6. ANALYTICAL METHODS

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

Toluene can be determined in biological fluids and tissues and breath using a variety of analytical methods. Representative methods are summarized in Table 6-1. Most analytical methods for biological fluids use headspace gas chromatographic (GC) techniques. Breath samples are usually collected on adsorbent traps or in sampling bags or canisters, and then analyzed by GC.

Because of its volatility, toluene is lost from biological samples, such as plant and animal tissue and body fluids, relatively easily. Therefore, samples must be collected and stored with care (e.g., at low temperatures in sealed containers) to prevent analyte loss. Blood samples are best stored at 4 EC or below, in full containers made from glass, Teflon, or aluminum components (Gill et al. 1988; Saker et al. 1991). Storage time must be limited to minimize losses. Contamination can occur during sampling or analysis since toluene is widely used as a solvent. Care must be taken to monitor for contamination.

Headspace techniques are usually used to separate toluene from biological fluids such as blood and urine. The headspace method involves equilibrium of volatile analytes such as toluene between a liquid and solid sample phase and the gaseous phase. The gaseous phase is then analyzed by GC. There are two main types of headspace methodology: static (equilibrium) headspace and dynamic headspace which is usually called the "purge and trap" method (Seto 1994). The static headspace technique is relatively simple, but may be less sensitive than the purge-and-trap method. The purge-and-trap method, while providing increased sensitivity, requires more complex instrumentation and may result in artifact

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Lyse; extraction with carbon disulfide	GC/FID	No data	No data	Benignus et al. 1981
Blood	Purge and trap	No data	7.5 µg/L	No data	Cocheo et al. 1982
Whole blood	Purge and trap	GC/MS	0.088 µg/L	91–147	Ashley et al. 1992
Blood	Purge and trap	cap. GC/FID	50 ng/L	50	Fustinoni 1996
Blood	Headspace extraction	cap. GC/ITD	0.04 µmol/L	No data	Schuberth 1994
Mother's milk	Purge and trap	cap. GC/FID	No data	63 (chloro-benzene)	Michael et al. 1990a Pellizzari et al. 1982
Urine	Purge and trap	cap. GC/FID	50 ng/L	59	Fustinoni 1996
Urine	Heated headspace extraction	cap. GC/FID	1 ng/mL	42.3	Lee et al. 1998b
Biofluids	Headspace extraction	GC/FID	No data	No data	Suitheimer et al. 1982
Adipose tissue	Evaporation at 150 EC into nitrogen, direct gas injection	GC/FID	No data	88–112	Carlsson and Ljungquist 1982
Brain tissue	Extraction with carbon disulfide; homogenization; centrifugation	GC/FID	No data	No data	Benignus et al. 1981
Breath	Collection in modified Haldane-Priestly tube; transfer to adsorption tube; thermal desorption	cap. GC/MS	1 nmole	No data	Dyne et al. 1997
Breath	Collection via spirometer into passivated canisters	cap. GC/MS	low µg/m^3	80–136	Thomas et al. 1991
Breath	Collection via spirometer into 1.8 L passivated canisters	cap. GC/MS	~2 µg/m^3	91–104	Thomas et al. 1992
Breath	Collection via spirometer onto charcoal traps; microwave desorption	cap. GC/MS-SIM	3 µg/m^3	No data	Riedel et al. 1996

cap. = capillary; FID = flame ionization detector; GC = gas chromatography; ITD = ion trap detection; MS = mass spectrometry; SIM = selected ion monitoring

formation (Seto 1994). Packed columns and capillary columns are used for chromatographic separation, followed by identification and quantitation using various detectors; flame ionization detection (FID), photoionization detection (PID), and mass spectrometry (MS) are used most often. Other sample preparation methods have been used, but less frequently. Solvent extraction permits concentration, thereby increasing sensitivity, but the extraction solvent can interfere with analysis. Direct aqueous injection is a very rapid method, but sensitivity is low and matrix effects can be a serious problem.

