

7. ANALYTICAL METHODS

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

Analytical methods have been developed to measure benzene levels in exhaled breath, blood, and various body tissues. The primary method of analyzing for benzene in exhaled breath, body fluids, and tissues is gas chromatography (GC) coupled with either flame ionization detection (FID), photoionization detection (PID), or mass spectrometry (MS). Rigorous sample collection and preparation methods must be followed when analyzing for benzene to prevent contamination of the sample. A summary of commonly used methods of measuring benzene in biological samples is presented in Table 7-1.

Breath samples are collected on a solid sorbent (EPA 1987b; Gruenke et al. 1986; Pellizzari et al. 1988; Wallace et al. 1985), in canisters (Thomas et al. 1991), or in a breath sampling tube and analyzed directly (Sherwood and Carter 1970). Samples collected on Tenax sorbent are subjected to a thermal desorption/cryofocussing step prior to analysis by capillary GC/MS (EPA 1987b; Pellizzari et al. 1988; Wallace et al. 1985). Techniques involving headspace analysis of benzene adsorbed on silica gel have also been used (Gruenke et al. 1986). MS detection generally provides the most sensitivity, from the low to sub-ppb. The selectivity of the methods is improved if capillary GC columns are used (Pellizzari et al. 1988). Extraction of benzene from blood is frequently accomplished by either purge-and-trap or headspace analysis. In purge-and-trap analysis, an inert gas such as helium or nitrogen is passed through the sample, and purged volatiles are trapped on an appropriate solid sorbent (Antoine et al. 1986; Ashley et al. 1992, 1994; Michael et al. 1980). Recent improvements in the method have resulted in excellent sensitivity (300 ppt) and acceptable precision and accuracy (Ashley et al. 1992, 1994). The purge-and-trap method has also been used to analyze breast milk for other volatile organic compounds and could be

7. ANALYTICAL METHODS

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath	Collection on Tenax GC; thermal desorption	HRGC/MS (IARC Method 5)	3 ppt	70–130 estimated	Pellizzari et al. 1988
Breath	Collection in sampling tube; direct injection of sample	GC/FID	100 ppb	100	Sherwood and Carter 1970
Breath	Collection on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/MS	1.6 ppb (5-L sample)	86–90	Wallace et al. 1986, 1985
Breath	Collection in bags, adsorption on silica gel; desorption to headspace vial; analysis of headspace gases	GC/MS-SIM	0.1 ppb	NR	Gruenke et al. 1986
Blood	Purge and trap	HRGC/MS	30 ppt	112–128	Ashley et al. 1992; 1994
Blood	Heparinization; transfer to isotonic saline in headspace vial; equilibration with heat	HRGC/PID	0.4 µg/L	NR	Pekari et al. 1989
Blood	Collection and transfer to headspace vial; analysis of headspace gases	GC/MS-SIM	2 µg/L	NR	Gruenke et al. 1986
Blood	Purging with nitrogen; collection on Tenax GC-silica gel	GC/MS	0.5 µg/L	NR	Antoine et al. 1986
Blood	Extraction with toluene + HCl; centrifugation; analysis of toluene layer	GC/FID	100 µg/L	98–100	Jirka and Bourne 1982
Blood	Extraction device coupled to MS	ITMS	90 ppt	NR	St-Germain et al. 1995
Blood	Centrifugation; dialysis; precipitation; acidic hydrolysis; Sep Pak cartridge column purification	HPLC	20 pmol/g globin	NR	Hanway et al. 2000
Urine	Incubation; analysis of headspace gases	GC/PID	0.51 nmol/L	>90	Kok and Ong 1994
Tissues (bone marrow, fat)	Homogenization with internal standard; centrifugation; analysis of supernatant	GC/MS-SIM	NR	NR	Rickert et al. 1979
Tissues (lung, liver)	Homogenization in buffer; centrifugation; analysis of supernatant	RID-preparative HPLC/UV	20 pg/g	NR	Bechtold et al. 1988

