7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring carbon tetrachloride, its metabolites, and other biomarkers of exposure and effect to carbon tetrachloride. 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 carbon tetrachloride is gas chromatography (GC). A number of devices are suitable for detection of carbon tetrachloride as it emerges from the GC, including flame ionization detector (FID), halogen-sensitive detector (HSD), or electron-capture detector (ECD). In general, HSD or ECD are preferable because of their high sensitivity for halogenated compounds. When absolute confidence in compound identity is required, gas chromatography/mass spectrometry (GC/MS) is the method of choice.

The most variable aspect of carbon tetrachloride analysis is the procedure used to extract carbon tetrachloride from the medium and prepare a sample suitable for GC analysis. As a volatile organic compound of relatively low water solubility, carbon tetrachloride is 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 carbon tetrachloride in biological and environmental samples are provided below.

7.1 BIOLOGICAL MATERIALS

Separation of carbon tetrachloride from biological samples may be achieved by headspace analysis, purge-and-trap collection from aqueous solution or slurry samples, solvent extraction, or direct collection on 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 adapted to biological samples such as blood or urine that are soluble in water (Pellizzari et al. 1985a; Peoples et al. 1979), and is readily adapted from techniques that have been developed for the analysis of carbon tetrachloride in water and waste water. For water-insoluble materials, the purge-trap approach is complicated by uncertainty of partitioning the analyte between sample slurry particles and water.

Historically, diethyl ether has been a widely used solvent for the extraction of volatile components from biological fluids (Zlatkis and Kim 1976). Homogenization of tissue with the extractant and lysing of cells improves extraction efficiency. When, as is often the case, multiple analytes are being determined using solvent extraction, selective extraction and loss of low-boiling compounds can cause errors. Highly purified solvents have largely eliminated problems with solvent impurities, although high costs, solvent toxicities, and restrictions on spent solvent disposal must be considered. Supercritical fluid extraction using pure carbon dioxide or carbon dioxide with additives offers some potential for the extraction of organic analytes such as carbon tetrachloride from biological samples (Hawthorne 1988).

Analytical methods for the determination of carbon tetrachloride in biological samples are summarized in Table 7-1.

7.2 ENVIRONMENTAL SAMPLES

The basic method for collection of carbon tetrachloride from the ambient atmosphere is adsorption on a solid phase, followed by removal by thermal or solvent elution for subsequent analysis. One of the most common adsorbents for carbon tetrachloride is Tenax® GC. Using Tenax® adsorbent, standard air containing 1.15 ppb by gas volume of carbon tetrachloride was determined with biases of -23.0, -34.7, -50.0, and -69.2% at collection volumes of 10, 20, 38, and 76 L of air, respectively (Crist and Mitchell 1986). Citing these large negative biases even when the sampled volume was less than 10% of the breakthrough volume, these authors conclude that Tenax® is not suitable for quantitative sampling for carbon tetrachloride (Crist and Mitchell 1986). For occupational monitoring of carbon tetrachloride in air, NIOSH (1984) recommends samplers containing activated carbon. The adsorbed carbon tetrachloride is extracted from the activated carbon with carbon disulfide, then determined by GC/FID. Studies have been conducted to improve analytical methods for detection of low-level volatile organic compounds.

Table 7-1. Analytical Methods for Determining Carbon Tetrachloride in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Alveolar air	Collect on Tenax-TA®; desorb thermally; inject by cryotrap	Capillary column GC/FID	No data	No data	Clair et al. 1991
Breath	Collect on Tenax-GC®; desorb thermally	Capillary column GC/MS	No data	No data	Pellizzari et al. 1985b
Adipose tissue	Purge from liquefied fat at 115 °C, trap on Tenax ®/silica gel, thermal desorption	GC/HSD	<1.3 µg/L	96 (90–100)	Peoples et al. 1979
Adipose tissue	Macerate in water; purge with inert gas; trap on Tenax-GC®; desorb thermally	Capillary column GC/MS	≈6 ng/g	≈50	Pellizzari et al. 1985a
Blood serum	Purge from water-serum mixture containing antifoam reagent at 115 °C, trap on Tenax®/silica gel, thermal desorption	GC/HSD	<1.3 µg/L	112 (108–124)	Peoples et al. 1979
Blood	Purge with inert gas, trap on Tenax-GC®; desorb thermally	Capillary column GC/MS	≈3 ng/mL	89.4	Pellizzari et al. 1985a
Biofluids	Dilute with water, sealed vial; collect headspace vapors	GC/FID	NR	No data	Suitheimer et al. 1982

