7. ANALYTICAL METHODS

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

Numerous methods for the determination of *o*-, *m*-, and *p*-cresol in urine have appeared in the literature. *o*-Cresol in urine is often measured to determine exposure to toluene or other aromatic compounds, of which cresol is a metabolite (DeRosa et al. 1987). The analytical methods summarized in Table 7-1 are sufficiently sensitive to detect the individual isomers of cresol at a concentration that may cause concern for human health. Humans normally excrete 16–29 mg of *p*-cresol daily as a result of the breakdown of tyrosine (Needham et al. 1984).

The isomers of cresol are excreted in the urine as their glucuronides and sulfates (Bieniek and Wilczok 1986). To analyze for cresols directly, they must first be separated from the biological carrier. This is usually accomplished by heating a urine sample with a concentrated mineral acid for 30 minutes to 1 hour (Angerer and Wulf 1985; DeRosa et al. 1987; Needham et al. 1984; Yoshikawa et al. 1986). The transfer of cresol from the aqueous hydrolysate to an organic solvent is accomplished by simple extraction with a volatile organic solvent such as methylene chloride or ethyl ether. Concentration of the extract by gentle removal of the solvent prepares the sample for the analysis stage.

The amount of cresol in the concentrated extract can then be determined by high performance liquid chromatography (HPLC) (DeRosa et al. 1987; Yoshikawa et al. 1986) or gas chromatography (GC) coupled to either a flame ionization detector (FID) or a mass spectrometer detection system (Angerer and

Table 7-1. Analytical Methods for Determining Cresols in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference	Isomer
Blood	Acidification with HCl followed by centrifugation at 3,000 rpm, filter to isolate free cresol	GC/MS	0.14 µg/mL	95.4	De Smet et al. 1998	*p*
Blood	Deprotonization with 2 mL acetonitrile, vortex, centrifugation at 1,000 rpm	GC/MS	0.016 µg/mL	>95	Boatto et al. 2004	*o*
Urine	Hydrolyze with sulfuric acid; extract with ethyl acetate	GC/FID	No data	78–97	Needham et al. 1984	*o, m, p*
Urine	Hydrolyze with HCl extract with isopropyl ether; remove solvent; dissolve residue in water; add B-cyclodextrin	HPLC/UV	1 ppm	97–102	Yoshikawa et al. 1986	*o, m, p*
Urine	Acidify; steam distill; extract with methylene chloride	GC/MS	No data	No data	Angerer and Wulf 1985	*o*
Urine	Hydrolyze with sulfuric acid; extract with CH_2Cl_2; concentrate	HPLC/UV	No data	No data	DeRosa et al. 1987	*o*
Urine	Collect sample with thymol; hydrolyze with HCl; extract with ethyl ether	GC/FID	2 µg/mL	94%	NIOSH 1994b	*o*
Expired air	Breath collected in Teflon bag; concentration on Tenax GC adsorbent; thermal desorption	GC/MS	No data	No data	Krotoszynski and O'Neill 1982	Not specified

FID = flame ionization detector; GC = gas chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry; UV = ultraviolet spectroscopy

Wulf 1985; Needham et al. 1984). Separation of the cresol isomers by GC is readily accomplished, and the use of an appropriate internal standard allows the determination of their concentrations. Although exact detection limits were not given for the above GC methods, a concentration of 10 ppm appears to be readily determined.

Reversed-phase chromatography columns have been used for the analysis of cresols with limited success. A reversed-phase support has been developed that allows complete separation of the three cresol isomers (Bassler and Hartwick 1989). Inclusion complexes of the cresols with ß-cyclodextrin cleanly separate the three isomers on commercially available columns (Yoshikawa et al. 1986). Detection limits down to 1 ppm can be obtained by this method.

In cases involving acute cresol poisoning, cresol levels in biological tissues or blood levels are occasionally determined (Boatto et al. 2004). Methods have been described that can determine the level of free (nonprotein bound) *p*-cresol (De Smet et al. 1998) and *o*-cresol (Boatto et al. 2004) in blood. These methods typically involve hydrolysis with hydrochloric acid or acetonitrile to separate cresol from proteins followed by centrifugation, solvent extraction, and analysis by GC/mass spectrometry (MS).

