7. ANALYTICAL METHODS

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

Detection and quantification of very low levels of DEHP are seriously limited by the presence of this compound as a contaminant in almost all laboratory equipment and reagents. Plastics, glassware, aluminum foil, cork, rubber, glass wool, Teflon sheets, and solvents have all been found to be contaminated (EPA 1988a; Giam et al. 1975; Williams 1973). While efforts have been made to reduce laboratory contamination (Giam et al. 1975; Thuren 1986), DEHP is still reported in laboratory blanks, even with thorough cleaning methods (EPA 1988a; Giam et al. 1975). Therefore, practical sample detection limits are often more than an order of magnitude higher than instrument or method detection limits. The EPA (1988a) reports that DEHP, along with other common phthalate and adipate esters, cannot generally be accurately or precisely measured at concentrations below about 2 ppb, due to blank contamination.

7.1 BIOLOGICAL MATERIALS

Laboratory contamination is a significant issue when measuring DEHP in biological materials and care must be taken to address this concern, as discussed in the introduction to Chapter 7.

Gas chromatography (GC) is the most common analytical method for detecting and measuring DEHP in biological materials (Ching et al. 1981a; EPA 1986f; Hillman et al. 1975; Jaeger and Rubin 1972; Sjoberg and Bondesson 1985). High performance liquid chromatography (HPLC) might also be employed (Kambia et al. 2001; Pollack et al. 1985a; Shintani 2000). The chromatography separates complex mixtures of organic compounds and allows individual compounds to be identified and quantified by a

detector. Detectors used to identify DEHP include the electron capture detector (ECD) (Mes et al. 1974; Vessman and Rietz 1974) and the flame ionization detector (FID) (Albro et al. 1984). When unequivocal identification is required, a mass spectrometer (MS) coupled to the GC column might be employed (Ching et al. 1981a; EPA 1986f; Hillman et al. 1975; Sjoberg and Bondesson 1985). Analytical methods for the determination of DEHP in various biological fluids and tissues are summarized in Table 7-1.

Prior to analysis, DEHP must be separated from the biological sample matrix and prepared for introduction into the analytical instrument. DEHP might be separated from the matrix by several methods including: extraction with an organic solvent such as chloroform, hexane, heptane, or acetonitrile (Ching et al. 1981a; Jaeger and Rubin 1972; Kambia et al. 2001; Sjoberg and Bondesson 1985); gel permeation chromatography (EPA 1986f); precipitation (Mes et al. 1974); solid phase extraction (Shintani 2000); and cleanup with Florisil® (EPA 1986f). Often, more than one of these procedures is required to separate the analyte from fats and other lipophilic materials.

Biological materials (blood fractions, urine, tissue) are often monitored for a chemical substance in order to evaluate the extent of human exposure to that substance. It appears that monitoring biological tissues for DEHP might underestimate exposure, because this compound is metabolized *in vivo* quickly and extensively (Albro et al. 1984; Liss et al. 1985; Sjoberg et al. 1985c). Therefore, in order to better estimate exposure levels, it is important to test for the metabolites of DEHP as well. The primary metabolite appears to be mono(2-ethylhexyl) phthalate (Niino et al. 2001; Sjoberg et al. 1985c); although other metabolites (2-ethylhexanoic acid, 2-ethyl-3-hydroxyhexanoic acid, and 3-ethyl-3-oxohexanoic acid) have been identified and can be measured in urine (Gunther et al. 2001; Wahl et al. 2001). However, since numerous metabolites have been identified (see Section 3.3.3), monitoring biological materials for total phthalates might often be appropriate (Albro et al. 1984). Monitoring total phthalates would not, of course, be specific for DEHP exposure.

Methods for analysis of individual phthalates in saliva, blood, urine, and/or feces involve separation of metabolites by HPLC combined with GC/MS (Niino et al. 2001; Sjoberg et al. 1985c) or GC/FID (Albro et al. 1984). Analysis for metabolites differs from analysis for DEHP mainly in sample preparation procedures (Albro et al. 1984; Sjoberg and Bondesson 1985). Metabolites from urine and/or feces are often treated with β-glucuronidase to remove conjugated glucuronic acid moieties. When GC methods