FID = flame ionization detection; GC = gas chromatography; HCl = hydrochloric acid; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; ITMS = ion trap mass spectrometry; MS = mass spectrometry; NR = not reported; PID = photoionization detection; RID = reverse isotope dilution; SIM = selected ion monitoring; UV = ultraviolet detection

7. ANALYTICAL METHODS

used for analyzing benzene (Michael et al. 1980; Pellizzari 1982). For headspace analysis, the samples are placed in a special vial, and the gas generated above the liquid sample under equilibrium conditions is analyzed (Gruenke et al. 1986; Pekari et al. 1989). Sensitivity is in the sub- to low-ppb range. A third method of sample preparation involves extraction of the blood sample with an organic solvent (Jirka and Bourne 1982) and analysis of the organic fraction. These methods are generally less sensitive, with reported detection limits usually in the low- to mid-ppb range. Selectivity is improved with use of high resolution gas chromatography (HRGC). Accuracy and precision could not be adequately compared given the limited data available.

Screening methods are available for analysis of benzene in feces and urine (Ghoos et al. 1994) and body fluids (Schuberth 1994). Both employ analysis by capillary GC with an ion trap detector (ITD). Benzene in urine has been determined by trapping benzene stripped from the urine on a Carbotrap tube, followed by thermal desorption GC/FID. Care must be taken when preparing benzene metabolite samples from urine and bodily fluids in order to protect against enzymatic and oxidative degradation. These samples are often treated to denature enzymes and avoid oxidation by hydroquinone. The detection limit is 50 ng/L and the average recovery is approximately 82% (Ghittori et al. 1993). Benzene in urine has also been determined using headspace analysis with capillary GC/PID. The detection limit is 40 ng/L (Kok and Ong 1994).

Methods are also available for determining metabolites of benzene in urine. A summary of available methods is shown in Table 7-2. Both GC/FID or GC/MS and high-performance liquid chromatography (HPLC) with ultraviolet detection (UV) have been used to measure urinary metabolites.

The primary metabolite of benzene is phenol. Phenol is excreted as glucuronide and sulphate conjugates in urine. Total phenolic metabolites (phenol, phenyl sulfate, and phenyl glucuronide) have been determined by hydrolyzing urine samples either enzymatically or by acid, then extracting the phenol with solvent. Phenol is then measured by GC or HPLC techniques. Enzymatic hydrolysis coupled with GC/FID has been reported; the detection limit is 1 mg/L and recovery is excellent (92–98%) (IARC 1988). Sulfate and glucuronide conjugates have been determined directly by HPLC/UV (Ogata and Taguchi 1987). The normal baseline levels of urinary phenolic metabolites from humans are usually 2–18 mg/L (Ong and Lee 1994). The available methods are sensitive enough to measure these relatively high amounts accurately.

7. ANALYTICAL METHODS

Table 7-2. Analytical Methods for Determining Metabolites of Benzene in Urine

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (phenol, phenyl sulfate, phenyl glucuronide)	Centrifugation	HPLC/UV	4 mg/L (PS); 5 mg/L (PG)	100.5 (PS); 101.8 (PG)	Ogata and Taguchi 1987
Urine (phenol, phenyl sulfate, phenyl glucuronide)	Digestion (enzymatic and with acid); extraction with diethyl ether	GC/FID (IARC Method 6)	1 mg/L	92–98	IARC 1988
Urine (phenols and cresols)	Hydrolysis with perchloric acid; extraction with diisopropyl ether	GC/FID	NR	NR	NIOSH 1974
Urine (phenols and cresols)	Hydrolysis with perchloric acid; saturation with NaCl; extraction with diisopropyl ether	GC/FID	2 mg/L	NR	Roush and Ott 1977
Urine (phenol)	Enzymatic reaction	HPLC/fluorometric detection	50 ppb	97.7	Jen and Tsai 1994
Urine (<i>trans,trans</i> -muconic acid)	Mixing with methanol; centrifugation	HPLC/UV	0.1 mg/L	NR	Inoue et al. 1989
Urine (muconic acid, phenol)	Mixing with formic acid; extraction (twice) with ethyl ether; evaporation of combined extracts	GC/MS	10 µg/L	NR	Bechtold et al. 1991
Urine (<i>trans,trans</i> -muconic acid)	Cleanup on anion exchange resin; derivitization	GC/MS	0.01 mg/L	93–106	Ruppert et al. 1995
Urine (S-phenylmercapturic acid)	Solid-phase extraction; hydrolysis; derivitization	HPLC	1 µg/L	NR	Einig and Dehnen 1995
Urine (<i>trans,trans</i> -muconic acid)	Cleanup on anion exchange resin	HPLC/UV	5 ppb	NR	Weaver et al. 2000

