7. ANALYTICAL METHODS

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

Di-*n*-butyl phthalate may be determined by high resolution gas chromatography with an electron capture detector (HRGC/ECD) (EPA 1984b), gas chromatography/mass spectrometry (GC/MS) (EPA 1994a), gas chromatography with a flame ionization detector (GC/FID) (NIOSH 1994), gas chromatography/Fourier transform infrared spectrometry (GC/FTIR) (EPA 1994b), high performance liquid chromatography/mass spectrometry (HPLC/MS) (Coldham et al. 1998), or high performance liquid chromatography/ion-trap mass spectrometry (HPLC/Ion Trap-MS) (Coldham et al. 1998). Prior to analysis, di-*n*-butyl phthalate must be separated from the biological or environmental sample matrix and prepared in a form suitable for introduction into the analytical instrument. Methods for extracting di-*n*-butyl phthalate from biological materials and environmental samples are discussed in Sections 7.1 and 7.2.

Phthalates are common environmental contaminants that frequently contaminate laboratory glassware, sampling equipment, and solvents used to extract di-*n*-butyl phthalate from various media for analysis (Staples et al. 1997). As a result, it is difficult to make accurate measurements at low levels (<10 ppb). Care must be taken to preclude environmental and other samples from contamination with di-*n*-butyl phthalate.

7.1 BIOLOGICAL SAMPLES

Since di-*n*-butyl phthalate is relatively nonvolatile and lipophilic, most methods for separating it from biological materials involve extraction into an organic solvent such as ether, heptane, or acetonitrile. In most cases, the material is homogenized in the solvent to improve extraction efficiency. Additional sample cleanup steps may be required to separate fats and other endogenous lipophilic materials in the samples that co-extract from the biological material (Walters 1986). Several analytical methods for the determination of di-*n*-butyl phthalate in biological materials are listed in Table 7-1.

Recently, Blount et al. (2000) summarized a methodology to detect di-*n*-butyl phthalate metabolites in urine. In humans or animals, di-*n*-butyl phthalate is metabolized to mono-*n*-butyl phthalate and oxidative products, which are excreted through the urine and feces. Human urine samples are processed by β-glucuronidase hydrolysis (to release the mono phthalate ester) followed by solid-phase extraction. The eluate is concentrated; mono-*n*-butyl phthalate is chromatographically resolved by reverse-phase HPLC, detected by negative ion atmospheric pressure chemical ionization (APCI) tandem mass spectrometry, and quantified by isotope dilution.

7.2 ENVIRONMENTAL SAMPLES

Separation of di-*n*-butyl phthalate from environmental samples such as water, soil, sediment, or wastes is also usually accomplished through extraction with an organic solvent. In some cases, di-*n*-butyl phthalate in water may be separated without solvents by adsorption onto a suitable polymer such as Tenax (Pankow et al. 1988). Analytical methods for the determination of di-*n*-butyl phthalate in environmental samples are given in Table 7-2.

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 di-*n*-butyl phthalate 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 di-*n*-butyl phthalate.

Table 7-1. Analytical Methods for Determining Di-*n*-butyl Phthalate in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Recovery	Reference
Aquatic organisms	Extract with acetonitrile and petroleum ether	HRGC/ECD	0.1 ng/g	68%	Thuren 1986
Adipose tissue	Extraction, bulk lipid removal, Florisil fractionation	HRGC/MS	10 ng/g	No data	Stanley 1986
Bile (cattle)	Extraction, fractionation by cation and anion exchange chromatography, β-glucuronidase/sulphatase hydrolysis	HPLC/Ion Trap-MS; HPLC/MS	No data	93.5±4.4%	Coldham et al. 1998
Blood plasma (cattle)	Extraction, fractionation by cation and anion exchange chromatography, β-glucuronidase/sulphatase hydrolysis	HPLC/Ion Trap-MS; HPLC/MS	No data	89.6±1.6%	Coldham et al. 1998
Blood serum	Extraction, bulk lipid removal, Florisil fractionation	HRGC/MS	10 ng/g	No data	Stanley 1986
Blood serum	Extraction with organic solvents (propanol, heptane)	GC/MS	No data	No data	Ching et al. 1981a
Cooked meat	Removal with nitrogen gas trap, extraction with diethyl ether	GC/MS	No data	No data	Ho et al. 1983
Urine (cattle)	Extraction, fractionation by cation and anion exchange chromatography, β-glucuronidase/sulphatase hydrolysis	HPLC/Ion Trap-MS; HPLC/MS	No data	76.2±2.3%	Coldham et al. 1998
Urine	β-glucuronidase hydrolysis, solid-phase extraction	HPLC/APCI-MS	0.6 ng/mL	>90%	Blount et al. 2000

APCI = negative ion atmospheric pressure chemical ionization; ECD = electron capture detector; GC = gas chromatography; HPLC = high performance liquid chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry

