

6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring trichloroethylene, its metabolites, and other biomarkers of exposure and effect to trichloroethylene. 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 may be included that modify previously used methods to obtain lower detection limits and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

Several methods are available for the analysis of trichloroethylene in biological media. The method of choice depends on the nature of the sample matrix; cost of analysis; required precision, accuracy, and detection limit; and turnaround time of the method. The main analytical method used to analyze for the presence of trichloroethylene and its metabolites, trichloroethanol and TCA, in biological samples is separation by gas chromatography (GC) combined with detection by mass spectrometry (MS) or electron capture detection (ECD). Trichloroethylene and/or its metabolites have been detected in exhaled air, blood, urine, breast milk, and tissues. Details on sample preparation, analytical method, and sensitivity and accuracy of selected methods are provided in Table 6- 1.

Several studies have analyzed breath samples for trichloroethylene. Preconcentration on Tenax@-GC cartridges, followed by thermal desorption onto a cryogenic trap connected to the gas chromatograph, was used to analyze exhaled air in several TEAM studies (Wallace et al. 1986a, 1986b, 1986c, 1986d). Vapors were thermally released directly onto the gas chromatograph column for separation and detection by electron impact mass spectrometry (EIMS). A similar study analyzed for trichloroethylene in expired air by directly injecting a portion of the collected sample from a Tedlar®

TABLE 6-1. Analytical Methods for Determining Trichloroethylene in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Exhaled air	Collected in Tedlar® bag; injected into GC	GC/ECD (both trichloroethylene and trichloroethanol)	5 ppb (trichloroethylene); 2 ppb (trichloroethanol)	NR	Monster and Boersma 1975
Exhaled air	Collected on Tenax®-GC, thermally desorbed; injected into GC	HRGC/MS	0.3 ppb	95–99	Wallace et al. 1986a
Blood	Digested with H ₂ SO ₄ : dimethylsulfate at 60°C for 4 hours; headspace gas injected into GC	GC/ECD (trichloroethylene, trichloroethanol, and trichloroacetic acid)	3 ppb (trichloroethylene); 60 ppb (trichloroethanol); 30 ppb trichloroacetic acid)	NR	Monster and Boersma 1975
Blood	Thermally decarboxylated; subjected to static headspace analysis	GC/ECD (for metabolite trichloroacetic acid)	2 ppb	101–109	Ziglio et al. 1984

TABLE 6-1. Analytical Methods for Determining Trichloroethylene in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Enzyme hydrolysis of sample; decarboxylation of trichloroacetic acid; headspace gas analyzed	GC/ECD	20 ppb	95 (trichloroethanol); 102 (trichloroethanol); 94 (trichloroacetic acid)	Christensen et al. 1988
Blood, plasma, and serum	Sample in sealed vial subjected to static headspace analysis	GC/ECD	100 ppb	NR	Ramsey and Flanagan 1982
Urine	Thermally decarboxylated; reacted with pyridine	Spectrophotometry (for metabolite trichloroacetic acid)	<800 ppb	93.5	Pekari and Aitio 1985a
Urine	Enzyme hydrolysis of sample; decarboxylation of trichloroacetic acid; headspace gas analyzed	GC/ECD	20 ppb	96 (trichloroethanol); 98 (trichloroacetic acid)	Christensen et al. 1988

TABLE 6-1. Analytical Methods for Determining Trichloroethylene in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Hydrolyzed with H ₂ SO ₄ ; extracted with isooctane; injected into GC	GC/ECD (for metabolite trichloro-ethanol)	75 ppb	98.2	Pekari and Aitio 1985b
Tissue	Mixed with a proteolytic enzyme; incubated at 65°C; headspace gas analyzed	GC/ECD	NR	NR	Ramsey and Flanagan 1982
Tissue	Homogenized with saline and isooctane at 4°C; headspace gas analyzed	GC/ECD	8.4 ppb	86–91	Chen et al. 1993
Human milk	Purged warm; trapped in Tenax®-GC; thermally desorbed	HRGC/MS	Qualitative identification	NR	Pellizzari et al. 1982

ECD = electron capture detector; GC = gas chromatography; HRGC = high-resolution gas chromatography; H₂S₄^O = sulfuric acid; MS = mass spectrometry; NR = not reported

bag into a gas chromatograph equipped with an ECD (Monster and Boersma 1975). Sensitivity was better with GC/MS, but precision was greater with GC/ECD. No recovery data were given for the GC/ECD technique, so accuracy could not be compared. GC/ECD was also used to measure trichloroethanol, a metabolite of trichloroethylene, in expired air (Monster and Boersma 1975). The sensitivity and precision were comparable to that of trichloroethylene measurement.