FID = flame ionization detection; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; MS = mass spectrometry; NaCl = sodium chloride; NR = not reported; PG = phenyl glucuronide; PS = phenyl sulfate; UV = ultraviolet detection

7. ANALYTICAL METHODS

Analysis of urinary *trans,trans*-muconic acid seems to be a better indicator than phenol for assessing exposure to low levels of benzene (Ducos et al. 1990). However, muconic acid is a minor metabolic route and background levels of muconic acid in urine are much lower than levels of phenolic metabolites and are frequently below the limit of detection of the method used to determine them (Inoue et al. 1989b). The detection of low levels of *trans,trans*-muconic acid in urine was difficult by earlier methods because of low recovery of *trans,trans*-muconic acid (37% with ether) by the commonly used solvent extraction method (Gad-El-Karim et al. 1985). An improved method for the determination of urinary *trans,trans*-muconic acid utilizes solid phase extraction with SAX sorbent in combination with the HPLC/UV for quantitation. The detection limit is 0.06–0.1 mg/L, and recovery is very good (90%) (Boogaard and van Sittert 1995; Ducos et al. 1990). Weaver et al. (2000) used an anion exchange column to extract the urine sample, which was then analyzed using HPLC-UV; detection limits were around 5 ppb. The relative standard deviation of the method was 5% in the concentration range 1–20 ng/L. *trans,trans*-muconic acid has been determined directly by HPLC/UV with similar sensitivity (detection limit=0.1 mg/L) (Inoue et al. 1989b). The detection limit and specificity for the determination of urinary *trans,trans*-muconic acid may be improved by using HPLC with diode array detector, GC/FID of the methylated product, or GC/MS of trimethylsilylated product (Bartczak et al. 1994). Both GC/FID and HPLC/diode array detection are capable of detecting urinary *trans,trans*-muconic acid at concentrations above 40 µg/L, but GC/MS is capable of detecting the metabolite at concentrations below 40 µg/L (Bartczak et al. 1994).

The metabolite, S-phenyl-mercaptic acid, may be an indicator of exposure to benzene. It can be detected at low levels (1 µg/L) in urine using solid phase extraction and determination by HPLC (Einig and Dehnen 1995). After purification by reverse phase cartridge chromatography, S-phenyl cysteine was detected with sensitivity of about 20 pmol/g globin using HPLC (Hanway et al. 2000).

7.2 ENVIRONMENTAL SAMPLES

Methods exist for determining benzene in air (ambient, occupational, and industrial), water, sediment, soil, foods, cigarette smoke, gasoline, and jet fuel. Most involve separation by GC with detection by FID, PID, or MS. HPLC/UV and spectrophotometry have also been used. Table 7-3 summarizes several of the methods that have been used to analyze for benzene in environmental samples.

Numerous methods exist for detecting and measuring benzene in ambient air. Air samples for benzene analysis may be preconcentrated by passing the sample through a trap containing a solid adsorbent (Bayer et al. 1988; EPA 1979, 1980a; Fung and Wright 1986; Gruenke et al. 1986; Harkov et al. 1985; Reineke

