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 hexachloroethane, its metabolites, and other biomarkers of exposure and effect to hexachloroethane. 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

Analytical methods are available for measuring hexachloroethane in blood, urine, feces, liver, kidney, and adipose tissues, and breath (Ashley et al. 1992; Fowler 1969a; Nolan and Karbowski 1978; Pellizzari et al. 1985a, 1985b). Gas chromatography (GC) is the usual method for detecting and measuring hexachloroethane in biological materials (Pellizzari et al. 1985a, 1985b). The chromatograph separates complex mixtures of organics and allows individual compounds to be identified and quantified by a detector. An electron capture detector (ECD) is often used to identify hexachloroethane (Fowler 1969b; Nolan and Karbowski 1978). A mass spectrometer (MS) coupled to the GC column provides unequivocal identification.

Blood samples for analysis of volatile organic compounds (VOCs) including hexachloroethane should be collected into containers from which VOC contamination has been reduced (Ashley et al. 1992). Potassium oxalate/sodium fluoride is the recommended anti-coagulant. Blood samples should be placed on ice or refrigerated shortly after collection, and Ashley et al. (1992) recommend that analysis for VOCs be completed within 14 days.

Hexachloroethane can be detected in tissues at levels as low as 0.001 μg/g (Nolan and Karbowski 1978) and recoveries range from 50 to 130%. Prior to analysis, hexachloroethane must be separated from the

biological sample matrix and prepared for introduction into the analytical instrument. Separation may be effected by purging with an inert gas (helium), and trapping on an adsorbent cartridge (Tenax GC®, followed by thermal desorption directly to the GC column (Pellizzari et al. 1985a, 1985b). Alternatively, hexachloroethane may be extracted from the matrix with hexane (Fowler 1969b; Nolan and Karbowski 1978). Details of selected analytical methods for hexachloroethane in biological samples are summarized in Table 6-l.

6.2 ENVIRONMENTAL SAMPLES

Determination of hexachloroethane in air, water, soil, wastes, and food is usually by GC analysis (APHA 1992; EPA 1982, 1990a, 1990b, 1990c; NIOSH 1994; Yurawecz and Puma 1986). Several representative methods for quantifying hexachloroethane in each of these media are summarized in Table 6-2. NIOSH (1994) has developed an approved method for analysis of hexachloroethane in air and EPA has developed approved methods for analysis of hexachloroethane in drinking water (EPA 1989, 1991c), water/wastewater (EPA 1982, 1990c), and soil/sediment/waste (EPA 1990a, 1990b) samples. The APHA (1992) method is equivalent to an EPA approved method.

Separation of hexachloroethane from environmental samples is usually by extraction with an organic solvent such as methylene chloride or acetonitrile (EPA 1982,1990a, 1990b, 1990c; Yurawecz and Puma 1986). A supercritical fluid extraction protocol has been developed for extraction of organics from soils and sediments (Lopez-Avila et al. 1991), which may be applicable to hexachloroethane. Air samples are drawn through a solid sorbent material and desorbed with carbon disulfide (NIOSH 1994). Cleanup procedures, with Florisil, for example, may be required for some environmental matrices (Yurawecz and Puma 1986). In addition, co-eluting compounds may be eliminated from extracts of drinking water by high performance liquid chromatography (HPLC) prior to GC/MS analysis, thus improving the quality of analytical results (Thruston 1978).

The electron capture detector (ECD) is most frequently used to identify hexachloroethane. A flame ionization detector (FID) may also be used (NIOSH 1994). When unequivocal identification is required, an MS coupled to the GC column may be employed.

TABLE 6-1. Analytical Methods for Determining Hexachloroethane in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Purge and trap on Tenax GC cartridge; desorb thermally	GC/MS	≈3 ng/mL[a]	≈80[a]	Pellizzari et al. 1985a
Blood	Purge and trap on Tenax GC cartridge; desorb thermally	GC/MS	0.028 ppb	93–41	Ashley et al. 1992
Adipose tissue	Macerate tissue in water; tap on Tenax GC cartridge; desorb thermally	GC/MS	≈6 ng/g[a]	≈50[a]	Pellizzari et al. 1985a
Breath	Collect on Tenax GC cartridge; dry over calcium sulfate; desorb thermally	Capillary column GC/MS	No data	≈70–130	Pellizzari et al. 1985b
Urine	Extract with hexane; successively wash with water, sodium hydroxide, hydrochloric acid, and water	GLC/ECD	No data	>90	Fowler 1969b
Feces	Macerate under warm hexane; successively wash with water, sodium hydroxide, hydrochloric acid, and water	GLC/ECD	No data	>90	Fowler 1969b

TABLE 6-1. Analytical Methods for Determining Hexachloroethane in Biological Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood, liver, kidney, fat	Extract with hexane	GC/ECD	0.001 µg/g	No data	Nolan and Karbowski 1978

[a]Typical or expected values for halocarbons by this method. Data were not reported for hexachloroethane.

