6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, and/or measuring, and/or monitoring chlorophenols, their metabolites, and other biomarkers of exposure and effect to these isomers. The intent is not to provide an exhaustive list of analytical methods. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used for environmental samples are the methods approved by federal agencies and organizations such as EPA and the National Institute for Occupational Safety and Health (NIOSH). Other methods presented in this chapter are those that are approved by groups such as the Association of Official Analytical Chemists (AOAC) and the American Public Health Association (APHA). Additionally, analytical methods may be included that modify previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

The principal methods used to analyze chlorophenols are gas chromatography with various detectors including flame ionization, electron capture and selected ion monitoring mass spectrometry, and high pressure liquid chromatography. Chlorophenols are highly polar with relatively low vapor pressures, which makes them difficult to measure directly using gas chromatography. To prevent adsorption problems and to improve peak shapes, chlorophenols are usually converted to less polar derivatives before analysis (Hajslova et al. 1988).

6.1 BIOLOGICAL MATERIALS

Methods for analysis of biological materials are summarized in Table 6-1. All methods require that the sample be extracted with an organic solvent. If the extraction of urine is completed under acid conditions, conjugates will be hydrolyzed so that total amounts (free + conjugates) of the various chlorophenols can be measured (Hargesheimer and Coutts 1983). Removal of other organic compounds, using XAD-4 resin (Edgerton et al. 1980; Wright et al. 1981) or a florisil column (Stein and Narang 1984), can improve the detection of chlorophenols. Techniques that require less chromatographic separation are the tandem mass spectrometry (MS/MS) methods described by Yost et al. (1984). These methods, especially the triple-stage quadrupole method, were suggested for rapid screening of a large number of samples. Yost et al. (1984) estimated that to analyze 25 samples by the more standard high-resolution gas chromatography/mass spectrometry method would require 186 hours, while the same number of samples could be analyzed in 27 hours by the triple-stage quadrupole tandem mass spectrometry method.

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

Sample Matrix	Preparation Method	Analytical Method	Sample Detection Limit	Percent Recovery	Reference
Urine	Hydrolyze sample using 70% perchloric acid; extract using diisopropyl ether; evaporate ether phase and dissolve residue in 50:50 acetonitrile/water solution	HPLC-uv/vis	4-CP, 0.2 mg/L	No data	Kusters and Lauwerys 1990
Urine	Hydrolyze sample using concentrated H_2SO_4; add NaOH until pH > 12; extract using methylene chloride; neutralize sample with H_2SO_4; derivatize by adding $NaHCO_3$ followed by acetic anhydride; extract using methylene chloride and concentrate	GC/SIM-MS	2-CP, 4-CP, 2,4-DCP, 2,4,5-TCP, 2,4,6-TCP, 2,3,4,6-TeCP, 2,3,4,5-TeCP, 1 pmol/mL	No data	Hargesheimer and Coutts 1983
Urine	Acid hydrolysis extraction, derivatization with acetic acid	GC/ECD	2,4-DCP, 8.3 µg/L; 2,4,5-TCP, 14.8 µg/L; 2,4,6-TCP, 8.5 µg/L; 2,3,4,5-TeCP, 7.1 µg/L; 2,3,4,6-TeCP, 4.9 µg/L	105 100 96 117 106	Angerer et al. 1981
Urine	Acid hydrolysis, elution through XAD-resin with 10% 2-propanol in hexane	GC/ECD	2,4-DCP, 2,4,5-TCP, 2,4,6-TCP, 1 ppb; 2,3,4,5- TeCP, 2,3,4,6-TeCP, 2,3,5,6- TeCP, 2 ppb	2,4-DCP, 70-103; 2,4,5-TCP, 76-94; 2,4,6-TCP, 70-110; 2,3,4,5-TeCP, 74-102; 2,3,4,6-TeCP, 81-102; 2,3,5,6-TeCP, 75-106	Edgerton et al. 1980