7. ANALYTICAL METHODS

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sample trapped on silica gel; thermal desorption	GC/MS	0.1 ppb	88–105	Gruenke et al. 1986
Air	Cryogenically trap; thermal desorption	GC/FID	NR	85–115	Singh et al. 1985
Air	Direct on-line analysis	GC/FID	NR	NR	Bayer et al. 1988
Air (ambient)	Direct injection of ambient air	GC/PID	0.25 ppb	NR	Clark et al. 1984
Air (ambient)	Direct analysis of ambient air	Electrochemical	NR	NR	Stetter et al. 1986
Air (ambient)	Sample collection in Tedlar bag; Cryogenically trap; thermally desorb	GC/PID	0.5 ppb	NR	Kowalski et al. 1985
Air (ambient)	Sample collection in stainless steel canisters or sorbent tubes; trap cryogenically; thermal desorption	HRGC/PID or HRGC/FID	5 ppt (PID) 24 ppt (FID)	97–104 (PID) 96–104 (FID)	Reineke and Bächmann 1985
Air (ambient)	Collect on charcoal (vapor badge or tube); desorb with carbon disulfide	GC/FID	0.3 ppb (estimated)	NR	Fung and Wright 1986
Air (ambient)	Cryogenically trap; thermal desorption	GC/PID/FID	1 ppt	70–130	Nutmagul and Cronn 1985
Air (ambient)	Collection in canisters; preconcentration using 2-stage trap	HRGC/ITD	sub-ppb level	8–13% bias	Kelly et al. 1993
Air (ambient)	Direct analysis	ALMS	250 ppb	NR	EPA 1985d
Air (ambient)	Sample collection onto on-column cryogenic sample loop or into stainless steel containers	GC/PID/FID	1 ppt	70–130	Nutmagul and Cronn 1985
Air (ambient)	Sample trapped on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC	<0.1 ppb	69–126	EPA 1979
Air (ambient)	Sample collected on Tenax GC; thermal desorption to on-column cryogenic trap	GC/FID	0.03 ppb	56–144	EPA 1980a
Air (ambient)	Sample trapped on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/FID; conf. by HRGC/MS	3 ppt	NR	Roberts et al. 1984
Air (consumer products)	Sample trapped on solid sorbent; thermal desorption	GC/MS	NR	NR	Bayer et al. 1988

7. ANALYTICAL METHODS

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (at waste sites and landfills)	Sample trapped on Tenax GC; thermal desorption	HRGC/FID/ECD; conf. by HRGC/MS	0.05 ppb	NR	Harkov et al. 1985
Air (occupational)	Sample trapped on charcoal; desorption with carbon disulfide	GC/FID (NIOSH Methods 1500 and 1501)	10–100 ppb	NR	NIOSH 1984
Air (occupational)	Sample collection on silica gel; desorption with ethanol	GC/FID	100 ppb	90	Sherwood and Carter 1970
Air (occupational)	Sample collection in Tedlar bag; direct injection	GC/PID (NIOSH Method 3700)	10 ppb	NR	NIOSH 1994
Air (occupational)	Sample collection on charcoal disk in miniature passive dosimeter; thermal desorption	GC/PID	60 ppb	85–115	Gonzalez and Levine 1986
Air (occupational; jet fuel fumes)	Sample collection on charcoal; desorption with methyl chloride-ethyl acetate	HPLC/UV	0.08 ppm	94–112	Dibben et al. 1989
Soil air	Sample collection on activated charcoal; desorption with carbon disulfide	GC/FID	NR	97–100	Colenutt and Davies 1980
Drinking water	Purge and trap	GC/MS	0.2 µg/L	NR	Brass et al. 1977
Drinking water	Purge and trap	HRGC/MS (EPA Method 524.2)	0.03–0.04 µg/L	97–99	EPA 1992a
Water	Purge and trap onto Tenax GC; thermal desorption to on-column cryogenic trap	HRGC/MS	0.1–10 µg/L	74–78	Michael et al. 1988
Water	Purge and trap on Tenax GC; thermal desorption to on-column cryogenic trap	HRGC	<1 µg/L	69–126	EPA 1979
Water	Purge and trap onto Tenax GC; thermal desorption	GC/MS	NR	85–125	Harland et al. 1985
Water	Solvent extraction with dichloromethane; concentration	GC/MS	15 µg/L	NR	Sporstøl et al. 1985
Water	Purge and trap on adsorbent column; thermal desorption	GC/PID (EPA Method 602)	0.2 µg/L	106	NEMI 2005a
Water	Purge and trap on activated carbon; desorption with carbon disulfide	GC/FID; conf. by GC/MS	NR	96–99	Colenutt and Thorburn 1980
Water	Purge and trap on Tenax; thermal desorption	GC/FID	0.001 µg/L	94–111	Hammers and Bosman 1986

