

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 di-*n*-octylphthalate, its metabolites, and other biomarkers of exposure and effect to di-*n*-octylphthalate. 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.

6.1 BIOLOGICAL MATERIALS

Very limited data were found regarding the measurement of di-*n*-octylphthalate and its metabolites in biological fluids. Table 6-1 summarizes the methods that are available. Analytical methods were located for measuring di-*n*-octylphthalate and its metabolites in urine, blood, and tissues (Albro and Moore 1974; Lanina et al. 1992; Oishi 1990). These methods include gas chromatography (GC) combined with mass spectrometry (MS) and high-performance liquid chromatography (HPLC) combined with an ultraviolet detector (UV). No comparisons can be made between methods since no data were given regarding sensitivity, recovery, or precision.

6.2 ENVIRONMENTAL SAMPLES

Table 6-2 summarizes the various methods available for measuring di-*n*-octylphthalate in environmental samples. GC/MS and GC combined with electron capture detection (ECD') can be used to measure di-*n*-octylphthalate in water, waste water, groundwater, soil, and solid waste (APHA 1992; Eichelberger et al. 1983; EPA 1981, 1986a, 1986b, 1986c, 1990b; Furtmann 1994; Lopez-Avila et al. 1989; Ritsema et al. 1989; Valkenburg et al. 1989).

TABLE 6-1. Analytical Methods for Determining Di-*n*-octylphthalate in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	Extract compound from urine using diethyl ether; wash with HCl, dry over anhydrous Na ₂ SO ₄ , filter; evaporate diethyl ether; dissolve evaporated residue with diethyl ether	HPLC/UV; GC/MS	NR	NR	Albro and Moore 1974; Albro et al. 1973
Blood	Extract compound from blood using heptane ^a	GC	NR	NR	Lanina et al. 1992
Blood and tissue samples	Mix blood sample with HCl and hexane; homogenize tissue sample and mix with HCl and hexane; evaporate hexane; dissolve evaporated residue with methanol ^{a,b}	HPLC	NR	NR	Oishi 1990

^aThis method was reported for determining "dioctylphthalate"; thus, the precise identity of the substance being determined may not necessarily be di-*n*-octylphthalate.

^bThis method was reported for determining only mono-*n*-octylphthalate not di-*n*-octylphthalate.

GC = gas chromatography; HCl = hydrochloric acid; HPLC = high-performance liquid chromatography; MS = mass spectrometry; Na₂SO₄ = sodium sulfate; NR = not reported; UV = ultraviolet detector

TABLE 6-2. Analytical Methods for Determining Di-*n*-octylphthalate in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Extract serial with methylene chloride at pH >11 (pH 7 for capillary column) and pH 2; analyze extracts separately for packed column, combined for capillary column; dry over anhydrous sodium sulfate; concentrate; inject	GC/MS	1–10 µg/L	70–81	Eichelberger et al. 1983
Water	Extract serial with methylene chloride at pH >11 and pH <2; concentrate	GC/MS	NR	83.5–105.4	Valkenburg et al. 1989
Tap water	Extract compound with solid-phase extraction technique that uses a membrane impregnated with reverse-phase particles; elute with acetonitrile	LC	Low ppb (µg/L)	82–93	Hagen et al. 1990
Water, groundwater, waste water, landfill leachate	Solid-phase extraction activating with methanol; dry; elute with ethyl acetate/DAIP	GC/MS	0.02 µg/L	94	Furtmann 1994

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water, waste water	Extract with methylene chloride at pH >11 and again at pH <2; dry; concentrate	GC/MS	2.5 µg/L	4–146	APHA 1992
Waste water	Extract sample with dichloromethane/hexane at pH 2, 7, and 10; elute from Florisil or alumina with ether/hexane	GC/ECD	0.1 ng/injection	>90	EPA 1981
Waste water	Add stable isotopically labeled analogs of the compounds to 1 L of waste-water sample; extract sample at pH 12–13, then at pH <2, with methylene chloride using continuous extraction techniques; dry extract over sodium sulfate; concentrate; add internal standard; inject	GC/MS	10 µg/L	NR	EPA 1990b (Method 1625)

