6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring diethyl phthalate in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify diethyl phthalate. Rather, the intention is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect diethyl phthalate in environmental samples are the methods approved by federal 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 refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL MATERIALS

No analytical methods were located for measuring diethyl phthalate in the serum, blood, or urine of humans or animals. Gas chromatography (GC), combined with electron capture detection (ECD) or mass spectroscopy (MS), has been used to measure diethyl phthalate in human semen (Waliszewski and Szmczymski 1990), animal fat (van Lierop and van Veen 1988), and animal tissues (Giam and Chan 1976), but the data are too limited to allow a comparison of methods. Because phthalates are so pervasive in plastics and elsewhere in the laboratory environment, rigorous control measures are needed to prevent contamination of the sample and to maintain a low background. These procedures include prewashing columns, use of equipment with purified solvents, and baking at high. temperatures to remove organic materials. Investigators have found that contamination from laboratory glassware limits the analysis of phthalate esters in the parts-per-billion to parts-per-trillion range and recommend concurrent controls for all analytical procedures (Lopez-Avila et al. 1990). Organochlorine pesticides and polychlorinated biphenyls (PCBs) cause interference in diethyl phthalate analysis by ECD, requiring their removal.

Preparation steps include extraction with petroleum ether, followed by Florisil® chromatography (Giam and Chan 1976; Waliszewski and Szymczymski 1990). The detection limit for semen was 0.04 mg/kg, and recovery was excellent (95%) (Waliszewski and Szymczymski 1990). Van Lierop and Van Veen (1988) recovered diethyl phthalate from fat by purging with nitrogen at high temperature, collecting the volatilized material on Tenax®, and extracting with hexane. This method was designed to avoid time-consuming methods for removing residual fat, but the low recovery (l-10%) and high detection limit (10 mg/kg) limit its usefulness to crude qualitative analysis.

Burns et al. (1981) described a method for measuring di-(2-ethylhexyl) phthalate in fish lipids with 79-86% recovery, and suggest it could also be used for measuring diethyl phthalate. Detection is by GC/ECD, with a background of 1 ng/injection. The ability to differentiate among different phthalate esters was sacrificed for excellent sensitivity (0.1 pg/injection) and good recovery (70-l 10%) in a method that involves hydrolyzing all phthalate esters to phthalic acid and converting the acid to bis(2,2,2-trifluoroethyl) phthalate, which has an increased ECD response (Takeshita et al. 1977). Table 6-l summarizes methods available for measuring diethyl phthalate in biological samples.

6.2 ENVIRONMENTAL SAMPLES

Diethyl phthalate in environmental samples is most commonly measured using GC with detection by MS preferred because it is less prone to interference than is ECD. Other detection methods include high-performance liquid chromatography (HPLC) or liquid chromatography with ultraviolet (UV) detection. As with biological samples, rigorous cleaning of reagents and equipment is necessary to prevent contamination. Methods that use a minimum number of steps and minimal amounts of solvents and column materials also help minimize contamination.

Diethyl phthalate can be collected by pumping an air sample through ethylene glycol (Thomas 1973) or directly through an activated Florisil® column (Giam and Chan 1976). Measurements in air can also be done by passive sampling on charcoal, which is less expensive than active sampling but requires much longer sampling times. Reproducibility for the passive sampling technique was

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Semen	Dry sample; extract with petroleum ether; concentrate; elute from Florisil® column with diethyl ether/petroleum ether; concentrate	GC/ECD	0.04 mg/kg	95%	Waliszewski and Szymczymski 1990
Fat	Purge sample with nitrogen at high temperature; collect on Tenax®; extract with hexane	GC/MS	≈10 mg/kg	1–10%	van Lierop and van Veen 1988
Muscle, liver	Homogenize sample in acetonitrile; extract with methylene chloride/ petroleum ether/salt water; concentrate; elute from Florisil® column with diethyl ether/petroleum ether	GC/ECD	≈30 ng/ injection	No data	Giam and Chan 1976

ECD = electron capture detection; GC = gas chromatograph(y); MS = mass spectrometry

l0-15% of the mean value, and the detection limit was estimated to be 0.2 μg/m^3 (Shields and Weschler 1987). The detection limit for the active sampling techniques was 10 ng per injection, with 90% recovery using ethylene glycol trapping (Giam and Chan 1976; Thomas 1973).

Solid phase extraction (SPE) methods using reverse-phase columns are particularly desirable for analyzing liquid samples because they eliminate the need for large solvent volumes and the resulting potential for contamination (Burkhard et al. 1991; Ritsema et al. 1989). SPE using a membrane impregnated with reverse-phase particles achieved 85-100% recovery (Hagen et al. 1990). Ritsema et al. (1989) found mass selective detection (MSD) much more selective than ECD and reported 85% recovery and a detection limit of 10 ng/L. Other methods involve hexane extraction followed by HPLC or GC. The detection limit of the UV detectors used for HPLC (20 ng/injection) is much higher than that of GC/ECD (0.5 ng/injection) (Payne and Benner 1981). Lopez-Avila et al. (1989) replaced the Florisil® column used in EPA method 3620 (EPA 1986b) with a smaller disposable Florisil®cartridge to reduce background and achieved 96% recovery when cartridges were not spiked with potential interfering agents. The cartridge could be used to separate diethyl phthalate from organochlorine pesticides, diesel hydrocarbons, and corn oil. In a later study, these investigators obtained a 55-70% recovery from a solid matrix, after alumina column cleanup (Lopez-Avila et al. 1991). Method 1625 of EPA's Industrial Technology Division is part of a group of broad-range methods for measuring pollutants in waste water. It includes continuous liquid-liquid extraction, followed by gel permeation chromatography and detection by GC/MS (Telliard 1990). EPA (198la) achieved over 100% recovery using Florisil® or alumina columns and GC/ECD, with a sensitivity of 0.13 ng/injection, but found the method inappropriate for certain waste waters because of high interference.