7. ANALYTICAL METHODS

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Permeation of benzene through a silicone polycarbonate membrane into an inert gas stream	GC/FID	7.2 µg/L	NR	Blanchard and Hardy 1986
Waste water	Purge and trap onto adsorbent column; thermal desorption	GC/MS (EPA Method 624)	4.4 µg/L	113	NEMI 2005b
Waste water	Addition of isotopically labeled benzene analog; purge and trap onto adsorbent column; thermal desorption	GC/IDMS (EPA Method 1624)	10 µg/L	65–141	NEMI 2005c
Water, industrial effluents	Purge and trap on Tenax; thermal desorption	GC/MS	<5 µg/L	95–106	Pereira and Hughes 1980
Landfill leachate	Purge sample and trap on Tenax-silica gel; thermally desorb	GC/FID/FID	1 µg/L	NR	EPA 1984b
Landfill leachate	Extract sample with pentane	GC/MS	1,000–10,000 µg/L	NR	Schultz and Kjeldsen 1986
Solid wastes	Purge and trap, direct injection, vacuum distillation, or headspace	PID (EPA Method 8021B)	0.009 µg/L	99	EPA 1994c
Soil	Sample mixed with NaOH solution; equilibration; analysis of headspace gases	HRGC/FID; conf. by HRGC/MS	0.02 ng/mL	75–98	Kiang and Grob 1986
Soil	Purge and trap on Tenax; thermal desorption to on-column cryogenic trap	HRGC	<0.1 ppb	69–126	EPA 1979
Soil	Purge and trap on Tenax; thermal desorption	GC/FID	1 ppt	52	Hammers and Bosman 1986
Soil	Supercritical fluid extraction	HRGC/FID	low ppb	77–81	Burford et al. 1994
Sediment and biota	Purge and trap on Tenax GC-silica gel; thermal desorption	HRGC/MS	NR	NR	Ferrario et al. 1985
Sediment	Purge and trap on Tenax; thermal desorption	GC/MS	NR	64	Harland et al. 1985
Fruits and vegetables	Mix with water and methanol; filter; distill azeotrope	GC/FID	NR	84–96	Kozioski 1985
Shellfish	Tissue homogenized; purge with inert gas and trap on Tenax GC-silica gel; thermally desorb	GC/MS	NR	NR	Ferrario et al. 1985

7. ANALYTICAL METHODS

Table 7-3. Analytical Methods for Determining Benzene in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Mainstream cigarette smoke	Collection on filters and impingers; [2H6]-benzene added to impinger	HRGC/IDMS-SIM	0.05 µg/cigarette	75–85 (trapping efficiency)	Byrd et al. 1990
Cigarette smoke	Mainstream smoke filtered and analyzed directly; side-stream smoke and smoke-polluted air filtered and collected in cryogenic methanol-filled impingers	HRGC/MS-SIM	0.1 µg/cigarette	NR	Brunnemann et al. 1989, 1990
Gasoline	Dilute sample with hexane	GC/FID	NR	NR	Poole et al. 1988
Gasoline	Dilute sample with methanol; elute benzene to analytical column with 50% methanol; back-flush guard column with 100% methanol	HPLC/UV	NR	NR	Ludwig and Eksteen 1988

ECD = electron capture detection; EPA = Environmental Protection Agency; FID = flame ionization detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IARC = International Agency for Research on Cancer; IDMS = isotope dilution mass spectrometry; ITD = ion trap mass spectrometry; LRS = laser Raman spectroscopy; MS = mass spectrometry; NaOH = sodium hydroxide; NIOSH = National Institute of Occupational Safety and Health; NR = not reported; PID = photoionization detection; SIM = selected ion monitoring; UV = ultraviolet detection