The detection of cresol in the expired air of humans has been accomplished by techniques used routinely for the analysis of other organic compounds in this sample matrix (Krotoszynski and O'Neill 1982). In this technique, the subject's breath is collected in a bag made of inert material. The sample is then concentrated by pumping the expired air through a sorbent tube that collects the organic compounds. The organics are liberated from the adsorbent tube by thermal desorption, which flushes the components of the mixture directly onto a GC. The amount of each cresol isomer is quantified by comparison of the signal strength to that of a suitable internal standard using a FID, and identification is accomplished by interpretation of the data provided by a mass spectrometer. No detection limits were given for this method.

7.2 ENVIRONMENTAL SAMPLES

Methods for determining cresols in environmental media are summarized in Table 7-2. Procedures for the determination of and *o*- and *p*-cresol in water, soil, and sediment samples at hazardous waste sites are outlined by EPA (2005a). The required quantitation limits for each of the isomeric cresols are 10 ppb for water samples and 330 ppb for soil and sediment samples in this monitoring program.

Table 7-2. Analytical Methods for Determining Cresols in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference	Isomer
Air	Pump air through adsorbent tube, desorb with methanol	HPLC/UV	0.3 ppt	90–110	Kuwata and Tanaka 1988	*o, m, p*
Air	Aerodispersive enrichment into water	HPLC/ED	No data	No data	Vecera and Janak 1987	*o*
Air	Samples collected on solid sorbent tube. Desorb with methanol	HPLC/UV	No data	No data	NIOSH 1994a	*o, m, p*
Air	Sample collected on solid sorbent tube, desorb with methanol	GC/FID	No data	No data	NIOSH 1994b	*o, m, p*
Air	Ambient air drawn through impingers containing 15 mL of 0.1M NaOH Phenolates solution adjusted to pH <4 with 5% sulfuric acid and diluted with water	HPLC/UV	1–5 ppbv	>80	EPA 1986	*o, m. p*
Water	Adjust pH to 2, extract with CH_2Cl_2, concentrate	GC/MS	No data	No data	EPA 2005a	*o, p*
Water	Solvent extraction, liquid chromatography prefractionation	GC/MS	No data	No data	Hites 1979	Not specified
Rain water	None; direct injection onto ion exchange column	HPLC/CD	No data	No data	DOE 1985	*o, m, p*
Rain water	Acidify, extract with CH_2Cl_2, concentrate. methylate	GC/MS	No data	>50	Kawamura and Kaplan 1986	*o, m, p*
Drinking water	1-L sample is extracted using a solid phase extraction cartridge	GC/MS	0.026 µg/L	85	EPA 2000b Method 528	*o*
Soil, air, water,	Samples are prepared for analysis by GC/MS	GC/MS	Not applicable	Not applicable	EPA 1998 Method 8270D	*o, m, p*
Water or leachate	Aqueous liquid waste or leachate is directly injected into a reverse phase HPLC column	HPLC/UV	2.6 mg/L (*o*-cresol) 0.9 mg/L (*m*-cresol) 2.1 mg/L (*p*-cresol)	89	DOE 1997a Method OH100R	*o, m, p*

Table 7-2. Analytical Methods for Determining Cresols in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference	Isomer
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	Not applicable	Not applicable	EPA 2001 Method 1625	*o*
Drinking water	Water samples are collected and analyzed via GC/MS	GC/MS	27 µg/L *o*-cresol 42 µg/L *p*-cresol	96	DOE 1997b Method OM100R	*o, p*
Aqueous samples	Samples are extracted and cleaned up (according to sample matrix) and the solvent appropriately exchanged; the phenols are then determined with or without derivatization	GC/MS	Not applicable	Not applicable	EPA 2000a Method 8041A	*o, m, p*
Effluent 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	*o,m,p*
Soil, sediment	Extract sample with CH_2Cl_2 using ultra sonic probe	GC/MS	330 ppb	No data	EPA 2005a	*o, p*
Bottom sediment	Wet sediment samples were dried and compounds were extracted using dichloromethane	GC/MS	41.2 µg/Lkg	86	USGS 1995 Method 0-5130-95	*p*
Water	Water samples were filtered using glass fiber filters; samples were extracted using SPE cartridges	GC/MS	0.27 µg/L	36	USGS 2002 Method 0-1433-01	*p*

Table 7-2. Analytical Methods for Determining Cresols in Environmental Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference	Isomer
Sediment	Extract rapidly stirred sediment slurry with CH_2Cl_2 or ether, concentrate	GC/MS	No data	No data	Goodley and Gordon 1976	Not specified
Breathing air	Draw air through XAD-s adsorbent tube, acetonitrile desorption	HPLC/ED	8 µg/m^3	No data	Nieminen and Heikkila 1986	*o, m, p*

CD = conductivity detector; ED = electrochemical detector; GC = gas chromatography; HPLC = high performance liquid chromatography; MS = mass spectrometry; SPE = solid phase extraction; UV = ultraviolet detector

For the determination of cresol in water, good laboratory practice (GLP) guidelines state that the aqueous sample be brought to pH 11 by the addition of sodium hydroxide (NaOH). The basic mixture is then extracted with methylene chloride either in a separatory funnel or a continuous liquid-liquid extractor. The aqueous phase is then acidified to pH 2 and reextracted with methylene chloride. This second extract is concentrated by evaporation and subjected to GC/mass spectrometry (MS) analysis for identification and quantification.

