

6. ANALYTICAL METHODS

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

Analysis of biological materials for methylene chloride is performed most frequently by gas chromatography with a flame ionization detector (GC/FID) (DiVincenzo et al. 1971; Engstrom and Bjurstrom 1977). Table 6-1 summarizes the data for specific methods for biological fluids and tissues.

Separation of methylene chloride from biological samples in preparation for GC/FID analysis is most often achieved by heating the sample in a sealed flask until the analyte concentration is in equilibrium in the sample matrix and the headspace vapor. The headspace vapor is then drawn off for analysis by gas chromatography (GC). Headspace sampling eliminates the need for a solvent extraction procedure, thus simplifying sample preparation and improving sensitivity (DiVincenzo et al. 1971). However, partitioning of the analyte between the headspace and the sample matrix depends on the nature of the matrix and must be determined separately for each different kind of matrix (Walters 1986). Acid hydrolysis of adipose tissue is required prior to headspace sampling (Engstrom and Bjurstrom 1977).

6.2 ENVIRONMENTAL SAMPLES

Several methods are available for the determination of methylene chloride in air, water, soil/sediments, and food. Table 6-2 summarizes several representative methods appropriate for quantifying methylene. In most analytical methods, methylene chloride is trapped on a solid sorbent such as activated charcoal. Air

Table 6-1. Analytical Methods for Determining Methylene Chloride in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Heat sample, collect headspace vapor	GC/FID	0.022 ppm	49.8±1.33	DiVincenzo et al. 1971
Urine	Heat sample, collect headspace vapor	GC/FID	No data	59±2.75	DiVincenzo et al. 1971
Urine	Heat sample, inject headspace air, loop	GC/MS	0.5 ppb	95%	Ghittori et al. 1993
Breath	Heat sample, inject into gas sample, loop	GC/FID	0.2±0.1 ppm	No data	DiVincenzo et al. 1971
Adipose tissue	Hydrolyze with acid, heat sample, collect headspace vapor	GC/FID	1.6 ppm ^a	No data	Engstrom and Bjurstrom 1977
Human milk	Purge with helium, trap on sorbent trap, desorb thermally	GC/MS	No data	No data	Pellizzari et al. 1982

^aLowest reported concentration

FID = flame ionization detector; GC = gas chromatography; MS = mass spectrometry

Table 6-2. Analytical Methods for Determining Methylene Chloride in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Adsorb on charcoal, desorb with carbon disulfide	GC/FID	25 ppb ^a	90–100 ^b	APHA 1977
Air	Adsorb on charcoal, desorb with carbon disulfide	GC/MS	No data	No data	Ghittori et al. 1993
Air	Adsorb on charcoal, desorb with carbon disulfide	GC/MS	No data	No data	Xiao et al. 1993
Air	Adsorb on charcoal, desorb with carbon disulfide	GC/FID	2,900 ppb 0.01 mg/sample	95.3	NIOSH 1994 [Method 1005]
Air	Extract with methylene chloride, purge with helium, desorb thermally	GC/MS	0.05 ppm	- 100	Savard et al. 1992
Air	Adsorb on charcoal, desorb with benzyl alcohol	GC/ECD	0.5 ppb	No data	Woodrow et al. 1988
Air	Purge with nitrogen, direct beam with mirrors to detect signal	RS-FTIR	0.57 ppm-m	99.7–100	Xiao et al. 1993
Air	Adsorb on activated charcoal, desorb with carbon disulfide	Toxic Gas Vapor Detector Tube	No data	No data	EMMI 1999a
Air	Adsorb on activated charcoal, desorb with carbon disulfide	GC/FID	No data	No data	EMMI 1999b
Air	Aspirate with a pump through detector tube	Toxic Gas Vapor Detector Tube	50 ppm	No data	EMMI 1999c
Stack gas effluents	Sorption onto Tenax [®] , thermal desorption	GC/MS	No data	50–150	EPA 1986e OSW 5041A

