7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring phenol, its metabolites, and other biomarkers of exposure and effect to phenol. 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 for the detection of phenol in biological materials are summarized in Table 7-1. Phenol is expected to be present in blood and urine in its free acid and conjugated forms (glucuronide and sulfate). The average urinary phenol concentration in unexposed individuals is 9.5±3.6 mg/L when corrected to a standard specific gravity of 1.024 (Piotrowski 1971). In exposed individuals, the urinary phenol level may vary from 10 to 200 mg/L (Tesarova and Packova 1983). The two common methods for quantifying conjugated phenol are chemical and enzymatic hydrolysis of the conjugate to the free phenol form. The chemical method uses acidic hydrolysis (Baldwin et al. 1981; Needham et al. 1984). Both the nature of the acid (sulfuric versus perchloric) and the temperature should be controlled carefully to obtain a quantitative yield and to avoid thermal decomposition of other phenolic or related compounds that may interfere with phenol quantification (Baldwin et al. 1981; Rick et al. 1982). The best available method appears to be specific enzyme hydrolysis or hydrolysis at ambient temperature with sulfuric acid. Enzymatic hydrolysis with an extract of *Helix pomatia* has also been used to liberate phenol from its conjugates (Ahmed and Hale 1994).

High-performance liquid chromatographic separation with electrochemical detection may provide the best sensitivity for phenol quantification in biological samples (Tesarova and Packova 1983). The use of gas chromatography with a flame ionization detector may be a more versatile method, if other non-ionic pollutants must be quantified. The advantages and disadvantages of different methods available for the

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Whole blood	Sample extracted with ethyl acetate, extract concentrated and analyzed (for free phenol), packed blood cells previously extracted for free phenol incubated with β-glucuronidase containing sulfatase at 37 °C, extracted with ethyl acetate after addition of normal saline solution, and extract concentrated and analyzed (for conjugated phenol)	GC-FID	<1 mg/mL	97% (free phenol); 103% (conjugated phenol)	O'Grodnick et al. 1983
Whole blood	Sample with spiked internal standard extracted with ethyl acetate and extract concentrated and analyzed	GC-FID	0.1 mg/L	>90% at concentrations above 0.5 mg/L	Handson and Hanrahan 1983
Urine	Sample mixed with phosphoric acid, passed through a pre-column at 165 °C for hydrolysis of conjugates (for free and conjugated phenol), and analyzed	GC-FID	NG	89% (for conjugates)	Baldwin et al. 1981
Urine	Sample heated under reflux with $HClO_4$, solvent extracted, concentrated, and separated by TLC; spot developed by *p*-nitro-benzenediazonium fluoroborate, removed quantitatively and solvent extracted (for free and conjugated phenol), and analyzed	Spectro-photometry	NG	NG	Bienick and Wilczok 1986
Urine	Acidified sample stream distilled, reacted with ammonia, *N*-chloro-succinimide, and sodium nitroprusside at basic pH (method probably for free phenol), and analyzed	Spectro-photometry	0.3 mg/L	>95%	Amlathe et al. 1987
Urine	Sample heated with HCl to 95 °C; diethyl ether added, mixture cooled to 0 °C and allowed to separate into two phases, clear ether layer used for analysis	GC-FID	0.5 µg/mL	94–95%	NIOSH 1994

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Sample incubated with glucuronidase and sulfatase at pH 5 and 3.7 °C, H_2SO_4 added and steam distilled (total phenol) and analyzed	HPLC-electro-chemical detector	2 ng/ inection	95–107%	Schaltenbrand and Coburn 1985
Urine	Sample hydrolyzed at room temperature and extracted with methyl *tert*-butyl ether (total phenol) and analyzed	GC-FID	NG	NG	Rick et al. 1982
Urine	Sample spiked with internal standard, hydrolyzed with H_2SO_4, and extracted with ethyl acetate (free and conjugated) and analyzed	GC-FID	NG	93–97% at 20–70 mg/L	Needham et al. 1984
Urine	Sample spiked with internal standard, distilled with H_2SO_4 in a special apparatus, distillate directly injected into GC (free and conjugated)	GC-FID	0.1 mg/L	99% at 5.9 mg/L	Van Roosmalen et al. 1981
Urine	Two spot urine samples (before and after exposure), hydrolyzed with HCl or perchloric acid, extracted with diethyl ether, and directly injected into GC	GC-FID	2 μg/mL	94%	NIOSH 1994

FID = flame ionization detector; GC = gas chromatography; H_2SO_4 = sulfuric acid; HCl = hydrochloric acid; $HClO_4$ = perchloric acid; HPLC = high performance liquid chromatography; NG = not given; TLC = thin layer chromatography

quantification of phenol and metabolites in biological and environmental samples have been discussed by Tesarova and Packova (1983).