A sensitive and reliable method for identification and quantitation of toluene in samples of whole blood taken from humans following exposure to volatile organic compounds (VOCs) has been developed by Ashley and coworkers at the Centers for Disease Control and Prevention (Ashley et al. 1992, 1996). The method involves purge-and-trap of a 10 mL blood sample with analysis by capillary GC/MS. Anti-foam procedures were used, as well as special efforts to remove background levels of VOCs from reagents and equipment (Ashley et al. 1992). The method is sensitive enough (ppt levels) to determine background levels of VOCs in the population and provides adequate accuracy (91–147% recovery) and precision (12% RSD) for monitoring toluene in the population. Most modern purge and trap methods provide detection limits in the ppt range for toluene in both blood and urine (Ashley et al. 1992; Fustinoni et al. 1996).

Few methods are available for the determination of methylbenzene in other body fluids and tissues. Toluene may be extracted from biological materials using solvents such as carbon disulfide (Benignus et al. 1981); homogenization of tissue with the extractant and lysing of cells improves extraction efficiency. Care must be taken to avoid loss of low-boiling compounds. Highly purified solvents may be used to minimize problems with solvent impurities. A modified dynamic headspace method for urine, mother's milk, and adipose tissue has been reported (Michael et al. 1980). Volatiles swept from the sample are analyzed by capillary GC/FID. Acceptable recovery was reported for model compounds, but detection limits were not reported (Michael et al. 1980). Supercritical fluid extraction using pure carbon dioxide or carbon dioxide with additives has good potential for the extraction of organic analytes such as toluene from biological samples.

Sensitive, reliable methods are available for measuring toluene in breath. Exhaled breath is collected in modified Haldane-Priestly tubes (Dyne et al. 1997), into passivated canisters (Thomas et al. 1992), or directly onto adsorbent traps (Riedel et al. 1996). The detection limits are in the low $\mu g/m^3$ range (Riedel et al. 1996; Thomas et al. 1991, 1992); accuracy, where reported, is good ($80%) (Riedel et al. 1996; Thomas et al. 1991, 1992).

Representative methods for determination of biomarkers of exposure to toluene are shown in Table 6-2. Measurement of toluene in blood (Kawai et al. 1993), urine (Kawai et al. 1996) and exhaled air (Lapare et al. 1993) provide reliable markers of exposure to toluene. Measurement of toluene metabolites is also utilized for monitoring toluene exposure in humans. Hippuric acid is formed in the body by the metabolism of toluene, and it is the glycine conjugate of benzoic acid. High performance liquid chromatography (HPLC) with ultraviolet (UV) detection is usually used for determination of hippuric acid in urine (Kawai et al. 1993; NIOSH 1984a). Other metabolites such as o-cresol (Kawai et al. 1996), benzylmercapturic acid (BMA) (Maestri et al. 1997), or S-p-toluylmercapturic acid (Angerer 1998a, 1998b) may also be measured. o-Cresol is a sensitive marker, but may arise from compounds other than toluene; the usefulness of BMA may be limited by variability among individuals.

6.2 ENVIRONMENTAL SAMPLES

Methods are available for determining toluene in a variety of environmental matrices. A summary of representative methods is shown in Table 6-3. Validated methods, approved by agencies and organizations such as EPA, ASTM, APHA, and NIOSH, are available for air, water, and solid waste matrices. GC is the most widely used analytical technique for quantifying concentrations of toluene in environmental matrices. Various detection devices used for GC include FID, MS, and photoionization detection (PID). Because of the complexity of the sample matrix and the usually low concentrations of VOCs in environmental media, sample preconcentration is generally required prior to GC analysis. Air samples may be collected and concentrated on adsorbent or in canisters for subsequent analysis. Methods suitable for determining trace amounts of methylbenzene in aqueous and other environmental media include three basic approaches to the pretreatment of the sample: gas purge-and-trap technique, headspace gas analysis, and extraction with organic solvent. Purge-and-trap is the most widely used method for the isolation and concentration of VOCs in environmental samples (Lesage et al. 1993). The purge and trap technique offers advantages over other techniques in that it allows facile isolation and concentration of target compounds, thereby improving overall limits of detection and recovery of sample.