Table 6-2. Analytical Methods for Determining Methylene Chloride in Environmental Samples(continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/HSD	No data	85	EPA 1989f
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/ELCD	0.01 ppb	97–100	EPA 1989g
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/MS	1.0 ppb	99	EPA 1989c
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	HRGC/MS	0.03–0.09 ppb	95–97	EPA1989b
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	HRGC/ELCD	0.01–0.05 ppb	97±28	APHA 1989a
Water	Purge with inert gas, trap on sorbent trap, desorb thermally	HRGC/MS	0.02–0.2 ppb	95±5	APHA 1989b
Water	Purge with helium, trap on sorbent trap, desorb thermally	GC/MS	No data	99–105	Michael et al. 1988
Water	Purge with helium, trap on sorbent trap, desorb thermally	GC/MS	0.099 ppb	85	APHA 1998a
Waste water	Purge with helium, trap on sorbent trap, desorb thermally	GC/MS or GC/PID or GC/ECD	0.5 ppb	80–120	APHA 1998b
Waste water	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/MS	0.25 ppb	25–162	EPA 1998h [Method 601]
Waste water	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/MS	2.8 ppb	D–221	EPA 1998i [Method 624]

Table 6-2. Analytical Methods for Determining Methylene Chloride in Environmental Samples(continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Solid/ Solid Waste	Purge with inert gas, trap on sorbent trap, desorb thermally	GC/MS	0.03 ppb	95	EPA 1996b [Method 8260]
Solid/ Solid Waste	Purge with inert gas, trap on sorbent trap, desorb thermally; or inject directly into GC	GC/PID/HECD	0.02 ppb	95±2.8	EPA 1996c [Method 8021]
Soil/sediment/ solid waste	Headspace extraction	GC/FID or GC/PID/ELCD	No data	No data	EPA 1986e OSW 5021
Soil/sediment	Extract with methanol, purge and trap	GC/ECD	No data	25–162	EPA 1986e OSW 8010B
Solid waste matrices	Purge and trap or direct injection	GC/ECD/PID	0.02 ppb	97	EPA 1986e OSW 8021B-PID
Solid waste matrices	Headspace extraction, purge and trap	GC/MS	5 ppb	0–0221	EPA 1986e OSW 8240B-S
Solid waste matrices	Purge and trap or direct injection	GC/MS	0.03 ppb	95	EPA 1986e OSW 8260B
Food	Equilibrate in heated sodium sulfate solution, collect headspace vapor	GC/ELCD	0.05 ppm	No data	Page and Charbonneau 1984
Food	Isolate solvent by closed system vacuum distillation with toluene as carrier solvent	GC/ELCD	7 ng	94	Page and Charbonneau 1984
Food	Isolate solvent by closed system vacuum distillation with toluene as carrier solvent	GC/ECD	7 ng	100	Page and Charbonneau 1984

Table 6-2. Analytical Methods for Determining Methylene Chloride in Environmental Samples(continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Purge with nitrogen, trap on sorbent trap, elute with hexane	GC/ELCD	1.2 ppb ^c	84–96	Heikes 1987
Food	Extract with acetone-water, back extract with isooctane	GC/ELCD	4 ppb	66	Daft 1987

^aLowest value for various compounds reported during collaborative testing of this method.

^bEstimated accuracy of the method when the personal sampling pump is calibrated with a charcoal tube in the line.

^cLowest reported concentration.

D = detected; ECD = electron capture detector; ELCD = electrolytic conductivity detector; FID = flame ionization detector; GC = gas chromatography; HECD = electrolytic conduction detector; HRGC = high resolution gas chromatography; HSD = halogen specific detector; MS = mass spectrometry; PID = photo ionization detector; RS-FTIR = remote sensing Fourier transform infrared

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samples are drawn directly through the sorbent (APHA 1977; NIOSH 1984). For water, soil, or solid chloride in each of these environmental media. The EPA and NIOSH methods are standardized to comply with regulatory requirements.

waste samples, methylene chloride is purged from the sample with an inert gas such as helium, and then passed through the sorbent (APHA 1989a, 1989b; EPA 1989c, 1989f, 1989g). Desorption is thermal or by carbon disulfide. Vacuum distillation or solvent extraction are sometimes used for separation of methylene chloride from food samples (Daft 1987; Page and Charbonneau 1977).