Table 7-2. Analytical Methods for Determining Di-*n*-butyl Phthalate in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Recovery	Reference
Air	Adsorption/solvent extraction with cellulose ester membrane	NIOSH 5020 GC/FID	1–20 mg/m^3 20 L air sample	No data	NIOSH 1994
Water	Extract with dichloromethane, exchange to hexane, concentrate	EPA 625 HRGC/ECD	2.5 µg/L	80±6%[a]	EPA 1984b
Water	Extract with dichloromethane, exchange to hexane, concentrate	EPA 606 HRGC/ECD	0.36 µg/L	80±6%[a]	EPA 1984b
Liquid	EPA Extraction Method 3510 using dichloromethane followed by cleanup	EPA 8061 GC/ECD	0.33 µg/L	76.5–108%	EPA 1996b
Groundwater	EPA Extraction Methods 3510 or 3520 using dichloromethane followed by cleanup	EPA 8250A GC/MS	2.5 µg/L[a]	76%	EPA 1994a
Wastes, non-water miscible	Extract with dichloromethane followed by cleanup	EPA 8410 GC/FT-IR	10 µg/L	No data	EPA 1994b
Wastes, non-water miscible	Extract with dichloromethane, cleanup, exchange to hexane	EPA 8060 GC/ECD or FID	36 mg/kg	96%	EPA 1986a
Wastes, non-water miscible	EPA Extraction Methods 3510 or 3520 using dichloromethane followed by cleanup	EPA 8270 GC/FT-IR	50 mg/kg	76%	EPA 1998l
Soil	Extraction using dichloromethane/acetone(1:1), followed by cleanup	EPA 8061 GC/ECD	0.33 µg/L	90–95%	EPA 1996b
Soil	Extract with dichloromethane, cleanup, exchange to hexane followed by cleanup	EPA 8060 GC/ECD or FID	2.4×10^{-4} mg/kg	96%	EPA 1986a

Table 7-2. Analytical Methods for Determining Di-*n*-butyl Phthalate in Environmental Samples *(continued)*

Sample matrix	Preparation method	Analytical method	Sample detection limit	Recovery	Reference
Soil	EPA Extraction Methods 3510 or 3520 using dichloromethane followed by cleanup	EPA 8250A GC/MS	1.7 mg/kg	96%	EPA 1994a
Soil/sediment	Extract with dichloromethane followed by cleanup	EPA 8410 GC/FT-IR	10 µg/L[b]	No data	EPA 1994b
Soil/sediment	EPA Extraction Methods 3510 or 3520 using dichloromethane followed by cleanup	EPA 8270 GC/FT-IR	0.66 mg/kg	76%	EPA 1998l

[a]Relative recovery, percent, ±standard deviation
[b]Detection limits for some samples may be several orders of magnitude higher depending upon the sample characteristics and extraction procedure employed.

ECD = electron capture detector; FID = flame ionization detector; FT-IR = Fourier transform-infrared spectrometer; GC = gas chromatography; MS = mass spectrometry

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. Sensitive and selective methods using high resolution GC or HPLC are available for the qualitative and quantitative measurement of di-*n*-butyl phthalate after it is separated from the biological matrix of tissue or fluid. However, methods for recovery of di-*n*-butyl phthalate from such samples have not been extensively developed, and additional work to improve and standardize sample extraction and preparation methods for biological fluids and tissues would be valuable in providing quantitative information concerning human exposure. The sensitivity of existing methods may not be high enough to measure background levels in the population, since an existing study failed to detect di-*n*-butyl phthalate in several samples (EPA 1986g). Because of the widespread use of di-*n*-butyl phthalate in laboratory equipment, cosmetics, and other consumer products, studies to determine background levels in the population must be done with care to avoid false positives from inadvertent contamination (see Staples et al. 1997). Since health effects appear to occur only after high levels of exposure, existing methods are probably capable of measuring body levels at which effects would be expected to occur in humans. HPLC/MS has been adapted to measure body levels of metabolites of di-*n*-butyl phthalate, primarily mono-*n*-butyl phthalate, which have the potential to be biomarkers of exposure (Blount et al. 2000).

No information was located concerning biomarkers of effect of di-*n*-butyl phthalate. Precise, accurate, reliable, and specific methods for measuring background levels of biomarkers of effect in the population, as well as the levels at which health effects may occur, are needed.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Good methods with adequate sensitivity and selectivity are available for detecting and quantifying di-*n*-butyl phthalate contamination in water, air, soil, and waste samples. Soil, water, and food are the media of most concern for human exposure to di-*n*-butyl phthalate. The basic methods of extraction followed by high resolution GC (with derivatization of sample) or HPLC-MS have

the potential to be sensitive enough to measure background levels of di-*n*-butyl phthalate and its degradation products in the environment, but care must taken to ensure that samples are representative, volumes are sufficient, contamination is avoided, preservation is adequate, and extraction and purification are complete. In measuring background levels in environmental media, contamination can pose a particular problem because of the extensive use of di-*n*-butyl phthalate in products found in laboratories. Existing methods should be sufficiently sensitive to measure levels of di-*n*-butyl phthalate at which health effects might occur.

7.3.2 Ongoing Studies

Examination of the literature suggests that studies are underway to improve means for determining di-*n*-butyl phthalate in biological samples and environmental media. Improvements continue to be made in chromatographic separation and detection. Current high level activity in the areas of supercritical fluid extraction and supercritical fluid chromatography (Smith 1988) includes di-*n*-butyl phthalate in biological samples and environmental media as an analyte. Fourier transform infrared flow cell detectors are promising for this application (Wieboldt et al. 1988).

A search of Federal Research in Progress reported no ongoing studies relating to analytical methods development (FEDRIP 2000).