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

Gas chromatography (GC) with an electron-capture detector (ECD) and/or GC with detection by mass spectrometry (MS) have been used to measure hexachlorobutadiene concentrations in human blood and adipose tissue (Bristol et al. 1982; LeBel and Williams 1986; Mes et al. 1985) and in rat liver tissue (Wang et al. 1991). In gas chromatography, samples dissolved in a volatile solvent are injected into a heated column with a stationary phase consisting of silica coated with a liquid phase. An inert gas carries the sample through the column, and the partitioning of hexachlorobutadiene between the mobile and stationary phases gives it a characteristic retention time which is used to identify it. Electron-capture detectors use a radioactive source such as ^{63}Ni to generate electrons that are captured by the chlorine atoms in hexachlorobutadiene. Reduction in electron flow by this capture produces a characteristic signal for hexachlorobutadiene. Identity of hexachlorobutadiene is confirmed by detection by mass spectroscopy, which provides specific identification by a characteristic ion fragmentation pattern.

Biological samples are prepared for analysis by extraction with organic solvents. This extract from blood may be used directly (Bristol et al. 1982; Kastl and Hermann 1983), but extracts from adipose or liver tissue are cleaned up by gel permeation chromatography (GPC), which separates

hexachlorobutadiene from higher molecular weight lipids, and/or by passage through a Florisil column which retains lipids and other contaminants (LeBel and Williams 1986; Mes et al. 1985). These methods provide 42- 122 % recovery and can detect < 1 μg/L hexachlorobutadiene in blood and 1 μg/kg hexachlorobutadiene in fat (Bristol et al. 1982; LeBel and Williams 1986; Mes et al. 1985; Wang et al. 1991). No information was located on methods for detection of hexachlorobutadiene metabolites or other biomarkers of hexachlorobutadiene exposure or effect.

Table 6-l summarizes the methods used for sample preparation and analysis of hexachlorobutadiene in biological samples.

6.2 ENVIRONMENTAL SAMPLES

Hexachlorobutadiene in environmental samples is also measured using GC coupled with ECD, MS, a halogen electrolytic conductivity detector (HECD), or a photoionization detector (PID) (APHA 1992a, 1992b; EPA 1982a, 1982c, 1986, 1989c, 1989d, 1990b, 1990d, 1990e). Several methods have been used for extraction of hexachlorobutadiene from environmental samples. Standard methods for analysis of air involve pumping the air through a material that will adsorb hexachlorobutadiene or through a cold trap to condense the hexachlorobutadiene (EPA 1990b; NIOSH 1990). Purge-and-trap methods are used to extract hexachlorobutadiene from water, soil, or solid waste (APHA 1992b; EPA 1989c, 1989d, 1989e, 1990e). Purge-and-trap methods involve bubbling an inert gas through the sample, trapping the hexachlorobutadiene in a tube containing a sorbent material, and then heating the sorbent tube and flushing the hexachlorobutadiene into a GC. Soil, sediment, and waste samples are mixed with water prior to purging (EPA 1990e). An alternative way to prepare water, soil, or solid waste samples for GC analysis is to extract with methylene chloride or some other organic solvent; for waste water, soil, and solid waste samples, the organic extracts are cleaned up by gel permeation chromatography (GPC) or Florisil adsorption chromatography (FAC) (APHA 1992a; EPA 1982a, 1982c, 1986). Purge-and-trap methods generally provide > 90% recovery, while organic extraction may have lower and more variable recovery rates (APHA 1992a, 1992b; EPA 1982a, 1982c, 1989c, 1990e).

Gas chromatographic methods with ECD and other detectors have a detection limit for hexachlorobutadiene of 0.02-0.05 μg/L in water (EPA 1982a, 1989c, 1989d, 1989e). Detection limits for soil and solid waste are usually higher, depending on matrix interferences, extraction, and

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Extract with hexane	GC/ECD and GC/MS	<1 μg/L	60–83	Bristol et al. 1982
Blood	Extract with hexane	GC/ECD	18 ng/L	85–122	Kastl and Hermann 1983
Adipose tissue	Extract with acetone/hexane, clean up with GPC and FAC	GC/ECD and GC/MS	No data	42–67	LeBel and Williams 1986
Adipose tissue	Extract with benzene/acetone, precipitate fat, clean up with FAC	GC/ECD and GC/MS	1 μg/kg [a] wet weight	No data	Mes et al. 1985
Rat liver tissue	Homogenize with sodium sulfate, digest with perchloric acid/acetic acid, extract with hexane, clean up with concentrated sulfuric acid and Florisil®, elute with hexane	Capillary column	0.0009 ppb	87	Wang et al. 1991

[a] Lowest concentration detected

ECD = electron capture detector; FAC = Florisil adsorption chromatography; GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry

clean up procedures (EPA 1986, 1990e). Detection by MS is most specific because identification is based on the characteristic mass ion as well as the retention time. Newer MS methods can achieve detection limits of 0.04-0.11 μg/L in water, comparable to ECD (EPA 1982a, 1989e).