Sampling techniques for air include collection in sample loops, on adsorbent, in canisters, and by cryogenic trapping. The analysis is normally performed by GC/FID, GC/PID, or GC/MS. Detection limits depend on the amount of air sampled, but values in the ppt range have been reported (Dewulf and Van Langenhover 1997).

Table 6-2. Analytical Methods for Determining Biomarkers of Toluene in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (Toluene)	Headspace	GC	No data	No data	Kawai et al. 1993
Urine (Toluene)	Headspace	GC/FID	2 µg/L	No data	Kawai et al. 1996
Urine (HA)	Extraction with ethyl acetate; evaporation; redissolve in water	HPLC/UV	30 mg/L	No data	NIOSH 1984b
Urine	Extraction with MTBE, elution with phosphate buffer/methanol/ formaldehyde	HPLC	0.1 mmol	101	Tardif et al. 1989
Urine (*o*-Cresol)	Hydrolysis; solvent extraction	HPLC/UV	0.5 mg/L	95[a]	Kawai et al. 1996
Urine (BMA)	Adsorbent column cleanup; derivatization	HPLC/Fl	0.5 µg/L	No data	Maestri et al. 1997
Breath	Collection in Tedlar bags	GC/FID	No data	No data	Lapare et al. 1993

[a]Extraction efficiency

BMA = benzylmercapturic acid; FID = flame ionization detector; Fl = fluorescence detector; GC = gas chromatography; HA = hippuric acid; HPLC = high performance liquid chromatography; MTBE = methyl tertiary butyl ether; UV = ultraviolet detection

Table 6-3. Analytical Methods for Determining Toluene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Workplace air	Sorption on activated carbon; extraction with carbon disulfide	GC/FID	0.01 mg	±11.4[a]	NIOSH 1994 Method 1501
Air	Sorption onto Tenax®; solvent extraction; thermal desorption	GC/MS	<0.88 ppb	111–163	Crist and Mitchell 1986
Air	Sorption onto Tenax®; thermal desorption	GC/MS	No data	93–94	EPA 1988a Method TO-1 Krost et al. 1982
Air	Collection in passivated canisters	GC/MS	low ppb	No data	EPA 1988b Method TO-14
Air	Collection on multisorbent tubes; thermal desorption	GC/MS	No data	No data	EPA 1997a Method TO-17
Air	Collection in sorbent sampler tubes	GC/FID	0.01 mg/sample	94	USEPA, EMMI 1997 NIOSH 1500
Air	Sorption on activated charcoal; extraction with carbon disulfide	GC/FID	0.01 mg/sample	No data	USEPA, EMMI 1997 NIOSH 4000
Stack gas effluents	Sorption onto Tenax®; thermal desorption	GC/MS	No data	50–150	USEPA, EMMI 1997 OSW 5041A
Vehicle exhaust	Direct	GC/FID	0.5 ppb	No data	Dearth et al. 1992
Drinking water	Purge and trap	cap. GC/PID	0.01–0.02 ppb	98–99	EPA 1991a Method 502.2
Drinking water	Purge and trap	GC/PID	0.02 ppb	94	EPA 1991b Method 503.1
Drinking water	Purge and trap	cap. GC/MS	0.08–0.11 ppb	100–126	EPA 1992a Method 524.2