Following separation of the organic compounds by GC, methylene chloride is detected by one of several types of instruments: a flame ionization detector (FID), electron capture detector (ECD), electrolytic conductivity detector (ELCD) or halogen specific detector (HSD). A mass spectrometer (MS) may be used for unequivocal identification. While the ELCD appears to be most sensitive, detection limits for all these methods are well below levels of health concern, but are not below EPA calculated cancer risk levels or all MRLs.

Several physical parameters may interfere with analytical accuracy. High sampling flow rates and high temperature and humidity may cause decreased adsorption of methylene chloride vapor on the solid sorbent (APHA 1977). In addition, methylene chloride is a common laboratory contaminant and is frequently found in laboratory blanks and in the environment (e.g., soil and water 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 methylene chloride 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 methylene chloride.

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

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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 methylene chloride may be evaluated by measuring the levels of this compound in blood (Ashley et al. 1994), urine, breath (DiVincenzo et al. 1971), adipose tissue (Engstrom and Bjurstrom 1977), and human milk (Pellizari et al. 1982). Sensitive methods such as gas chromatography with a flame ionization detector (GC/FID) and gas chromatography with a mass spectrometry detector (GC/MS) are available for the determination of methylene chloride in biological materials. However, additional data on detection limits and accuracy are needed to determine whether these methods are sufficiently sensitive and specific to identify individuals with low-level exposures.

Neurological or neurobehavioral effects are characteristic markers of effects of methylene chloride. These effects can occur in people exposed to low levels. No specific tests are known that measure specific biomarkers of methylene chloride effects. The tests are standard for a wide range of chemicals and are not very sensitive. Therefore, other methods are needed to identify specific biomarkers of methylene chloride exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Air is the environmental medium of most concern for human exposure to methylene chloride. Exposure from drinking water may also be of concern in some areas, such as near hazardous waste sites. Existing analytical methods can measure methylene chloride in these and other environmental media at background levels. Analytical methods such as gas chromatography with flame ionization detector (GC/FID), electron capture detector (GC/ECD), and mass spectrometry detector (GC/MS) are available for determining methylene chloride in environmental media. High resolution gas chromatography with mass spectrometry (HLGC/MS) and with electrolytic conductivity detector (HLGC/ELCD) as well as Remote Sensing Fourier Transform Infrared (RS-FTIR) spectroscopy are also reliable. Exposure to methylene chloride may be evaluated by measuring the levels of this compound in air (APHA, NIOSH), water (APHA 1989a, 1989b; EPA 1989a, 1989b, 1989c, 1989d, 1989e, 1989f, 1989g), waste water (EPA 1998a, 1998b, 1998c, 1998d, 1998e, 1998f, 1998g, 1998h, 1998i, 1998j, 1998k, 1998l), soil/solid waste

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(EPA 1996a, 1996b, 1996c, 1996d), and food (Daft 1987; Heikes 1987; Page and Charbonneau 1977, 1984). The accuracy and precision of the methods are well documented and mass spectrometry provides adequate specificity. Development of methods to improve the accuracy, sample preparation, and transfer techniques are needed to monitor environmental media, especially how to preclude false positives.

6.3.2 Ongoing Studies

The following information was found as a result of a search of Federal Research in Progress (FEDRIP 1996).

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 methylene chloride 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.

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 methylene chloride and other phenolic compounds in urine. These methods use high resolution gas chromatography and magnetic sector mass spectrometry which gives detection limits in the low parts per trillion (ppt) range.

Other on-going studies to improve analytical methods for methylene chloride and related compounds include the EPA "Master Analytical Scheme" being developed for organic compounds in water (Michael et al. 1988), the research in supercritical fluid extraction (King 1989) which is applicable to organohalide analytes, and the development of whole column cryotrapping techniques (Pankow and Rosen 1988). These improvements are designed to overcome problems with purge and trap sample preparation and increase sensitivity, reliability, and speed of the analyses.