7. ANALYTICAL METHODS

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood serum	Extract with propanol/ heptane/ sulfuric acid dissolve in benzene methylate fatty acids, redissolve in acetone	GC/MS	3 µg/mL[a]	No data	Ching et al. 1981a
Blood plasma	Extract with acetonitrile and hexane	GC/MS	0.15 µg/mL	93	Sjoberg and Bondesson 1985
Blood plasma	Mix 1:1 with 1 M NaOH, extract with hexane, reduce to dryness, resuspend in acetonitrile	HPLC/UV	20 ng/mL	>97	Kambia et al. 2001
Blood	Extract with ethyl acetate	HPLC/UV	0.345 µg/mL	No data	Pollack et al. 1985a
Blood	Mix blood 1:1 with 10 mM acetate buffer (pH 3), extract with SPE (elute with acetonitrile/acetic acid)	HPLC/UV	No data	98–102	Shintani 2000
Urine	Samples deconjugated with β-glucoronidase, purified using two-step solid phase extraction	HPLC-APCI-MS/MS	1.2 ng/mL (MEHP)	78–91%	Blount et al. 2000b
Tissue	Extract with chloroform/methanol	GC/MS	0.02 µg/g	No data	Hillman et al. 1975
Tissue	Extract with chloroform/methanol	GC	5 µg/g	60–90	Jaeger and Rubin 1972
Adipose tissue	Extract with methylene chloride, remove bulk lipid by gel permeation chromatography, fractionate on Florisil (elute with diethyl ether/ hexane)	HRGC/MS	9 ng/g	No data	EPA 1986f

[a]Lowest concentration reported.

GC = gas chromatography; HPLC = high performance liquid chromatography; HPLC-APCI-MS/MS = high performance liquid chromatography - atmospheric pressure chemical ionization - tandem mass spectrometry; HRGC = high resolution gas chromatography; MS = mass spectrometry; SPE = solid phase extraction; UV = ultraviolet

are employed for metabolite identification, carboxyl groups are first converted to methyl esters using diazomethane (Albro et al. 1983, 1984). Another method involves the oximation of the DEHP metabolites with *O*-(2,3,4,5,6-pentafluorobenzyl)-hydroxyamine hydrochloride before sample purification, followed by the conversion of the metabolites to their *tert*-butyldimethylsilyl derivatives for GC/MS analysis (Wahl et al. 2001).

Application of LC-MS/MS techniques to the analysis of phthalate ester metabolites in urine have also been developed. For example, Blount et al. (2000b) have developed an assay to quantify the monoester metabolites (including MEHP) of eight phthalate diesters in urine, utilizing HPLC coupled with atmospheric pressure chemical ionization and tandem mass spectrometric (APCI-MS/MS) detection techniques. Urine samples were treated with β-glucuronidase to release the free phthalate monoesters followed by a two-step solid phase extraction procedure. After evaporative concentration of the eluant, the analytes in the purified samples are further separated on a phenyl reverse phase HPLC column and quantified by APCI-MS/MS, following careful optizimation of the APCI-MS/MS instrument. The limits of detection for MEHP were determined to be 1.2 ng/ml urine with recovery efficiencies of between 78 and 91%.

7.2   ENVIRONMENTAL SAMPLES

Laboratory contamination is a significant issue when measuring DEHP in environmental samples and care must be taken to address this concern, as discussed in the introduction to Chapter 7.

Determination of DEHP in air, water, soil/sediments, and food is usually by GC analysis (Cartwright et al. 2000; EPA 1982a, 1982b, 1986c, 1986d, 1988a; Ishida et al. 1981; NIOSH 1985b; Otake et al. 2001; Rudel et al. 2001; van Lierop and van Veen 1988; Williams 1973). An HPLC method for food has also been developed (Giust et al. 1990). Several representative methods appropriate for quantifying DEHP in each of these media are summarized in Table 7-2. The EPA has developed methods for analysis of drinking water (EPA 1988a), waste water (EPA 1982a, 1982b), and soil/sediment (EPA 1986c, 1986d) samples. Many of the APHA (1989) methods for water are equivalent to the EPA methods. Determination of DEHP in polyvinyl chloride (PVC) plastics might also be of interest and can be accomplished by GC analysis as described in the American Society for Testing and Materials (ASTM) Method D 3421-75 (Stringer et al. 2000).