The level of phenol detected in blood or urine may not accurately reflect actual phenol exposure because phenol may also appear as a metabolite of benzene or other drugs. It has been shown that under certain acidic conditions used for the hydrolysis of conjugated phenols, acetyl salicylic acid (aspirin) may produce phenol (Baldwin et al. 1981) and yield spuriously higher values for phenol in blood and urine.

For occupational exposure, it is recommended that urine samples be collected at the end of an 8-hour work shift (ACGIH 2001). Small amounts of thymol can be used as a preservative, and the urine can be stored for 4 days if refrigerated, or at least 3 months if frozen.

7.2 ENVIRONMENTAL SAMPLES

Analytical methods for detecting phenol in environmental samples are summarized in Table 7-2. The accuracy and sensitivity of phenol determination in environmental samples depends on sample preconcentration and pretreatment and the analytical method employed. The recovery of phenol from air and water by the various preconcentration methods is usually low for samples containing low levels of phenol. The two preconcentration methods commonly used for phenols in water are adsorption on XAD resin and adsorption on carbon. Both can give low recoveries, as shown by Van Rossum and Webb (1978). Solvent extraction at acidic pH with subsequent solvent concentration also gives unsatisfactory recovery for phenol. Even during carefully controlled conditions, phenol losses of up to 60% may occur during solvent evaporation (Handson and Hanrahan 1983). The *in situ* acetylation with subsequent solvent extraction as developed by Sithole et al. (1986) is probably one of the most promising methods.

Capillary columns may provide the best method for the separation of phenols prior to their quantification (Eichelberger et al. 1983; Shafer et al. 1981; Sithole et al. 1986). Of the various methods available for detection, the two commonly used methods that are most sensitive are mass spectrometry and flame ionization detection. Although electron capture detectors provide good sensitivities for higher chlorine-substituted phenols, they are poor for phenol itself (Sithole et al. 1986). The best method for the quantification of phenol may be mass spectrometric detection in the selected ion mode, but the loss of qualitative information may be significant (Eichelberger et al. 1983).

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urban air	Sample collected in bubbler containing NaOH, derivatized as nitrobenzeneazo compound	HPLC-UV	0.05 ppb for 150-L sample; 58–60% at 0.33–0.5 µg phenol	72.3% at 10–50 µg phenol	Kuwata et al. 1980
Air	Sample collected on a solid sorbent tube, desorbed using methanol	GC-FID	0.25 ppm for 20-L sample	NG	NIOSH 1994b
Air	Sample collected on a thermal desorption tube	GC-MS	NG	NG	NIOSH 1996
Occupational air	Sorption on activated carbon, desorption by solvent and derivatized to trimethylsilyl product	GC-FID	0.5 mg/m^3 (0.13 ppm)	96–102% at 2.5–100 mg/m^3	Yrjanheikki 1987
Occupational air	Sorption on XAD-2, desorption by acetonitrile and concentrated if necessary	HPLC-electro-chemical detector and HPLC-UV	8 µg/m^3 (2.04 ppb) with 12 L air (electro-chemical); 0.16 mg/m^3 (0.04 ppm) with 12 L air (UV)	NG	Nieminen and Heikkila 1986
Occupational air	Sample collected with a thermal desorption tube using a sorbent capable of capturing a C_6 organic compound	GC-MS	100 ng/tube or less	NA	NIOSH 1994
Total particulate matter in cigarette smoke	Extract particulate matter with NaOH, buffer to pH 4.6	HPLC-fluorescence spectrophoto-meter	0.3 mg/L	91% at 20–30 µg	Tomkins et al. 1984
Industrial emission, auto exhaust, and tobacco smoke	Sample collected in NaOH bubbler and derivatized to *p*-nitrobenzene-diazonium tetrafluoroborate	HPLC-UV	0.05 ppb for 150-L sample	NG	Kuwata et al 1980
Drinking water, waste water, and natural water	Direct distillation of solvent-cleaned sample (if necessary) at acidic pH, react with 4-amino-antipyrine and potassium ferricyanide at pH 8, extract in chloroform	Spectrophoto-metric	1 µg/L for 500-mL sample	NG	APHA/ AWWA/ WPCF 1985
Waste water and natural water	None	GC-FID	<1 mg/L	NG	APHA/ AWWA/ WPCF 1985
Water	1-L sample acidified and extracted with methylene chloride	GC-FID (Method 604)	0.14 µg/L	44%	EPA 2001a