7. ANALYTICAL METHODS

and Bächmann 1985; Roberts et al. 1984). Commonly used adsorbents are Tenax resins (e.g., Tenax TA, Tenax GC), silica gel, activated carbon, and carbonaceous polymeric compounds. Benzene in ambient air can be collected in stainless steel canisters (EPA 1988a; Kelly et al. 1993) or Tedlar bags (Kowalski et al. 1985) and can be analyzed with or without preconcentration. Preconcentration of benzene can be accomplished by direct on-column cryogenic trapping (EPA 1985c; Kowalski et al. 1985; Nutmagul and Cronn 1985; Reineke and Bächmann 1985; Singh et al. 1985), or samples may be analyzed directly without preconcentration (Bayer et al. 1988; Clark et al. 1984).

The most common methods of analysis for benzene in air are GC/PID, GC/FID, and GC/MS. The limit of detection for GC/FID and GC/PID ranges from low ppb to low ppt. GC/MS is generally considered to be more reliable than GC/FID or GC/PID in identifying benzene in samples, particularly those containing multiple components having similar GC elution characteristics. Benzene has been quantified in ambient air samples at sub-ppb levels by GC/MS (Gruenke et al. 1986) and ion trap mass spectrometry (Kelly et al. 1993). The ion trap detector has the advantage of remaining largely unaffected by water vapor in the sample. A continuous monitoring instrument using Fourier transform infrared (FTIR) spectroscopy is being developed for measuring the levels of benzene or other toxic chemicals in exhaust emissions from hazardous waste incinerators (DOE 1992).

Several analytical methods are available for determining atmospheric levels of benzene in the workplace. The OSHA recommended procedure involves the collection of the sample vapors on charcoal adsorption tubes, and then desorption followed by GC/MS analysis (OSHA 1985). Samples desorbed from charcoal are also analyzed by GC/FID (NIOSH 1984) or HPLC/UV (Dibben et al. 1989). Detection limits are in the ppb range (Dibben et al. 1989; NIOSH 1984). Passive dosimeters are also utilized, with GC/PID quantitation; detection limits are in the ppb range (Gonzalez and Levine 1986). Other acceptable methods include portable direct reading instruments and real-time continuous monitoring systems; these methods generally have a sensitivity in the ppm range.

The most frequently used analytical methods for water samples containing benzene are GC/MS, GC/FID, and GC/PID (Blanchard and Hardy 1986; Colenutt and Thorburn 1980; DOI 1984; EPA 1984, 1992; Hammers and Bosman 1986; Harland et al. 1985; Lysyj et al. 1980; Michael et al. 1988; Pereira and Hughes 1980; Sporstol et al. 1985). Benzene is usually isolated from aqueous media by the purge-and-trap method (Brass et al. 1977; Colenutt and Thorburn 1980; DOI 1984; EPA 1979, 1984, 1992; Hammers and Bosman 1986; Harland et al. 1985; Michael et al. 1988). An inert gas such as nitrogen is used to purge the sample. The purged benzene is trapped on an adsorbent substance, such as Tenax GC

7. ANALYTICAL METHODS

or activated charcoal, and thermally desorbed. Recovery, where reported, ranges from acceptable ($\approx 70\%$) (EPA 1979, 1984; Michael et al. 1988) to very good ($\geq 90\%$) (Colenutt and Thorburn 1980; EPA 1984, 1992; Hammers and Bosman 1986). Detection limits in the sub-ppb to ppt range may be attained with HRGC/MS techniques (EPA 1992a); Michael et al. 1988). Liquid-liquid extraction procedures (Harrison et al. 1994; Schultz and Kjeldsen 1986; Sporstol et al. 1985) are less commonly used, having been replaced by more sensitive purge-and-trap methods. Interference from contamination can occur with all methods if extreme care is not used in the handling of samples and cleaning of all equipment.