The method most frequently used to determine the presence of trichloroethylene or its metabolites in biological tissues and fluids is headspace analysis, followed by GC/MS or GC/ECD (Christensen et al. 1988; Monster and Boersma 1975; Pekari and Aitio 1985a, 1985b; Ziglio et al. 1984). In headspace analysis, the gaseous layer above the sample is injected into the gas chromatograph. Headspace gases can be preconcentrated prior to GC analysis (Michael et al. 1980) or injected directly into the gas chromatograph (Collins et al. 1986; Ramsey and Flanagan 1982). Analysis of blood and urine for the trichloroethylene metabolites TCA, trichloroethanol, and trichloroethanol-B-glucuronide has been done primarily by headspace GC/ECD (Christensen et al. 1988). Trichloroethanol-pglucuronide in the samples was first hydrolyzed to trichloroethanol by β -glucuronidase, then TCA was decarboxylated to chloroform. A headspace sample was then analyzed for trichloroethanol and chloroform. The method had relatively high accuracy and acceptable precision. Detection limits were generally in the low-ppb range. Whole tissue analysis has been performed by GC/ECD after enzyme treatment (Ramsey and Flanagan 1982) and after homogenization in the presence of an extractive solvent (Chen et al. 1993).

Purge-and-trap methods have also been used to analyze biological fluids for the presence of trichloroethylene. Breast milk and blood were analyzed for trichloroethylene by purging onto a Tenax® gas chromatograph to concentrate the volatiles, followed by thermal desorption and analysis by GC/MS (Antoine et al. 1986; Pellizzari et al. 1982). However, the breast milk analysis was only qualitative, and recoveries appeared to be low for those chemicals analyzed (Pellizzari et al. 1982). Precision (Antoine et al. 1986) and sensitivity (Pellizzari et al. 1982) were comparable to headspace analysis.

6.2 ENVIRONMENTAL SAMPLES

Analysis of environmental samples is similar to that of biological samples. The most common methods of analyses are GC coupled to MS, ECD, a Hall's electrolytic conductivity detector (HECD),

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or a flame-ionization detector (FID). Preconcentration of samples is usually done by sorption on a solid sorbent for air and by the purge-and-trap method for liquid and solid matrices. Alternatively, headspace above liquid and solid samples may be analyzed without preconcentration. Details of commonly used analytical methods for several types of environmental samples are presented in Table 6-2.

The primary methods of analyzing for trichloroethylene in air are GC combined with MS and GC with ECD. Air samples are usually pumped through a sample collection column, with Tenax®GC and coconut charcoal, the most common adsorbents. Trichloroethylene is thermally desorbed from the collection column and concentrated on a cryogenic trap column located on the gas chromatograph. Vapors are heat-released from the trapping column directly to the gas chromatograph (Krost et al. 1982; Wallace et al. 1986a, 1986b, 1986c, 1986d). Grab-samples of air can also be obtained and preconcentrated on a cryogenic column (Makide et al. 1979; Rasmussen et al. 1977). The limit of detection for both GC/ECD and GC/MS is in the low- to sub-ppb range (Krost et al. 1982; Makide et al. 1979; Rasmussen et al. 1977; Wallace et al. 1986a, 1986d). With careful technique, precision for both is acceptable (Krost et al. 1982; Rasmussen et al. 1977; Wallace et al. 1986a, 1986b, 1986c, 1986d). Accuracy of the two analytical methods could not be compared because no recovery data were located for GC/ECD. The detection and measurement of trichloroethylene in air can also be adequately performed using infrared spectrometry instead of GC (Xiao et al. 1990).

Trichloroethylene can be detected in drinking water, groundwater, waste water, and leachate from solid waste. In most methods, trichloroethylene is liberated from the liquid matrix by purging with an inert gas and concentrated by trapping on a suitable solid sorbent. Trichloroethylene is thermally desorbed and backflushed onto the gas chromatograph column with an inert gas. Detection of trichloroethylene is generally by HECD (or other halogen-specific detector) or MS (APHA 1985; EPA 1982b, 1982c; Otson and Williams 1982; Wallace et al. 1986a, 1986c, 1986d). The limit of detection is in the subppb range for halogen-specific detectors (APHA 1985; EPA 1982b, 1982c) and in the low-ppb range for MS (EPA 1982b). An experiment with a purge-closed loop sample extraction system, followed by GC/ECD, GC/HECD, or GC/FID analysis, yielded a sensitivity and reproducibility comparable to headspace analysis (Otson and Williams 1982; Wang and Eenahan 1984).