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	1-L sample is adjusted to pH >11 and extracted with methylene chloride	GC-MS (Method 635)	1.5 µg/L	56%	EPA 2001c
Water	Direct distillation or distillation of solvent-cleaned sample at acidic pH, react with 4-amino antipyrine and potassium ferricyanide at pH 10 or extract colored complex in chloroform	Spectrophoto-metric (ASTM Method D-1783)	<5 µg/L (chloroform extract); <0.1 mg/L (direct)	NG	ASTM 1978
Water, waste water	Acidified sample extract with solvent, concentrated or derivatized to pentafluoro-benzylbromide product	GC-FID; GC-ECD (for derivatized EPA Method 604)	0.14 µg/L (FID); 2.2 µg/L (ECD)	41% (FID); NG (ECD)	EPA 1982
Water, waste water	Sample extracted in acidic pH, extract concentrated	GC-MS (EPA Method 625); HRGC-MS (EPA Method 625.1)	1.5 µg/L (GC-MS); 1–10 µg/L (HRGC-MS)	36% (GC-MS) at 10–1,500 µg/L; 25% (GC-MS) at 8.3 µg/L; 42% (HRGC-MS) at 20 µg/L	Eichelberger et al. 1983; EPA 1982
Water	Sample passed through graphitized carbon black, eluted with methylene chloride	Ion-suppression; reversed phase LC with UV detection	50–100 ng/L	91–97%	Di Corcia et al. 1996
Water	Sample passed through a mixed XAD-4/8 column, solvent eluted and concentrated	GC-MS	NG	46-70% (distilled water); 9% (tap water)	Van Rossum and Webb 1978
Waste water	Distillation of acidified solution, reacted with ammonia, *N*-chloro-succinimide, and sodium nitro-prusside at basic pH	Spectrophoto-metric	<0.3 mg/L	96.7% at 3 mg/L	Amlathe et al. 1987
Potable water and raw source water	Sample acetylated *in situ* by addition of acetic anhydride, solvent extracted and concentrated; alternatively, extracted acidic sample derivatized by pentafluorobenzyl bromide and cleaned up by column chromatography	HRGC-ECD (for pentafluoro-benzyl derivative); HRGC-MS (for acetyl derivative)	<50 ng/L (pentafluoro-benzyl); <50 ng/L (acetyl derivative)	10–64% (penta-fluoro-benzyl derivative); 70–132% (acetyl derivative)	Sithole et al. 1986

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water	1-L sample is extracted using a solid phase extraction cartridge	GC-MS (Method 528)	0.026 µg/L	85	EPA 2000a
Water	The sample is extracted at pH 12–13, then at pH <2 with methylene chloride using continuous extraction techniques; the extract is dried over sodium sulfate and concentrated to a volume of 1 mL	GC-MS (Method 1625)	Not applicable	Not applicable	EPA 2001b
Drinking water	Water samples collected and analyzed via GC-MS	GC-MS (Method OM100R	Not applicable	Not applicable	DOE 1997
Aqueous samples	Samples extracted and cleaned up (according to sample matrix) and the solvent appropriately exchanged; the phenols are then determined with or without derivatization	GC-MS (Method 8041A	Not applicable	93%	EPA 2000b
Sediment	Homogenized sample solvent extracted at acidic pH, fractionated by GPC and fractions concentrated	HRGC-MS	NG	112–128% at 400 ng/g	Lopez-Avila et al. 1983
Groundwater	Solvent extraction in acidic pH, extract concentrated	GC-MS (EPA-CLP Method)	10 µg/L	NG	EPA 1987
Soil, sediment	Sample mixed with anhydrous powdered Na_2SO_4, solvent extracted ultrasonically, extract subjected to GPC if necessary, extract concentrated	GC-MS (EPA-CLP Method	330 µg/kg	NG	EPA 1987
Water and waste water	Phenols separated on a Nova-Pak Phenyl column eluted with ammonium acetate:acetonitrile	LC-ED	0.5 mg/L	91–100%	Paterson et al. 1996
Groundwater	Solvent extraction, column chromatographic cleanup, concentration of extract	GC-MS (EPA Method 8250A)	1.5 mg/L	0.43c+ 1.26)/cx 100 where c is the actual concentra-tion	EPA 1994b
Bottom sediment	Wet sediment samples dried and compounds extracted using dichloromethane	GC-MS (Method 0-5130-95)	23.5 µg/kg	84	USGS 1995
Water	Water samples filtered using glass fiber filters; samples extracted using SPE cartridges	GC-MS (Method 0-1433-01)	0.11 µg/L	93	USGS 2002