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

Sample Matrix	Preparation Method	Analytical Method	Sample Detection Limit	Percent Recovery	Reference
Urine	Acid hydrolysis, extract with diethyl ether, derivatize with diazoethane, sample cleanup with silica gel chromatography	GC/ECD	2,4,5-TCP, 5 ng/mL	Median 62	Kutz et al. 1992
Urine	Distilled with H_2SO_4, extracted with isopropyl ether	GC-FID	2-CP, 1 mg/L; 2,4-DCP, 1 mg/L; 2,4,6-TCP, 1 mg/L; 2,3,5,6-TeCP, 1 mg/L	93-96 99-101 98-102 97-102	Van Roosmalen et al. 1980
Urine	Hydrolysis with H_2SO_4, extract with benzene, derivitization with diazo-ethane, sample cleanup with silica gel chromatography, elution with 80:20 benzene:hexane	GC PCI MS/MS	2,4-DCP, 2,4,5-TCP, 2,4,6-TCP, 1 ppb	2,4-DCP, 80; 2,4,5-TCP, 67; 2,4,6-TCP, 97	Holler et al. 1989
Urine	Acid hydrolysis, elution through XAD-4 resin with 2-propanol-hexane	HPLC/MS	2-CP, 4-CP, 5-25 ppm	No data	Wright et al. 1981
Blood/urine	Samples acidified with HCl and extracted with chloro-form/methanol, chloroform, then hexane	TSQ GC MS/MS	2,4,5-TCP, 0.25 pg/sample	No data	Yost et. 1984
Blood/urine	Samples acidified with HCl and extracted with chloroform/methanol, chloroform, then hexane	MIKES GC MS/MS	2,4,5-TCP, 100 pg/sample	No data	Yost et al. 1984

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

Sample Matrix	Preparation Method	Analytical Method	Sample Detection Limit	Percent Recovery	Reference
Biological tissue	Extract sample with hexane, followed by hexane:ethyl ether (1:1); concentrate organic phase; cleanup on florisil column	GC/ECD	2,4,6-TCP, 2,4-DCP, 2,4,5-TCP, 2,3,4,5-TeCP, 2,3,4,6-TeCP, 4 ng/g fat, 1 ng/g tissue, 0.2 ng/mL blood	86-105	Stein and Narang 1984
Biological tissue	H_2SO_4 extraction of tissue, hydrolysis of conjugates with KOH, extract with diethyl ether, add acetic anhydride to form acetyl derivatives, extract with *n*-hexane	GC/SIM-MS	2,4,6-TCP, 2,3,4,6-TeCP, 0.01 µg/g	80 (minimum recovery of internal 2,4,6-tribromophenol standard)	Mussalo-Rauhamaa et al. 1989
Fish muscle	Add sodium sulfate and ascorbic acid to crushed sample; extract with t-butyl methyl ether/dichloromethane (1:1); concentrate and derivatize using acetic anhydride	GC/SIM-MS	2-CP, 0.01 µg/g	65	Morales et al. 1992

CP = chlorophenol; DCP = dichlorophenol; ECD = electron capture detection; FID = flame ionization detection; GC = gas chromatography; HCl = hydrochloric acid; HPLC = high performance liquid chromatography; H_2SO_4 = sulfuric acid; KOH = potassium hydroxide; MIKES = mass-analyzed ion kinetic energy spectrometry; MS = mass spectrometry; $NaHCO_3$ = sodium bicarbonate; NaOH = sodium hydroxide; PCI = positive chemical ionization; SIM-MS = selected ion monitoring mass spectrometry; TCP = trichlorophenol; TeCP = tetrachlorophenol; TSQ = triple-stage quadrupole tandem mass spectrometry

Edgerton (1981) studied the stability of 2,4,6-TCP and 2,3,5,6-TeCP in human urine and found that the compounds were stable for up to 40 days if frozen at -4°C. A loss of these compounds occurred if the specimens were thawed and refrozen.

6.2 ENVIRONMENTAL SAMPLES

Methods for analysis of environmental samples are summarized in Table 6-2. All methods require extraction of chlorophenols with an organic solvent, and most methods derivatize the chlorophenols before analysis. Samples for chlorophenol determination should be collected into amber glass containers and stored in the refrigerator (APHA 1992). It is also recommended that samples be extracted within 7 days of collection and analyzed within 40 days of extraction. Using gas chromatography with an electron capture detector, Hajslova et al. (1988) compared detection limits and percentage of recovery for 2,4-DCP, 2,4,5-TCP, 2,4,6-TCP, and 2,3,5,6-TeCP extracted from water with or without derivatization (acetates, methyl, and pentafluorobenzyl derivatives). All three types of derivatization lowered sample detection limits and increased the percentage of recovery for the tri- and tetrachlorophenols. The detection limit of 2,4-DCP was lowered from 5 to 0.03 mg/L only following derivatization with pentafluorobenzyl bromine, although derivatization decreased recovery from 104 to 85%.

The APHA approved method for analyzing phenols including 2-CP, 2,4-DCP, and 2,4,6-TCP uses gas chromatography and a flame ionization detector (APHA 1992). If there are interfering substances in the sample, APHA (1992) recommends derivitization of the sample with pentafluorobenzyl bromide, followed by clean-up through a silica gel column. Gas chromatography with an electron capture detector is then used to analyze the derivatized chlorophenols.