Solid samples, such as soil, sediment, and foods, are most frequently prepared for analysis using the purge-and-trap method (EPA 1979, 1994c; Ferrario et al. 1985; Hammers and Bosman 1986; Harland et al. 1985), although supercritical fluid extraction has recently been utilized (Burford et al. 1994). Detection and quantitation of benzene may be GC/FID, GC/PID, or GC/MS. Detection limits as low as 1 ppt have been reported, but recoveries and precision have frequently been low. Improvements in the method, including analysis by HRGC/PID, have resulted in low detection limits (9 ppt) and excellent recovery (99%) for benzene (EPA 1994c). Screening methods are available for benzene; some may be used at field sites. Immunoassay may be used as a screening and semiquantitative tool (Van Emon and Gerlach 1995).

Methods exist for detection of benzene in other environmental media such as cigarette smoke, gasoline, and jet fuel and its fumes (Brunnemann et al. 1989; Byrd et al. 1990; Ludwig and Eksteen 1988; Poole et al. 1988). HPLC/UV, GC/FID, and GC/MS separation and detection techniques have been used for these analyses. Sensitivity and reliability of these methods cannot be compared because of the lack of data. Few methods have been reported for measurement of benzene in foods; performance data are generally lacking.

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 benzene 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 benzene.

7. ANALYTICAL METHODS

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. Methods exist for measuring benzene in breath (Gruenke et al. 1986; Pellizzari et al. 1988; Sherwood and Carter 1970; Wallace et al. 1986), blood (Antoine et al. 1986; Ashley et al. 1992, 1994; Gruenke et al. 1986; Jirka and Bourne 1982; Pekari et al. 1989), and tissues (Bechtold et al. 1988; Rickert et al. 1979). The methods for breath are sensitive and accurate for determining exposure levels of benzene at which health effects have been observed to occur, as well as for background levels in the general population. The methods are relatively precise and selective. Methods for determining benzene in blood are sensitive; based on the limited recovery data available, they appear to be accurate. More information on the performance obtained with different methods would be helpful. The application of GC/MS techniques to the analysis of blood specimens has resulted in a rapid, cost-effective, clinical screening test for common volatile organic compounds, including benzene (DeLeon and Antoine 1985). This test, the VOST (Volatile Organics Screening Test), has demonstrated the presence (down to 0.1 ppb) of a variety of toxic volatile organics in the blood of environmentally sensitive patients and has provided preliminary baseline concentration levels for the test population (DeLeon and Antoine 1985). The data on determination of benzene in urine and tissue samples are very limited. In general, the available methods have limits of detection that are too high to be useful in other than acute exposure situations. Methods that could be used to measure low levels in human tissues would be useful for determining the relationship between chronic low-level exposure and the effects observed in specific tissues.

Methods are available for measuring phenolic benzene metabolites in urine (Bechtold et al. 1991; IARC 1988; Jen and Tsai 1994; Jongeneelen et al. 1987; NIOSH 1974; Ogata and Taguchi 1987). Available methods for determining most benzene metabolites in urine are sufficiently sensitive and reliable to allow measurement of background concentrations in nonoccupationally exposed individuals. However, the phenolic metabolites are not unique to benzene. Improved methods to detect phenolic metabolites are not needed. Sensitive assays have been developed for detection of urinary *trans,trans*-muconic acid

7. ANALYTICAL METHODS

(detection limit 10 µg/L) (Bechtold et al. 1991; Ruppert et al. 1995). Since urinary *trans,trans*-muconic acid concentration can be correlated with benzene exposure, this may provide a useful biomarker of exposure on an individual basis (Bechtold et al. 1991; EPA 1992b; Weaver et al. 2000). In addition, information is needed to assess the effect of co-exposure to other chemicals (e.g., toluene) on urinary muconic acid levels. Also needed are specific biomarkers of cumulative exposure to benzene, based on albumin or hemoglobin adducts, and lymphocyte DNA adducts of N-7-phenylguanine. It would also be useful to develop specific biomarkers of acute- and chronic-duration exposure to benzene based on adducts of muconaldehyde. The levels of such biomarkers formed *in vivo* would be useful later for correlation with toxic effects of acute- or chronic-duration exposure to benzene. S-phenyl-mercapturic acid has also been useful in biological monitoring of benzene exposure in humans and animals. S-phenyl-mercapturic acid can be measured with a sensitivity of about 20 pmol/g globin using HPLC (Hanway et al. 2000). S-phenyl-mercapturic acid levels can also be correlated to environmental benzene exposure (Popp et al. 1994), which may indicate its utility as a biomarker.