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil, sludge, or solid waste	Extracted by soxhlet or sonication, extract subjected to column chromatographic cleanup and concentrated	GC-MS (EPA Method 8250A)	1.5 mg/kg	0.43c+ 1.26)/cx 100 where c is the actual concentra-tion	EPA1994b
Groundwater	Solvent extraction, column chromatographic cleanup, concentration of extract	HRGC-MS (EPA Method 8270B)	10 µg/L	0.43c+ 1.26)/cx 100 where c is the actual concentra-tion	EPA1994c
Soil, sludge, or solid waste	Extracted soxhlet or sonication, extract subjected to column chromatographic cleanup, concentrated	HRGC-MS (EPA Method 8270C)	660 µg/kg	0.43c+ 1.26)/cx 100 where c is the actual concentra-tion	EPA1994c
Soil, air, water,	Soxhlet extraction with acetone/hexane	GC-MS (Method 8270D)	10 µg/L	46%	EPA 1998
Honey	Sample dissolved in water, steam distilled; distillate cleaned up by column chromatography	HPLC-UP	0.1 ppm (for 10-g sample	98% at 111 µg added phenol	Sporns 1981

C_6 = 6 carbon; ECD = electron capture detector; ED = electrochemical detection; FID = flame ionization detector; GC = gas chromatography; GPC = gel permeation chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; LC = liquid chromatography; MS = mass spectrometry; Na_2SO_4 = sodium sulfate; NaOH = sodium hydroxide; NG = not given; SPE = solid-phase extraction; UV = ultraviolet detection

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 phenol 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 phenol.

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. Measurement of total phenol in urine serves as a biomarker of exposure for persons occupationally exposed to phenol (ACGIH 2001).

Effect. Specific biomarkers used to characterize effects caused by phenol have not been identified. Dark urine has been reported in persons occupationally exposed to phenol (inhalation, dermal) (ACGIH 2001; Merliss 1972) and following oral exposure (Baker et al. 1978; Kim et al. 1994). The dark urine may be a result of an oxidation product of phenol, or hemoglobin and hemoglobin breakdown products. Further research is required to identify the cause of the dark urine and relate it to exposure concentration.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The analytical methods available (Amlathe et al. 1987; Baldwin et al. 1981; Bieniek and Wilczok 1986; Handson and Hanrahan 1983; Needham et al. 1984; O'Grodnick et al. 1983; Rick et al. 1982; Schaltenbrand and Coburn 1985; Van Roosmalen et al. 1981) are adequate for the quantification of phenol and its conjugates in biological samples. The study of the levels of parent compound in human

blood, urine, or other biological matrices can be useful in deriving a correlation between the levels of this compound found in the environment and those found in human tissue or body fluid.

The changes in metabolite concentrations in human blood, urine, or other appropriate biological media over time may be useful in estimating phenol's rate of metabolism in humans. In some instances, the quantification of metabolites may be useful in correlating the exposure doses to the human body burden. Studies that correlate phenol exposure with levels of metabolites in human biological matrices are not available for this compound, although analytical methods for the quantification of the metabolites are available.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The analytical methods available (Eichelberger et al. 1983; EPA 1982a, 1986b, 1987; Kuwata et al. 1980; Nieminen and Heikkila 1986; NIOSH 1994; Sithole et al. 1986; Tomkins et al. 1984; Van Rossum and Webb 1978; Yrjanheikki 1978) are adequate for the quantification of phenol in environmental materials. Knowledge of the levels of this compound in environmental media, such as air, water, and food, can be used to indicate exposure of humans to this compound through the inhalation of air and ingestion of drinking water and foods containing phenol.

Although the products of environmental biotic and abiotic degradation of phenol have been identified adequately, no systematic study measuring the concentrations of the degradation products in the environment was found. Analytical methods are available for determining the levels of the degradation products such as hydroxylated phenol. Knowledge of the levels of degradation products would allow the development of a monitoring program designed to assess the ambient concentrations of phenol degradation products in the environment. Such a program could provide information concerning both human and environmental exposure to phenol since it might allow an estimation of the concentration of phenol in the environment prior to degradation.

7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2006) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 7.3.1. Two studies pertaining to analytical procedures for phenol detection were found in this database. Research being done at Mount Sinai School of Medicine at New York University and supported by National Institute of Environmental Health Sciences is looking into more accurate methods for quantitative

detection to toxicants in children and infants. A study from A.D. Grosso sponsored by the Center for Biologics and Evaluation—Quality Control is looking at chromatographic determination of phenol used as an antimicrobial preservative in vaccines and allergenic extracts.

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 phenol 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.

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 phenol and other phenolic compounds in urine. These methods use high-resolution gas chromatography and magnetic sector mass spectrometry, which give detection limits in the low parts per trillion (ppt) range.