7. ANALYTICAL METHODS

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

As is true for most volatile organic compounds, the preferred analytical technique for dibromochloromethane and bromoform is gas chromatography (GC) (Ashley et al. 1996; Djozan and Assadi 1995; Fishbein 1985). A number of devices are suitable for detection and quantification of dibromochloromethane and bromoform as they emerge from the GC, including flame ionization detection (GC/FID), halogen-sensitive detection (GC/HSD) or electron-capture detection (GC/ECD). In general, HSD or ECD are preferable because of their high sensitivity for halogenated compounds. When absolute confidence in compound identity is required, mass spectrometry (GC/MS) is the method of choice.

The most variable aspect of analyses of this sort is the sample preparation procedure used to separate dibromochloromethane and bromoform from the test medium in order to prepare a sample suitable for GC analysis. As volatile organic compounds of relatively low water solubility, both dibromochloromethane and bromoform are easily lost from biological and environmental samples, so appropriate care must be exercised in handling and storing such samples for chemical analysis. Brief summaries of the methods available for extraction and detection of these compounds in biological and environmental samples are provided below.

7.1 BIOLOGICAL MATERIALS

Separation of dibromochloromethane and bromoform from biological samples is most often achieved by headspace analysis, purge-and-trap collection, solvent extraction, or direct collection on adsorbent resins.

Headspace analysis offers speed, simplicity, and good reproducibility, but partitioning of the analyte between the headspace and the sample matrix is dependent upon the nature of the matrix and must be determined separately for each different kind of matrix (Walters 1986).

Purge-and-trap collection is well suited to biological samples such as blood or urine that are readily soluble in water (Ashley et al. 1996; Peoples et al. 1979). This method consists of bubbling an inert gas through a small volume of the sample and collecting the vapor in a trap packed with sorbent. The analytes are then removed from the trap by heating it and backflushing the analytes onto a gas chromatographic column. The two materials most widely used for adsorption and thermal desorption of volatile organic compounds collected by the purge and trap technique are Carbotrap®, consisting of graphitized carbon black, and Tenax®, a porous polymer of 2,6-diphenyl-p-phenylene oxide (Fabbri et al. 1987).

For water-insoluble materials such as fat or other tissues, the most common separation procedure is extraction with an organic solvent such as diethyl ether (Zlatkis and Kim 1976). Homogenization of tissue with the extractant and lysing of cells usually improves solvent extraction efficiency.

Analytical methods for the determination of bromoform and dibromochloromethane in biological materials are summarized in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

Dibromochloromethane and bromoform may be isolated from environmental samples using the same methods and principles as those used for biological materials, followed by gas chromatographic analysis. The most convenient procedure for most liquid and solid samples is the purge-and-trap method. Arthur et al. (1992) used solid phase micro extraction to separate volatile halogenated compounds from water samples. The organic analytes partition between the water sample and the stationary phase coating of a fused silica fiber before they are thermally desorbed in a GC injector. Djozan and Assadi (1995) introduced a gas stripping cryogenic trapping technique for separating volatile halogenated compounds from drinking water samples. In this method, a purified gas passes through the water sample in a stripping column where it removes volatile compounds from a water sample. The effluent from the column is dried, trapped in a cold trapping coil, and then released to the GC system by warming. Membrane inlet mass spectrometry (MIMS) is another technique for separating organohalogen compounds from drinking water (Bocchini et al. 1999). With this method, volatile organic compounds

