hexanedione; HPLC = high performance liquid chromatography; MS = mass spectrometry; SIM = selected ion monitoring; SPE = solid-phase extraction; UV = ultraviolet (detection)

Table 6-3. Analytical Methods for Determining n-Hexane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Occupational air (breathing zone)	Collection on charcoal adsorbent; CS₂ desorption	GC/FID	0.006–1.1 ppb (50 L sample)	Not reported	Rosenberg et al. 1991
Occupational air (breathing zone)	Collection on charcoal tubes; DMF desorption	dual column cap. GC/FID	≈0.5 µg/sample (for 1–4 L sample)	Not reported	Hakkola and Saarinen 1996
Occupational air	Collection in canisters	cap. GC/FID	Not reported	87.8 (collection efficiency)	Tang et al. 1996
Occupational air	Collection on charcoal adsorbent; thermal desorption	cap. GC/FID	≈0.6 ppb (0.5 L sample)	86.4 (collection efficiency)	Tang et al. 1996
Ambient air	Collection on Tenax adsorbent; thermal desorption and cryofocussing	cap. GC/MS	Not reported	75–98 (all target compounds)	Krost et al. 1982
Ambient air	Collection in canisters; cryofocus	cap. GG/FID or GC/MS	Not reported	Not reported	EPA 1988 (Method TO-14)
Ambient air	Collection on dual multisorbent traps; thermal desorption	cap. GC/FID	0.14 ppb	Not reported	Oliver et al. 1996
Ambient air	Collection in canisters	cap. GC/FID	low-ppt range	Not reported	McLaren et al. 1996; Lai et al. 1993
Indoor air	Collection on Tenax adsorbent; thermal desorption	cap. GC/MS	0.014–0.06 ppb	Not reported	Kostianen 1995
Emissions from vegetation	Collection in canisters; cryogenic concentration	cap. GC/FID	0.1–0.02 µg/m²/h	Not reported	Fukui and Doskey 1996
Vehicle emissions	Collection in foil bags	GC	≈30 µg/m³	Not reported	Cooper et al. 1996

Table 6-3. Analytical Methods for Determining n-Hexane in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Emissions from consumer products	Collection on charcoal adsorbent; CS_2 desorption	cap. GC/FID	Not reported	Not reported	Wadden et al. 1995
Stack gas effluents	Collection on volatile organic sampling train (VOST)	GC/MS	Not reported	Not reported	EPA 1994i (Method 0030 - collection); EPA 1994k (Method 5041 - analysis)
Water	Purge and trap	GC/FID	≈0.05 µg/L	Not reported	Biziuk et al. 1996
Water	Purge and trap	cap. GC/MS	low- to sub-ppb levels (µg/L)	90–120	Michael et al. 1988
Water	Purge and trap	cap. GC/FID	35–1,760 µg/L (gasoline)	77	Belkin and Hable 1988
Water	Distillation; purge and trap	GC/FID	Not reported	83–87	Kozloski 1985
Water, soil	Headspace extraction	GC/MSD	0.5 µg/L	Not reported	Roberts and Burton 1994
Soil	Supercritical fluid extraction	cap. GC/FID	Not reported	86–90 (trapping efficiency)	Yang et al. 1995
Sediment	Elevated temperature dynamic headspace extraction	cap. GC/FID, GC/ITD	20 ng/kg	Not reported (bias 2–16%)	Bianchi et al. 1991

cap. = capillary; CS_2 = carbon disulfide; DMF = dimethylformamide; FID = flame ionization detector; GC = gas chromatography; ITD = ion trap detector; MS = mass spectrometry; MSD = mass selective detector

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1996), then capillary GC/FID analysis (Hakkola and Saarinen 1996; Tang et al.1996). Samples may also be collected in canisters with subsequent capillary GC/FID analysis (Tang et al.1996). Detection limits are in the low ppb ($\mu g/m^3$) range (Hakkola and Saarinen 1996; Rosenberg et al.1992; Tang et al.1996). Collection efficiencies of 87.8% (8.6% RSD) for canisters and 86.4% (9.4% RSD) for carbon adsorbent have been reported (Tang et al.1996). Both methods showed a loss of 10% or less after storage for 14 days (Tang et al.1996). Passive samplers are finding increased use, due in part to their small size and ease of use. The compounds on the adsorbent in the sampler is desorbed by solvent and the extract analyzed by GC techniques. Detection limits are reported to be in the low ppb range (Otson et al.1994) and appear to be comparable to other monitoring methods (Bartolucci et al.1986; Gentry and Walsh 1987). All these methods provide detection well below the Minimal Risk Level (MRL) of 0.6 ppm for the health effects in humans for chronic-duration inhalation exposure to *n*-hexane (Appendix A).

Gas purge-and-trap is the most widely used method for the isolation and concentration of volatile organic compounds in environmental samples (Lesage 1993). The purge-and-trap technique offers advantages over other techniques because it allows isolation and concentration of target compounds, thereby improving overall limits of detection (LODs) and recovery. A potential problem of this technique is interference by impurities in the stripping gas. Detection limits of less than 1 μg of *n*-hexane per liter of sample (1 ppb) have been achieved (Michael et al.1988).