Table 6-3. Analytical Methods for Determining Toluene in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water/ waste water	Purge and trap	GC/PID	0.2 ppb	77	EPA 1982b Method 602
Water/ waste water	Purge and trap	GC/MS	6.0 ppb	98–101	EPA 1982c Method 624
Water/ waste water	Addition of isotopically labeled analog; purge and trap	GC/MS	10 ppb	No data	EPA 1984 Method 1624
Industrial effluents	Purged with inert gas onto Tenax®; thermally desorbed	GC/IDMS	20 ppb	No data	Colby et al. 1980
Drinking water, waste water	Purged with inert gas onto Tenax®; thermally desorbed, cryofocused	GC/MS	1 ppb	74–107	Michael et al. 1988
Groundwater	Solid-phase microextraction	GC/FID	2 ppb	No data	Arthur et al. 1992
Water	Purge and trap	GC/MS	0.047 ppb	106	USEPA, EMMI 1997 APHA 6210-B
Water	Direct aqueous injection	GC	1.0 ppm	No data	USEPA, EMMI 1997 ASTM D3695
Water	Purge and trap	GC	0.5 ppb	80–120	USEPA, EMMI 1997 APHA 6220-B
Water	Dilution in appropriate solvent	FS	2.1 ppm	No data	USEPA, EMMI 1997 ASTM D4763
Groundwater, aqueous sludges, waste solvents, acid and caustic liquors, soils, sediments	Purge and trap	GC/MS	5 ppb	47–150	USEPA, EMMI 1997 OSW8240B-W

Table 6-3. Analytical Methods for Determining Toluene in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater, aqueous sludges, waste solvents, acid and caustic liquors, soils, sediments	Purge and trap or direct injection	GC/EC or GC/PID	0.01 ppb	99	USEPA, EMMI 1997 OSW 8021B-PID
Solid waste	Purge-and-trap	cap. GC/PID	0.01 ppb	99	EPA 1996a Method 8021B
Solid waste	Purge-and-trap	GC/PID	0.08–0.11 ppb	100–102	EPA 1996b Method 8260B
Soil	Methanol extraction; SPE	cap. GC	sub-ppm	>90	Meney et al. 1998
Soil (screening)	Filter	immunoassay	7 ppm	No data	EPA 1996c Method 4030
Soils and sediments	Headspace extraction	GC/PID	0.2 ppb	46–148	USEPA, EMMI 1997 OSW 8020A
Soids and other solid matrices	Headspace extraction	GC/FID GC/PID/ELCD	No data	No data	USEPA, EMMI 1997 OSW 5021
Solid waste matrices	Purge and trap or direct aqueous injection or concentration by azeotropic distillation or automated static headspace	GC/MS	0.11 ppb	102	USEPA, EMMI 1997 OSW 8260B
Plant cuticle	Headspace extraction	GC/FID	No data	No data	Keymeulen et al. 1997
Food	Headspace extraction, 1 hour at 90 EC	GC	No data	No data	Walters 1986

Table 6-3. Analytical Methods for Determining Toluene in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Foods	Purge and trap	cap. GC/MS	8 ppb	54–76[b]	Heikes et al. 1995
Bottled water	Headspace extraction	GC/MS	0.5-1 ppb	No data	Page et al. 1993
Olive oil	Homogenization; headspace	cap. GC/MS	No data	No data	Biedermann et al. 1995

[a]Reported accuracy
[b]Intralaboratory accuracy. Single lab accuracy is reported as 100–106% recovery.

cap. = capillary; FID = flame ionization detector; FS = fluorescence spectroscopy, ELCD; GC = gas chromatography; IDMS = isotope dilution mass spectrometry; MS = mass spectrometry; PID = photoionization detector; SPE = solid-phase extraction

Toluene may be determined in occupational air using collection on adsorbent tubes, solvent desorption and GC/FID analysis (NIOSH 1994). Detection limits depend upon the amount of air sampled; accuracy is very good (11.4% bias) (NIOSH 1994). Passive samplers are also used (Ballesta et al. 1992; Periago et al. 1997); however, little performance data are available.

Gas purge and trap is the most widely used method for the isolation and concentration of VOCs in environmental samples (Lesage et al. 1993). The purge and trap technique offers advantages over other techniques in that it allows facile isolation and concentration of target compounds, thereby improving overall limits of detection and recovery of sample. Detection limits of less than 1 µg of methylbenzene per liter of sample have been achieved. Very low detection limits for drinking water are reported for the purge and trap method with GC/PID (0.01–0.02 ppb) (EPA 1991a, 1991b). Accuracy is very good (94–99% recovery) (EPA 1991a, 1991b). While the analytical method is selective, confirmation using a second column or GC/MS is recommended (EPA 1992a). Good sensitivity (0.08–0.11 ppb) and accuracy (100–126% recovery) can also be obtained using capillary GC/MS detection (EPA 1992a). Purge-and-trap methodology may be applied to waste water as well (EPA 1982b, 1982c, 1984). Sensitivity is in the low ppb range and recovery is good (77–101%) (EPA 1982b, 1982c, 1984).