Table 7-1. Analytical Methods for Determining Bromoform and Dibromochloromethane in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Adipose tissue	Extraction, bulk lipid removal, Florisil fractionation	HRGC/MS	0.1 µg/g	NR	Mack and Stanley 1985
Adipose tissue	Heated dynamic headspace purge-and-trap	HRGC/MS	1 ng/g (DBCM) 2 ng/g (TBM)	NR	Stanley 1986
Adipose tissue	Purge from liquified fat at 115 °C, trap on Tenax/silica gel, thermal desorption	GC/HSD	<2 µg/L	83–107 (TBM) 90–118 (DBCM)	Peoples et al. 1979
Blood	Purge from blood onto Tenax, thermal desorption onto column maintained at -20 °C	GC/MS	≈0.1 ng/mL	NR	Antoine et al. 1986
Blood	Extract with n-pentane	HRGC/ECD	0.1 µg/L (DBCM)	NR	Kroneld 1985
Blood	Purge from blood at 30 °C, trap onto Tenax, thermal desorption	GC/MS	0.021µg/L (TBM)	102–108 (TBM)	Ashley et al. 1992
Blood	Purge from blood at 30 °C, trap onto Tenax, thermal desorption	GC/MS	0.017µg/L (DBCM)	91–104 (DBCM)	Ashley et al. 1992
Blood, tissue	Macerate tissue in water, warm blood or tissue, pass inert gas through, trap on Tenax, thermal desorption	GC/MS	3 ng/mL (blood) 6 ng/g (tissue)	NR	Pellizzari et al. 1985b
Blood serum	Purge from water-serum mixture containing antifoam reagent at 115 °C, trap on Tenax/silica gel, thermal desorption	GS/HSD	<2 µg/L	79–100 (TBM) 78–100 (DBCM)	Peoples et al. 1979
Breath	Trap on Tenax, dry over calcium sulfate, thermal desorption	GC/MS	1–5 µg/m^3	92±15 (TBM) 93±13 (DBCM)	Wallace et al. 1986b

µg = microgram; DBCM = dibromochloromethane; ECD = electron capture detector; g = gram; GC = gas chromatography; HRGC = high resolution gas chromatography; HSD = halide specific detector; L = liter; m^3 = cubic meters; mg = milligram; mL = milliliter; MS = mass spectrometry; ng = nanogram; NR = not reported; TBM = bromoform

from the drinking water sample diffuse through a hollow-fiber membrane into a mass spectrometer. The advantages of this method are that sample pre-treatment is not required, response times are fast, and trace analysis of the pollutants can be preformed on-line. Halocarbons can also be removed from water by adsorption on synthetic polymers contained in cartridges, followed by thermal desorption of the analyte (Pankow et al. 1988). Among the products used for this purpose are Tenax-GC® and Tenax-TA®. A similar procedure is used for air, in which the air is passed through an adsorbent canister, followed by thermal desorption (Pankow et al. 1998).

Analytical methods for the determination of dibromochloromethane and bromoform in environmental samples are given in Table 7-2.

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 bromoform and dibromochloromethane is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (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 bromoform and dibromochloromethane.

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 specific methods exist for the determination of dibromochloromethane and bromoform in blood, expired air, and adipose tissue. These methods are presumably sensitive enough to

