7. ANALYTICAL METHODS

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

7.1 BIOLOGICAL MATERIALS

Trichlorobenzenes are typically analyzed for in blood, adipose tissue, and breath samples using gas chromatographic (GC) analysis. Detection limits are typically in the ng/g range. Representative methods are summarized in Table 7-1. For analytes such as trichlorobenzenes, electron capture detection (ECD) is often employed since it is highly sensitive to halogenated compounds and is less costly than mass spectrometer (MS) detection systems. However, the specific identification of a compound is determined only by its retention time, which can be subject to shifts or to interference from other non-targeted compounds. GC using a quadrupole mass spectrometer operating in either the select ion monitoring (SIM) mode or the SCAN mode allows for positive identification of the compound.

Blood or tissue samples are typically prepared by homogenizing and extracting the sample using a solvent such as benzene or acetone, followed by centrifugation and filtration (Mes 1992). In order to eliminate potential interferences from the sample matrix, cleanup and fractionation of the filtrate is accomplished with a Florisil® column using hexane as the eluting solvent prior to introduction to the GC. Average recoveries of the trichlorobenzenes from fortified adipose tissue or blood have been reported to exceed 75% (Bristol et al. 1982; Mes 1992). Alternatively, a purge-and-trap method can be employed in which blood samples are warmed while passing an inert gas over the heated samples (Pellizari et al. 1985a). Trichlorobenzene vapors are trapped onto an adsorbent polymeric resin (Tenax®) and released by thermal desorption, followed by analysis with GC/MS or GC/ECD.

Table 7-1. Analytical Methods for Determining Trichlorobenzenes in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Blood samples were homogenized with benzene, centrifuged, and filtered	GC/ECD and GC/MS	1.17 ng/g (1,2,3-TCB); 4.02 ng/g (1,3,5-TCB)	75–90	Mes 1992
Blood	Trichlorobenzenes are volatilized from blood by warming the sample and collecting in Tenax cartridges	GC/MS	3 ng/L	80	Pellizzari et al. 1985a
Blood	Samples of whole blood were extracted with hexane, centrifuged, and analyzed by GC/ECD and GC/MS	GC/ECD and GC/MS	0.33–0.55 ng/g	77.2–93.4 (1,3,5-TCB); 80.4–88.4 (1,2,3-TCB)	Bristol et al. 1982
Adipose tissue	Blood samples were homogenized with benzene, centrifuged, and filtered with glass wool	GC/ECD and GC/MS	1.68 ng/g (1,2,3-TCB); 0.90 ng/g (1,3,5-TCB)	79–95	Mes 1992
Breath	Collection on Tenax tubes, dried using calcium sulfate and desorb to GC inlet	GC/MS	No data	No data	Pellizzari et al. 1985b

ECD = electron capture detector; GC = gas chromatography; MS = mass spectrometry; TCB = trichlorobenzene

Trichlorobenzenes can be analyzed in human breath by collecting exhaled air samples onto a Tenax® GC cartridge, followed by drying with calcium sulfate and thermal desorption to a GC/MS (Pellizari et al. 1985b). Detection limits and recoveries for trichlorobenzene were not reported; however, the detection limit and quantification limit for m-dichlorobenzene was 0.27 and 1.37 μg/m^3, respectively, suggesting that similar limits will be observed for trichlorobenzenes (Pellizari et al. 1985b).

7.2 ENVIRONMENTAL SAMPLES

Methods are available for determining the level of trichlorobenzenes in a variety of environmental matrices. A summary of representative methods is shown in Table 7-2. Validated methods, approved by EPA and NIOSH, are available for air, water, soil, and sediment. GC using ECD or GC/MS is the most widely used analytical technique for identifying and quantifying trichlorobenzenes in environmental matrices.

NIOSH Method 5517 summarizes a method to analyze ambient air for the presence of trichlorobenzenes. Air samples are collected by a sampling pump at a flow rate between 0.01 and 0.2 L/minute for a total sample size of 3–12 L, and adsorption occurs onto a charcoal tube or Tenax® GC cartridge. Identification and quantification is accomplished using GC/ECD following ultrasonic desorption from the charcoal or Tenax® cartridge (NIOSH 1994). EPA Method TO-14 is similar to the previously described NIOSH method; however, collection of ambient air samples is performed using specially prepared passivated stainless steel canisters rather than adsorbant tubes. The collection of ambient air samples in these canisters provides a number of advantages as compared to the adsorbant tubes including: (1) convenient integration of ambient samples over a specific time period (e.g., 24 hours); (2) remote sampling and central analysis; (3) ease of storing and shipping samples; (4) unattended sample collection; (5) analysis of samples from multiple sites with one analytical system; (6) collection of sufficient sample volume to allow assessment of measurement precision and/or analysis of samples by several analytical systems; and (7) storage stability for many volatile organic compounds (VOCs) over periods of up to 30 days (EPA 1999). Detection limits for trichlorobenzenes in ambient air are typically in the low ppbv range.