Soil, sediment, and solid waste are more difficult to analyze. Volatilization during sample handling and homogenization can result in analyte loss. Purge-and-trap methods with capillary GC/PID or GC/MS analysis provide detection limits of approximately 0.5 ppm for wastes and 5 µg/g for soil and sediment (EPA 1982b, 1982c, 1984).

No methods were found for the determination of toluene in fish and biota. Few methods are available for the determination of toluene in food. A purge and trap extraction method is available for determining toluene in a variety of foods. The quantitation limit is 8 ppb; single lab recovery is very good (100–106%) and precision is good (9.8–25% RSD). Both intra- and inter-laboratory studies were conducted, and precision was found to be #25% RSD (Heikes et al. 1995).

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 toluene 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 toluene.

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. Although toluene and its metabolites can be measured in body fluids using a number of techniques (Kawai et al. 1993, 1996; NIOSH 1984a,), the metabolites have limited value as biomarkers. A number of common food materials produce the same metabolites, thus measurement of toluene metabolites can be used to confirm a known exposure but cannot be used to determine whether or not exposure occurred in a poorly defined situation. It is also very difficult to quantify the magnitude of exposure from levels of either toluene or its metabolites in biological samples. Despite these limitations, end-of-shift concentrations of 2.5 g hippuric acid/g creatinine and 1.0 mg/L toluene *in venous* blood have been established as biological exposure indices for toluene (ACGIH 1992a). A technique that could accurately quantify exposure to toluene would be useful.

For occupational health monitoring and animal studies, there is also a need for improved and more sensitive methods to determine toluene metabolites. Additional work to develop sensitive methods for analysis of the cresol metabolites and to correlate these metabolites with specific exposure conditions would be valuable.

It is equally difficult to monitor the effects of toluene exposure. Magnetic resonance imaging (MRI) and BAER evaluations of the brain have some value in determining the neurological damage resulting from long-term exposures to high levels of toluene, but have no known value for determining the effects of low-level and/or short-term exposures.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. There are methods available for the determination of toluene and its metabolites in environmental samples. Sensitive techniques for air allow detection of toluene at levels as low as 0.88 ppb (Crist and Mitchell 1986). In drinking water, detection limits of 0.01–0.02 µg/L are possible using GC/MS or GC/PID (EPA 1991a, 1991b). For waste water, detection limits in the low ppb range are achievable (EPA 1982b, 1982c, 1984). These techniques are adequate to measure both background toluene levels and the levels of toluene in environmental media that could cause health effects. However, when toluene is present in combination with other volatile materials, interference from the companion volatiles often raises the detection limit and decreases the accuracy and precision of the technique. Improved methods for separation of toluene from other volatiles would be useful.

Very little work has been conducted on measuring the levels of toluene metabolites in the environment. Although techniques for measuring these substances exist, they have not routinely been applied to environmental media. Research on measuring the levels of metabolites in soil and water would be valuable especially in studying the end products of microbial degradation. Few methods are available for monitoring toluene in foods; reliable methods are needed for evaluating the potential for human exposure that might result from toluene ingestion.

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

The U.S. EPA is conducting a pilot program for comprehensive monitoring of human exposure. The National Human Exposure Assessment Study (NHEXAS) is being conducted in three regions of the United States in order to establish relationships between environmental concentrations, exposure, dose, and health response and to determine the incidence and causes of high exposures, especially for biologically susceptible persons. One of the aims of the pilot study is to test measurement methodology for a variety of pollutants, including toluene, in air and water. As an adjunct to this pilot study, the U.S. EPA and the State of Minnesota are conducting a study of children's exposure to toxic chemicals, including toluene.