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 the chlorophenols is available. Where adequate information is not available, ATSDR, in conjunction with the NTP, is required to assure the initiation of a program of research designed to determine the health effects (and techniques for developing methods to determine such health effects) of the chlorophenols.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Wastewater	Acidify and extract sample with methylene chloride; during concentration the extract is exchanged to 2-propanol	GC/FID	2-CP, 0.31 mg/L; 2,4-CP, 0.39 mg/L; 2,4,6-TCP, 0.46 mg/L	No data	APHA 1992 (EPA method 604
Freshwater, seawater, and wastewater	Add 0.5 M Na_2PO_4 buffer solution to sample; add hexane and acetic anhydride and shake	GC/ECD	2,4-DCP, 2 ng/L; 2,4,6-TCP, 2,3,4,6-TeCP, 1 ng/L	99-105	Abrahamsson and Xie 1983
Water	Add ethanol and 2-fluorophenol to groundwater sample and acidify; extract with toluene; separate organic layer and analyze	GC/FID	2,4-DCP, 0.3 ng/sample; 2,4,6-TCP, 0.5 ng/sample	No data	Bengtsson 1985
Water	Acetone, HCl extraction; partition with $NaSO_4$; dry over dichloromethane	GC/ECD	2,4,6-TCP, 1 µg/L; TeCP, 0.5 µg/L	74.8-77.7 46.7-61.4	Woodrow et al. 1986
Water	Adjust sample to pH 11 with 0.1 M NaOH; cleanup on borosilicate column	HPLC/ Dual UV detection	2-CP, 2,4-DCP, 2,4,6-TCP, ng/L range	85 79 95	Alarcon et al. 1987
Water	Add toluene to water sample and shake; separate organic extract, add HFB, shake vigorously and centrifuge; separate toluene phase and analyze	GC/ECD	2,4,6-TCP, 1.1 pg/sample	19.3 (cv)	Bengtsson 1985

TABLE 6-2. Analytical Methods for Determining Chlorophenols in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Add acetone and NaOH to sample; extract with hexane; acidify with H_2SO_4; extract with hexane:diethyl ether (1:1) and concentrate	GC/ECD	Free CPs: 2,4-DCP, 5 µg/L; 2,4,5-TCP, 1 µg/L; 2,4,6-TCP, 1 µg/L; 2,3,5,6-TeCP, 2 µg/L	 104 85.3 87.1 70	Hajslova et al. 1988
	Also derivatize above sample by acetylation, methylation, and pentafluorobenzylation		Acetates: 2,4-DCP, 5 µg/L; 2,4,5-TCP, 0.5 µg/L; 2,4,6-TCP, 0.5 µg/L; 2,3,5,6-TeCP, 0.2 µg/L	 92.2 94.2 91.7 76	
			Methyl derivatives: 2,4-DCP, 5 µg/L; 2,4,5-TCP, 0.5 µg/L; 2,4,6-TCP, 0.5 µg/L; 2,3,5,6-TeCP, 0.2 µg/L	 87 91 90 83	
			PFB derivatives: 2,4-DCP, 0.03 µg/L; 2,4,5-TCP, 0.03 µg/L; 2,4,6-TCP, 0.03 µg/L; 2,3,5,6-TeCP, 0.05 µg/L	 85 98.1 87 106	

TABLE 6-2. Analytical Methods for Determining Chlorophenols in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Acidify water sample and extract with methylene chloride; separate aqueous layer and add 0.1 M tetrabutyl ammonium chloride; adjust pH to 14 and extract with methylene chloride; combine acidic and basic extracts and analyze	HPLC/Dual UV detection	2-CP, 4.2 ng/sample; 2,4,6-TCP, 12.6 ng/sample	97	Realini 1981
Water	Acidify water sample to pH 1.5 with phosphoric acid; extract with petroleum ether and concentrate organic phase	TLC	2,4,6-TCP, 0.1 µg/L	75-95	Zigler and Phillips 1967
Industrial waste	Lyophilize sample and add dichloromethane; derivatize to carbamates; separate organic phase and concentrate; place extract on or between glass wool plug of glass tubing connected to GC and slowly volatilize	GC/MS	2,4,6-TCP, 10-100 µg/L	No data	Hunt et al. 1985
Sediment	Without pretreatment: Add 0.1 M Na_2CO_3 solution to sample and shake; derivatize sample by adding acetic anhydride; extract with hexane	GC/ECD	2,4,6-TCP, ~0.1 ng/g	94	Xie 1983