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collected in stainless steel canister; preconcentrated in cooled adsorbent; thermally desorbed	GC/ECD	1 ppt	NR	Makide et al. 1979
Air	Adsorbed on Tenax®-GC thermally desorbed to on-column cold trap; heat-released	HRGC/MS	1.9 ppt	NR	Krost et al. 1982
Air	Collected in stainless steel canister; preconcentrated by cryogenic trapping; thermally desorbed	GC/ECD	0.3 ppt	NR	Rasmussen et al. 1977
Air	Adsorbed on Tenax®-GC; thermally desorbed to on-column cold trap; heat-released	HRGC/MS	15 ppt	95-99	Wallace et al. 1986a
Water	Purged and trapped on Tenax®-GC; thermally desorbed	HRGC/HSD	0.5 ppb	91	APHA 1985
Water	Purged and trapped on coconut charcoal/Tenax/silica gel; thermally desorbed	GC/MS	1.9 ppb	101	EPA 1982b

TABLE 6-2 (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Purged and trapped on coconut charcoal/Tenax®/silica gel; thermally desorbed	GC/HSD	0.12 ppb	106	EPA 1982b
Water	Equilibrated in sealed vial at room temperature; headspace gas injected into GC	GC/ECD	.04 ppb	105	Dietz and Singley 1979
Water	Purged at room or elevated temperature; trapped in closed loop; injected into GC	GC/ECD	0.2 ppb	104	Wang and Lenahan 1984
Water	Purged and trapped on Tenax®-GC; thermally desorbed	GC/HECD; GC/FID	<0.1 ppb (HECD); 0.1 ppb (FID)	98 (HECD); 79 (FID)	Otson and Williams 1982
Water	Purged and trapped on Tenax®-GC; thermally desorbed	GC/HECD	.05 ppb	50-90	Wallace et al. 1986a
Water	Sample directly injected	GC/UV	1 ppb	39	Motwani et al. 1986
Soil	Equilibrated in sealed vial; headspace gas injected into GC	GC/PID	100 ppb	NR	Hewitt et al. 1992

TABLE 6-2 (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Equilibrated in sealed vial; headspace gas injected into GC	GC/FID	NR	80	Pavlostathis and Mathavan 1992
Liquid and solid waste	Equilibrated in sealed vial; headspace gas injected into GC	GC/HSD	0.03 ppb	106	EPA 1982c
Building materials and consumer products ^a	Collected by adsorption onto sorbent; thermally desorbed	HRGC/MS	0.02 ppt	NR	Wallace et al. 1987
Food	Undigested or H ₂ SO ₄ -digested samples at 90°C subjected to static headspace analysis	HRGC/ECD; GC/MS	0.23 ppb	90–100	Entz and Hollifield 1982
Food	Extraction with isoctane; clean-up on Florisil column if needed	GC/ECD; GC/HECD	6 ppb (GC/ECD); 13 ppb (GC/ HECD)	>50	Daft 1988

TABLE 6-2 (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Grains, grain-based foods	Purged and trapped on Tenax®/XAD-4 resin; desorb with hexane	GC/ECD	Low- to sub-ppb	86–100	Heikes and Hopper 1986

^aSample is air from an environmental chamber containing the building material or consumer product.

ECD = electron capture detector; FID = flame ionization detection; GC = gas chromatography; HECD = Hall electrolytic conductivity detector; HRGC = high-resolution gas chromatography; HSD = halogen-specific detector; H₂SO₄ = sulfuric acid; MS = mass spectrometry; NR = not reported; PID = photoionization detection; UV = ultraviolet detection

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Headspace analysis has also been used to determine trichloroethylene in water samples. High accuracy and excellent precision were reported when GC/ECD was used to analyze headspace gases over water (Dietz and Singley 1979). Direct injection of water into a portable GC suitable for field use employed an ultraviolet detector (Motwani et al. 1986). While detection was comparable to the more common methods (low ppb), recovery was very low. Solid waste leachates from sanitary landfills have been analyzed for trichloroethylene and other volatile organic compounds (Schultz and Kjeldsen 1986). Detection limits for the procedure, which involves extraction with pentane followed by GC/MS analysis, are in the low-ppb and low-ppm ranges for concentrated and unconcentrated samples, respectively. Accuracy and precision data were not reported.

Analysis of soils and sediments is typically performed with aqueous extraction followed by headspace analysis or the purge-and-trap methods described above. Comparison of these two methods has found them equally suited for on-site analysis of soils (Hewitt et al. 1992). The major limitation of headspace analysis has been incomplete desorption of trichloroethylene from the soil matrix, although this was shown to be alleviated by methanol extraction (Pavlostathis and Mathavan 1992).