Effect. Methods for determining benzene in breath, blood, and tissues and for determining its metabolites in urine could also be used as biomarkers of effect. However, efforts to correlate these measures with observed toxic effects of benzene exposure have been unsuccessful. Other biomarkers of effect (e.g., complete blood cell counts, red and white blood cell counts, chromosomal aberrations, sister chromatid exchanges, and examination of bone marrow) have been suggested for benzene, but they are not specific for benzene exposure. Further development of methods for determining reliable unique biomarkers of effect for benzene would be useful.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining benzene in air (Clark et al. 1984; Gruenke et al. 1986) and water (Brass et al. 1977; EPA 1979, 1984; Hammers and Bosman 1986; Pereira and Hughes 1980), the media of most concern for human exposure, are sensitive enough to measure background levels in the environment and levels at which health effects might occur. Their reliability is limited primarily by the ubiquitous presence of benzene in the environment, which makes contamination a constant problem. The accuracy and precision of some methods for water analyses (e.g., GC/MS) need to be improved to produce more reliable results. Methods for soil and other solid media appear to have the same problems as those for air and water. In addition, there is a lack of information on methods for determining benzene in media such as shellfish, fish, foods, and plants. Although exposure to benzene via ingestion of food is believed to be minimal, standardized methods for these media are needed to better assess the extent of benzene contamination in the environment and the resulting risk of exposure.

7. ANALYTICAL METHODS

7.3.2 Ongoing Studies

The information in Table 7-4 was found as a result of a search of the Federal Research in Progress database (FEDRIP 2005).

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 benzene 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.

7. ANALYTICAL METHODS

Table 7-4. Ongoing Studies on Benzene, Analytical Methods^a

Investigator	Affiliation	Research description	Sponsor
Fales, HM		Analysis of proteins, peptides, and metabolites for carcinogen exposure using mass spectrometry	USDA
Kalman, DA	University of Washington Seattle, Washington	Study of the use of human dosimetry for assessment of exposure to volatile compounds	NIEHS
Kavanaugh, TJ		Study of glutathione biosynthesis as a biomarker of toxic exposure	NSF
O'Brien, RJ		Assessment Environmental Health Breath using PF-GC	NIEHS
Pavel, K	VOC Technologies Inc.; Portland, Oregon	Assessment Environmental Health Breath using PF-GC	NIEHS
Rappaport, SM	University of North Carolina; Chapel Hill, North Carolina	Development and application of biomarkers in the study of exposure to carcinogens	NIEHS
Sabri, MI	Oregon Health Science University; Portland, Oregon	Study of biomarkers of neurotoxicant exposure and neurodegeneration	NIH
Smejtek, PK	VOC Technologies Inc.; Portland, Oregon	A PF-GC for Environmental Health Breath Assessment	NIEHS
Smith, MT	University of California at Berkeley	Study of biomarkers of benzene exposure and genotoxicity	NIEHS
Spencer, PS	Oregon Health Sciences University; Portland, Oregon	Study of neurotoxic chemicals and biomarkers at superfund sites	NIH
Stewart, P		Use of biomarkers to study occupational exposure of carcinogens in order to enhance exposure assessment	Division of cancer epidemiology and genetics
Turtletaub, KW	University of California at Berkeley	Study and development of biomarkers for exposure using accelerator mass spectrometry	NIEHS
Weaver, VM		Use of biomarkers to study benzene exposure in inner city residents	USDA
Wiencke, JK		Use of molecular biomarkers of occupational benzene exposure	USDA

^aFEDRIP 2005

NIEHS = National Institute of Environmental Health Sciences; NIH = National Institutes of Health; NSF = National Science Foundation; USDA = U.S. Department of Agriculture