Table 7-2. Analytical Methods for Determining Bromoform and Dibromochloromethane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Drinking water	Solvent extraction with pentane, direct injection of extract	HRGC/ECD	<0.5 µg/L	NR	Fayad and Iqbal 1985
Drinking water	Gas stripping and cryogenic trapping system	GC/FID	0.05 µg/L (DBCM)	75±7.7 (DBCM)	Djozan and Assadi 1995
Drinking water	Adsorption to and diffusion through a polymeric hollow-fiber membrane	MIMS	0.5 µg/L (TBM)	NR	Bocchini et al. 1999
Drinking water	Adsorption to and diffusion through a polymeric hollow-fiber membrane	MIMS	0.5 µg/L (DBCM)	NR	Bocchini et al. 1999
Drinking water	Purge and trap, thermal desorption	GC/MS	0.1µg/L	89–90 (TBM)	Eichelberger et al. 1990
Drinking water	Purge and trap, thermal desorption	GC/MS	0.1µg/L	95–100 (TBM)	Eichelberger et al. 1990
Air	Coconut shell charcoal sorption, carbon disulfide desorption	GC/FID	10 µg/sample (TBM)	14.0 (TBM)	NIOSH 1994
Air	Carbotrap/Carboxen filled glass cartridge adsorption/thermal desorption	GC/MS	0.02 ppbv (TBM)	95–102 (TBM)	Pankow et al. 1998
Air	Carbotrap/Carboxen filled glass cartridge adsorption/thermal desorption	GC/MS	0.04 ppbv (DBCM)	96–99 (DBCM)	Pankow et al. 1998
Water	Purge and trap	GC/MS	10 µg/L	NR	EPA 1980b
Water	Purge and trap	GC/HSD	0.20 µg/L (TBM)	89±9 (TBM)	EPA 1982a
			0.09 µg/L (DBCM)	98±7 (DBCM)	
Water	Purge and trap	GC/MS	4.7 µg/L (TBM)	105±16 (TBM)	EPA 1982b
			3.1 µg/L (DBCM)	104±14 (DBCM)	
Water	Purge and trap	GC/HSD	0.5 µg/L	97 (DBCM)	APHA 1985a
				101 (DBCM)	
Water	Purge and trap	GC/MS	<2 µg/L	82 (TBM)	APHA 1985b
Water	Solvent extraction (isooctane)	GC/ECD	2 µg/L	NR	ASTM 1988
Water	Solid phase micro extraction	GC/MS	4.7 µg/L (TBM)		Arthur et al. 1992

Table 7-2. Analytical Methods for Determining Bromoform and Dibromochloromethane in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Accuracy (percent recovery)	Reference
Water	Solid phase micro extraction	GC/MS	3.1 µg/L (DBCM)		Arthur et al. 1992
Contaminated soil	Purge and trap	GC/HSD	2 µg/kg (TBM)	96[b] (TBM)	EPA 1986a
			0.9 µg/kg (DBCM)	94[b] (DBCM)	
Wastes, nonwater miscible	Purge and trap	GC/HSD	250 µg/kg (TBM)	96[b] (TBM)	EPA 1986a
			113 µg/kg (DBCM)	94[b] (DBCM)	
Solid waste	Purge and trap	GC/MS	5 µg/kg	118[b] (TBM)	EPA 1986b
				101[b] (DBCM)	

[a]Value refers to both DBCM and TBM unless noted otherwise.
[b]This recovery is typical at concentrations of around 100 µg/L or higher. Recoveries may deviate at lower concentrations.

µg = microgram; DBCM = dibromochloromethane; ECD = electron capture detector; FID = flame ionization detector; g = gram; GC = gas chromatography; HRGC = high resolution gas chromatography; HSD = halide specific detector; kg = kilogram; L = liter; MS = mass spectrometry; NR = not reported; TBM = bromoform

measure levels in humans exposed to doses of the chemicals that produce sedation or cause injury to liveand kidney. However, data on this are lacking due to absence of cases. The methods are also suitable for measuring background levels in the general population, although increased sensitivity would be useful for analysis of expired air and adipose tissue. The major limitation to these methods is that only recent exposures can be detected, so work to identify and quantify a more stable biomarker of exposure (e.g., a halomethyl adduct) would be valuable.

Effect. No chemical or biochemical biomarkers of effect are recognized, aside from nonspecific indices of hepatic or renal dysfunction, Efforts to identify a specific biomarker of effect (in particular, an effect such as alkylation of DNA that may be related to cancer risk) would be valuable in evaluating potential health risk to exposed humans.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Reliable and specific methods exist for measuring parent dibromochloromethane and bromoform in air, water, soil and solid wastes. Humans could be exposed to these compounds by contact with any of these media, although ingestion of or dermal contact with contaminated water appears to be the most likely route near a chemical waste site. Existing methods are readily able to detect concentration values in environmental media that are likely to lead to significant noncancer health effects, but might not be sensitive enough to measure levels that pose low levels of cancer risks. However, since no chemical-specific cancer potency values are available for these components, this is not certain.

7.3.2 Ongoing Studies

No ongoing studies on analytical methods were identified in a search of the Federal Research in Progress database (FEDRIP 2004).