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater, soil, solid waste	Extract sample with methylene chloride; concentrate solvent; exchange to hexane	GC/ECD	30 µg/L (groundwater); 2 mg/kg (soil); 300 mg/kg (solid waste)	D ^a -114	EPA 1986c (Method 8060)
Groundwater, soil, solid waste	Extract with methylene chloride	GC/MS	25 µg/L (groundwater); 2 mg/kg (soil); 250 mg/kg (solid waste)	4-146	EPA 1986b (Method 8250)
Groundwater, soil	Extract with methylene chloride; gel permeation cleanup	GC/MS	660 µg/kg (soil); 10 µg/L (groundwater)	4-146	EPA 1986a (Method 8270)
Water/SPM	Collect water samples on disposable octyl-bonded silica solid-phase extraction columns; dry; elute with hexane/ether; SPM collected by continuous flow centrifugation; extract with acetone/water/benzene	GC/ECD; GC/MS	0.1 µg/L (water); 0.1 mg/kg (suspended particulate)	83 (water); 82 (suspended particulate)	Ritsema et al. 1989

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil, sediment, citrus leaves, coal, coal fly ash	Clean up sample using disposable Florisil cartridge; elute with methylene chloride in hexane and hexane/acetone	GC/ECD	NR	80–104	Lopez-Avila et al. 1989

^aDetected, result greater than zero

DAIP = diallylphthalate; ECD = electron capture detection; GC = gas chromatography; LC = liquid chromatography; MS = mass spectrometry; NR = not reported; SPM = suspended particulate matter

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Generally, sample preparation procedures involved extraction with methylene chloride at pH <2 and pH >11. The removal of interferences such as organochlorine pesticides and polychlorinated biphenyls has been approached through the addition of a clean-up step using Florisil columns (EPA 1981) and more recently solid-phase extraction alone with Florisil or other solid-phase matrices have been used (Furtmann 1994; Lopez-Avila et al. 1989; Ritsema et al. 1989). Sensitivity is in the low-ppb ($\mu\text{g/L}$) range for water samples using GC/MS (APHA 1992; Eichelberger et al. 1983; EPA 1986a, 1986b, 1990b; Furtmann 1994; Valkenburg et al. 1989). GC/MS provided slightly better sensitivity than did GC/ECD. For water samples, recoveries are good for GC/MS and GC/ECD (Eichelberger et al. 1983; EPA 1981; Furtmann 1994; Ritsema et al. 1989; Valkenburg et al. 1989). Precision was adequate (<13-21% relative standard deviation [RSD]) (Eichelberger et al. 1983; Valkenburg et al. 1989). For soil and solid waste samples, sensitivity was in the ppm ($\mu\text{g/L}$) range using GC/MS and GC/ECD. For the standard analytical methods approved by EPA (Test Methods 1625, 8060, 8250, and 8270) and APHA, recovery and precision varied greatly for both water and soil samples (APHA 1992; EPA 1986a, 1986b, 1986c, 1990b).

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 di-*n*-octylphthalate 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 di-*n*-octylphthalate.

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.

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6.3.1 Identification of Data Needs**Methods for Determining Biomarkers of Exposure and Effect**

Exposure. Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available for measuring di-*n*-octylphthalate and/or its metabolites (primarily the corresponding phthalate monoesters) in urine, blood, and tissues (Albro and Moore 1974; Lanina et al. 1992; Oishi 1990); however, the data are very limited. More information on the accuracy, precision, and sensitivity of these methods is needed to evaluate the value of using the levels of di-*n*-octylphthalate and its metabolites (particularly in urine) as indicators of exposure. The lack of data for these methods makes it difficult to assess whether these methods are sufficiently sensitive to measure levels at which health effects might occur, as well as background levels in the population.

Effect. No biomarkers of effects caused by di-*n*-octylphthalate have been identified in humans or animals,

Methods for Determining Parent Compounds and Degradation Products in

Environmental Media. Methods exist for measuring di-*n*-octylphthalate in water, groundwater, waste water, soil, and solid waste (APHA 1992; Eichelberger et al. 1983; EPA 1981, 1986a, 1986b, 1986c, 1990b; Furtmann 1994; Lopez-Avila et al. 1989; Ritsema et al. 1989; Valkenburg et al. 1989); however, the database is limited. More information on the accuracy and precision of these methods is needed to accurately compare them. No data were located for measuring di-*n*-octylphthalate in air. The lack of data on background levels in the environment, as well as levels at which health effects might occur, prevents an evaluation of whether the methods are sensitive enough. Research investigating the relationship between environmental levels and observed health effects could increase confidence in existing methods and indicate where improvements are needed. Analytical methods are needed for determining degradation products in all environmental media.

6.3.2 On-going Studies

No on-going analytical methods studies were located.