ECD = electron capture detector; GC = gas chromatography; GLC = gas liquid chromatography; MS = mass spectrometry

TABLE 6-2. Analytical Methods for Determining Hexachloroethane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect on activated coconut shell charcoal in glass tube; desorb with carbon disulfide	GC/FID	0.01 mg/sample	98	NIOSH 1994
Water/waste water	Extract with methylene chloride; exchange to hexane; Florisil cleanup, if required	GC/ECD	0.03 µg/L	99	EPA 1982
Waste water	Extract continuously with methylene chloride under alkaline and then acidic conditions	Isotope dilution, capillary column GC/MS	10 µg/L	No data	EPA 1990c
Water/waste water	Extract with methylene chloride under alkaline and then acidic conditions	Packed column GC/MS	1.6 µg/L	40–113	APHA 1992
Water/soil/ wastes	Extract with methylene chloride; exchange to hexane; Florisil or GPC cleanup, if required	Capillary column GC/ECD	1.6 µg/L[a]	83–96	EPA 1990a
Water/soil/ wastes	Extract with methylene chloride; exchange to hexane; Florisil or GPC cleanup, if required	Packed column GC/ECD	0.03 µg/L[a]	≈74	EPA 1990b

TABLE 6-2. Analytical Methods for Determining Hexachloroethane in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food (fish, milk, butter, corn oil)	Extract with acetonitrile; cleanup with Florisil; elute with petroleum ether and ethyl ether/petroleum ether	GC/ECD	No data	≥80	Yurawecz and Puma 1986

[a]Method detection limit (MDL) in reagent water. Estimated quantitation limits for other matrices are: 10 MDL in groundwater, 670–10,000 MDL in soil, and 100,000 MDL in nonaqueous wastes.

ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry

6. ANALYTICAL METHODS

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 hexachloroethane 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 hexachloroethane.

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 The presence of hexachloroethane in exhaled air, blood, and tissues can be determined using GC/MS (Ashley et al. 1992; Pellizzari et al. 1985a, 1985b). Separation by GC with electron capture detection and liquid chromatography have also been used to identify hexachloroethane in blood, tissues, urine, and/or fecal matter (Fowler 1969b; Gorzinski et al. 1985; Jondorf et al. 1957; Mitoma et al. 1985; Nolan and Karbowski 1978). These methods are sufficiently sensitive and selective to measure low levels of hexachloroethane and levels that may result in adverse effects. Since the metabolites of hexachloroethane are themselves xenobiotic compounds or are the metabolites of other xenobiotics, the parent compound serves as the only true biomarker of exposure. Endogenous production of hexachloroethane following carbon tetrachloride exposure necessitates the need for an exposure history even when hexachloroethane is detected in body tissues or fluids (Fowler 1969a). Additional studies correlating levels of hexachloroethane in various biological media with environmental exposures would be useful.

No data were located regarding methods that identify biomarkers of hexachloroethane's toxic effects. Although hexachloroethane-induced hepatic damage can cause increases in serum levels of liver enzymes,

these enzyme changes are not specific to hexachloroethane exposure (Fowler 1969b; Weeks et al. 1979). In male rats, exposure to hexachloroethane is associated with the presence of granular and cellular casts in the urine (NTP et al. 1989). These effects are related to the formation of hyaline droplets in the male rat kidney. The formation of hyaline droplets is unique in male rats and is not indicative of the toxic effect of hexachloroethane. Therefore, they are not useful as biomarkers of effect. There is a need to identify compound-specific biomarkers for the effects of hexachloroethane exposure at this time.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods are available to detect and quantify hexachloroethane in air, water, soil, wastes, and food (APHA 1992; EPA 1982,1990a, 1990b, 1990c; NIOSH 1994; Yurawecz and Puma 1986). 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 hexachloroethane at levels sufficiently low to meet regulatory requirements and evaluate health effects (EPA 1982, 1990a, 1990b, 1990c; NIOSH 1994).

Methods are also available to measure degradation products of hexachloroethane in environmental samples, but these products (e.g., tetrachloroethylene) are released to the environment from many other sources and are therefore not useful determinants of the environmental impact of this chemical.

6.3.2 On-going Studies

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control, is developing methods for the analysis of hexachloroethane and other volatile organic compounds in blood. These methods use purge and trap methodology, high resolution gas chromatography, and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion (ppt) range.

On-going studies to i,mprove analytical methods for hexachloroethane and related compounds include the EPA “Master Analytical Scheme” being developed for organic compounds in water (Michael et al. 1988) and the research in supercritical fluid extraction (Lopez-Avila et al. 1991; Wieboldt et al. 1988). Research continues on improving extraction, concentration, and elution techniques, and detection devices (Eichelberger et al. 1983,1990; Ho et al. 1993; Pankow and Rosen 1988; Valkenburg and Munslow

1989). These improvements are designed to overcome problems with sample preparation and increase sensitivity and reliability of the analyses.