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect on cellulose membrane filter, desorb with carbon disulfide	GC/FID	0.01 mg/ sample	107	NIOSH 1985a
Air	Collect on charcoal, ultrasonic solvent extraction of charcoal with toluene	GC/MS	0.17 µg/ sample	98	Otake et al. 2001
Air	Collected on XAD-2 resin sandwiched between polyurethane foam plugs, Soxhlet extracted with 6% ether/hexane	GC/MS	1.0691 µg/ extract	114	Rudel et al. 2001
Water	Extract in LSE cartridge, elute with methylene chloride	HRGC/MS	2 µg/L[a]	95–100	EPA 1988a
Waste water	Extract with methylene chloride, exchange to hexane	GC/ECD	2 µg/L	85±4	EPA 1982b
Waste water	Extract with methylene chloride at pH>11 and again at pH<2	GC/MS	2.5 µg/L	82	EPA 1982a
Waste water	Continuous liquid-liquid extraction (methylene chloride as extraction solvent)	GC/MS	0.05–0.20 µg/L	No data	Brown et al. 1999
Soil	Extract with methylene chloride, clean-up, exchange to hexane	GC/ECD	1.3 mg/kg	D[b]–158	EPA 1986d
Soil	Extract from sample, clean-up	HRGC/MS	660 µg/kg	8–158	EPA 1986d
Soil	Ultrasonic solvent extraction with ethyl acetate and unltracentrifuged	GC/FID	0.1 µg/mL	73.3	Cartwright et al. 2000
Sediment	Extraction from sample using SFE, purification on silica gel column, exchanged into hexane	GC/MS	0.81 µg/g	70-85	McDowell and Metcalfe 2001

Table 7-2. Analytical Methods for Determining DEHP in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sewage sludge	Ultrasonic solvent extraction into methanol/dichloro-methane, cleanup with reverse phase extraction cartridge, dissolved in methanol	LC-APCI-MS	50 ng/g	78	Petrovi*f* and Barceló 2000
Food	Extract with chloroform/methanol, dry with sodium sulfate, dissolve in ethyl ether	GC/FID	0.01–1.0 ppm	58–90	Ishida et al. 1981
Food	Extract with hexane acetonitrile, petroleum ether, dry with sodium sulfate, elute with ethyl ether/petroleum ether	GC/FID	15 ppb[a]	65–70	Williams 1973
PVC plastic toys	Cooled in liquid nitrogen, grated, sonicated in hexane	GC/MS	No data	87.9	Stringer et al. 2000
Food	Extract with acetonitrile, methylene chloride/petroleum ether, dry with sodium sulfate, clean-up on Florisil	GC/ECD	1 ppb	70–100	Giam et al. 1975
Food	Extract with acetonitrile and petroleum ether, dry with sodium sulfate clean-up with Florisil	GC/ECD	0.1 μg[a]	91	Thuren 1986

[a]Limited by laboratory contamination; see text.
[b]Detected, result greater than zero.

ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; HRGC = high resolution gas chromatography; LC-APCI-MS = liquid chromatography-atmospheric pressure chemical ionization-mass spectrometry; LSE = liquid-solid extraction; MS = mass spectrometry; PVC = polyvinyl chloride; SFE = supercritical fluid extraction

Separation of DEHP from environmental samples is usually by extraction with an organic solvent such as acetonitrile, chloroform, ethyl acetate, hexane, or methylene chloride. Air samples are drawn through a solid sorbent material (e.g., charcoal or XAD-2 resin) and desorbed with carbon disulfide (NIOSH 1985b) or ether/hexane (Rudel et al. 2001). A purge and trap method might be used for separation of DEHP from the fat in foods (van Lierop and van Veen 1988). Detector options are identical to those mentioned above (Section 7.1). Detection limits for these methods are generally in the ppb range.

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

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 to DEHP might be evaluated by measuring the levels of this compound or its metabolites in blood, adipose tissue, and urine. Sensitive analytical methods, including GC/MS and HPLC, are available for these determinations (Ching et al. 1981a; EPA 1986f; Hillman et al. 1975; Jaeger and Rubin 1972; Kambia et al. 2001; Pollack et al. 1985a; Shintani et al. 2000; Sjoberg and Bondesson 1985). However, development of improved methods for sample extraction and of better ways to reduce laboratory contamination levels of DEHP would be valuable in reducing practical detection limits or degradation of DEHP during sample isolation and workup.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media.   Food and water are the media of most concern for human exposure to DEHP. Existing analytical methods can measure this compound in all environmental media at ppb levels (EPA 1982a, 1982b, 1986c, 1986d, 1988a; Giust et al. 1990; Ishida et al. 1981; NIOSH 1985b; van Lierop and van Veen 1988, 1989; Williams 1973). However, ubiquitous laboratory contamination with this compound prevents accurate determinations. Research efforts pertaining to solving contamination problems are a major research need.

7.3.2 Ongoing Studies

No ongoing studies in analytical chemistry were identified.