Trichlorobenzenes in water, soil, and sediment are typically analyzed by GC/MS or GC/ECD after extraction from the environmental matrix. EPA Method 8260B is used to quantitatively analyze a variety of VOCs, including trichlorobenzenes, with GC/MS. Analysis can be performed on contaminated groundwater and surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes,

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection at flow rate of 0.01 and 0.2 L/minute onto sorbent tubes and desorb with hexane	GC/ECD	0.002 mg/m^3	No data	NIOSH 1994 (NIOSH method 5517)
Air	Collection in passivated stainless steel canisters	GC/ECD or GC/MS	ppbv level	No data	EPA 1999 (Method TO-14)
Groundwater and surface water, aqueous sludges	Closed-system purge-and-trap process	GC/MS	0.03–0.04 μg/L[a]	>100	EPA 1996b (Method 8260B)
Water and waste samples	Continuous liquid-liquid extraction or separatory funnel extraction using methylene chloride	GC/ECD	39 ng/L (1,2,3-TCB); 130 ng/L (1,2,4-TCB); 12 ng/L (1,3,5-TCB)	No data	EPA 1994 (Method 8121)
Groundwater	Continuous liquid-liquid extraction or separatory funnel extraction using methylene chloride	GC/MS	10 μg/L	>70%	EPA 2007 (Method 8270D)
Soil and sediment	Soxhlet Extraction using methylene chloride/acetone (1:1) as the extraction solvent or ultrasonic extraction with GPC cleanup	GC/ECD	No data	96% (1,2,3-TCB); 59% (1,2,4-TCB); 102% (1,3,5-TCB)	EPA 1994 (Method 8121)
Soil and sediment	Closed-system purge-and-trap process	GC/MS	0.44 μg/kg	11.4–75.4	EPA 1996b (Method 8260B)
Sediment	Soxhlet Extraction using dichloromethane. Cleanup with GPC	GC/MS	50 μg/kg	No data	Lopes and Furlong 2001
Fruit and vegetables	Homogenize in a blender followed by Soxhlet extraction using hexane/acetone (2:1)	GC/ECD	0.007–0.01 μg/kg	76.5–125 (1,2,3-TCB); 76.0–85.5 (1,2,4-TCB); 77.0–80.2 (1,3,5-TCB)	Wang and Jones 1994

[a]Value is dependent upon the GC column used and isomer measured.

ECD = electron capture detector; GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry; TCB = trichlorobenzene

mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments (EPA 1996b). Purge-and-trap methods such as EPA Method 5035 are usually employed in conjunction with Method 8260B for sample preparation. Detection limits in water and waste samples are generally <1 ppb for trichlorobenzenes; however, the actual value depends upon the specific isomer and GC column used for the analysis. Detection limits in soil and sediment are also <1 ppb. Wang and Jones (1994) described an analytical method using GC/ECD to analyze levels of trichlorobenzenes in fruits and vegetables. Soxhlet extraction using a hexane/acetone (2:1) solvent mixture followed by cleanup using a Florisil® column was used for the sample preparation. Recoveries in fortified samples exceeded 75% for each isomer, and detection limits in the sub-ppb range were reported.

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 trichlorobenzenes is available. Where adequate 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 trichlorobenzenes.

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. There are no unique biomarkers of exposure for trichlorobenzenes other than the parent compound. Methods are available that can detect trichlorobenzenes in blood (Bristol et al. 1982; Mes 1992; Pellizzari et al. 1985a), adipose tissue (Mes 1992), and exhaled breath (Pellizzari et al. 1985b). No data needs are identified. Trichlorobenzenes detected in humans may arise from direct exposure to the parent compound, but may also arise from the metabolism of other chlorinated compounds such as tetrachlorobenzenes, pentachlorobenzene, or hexachlorobenzene.

Effect. There are no unique biomarkers of effect for trichlorobenzenes.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Trichlorobenzene levels in air, water, and food are of most concern for human health. Methods are available to determine levels of trichlorobenzene in air (EPA 1999; NIOSH 1994), water (EPA 1996b), soil (EPA 1996b), sediment (EPA 1996b), and foods (Wang and Jones 1994). These methods are sensitive enough to detect trichlorobenzene levels in environmental matrices that may be a concern to human health. No data needs are identified.

7.3.2 Ongoing Studies

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 trichlorobenzenes and other volatile organic compounds in blood. These methods use purge and trap methodology, high-resolution gas chromatography, and magnetic sector mass spectrometry, which give detection limits in the low parts per trillion (ppt) range.

No ongoing studies pertaining to trichlorobenzenes were identified in Toxline (2013).