FID = flame ionization detector; GC = gas chromatography; HSD = halogen-selective detector; MS = mass spectrometry; NR = not reported

Methods have been evaluated that do not require the use of sorbents, thereby reducing associated uncertainties due to their adsorption/desorption efficiencies. The use of cryogenic preconcentration techniques to increase the sample content of trace volatile toxic organic compounds in a gas matrix for analysis by GC has been evaluated (Rhoderick and Miller 1990). The authors revealed that a linear multipoint calibration range from 1 to 15 ppb can be obtained by using a single standard, cryogenic trapping, a constant flow rate and varied trapping timer. Acceptable methods for the determination of carbon tetrachloride in ambient air are detailed in EPA Compendium Method TO-14A (EPA 1999).

Purge and trap methods are standard for the determination of carbon tetrachloride in water, with analyte measurement by GC using halogen-specific detection, electron-capture detection, or mass-spectrometry (APHA 1992a, 1992b; ASTM 1987; Bellar 1989; Eichelberger and Buddle 1989a, 1989b; EPA 1982a, 1982b; Ho 1989). The APHA (1992a, 1992b) methods for carbon tetrachloride have been accepted by EPA as equivalent to EPA-developed methods. Analyte measurement using an ion trap detector that functions as a mass spectrometer has also been evaluated (Eichelberger et al. 1990). This method is sufficiently sensitive to measure the analytes below the regulatory levels. Headspace sampling, coupled with whole column cryotrapping chromatography and mass spectrometry, have been used in the analysis of volatile priority pollutants in water and waste water (Gryder-Boutlet and Kennish 1988). The advantage of headspace sampling over other methods of analysis include minimal sample preparation, injection of a larger sample preparation and, and shorter analysis timer, because all of the compounds being analyzed are volatile. Carbon tetrachloride can also be determined in solid wastes by purge and trap collection followed by GC (EPA 1986a, 1986b). A modified open-loop dynamic headspace technique has been applied for stripping and trapping volatile organic compounds from estuarine sediments (Bianchi et al. 1991). This method is capable of quantifying volatile organic compounds at detection limits between 10 and 100 ng/kg.

Analytical methods for the determination of carbon tetrachloride in environmental samples are summarized 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 carbon tetrachloride is available. Where adequate

Table 7-2. Analytical Methods for Determining Carbon Tetrachloride in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Coconut shell carbon sorption, carbon disulfide desorption	GC/FID	10 µg/sample	No data	NIOSH 1984
Air	Adsorption on Tenax® GC, thermal desorption	GC/MS	<1.15 ppb[a]	23–77	Crist and Mitchell 1986
Air	Sorption	GC/CLMD	0.003 ng/ sample	No data	Yamada et al. 1982
Air	Charcoal sorption, carbon disulfide desorption	GC/HSD	No data	No data	ASTM 1987
Water	Purge and trap	GC/MS	No data	No data	ASTM 1987
Water	Extract with n-pentane	GC/ECD	0.4 µg/L	No data	Garcia et al. 1992
Water	Purge and trap	GC/HSD	0.12 µg/L	82.5	EPA 1982a
Water	Purge and trap	GC/MS	2.8 µg/L	102	EPA 1982b
Drinking water	Purge with inert gas; trap on sorbent tube; desorb thermally	Capillary column GC/HSD	0.01 µg/L	92	Ho 1989
Drinking water	Purge with inert gas; trap on sorbent tube; desorb thermally	Packed column GC/HSD	0.003 µg/L	90	Bellar 1989
Drinking water	Purge with inert gas; trap on sorbent tube; desorb thermally	Capillary column GC/HSD	0.08 µg/L	92	EPA 1989b
Drinking water	Purge with inert gas; trap on sorbent tube; desorb thermally	Packed column GC/HSD	0.3 µg/L	88	EPA 1989b
Water	Purge and trap	GC/ITD	0.1 µg/L	No data	Eichelberger et al. 1990
Water	Solvent extraction (isooctane)	GC/ECD	1 µg/L[b]	No data	ASTM 1988
Soil	Purge and trap	GC/HSD	1.2 µg/kg	43–143	EPA 1986b
Wastes, non-water miscible	Purge and trap	GC/HSD	150 µg/kg	43–143	EPA 1986b
Solid waste	Purge and trap	GC/MS	5 µg/kg	70–140	EPA 1986a
Grain	Extract with acetone/water (5/1); dry; inject acetone solution	GC/ECD	NR	No data	AOAC 1984