Table 6-2 summarizes some of the methods used for sample preparation and analysis of hexachlorobutadiene in environmental samples.

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 hexachlorobutadiene 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 hexachlorobutadiene.

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. Hexachlorobutadiene can be measured in human blood and adipose tissue, with detection limits < 1 μg/L in blood and 1 μg/kg wet weight of adipose tissue (Bristol et al. 1982; LeBel and Williams 1986; Mes et al. 1985). No hexachlorobutadiene was detected in blood from controls or residents near a hazardous waste site (Bristol et al. 1982), indicating that the method was not sensitive enough to measure background levels of hexachlorobutadiene in the general population. It is likely that this method would be sensitive enough to measure levels at which biological effects occur. Hexachlorobutadiene was detected in adipose tissue of victims of accidental and nonaccidental deaths, with about twice as much

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sorb on Amberlite XAD-2®, desorb with hexane	GC/ECD	0.02 µg/sample	85–100	NIOSH 1990
Indoor air	Collect in stainless steel canister, concentrate sample in cryogenic trap	GC/ECD or GC/MS	0.2 ppbv	90–110	EPA 1990b
Water	Adjust to pH > 11, extract with methylene chloride, dry, concentrate	GC/MS	0.9 µg/L	24–116	APHA 1992a
Water	Purge and trap	GC/PID and GC/HECD	No data	98–99	APHA 1992b
Water	Purge and trap	GC/MS	0.04–0.11 µg/L	88–91	Eichelberger et al. 1990; EPA 1989e
Water	Purge and trap	GC/PID	0.02 µg/L	No data	EPA 1989d
Water	Purge and trap	GC/PID and GC/HECD	0.05–0.09 µg/L	92–99	EPA 1989c
Waste water	Extract with methylene chloride, dry, concentrate into hexane, clean up with FAC	GC/ECD	0.05 µg/L	86–106	EPA 1982a

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Waste water	Extract with methylene chloride, dry, concentrate	GC/MS	0.9 µg/L	20–76	EPA 1982c
Soil/solid waste	Extract with organic solvent, clean up with GPC	GC/MS	0.66–50 mg/kg wet weight	No data	EPA 1986
Soil/solid waste	Purge and trap	GC/MS	0.05–2.5 mg/kg wet weight	93–107	EPA 1990e

ECD = electron capture detection; FAC = florisil adsorption chromatography; GC = gas chromatography; GPC = gel permeation chromatography; HECD = halogen electrolytic conductivity detector; MS = mass spectroscopy; PID = photoionization detector; ppbv = parts per billion by volume

in accident than nonaccident victims (Mes et al. 1985). This indicates that the GC/ECD and GC/MS method is sensitive enough to measure background levels of hexachlorobutadiene in the general population as well as levels at which biological effects occur. No data were located concerning methods to measure hexachlorobutadiene metabolites in biological samples; such methods would be useful if it were established that hexachlorobutadiene metabolite levels were reliable markers of exposure to hexachlorobutadiene.

No data were located concerning methods to measure biological markers of hexachlorobutadiene effects. Research into biomarkers of effect would be most useful if performed in conjunction with development of sensitive, specific, and reliable methods for measuring the biomarker(s) of effect.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for detection of hexachlorobutadiene in air, water, soil, solid waste, and food are all based on gas chromatography (APHA 1992a, 1992b; EPA 1982a, 1982c, 1986, 1989c, 1989d, 1989e, 1990b, 1990e). Existing methods for analysis of air and water appear to be sufficiently sensitive, specific, and reliable to measure background levels in the environment. Matrix interference and contamination by co-eluting chemicals may limit the sensitivity and specificity of methods for analysis of hexachlorobutadiene in soil and solid waste (EPA 1986, 1990e). Supercritical fluid extraction, which uses carbon dioxide liquified above 31°C at high pressure, might provide efficient extraction of hexachlorobutadiene from large samples (Walters 1990). Supercritical fluid chromatography may provide an alternate approach to GC for analysis of hexachlorobutadiene and other compounds from complex environmental samples (Pospisil et al. 1991). An immunoassay for heptachlor has been developed which shows 1.6 % cross-reactivity with hexachlorobutadiene (Stanker et al. 1990). Development of an immunoassay specific for hexachlorobutadiene could provide a rapid, inexpensive, and sensitive method for detecting hexachlorobutadiene in environmental samples. No data were located concerning methods to measure hexachlorobutadiene degradation products in the environment. Degradation products are likely to be compounds that could be separated either by GC or by high performance liquid chromatography (HPLC) (for oxidized, polar degradation products). Mass spectrometry would be likely to be the most specific method to identify such products. Development of methods to measure hexachlorobutadiene degradation products would be useful for assessing the fate of hexachlorobutadiene in the environment.

6.3.2 On-going Studies

No information was located concerning on-going studies for improving methods of analysis of hexachlorobutadiene, its metabolites, or other biomarkers of exposure and effect to hexachlorobutadiene in biological materials or environmental samples.