Several procedures for determination of trichloroethylene in food were located. GC/ECD and GC/halogen-specific detector (HSD) are most commonly used to analyze solid samples for trichloroethylene contamination. Extraction, purge-and-trap, and headspace analysis have all been used to prepare samples. Analysis of headspace gases by GC coupled with ECD, MS, or HSD has proven relatively sensitive (low- to sub-ppb range) and reproducible for a variety of foods (Boekhold et al. 1989; Entz and Hollifield 1982; EPA 1982b). GC/MS has also been used to analyze building materials and consumer products (Wallace et al. 1987). GC/HSD of headspace gases is the EPA-recommended method for solid matrices (EPA 1982c). Foods have also been analyzed for trichloroethylene by GC/ECD/HECD following isooctane extraction. Sensitivity was comparable to headspace methods, but recovery (>50%) and precision (18-59%) were not as good (Daft 1988). In both preparation techniques, increased lipid content of the matrix adversely affected accuracy and precision. A purge-and-trap technique proved useful for analyzing grains and grain-based foods with high sensitivity and good recovery (Heikes and Hopper 1986).

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 trichloroethylene 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 trichloroethylene.

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

Exposure. Methods are available for monitoring exposure to trichloroethylene by measuring trichloroethylene in breath and blood; trichloroethanol in breath, blood, and urine; and TCA in blood and urine (Christensen et al. 1988; Monster and Boersma 1975; Pellizzari et al. 1982; Ramsey and Flanagan 1982; Wallace et al. 1986a, 1986b, 1986c, 1986d). Available methods are sensitive for measuring levels of trichloroethylene and its metabolites at which health effects have been observed to occur, for example, in workers known to be overexposed to trichloroethylene (Christensen et al. 1988; Monster and Boersma 1975; Ziglio et al. 1984). These methods have also been used to measure background levels of trichloroethylene and its metabolites in individuals believed not to have been exposed to higher-than-expected levels of trichloroethylene (e.g., office workers and housewives). The methods are generally reliable, although increased precision for most methods would increase reliability. However, trichloroethylene is pervasive in the environment, and background levels for the general population are ill defined. Levels may vary considerably within the environment, making it difficult to differentiate between normal background exposure and excess exposure. Further research

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on the relationship between levels found in living environments and levels found in biological media would help in better defining background levels of the chemical. This would also aid in determining if improved methods of monitoring exposure are needed.

Effect. Existing methods for measuring biomarkers of effect are the same as those for exposure. These methods are sensitive for measuring levels of trichloroethylene and its metabolites at which health effects have been observed, for example, in workers known to be overexposed to trichloroethylene. Improved methods of tissue analysis, giving greater sensitivity and reproducibility, would also help in determining the quantitative relationship between observed toxic effect on specific organs and levels of trichloroethylene in these organs. Trichloroethylene is known to affect the kidney. To determine the potential for human kidney damage resulting from workplace air exposure to trichloroethylene, urinary total protein and β_2 -microglobulin can be measured. To detect renal glomerular dysfunction, urinary total protein is analyzed by the Coomassie blue dye binding method using a protein assay. To detect renal tubular dysfunction, an enzyme immunoassay is used to measure β_2 -microglobulin (Nagaya et al. 1989b).

Methods for Determining Parent Compounds and Degradation Products in Environmental

Media. Existing methods for determining trichloroethylene in air and water, the media of most concern for human exposure, are sensitive, reproducible, and reliable for measuring background levels in the environment (see Table 6-1). These methods can also be used to measure levels of trichloroethylene and its metabolites at which health effects occur. Research investigating the relationship between levels measured in air and water and observed health effects could increase our confidence in existing methods and/or indicate where improvements are needed. Methods for solid matrices vary in accuracy and precision depending on the method and the matrix (e.g., sludge, soil, sediment, building material). No detailed descriptions of methods specifically for soil were located. Soil analyses presumably were done using a method for solid waste (e.g., EPA Method 8010). Data specifically for soil might be useful in evaluating the reliability of soil data and in determining if additional methods are needed. Improved methods of detecting trichloroethylene in plants and foods, especially those with higher fat content, would aid in determining the contribution of trichloroethylene exposure from these sources. This would be especially important in determining the potential for contamination of populations living adjacent to hazardous waste sites and other potential sources of exposure to higher than background levels of trichloroethylene.

6.3.2 On-going 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 trichloroethylene 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.

A method of monitoring chemicals expired by rats exposed to trichloroethylene and other fumigants is being developed at the University of California, Davis. Investigators plan to determine possible relationships between chemicals produced and internal damage produced by exposure to trichloroethylene. Researchers at New York University Medical Center are investigating methods for detecting human chemical exposure *in vivo* using noninvasive sampling procedures.