[a]Persistent negative bias in recovery suggests Tenax® sorption is not suitable for collection of carbon tetrachloride.
[b]Approximate detection limit

CLMD = chemiluminescence detection; ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; HSD = halogen-selective detector; ITD = ion trap detector; MS = mass spectrometry; NR = not reported

information is not available, ATSDR, in conjunction with 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 carbon tetrachloride.

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. Covalent adducts between reactive carbon tetrachloride metabolites (e.g., the trichloromethyl radical) and cellular proteins, lipids and nucleic acids are known to occur, but at present these can only be measured using radiolabeled carbon tetrachloride. Development of immunological or other methods to detect such adducts in humans exposed to carbon tetrachloride could be of value in estimating past exposures to carbon tetrachloride.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Analytical methods are available for measuring carbon tetrachloride in air, water, soil, and solid waste, and most of these methods have good sensitivity and specificity (APHA 1992a, 1992b; ASTM 1987; Bellar 1989; Eichelberger and Buddle 1989a, 1989b; EPA 1982a, 1982b; Ho 1989). However, the estimated 10-6 cancer risk levels for carbon tetrachloride are quite low (0.01 ppb in air and 0.3 ppb in drinking water) (IRIS 2003), so improvements in sensitivity would be valuable. It is desirable to have means to measure organohalides such as carbon tetrachloride *in situ* in water and other environmental media. One approach to doing this has been demonstrated by the *in situ* analysis of chloroform-contaminated well water using remote fiber fluorimetry (RFF) and fiber optic chemical sensors (FOCS) (Milanovich 1986). With this approach, fluorescence of basic pyridine in the presence of an organohalide (Fujiwara reaction) is measured from a chemical sensor immersed in the water at the end of an optical fiber. Carbon tetrachloride undergoes a Fujiwara reaction, so its determination might be amenable to this approach. Another *in situ* method for field monitoring of carbon tetrachloride has been described by Kirtland et al. (2003); this method uses isotopic labeling and detection of metabolites by gas chromatography.

7.3.2 Ongoing Studies

The EPA is funding on-going research to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988), which includes carbon tetrachloride as an analyte. The overall goal is to detect organic compounds at 0.1 µg/L in drinking water, 1 µg/L in surface water, and 10 µg/L in effluent waters. Analytes are to include numerous semivolatile compounds and some compounds that are only "semisoluble" in water, as well as volatile compounds. A comprehensive review of the literature leading up to these efforts has been published (Pellizzari et al. 1985a).

Improvements in analytical technology to identify groundwater contaminants revealed that soil gas analysis may enhance the effectiveness of traditional sampling and analysis (Kerfoot 1990). Carbon tetrachloride has properties that make it amenable to detection by soil gas analysis.

Researchers have coupled two GC capillary columns with different lengths and polarities in series to optimize separation of complex mixtures of volatile organics in air samples (Clair et al. 1991). Atomic emission detectors (AEDs) and mass selective detectors (MSDs) are also being used to enhance selectivity and sensitivity for air analyses (Yamashita et al. 1992).

The Environmental Health Laboratory Sciences Division of the Center for Environmental Health and Injury Control, Centers for Disease Control, is developing methods for the analysis of carbon tetrachloride and other volatile organic compounds in blood (Ashley et al. 1992). These methods use purge and trap methodology, high resolution gas chromatography, and magnetic mass spectrometry which gives detection limits in the low parts per trillion (ppt) range. Also useful is the ability to test for carbon tetrachloride and other volatile organic compounds in expired air (Wallace 1996).