TABLE 6-2. Analytical Methods for Determining Chlorophenols in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediment	Pretreatment hexane extraction to remove neutral and basic impurities for heavily polluted samples; add 0.1 M Na_2CO_3 solution to sample and shake; separate aqueous phase, derivatize with acetic anhydride, and extract with hexane	GC/ECD	2,4-DCP, 2,4,6-TCP, 2,3,4,6-TeCP, ~0.1 ng/g	92	Xie 1983
Soil leachate	Acidify sample and extract with dichloromethane; derivatize with 10% PFC solution in toluene	GC/ECD	2,4,6-TCP, >5 ng/L	73	Buisson et al. 1984
Soil	Add distilled water to soil sample followed by 50% H_2SO_4 and stir; add toluene: methylene chloride (19:1) and reflux; separate organic layer and concentrate	GC/ECD	2,4,6-TCP, µg/g range	98	Narang et al. 1983
Wood dust	Collect dust sample by suction on a membrane filter; extract with diethyl ether	GC/ECD	2,4,6-TCP, 1-4 µg/m^3	No data	Kauppinen and Lindroos 1985
Air	Draw air sample through an impinger containing toluene (at 0°C); derivatize sample by acetylation and extract with hexane	GC/ECD	2,4,6-TCP, 13 µg/m^3	No data	Kauppinen and Lindroos 1985
Air	Collect in toluene in impingers; extract into borax solution	GC/ECD	TCP, TeCP, 2-5 µg/L	No data	Kauppinen and Lindroos 1985

TABLE 6-2. Analytical Methods for Determining Chlorophenols in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Spent bleach liquors (bleach plant)	Extract sample continuously with diethyl ether at pH 2 and shake with Na_2CO_3 solution; derivatize with diazomethane and analyze	GC/ECD and GC/MS	2,4,6-TCP, 41 pg/sample	2.6 RSD	Lindstrom and Nordin 1976
Sample formulation	Dissolve sample in acetonitrile and inject into HPLC column	HPLC/ UV detection	2-CP, 2.7 ng/sample; 2,4,6-TCP, 11.3 ng/ sample	0.06 RSD	Buckman et al. 1984

CP = chlorophenol; cv = coefficient of variation; DCP = dichlorophenol; ECD = electron capture detection; FID = flame ionization detection; GC = gas chromatography; HCl = hydrochlororic acid; HFB = heptafluoro butyric anhydride; HPLC = high performance liquid chromatography; H_2SO_4 = sulfuric acid; MS = mass spectrometry; Na_2CO_3 = sodium carbonate; NaOH = sodium hydroxide; Na_2PO_4 = sodium phosphate; $NaSO_4$ = sodium hydroxide; PFB = pentafluoro benzyl bromide; PFC = pentafluoro benzyl chloride; RSD = relative standard deviation; TCP = trichlorophenol; TeCP = tetrachlorophenol; TLC = thin-layer chromatography spectrometry; UV = ultra-violet

6. 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.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect

Exposure. Analytical methods are available to determine levels of chlorophenols in urine (Angerer et al. 1981; Hargesheimer and Coutts 1983; Kusters and Lauwerys 1990; Van Roosmalan et al. 1980; Wright et al. 1981) and other biological samples, including blood and tissue (Morales et al. 1992; Stein and Narang 1984). Chlorophenols, especially the lower chlorinated compounds, are metabolites of a number of other compounds including pesticides. Therefore, the value of urinary chlorophenols as a measure of exposure to chlorophenols, per se, at hazardous waste sites, where exposure to many compounds can occur, is not clear. Further research on the relationship between low-level exposure and levels of chlorophenols in biological media would be helpful in assessing the risks and health effects of chronic low-level exposure.

Effect. There are no specific markers of the biological effects of chlorophenols. Acute exposure to monochlorophenols results in myelonic convulsions (Angel and Rogers 1972; Borzelleca et al. 1985a, 1985b; Farquharson et al. 1958), and exposure to chlorophenols also results in effects on the immune system (Exon et al. 1984) and on reproduction (Exon and Koller 1985). Further studies are needed to relate levels of chlorophenols in biological media to observed effects. One would doubt that these biological effects (myelonic convulsions) are specific enough to be a food biomarker of effect.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Although there is limited information available about determining levels of chlorophenols in air (Kauppinen and Lindroos 1985), the relatively low vapor pressure of these compounds suggests that, under environmental conditions, exposure through air would be minimal. Sufficient information is available concerning the measurement of the chlorophenols in water (Abrahamsson and Xie 1983; Alarcon et al. 1987; APHA 1992; Bengtsson 1985; Hajslova et al. 1988; Realini 1981; Woodrow et al. 1986; Zigler and Phillips

1967), soil (Buisson et al. 1984; Narang et al. 1983) and sediment (the media of concern for human exposure) (Xie 1983).

Current analytical methods are sensitive enough to measure background levels in environmental media. The precision, accuracy, reliability, and specificity of these methods are sufficiently documented
.

6.3.2 On-going Studies

No on-going studies regarding the development analytical methods for the chlorophenols were identified.