Ambient air may also be collected on adsorbent traps (Krost et al.1982; Oliver et al.1996) or in stainless steel canisters (Anlauf et al.1985; EPA 1988; McLaren et al.1996). Detection limits, where reported, are in the low ppb to low parts per trillion (ppt) range (Anlauf et al.1985; McLaren et al.1996; Oliver et al. 1996). Recovery of 75-98% (for all compounds tested) has been reported for collection on Tenax adsorbent (Krost et al.1982). Passive monitors are utilized for ambient air and indoor air (Cao and Hewitt 1993; Fellin and Otson 1994; Otson et al.1994). Detection limits in the low ppb range have been reported (Otson et al.1994).

Dynamic headspace-extraction stripping and purge-and-trap methodology are used most often for determination of *n*-hexane in water and hazardous wastes. Dynamic headspace extraction techniques have been applied to water samples (Roberts and Burton 1994) and sediment (Bianchi et al.1991). Detection limits of 0.5 $\mu g/L$ were reported for lake water (Roberts and Burton 1994) and 20 ng/kg (ppt) for sediment (Bianchi et al.1991). Supercritical fluid extraction (SFE) is a relatively new technique that has been applied to *n*-hexane in soil (Yang et al.1995). Membrane extraction of *n*-hexane from water samples has

been developed to provide online, continuous monitoring (Wong et al.1995; Xu and Mitra 1994). The extract is analyzed directly by MS (Wong et al.1995) or subjected to conventional capillary GC/FID analysis (Xu and Mitra 1994). Detection limits are in the low ppb range (Wong et al.1995; Xu and Mitra 1994).

No methods were located for determination of *n*-hexane in biota or foods.

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 hexane 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 hexane.

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. Exposure to *n*-hexane is evaluated by measuring the levels of this compound in blood, urine, and exhaled breath and by measuring the levels of 2,5-hexanedione, a neurotoxic metabolite of *n*-hexane, in urine. The available methods are sufficiently sensitive and reliable for monitoring occupational exposures to *n*-hexane. They may not be sensitive enough to monitor levels at which biological effects occur. Few methods are available for measuring *n*-hexane in blood (Brugnone et al.1991; Schuberth 1994). Development of these methods or other established methods for the determination of volatile organic compounds in blood (Ashley et al.1992, 1994) with improved specificity and sensitivity would be valuable in measuring low-level exposures to *n*-hexane and background exposures in the general population. Measurement of *n*-hexane levels in urine

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has been used as a noninvasive method for evaluating exposure to the compound. Available methods are reliable (Imbriani et al.1984; Kawai et al.1993); however, this measurement is not as sensitive as measuring *n*-hexane in blood for evaluating low-level exposures to *n*-hexane (Kawai et al.1993). Measurement of *n*-hexane in breath is another noninvasive method for evaluating exposure to *n*-hexane, particularly occupational exposures (Brugnone et al.1991; Periago et al.1991). Development of simple, reliable field methods and standardization of the sampling methodology would be helpful for exposure monitoring. Methods for determining *n*-hexane in tissues are generally lacking. Development of a method for monitoring this compound in other biological matrices, particularly mother's milk, would be useful.

Urine may be analyzed for the neurotoxic metabolite, 2,5-hexanedione, to evaluate *n*-hexane exposure; however, results for various preparation methods are not comparable. Urinalysis for metabolites provides adequate sensitivity when exposure is relatively high but may not provide adequate sensitivity for evaluating low exposures (Kawai et al.1992). Standardization of urinalysis methods and development of methods with greater sensitivity are needed.

The neurotoxic effects of *n*-hexane have been associated with the pyrrolidation of protein (Graham et al. 1995) by 2,5-hexanedione. Therefore, the development of analytical methods to determine this potential biomarker of effect of *n*-hexane in hair and the subsequent crosslinking of the blood proteins, spectrin and hemoglobin, would be useful.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Air is the medium of most concern for human exposure to *n*-hexane. Exposure from drinking water may also be of concern in some areas, such as near hazardous waste sites. Existing methods are sufficiently sensitive and reliable to monitor environmental air (Hakkola and Saarinen 1996; Rosenberg et al.1992; Tang et al.1996). Methods for measuring ambient air provide the sensitivity required for monitoring *n*-hexane at low levels at which biological effects are expected to occur and at low and background levels. Additional performance data would be helpful. Available methods for determination of *n*-hexane in water provide adequate sensitivity for measurement of sub-ppb levels of *n*-hexane (Biziuk et al. 1996; Michael et al.1988). Additional performance data would allow better comparability of results.

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6.3.2 Ongoing Studies

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of *n*-hexane and other volatile organic compounds in blood. These methods use purge-and-trap methodology, high resolution gas chromatography, and magnetic sector MS which gives detection limits in the low ppt range.