In sediment and soil samples, the isomers of cresol are determined by transferring a small portion of the solid sample (1 g) to a vial and adding methylene chloride. The contaminants are extracted from the sample with the aid of an ultrasonic probe. The methylene chloride extract is filtered, concentrated, and subjected to GC/MS analysis for quantitation.

No other standardized methods for the determination of the three isomers of cresol were located (EPA 1988a). However, numerous methods for their determination have appeared in the open literature. Methods for the determination of cresols in ambient air (Kolber et al. 1981; Kuwata and Tanaka 1988; Vecera and Janak 1987), breathing air (Heikkila et al. 1987; Leuenberger et al. 1985; Nieminen and Heikkila 1986), surface water (Goodley and Gordon 1976; Hites 1979; McKnight et al. 1982; Sheldon and Hites 1979), groundwater (Goerlitz et al. 1985; Hutchins et al. 1984; Sawhney and Kozloski 1984; Stuermer et al. 1982) rain water (DOE 1985; Kawamura and Kaplan 1986; Leuenberger et al. 1985), and sediment samples (Goodley and Gordon 1976; Hites and Lopez-Avila 1980) are available.

The greatest difference between these methods is the procedure used in the sample preparation step. This step of the analysis varies widely between experimental techniques and may involve the use of highly specialized equipment. After the sample preparation step, however, the consensus is that separation of the isomers is best accomplished by using either GC or HPLC.

Cresols degrade rapidly in the environment (see Section 6.3.2). The degradation products are also removed rapidly. The products resulting from the degradation of the three isomers of cresol in the environment are not unique to these compounds.

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 cresols 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 cresols.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. There are no known biomarkers of exposure that are unique to cresols. In addition, *o*-cresol has been used as a biomarker of toluene exposure, and the isomers of cresol may appear as a result of exposure to other aromatic compounds (Needham et al. 1984). The methods presently available are capable of determining low levels of the cresol isomers in biological media, and background levels in the population could be established using existing techniques (Angerer and Wulf 1985; DeRosa et al. 1987; Krotoszynski and O'Neill 1982; Needham et al. 1984; Yoshikawa et al. 1986). Before a complete discussion on determining biomarkers of exposure for cresol can be undertaken, biomarkers unique to this compound must first be established.

Effect. Correlations of exposure and resulting biological effects are confounded by the metabolic formation of cresol after exposure to other organic compounds. Although the analytical methods for determining cresol in biological materials appear to provide the necessary precision and accuracy, their reliability in determining biomarkers of exposure and effect cannot, at this time, be ascertained. Before a complete discussion on determining biomarkers of effect for cresol can be undertaken, biomarkers unique to this compound must first be established.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Numerous methods for the determination of cresol in environmental matrices have appeared in

the literature (DOE 1985; EPA 2005a; Goodley and Gordon 1976; Hites 1979; Kawamura and Kaplan 1986; Kuwata and Tanaka 1988; Nieminen and Heikkila 1986; Vecera and Janak 1987). These procedures are capable of both identifying areas that have been contaminated with cresol and determining if the contaminated areas constitute a concern for human health. Human exposure to cresol is likely to occur by inhalation or ingestion of contaminated water. Standardized methods for the determination of the isomeric cresols exist for both of these matrices. These methods are both reproducible and sensitive. In addition, acceptable methods for the determination of cresol in other environmental media have appeared in the literature. No data needs are identified at this time.

Although the isomeric cresols degrade readily in the environment, their degradation products (Bayly and Wigmore 1973; Masunaga et al. 1983, 1986) are not unique to these compounds (see Section 6.3.2). As a result, the determination of these intermediates cannot be accurately extrapolated back to levels of cresol contamination in the environment.

7.3.2 Ongoing Studies

No information regarding ongoing studies was found as a result of a search of the Federal Research in Progress database (FEDRIP 2006).

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 cresols 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 cresols 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.