Sludge, sediment, and soil samples are extracted with moderately nonpolar solvents ands.leaned up by liquid chromatography (Ritsema et al. 1989; Russell and McDuffie 1983). Soxhlet® extraction or extraction using ultrasonication was sometimes used to improve efficiency; some authors found ultrasonic extraction resulted in lower blanks and slightly higher efficiency than Soxhlet® extraction (Peterson and Freeman 1982a; Zurmiihl 1990). Recovery was over 80% for the few techniques where recovery was reported. Sensitivity was reported for only a few of the methods, where it ranged from 0.1 to 5.3 μg/kg. Diethyl phthalate has been detected in cosmetic preparations using liquid

chromatography and UV detection, or by direct injection onto the gas chromatograph with flame ionization detection (FID) (Hancock et al. 1966). Diethyl phthalate was detected in pharmaceutical tablets by thin-layer chromatography (TLC) and HPLC/UV (Cafmeyer and Wolfson 1991). Table 6-2 summarizes methods available for measuring diethyl phthalate in environmental samples.

High temperature continuous counter-current gas-liquid chromatography is an effective methodology for separating mixtures of high boiling point organics (Watabe et al. 1992). At a column temperature of 200°C dimethyl and diethyl phthalates were separated to purities of 99.96% and 99.69% respectively.

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 diethyl phthalate 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 diethyl phthalate.

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 or eliminate 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 may be proposed.

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collect on charcoal pad; extract with carbon disulfide; concentrate. Method of CC/MS quantitation not described.	GC/MS	≈ 0.2 µg/m^3	No data	Shields and Weschler 1987
Air	Pump sample through ethylene glycol; extract with hexane; concentrate; elute from Florisil® with diethyl ether	GC/MS	10 ng/injection	>90%	Thomas 1973
Tap water	Collect sample by reverse phase SPE C18 PTFE membrane; elute with acetonitrile	HPLC/UV	<10 µg/L	85–99%	Hagen et al. 1990
Waste water	Extract sample with dichloromethane/hexane; elute from Florisil® or alumina with ether/hexane	GC/ECD; GC/FID	0.1 ng/injection 31 ng/injection	109–116%	EPA 1981a
Effluent	Filter sample; elute from SPE column with methanol/ water; concentrate eluate; separate over reverse-phase HPLC column with methanol/water and collect eluates; concentrate and analyze by CC/MS	GC/MS	1 µg/L	No data	Burkhard et al. 1991

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water/suspended particulate matter (SPM)	Water samples collected on reverse-phase SPE column, dried, eluted with hexane/ether; SPM collected by continuous flow centrifugation, extracted with acetone/water/hexane, and wash hexane phase with water	GC/MS/SIM	10 ng/L (water) 0.1 mg/kg (SPM)	85% (water) 67% (SPM)	Ritsema et al. 1989
Water, sediment	Water samples extracted with hexane, concentrated, for GLC extracted with isooctane; sediment samples Soxhlet® extracted with acetonitrile	HPLC/UV	20 ng/injection	94-96%	Payne and Benner 1981
		GC/ECD	0.5/injection	95-98%	
		HPLC/UV	20/injection	90-94%	
Soil, sandy loam	Extraction by sonication with methylene chloride-acetone (1+1), concentrate into hexane; with comparative evaluation of sample clean-up on alumina or Florisil® columns	GC/ECD & GC/FID (dual columns, dual detector)	6-60 µg/kg (typical values)	55-70%	Lopez-Avila et al. 1991

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sewage sludge	Homogenize, freeze-dry and grind sample; extract with methylene chloride using ultrasonication, clean-up on activated alumina and activated Florisil® columns. Transfer into hexane and add to activated alumina column, wash with hexane, hexane/10% CH_2Cl_2 and elute with hexane 15% CH_2Cl_2; add latter to activated Florisil® columns, wash with CH_2Cl_2 and elute with CH_3Cl_2/5% acetone	GC/ECD	No data	80%	Zurmuhl 1990
Landfill leachate, sediment, soil	Extract leachate with methylene chloride; dry; dissolve in hexane; elute from alumina column, after washing, with benzene; elute from silica column in acetone/benzene. Extract soil and sediment samples with hexane/acetone; dry; dissolve in hexane; clean up as above	GC/ECD	1–3 ng/g (soil and sediment) 0.1 µg/L (leachate)	No data	Russell and McDuffie 1983

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediment	Dry sample; utrasonically extract with methylene chloride concentrate	GC/MS/SIM	5.3 ng/g	86%	Peterson and Freeman 1982a

ECD = electron capture detection; FID = flame ionization detector; GC = gas chromatograph(y); GLC = gas liquid chromatography; HPLC = high-performance liquid chromatography; MS = mass spectrometry; SIM = selected ion monitoring; SPE = solid-phase extraction; SPM = suspended particulate matter; UV = ultraviolet detection

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. No methods were located for measuring diethyl phthalate or its metabolites in animal or human serum, blood, or urine. Methods are available for determining diethyl phthalate in human semen and animal fat and muscle (Giam and Chan 1976; Van Lierop and Van Veen 1988; Waliszewski and Szymczymski 1990). Because the available data indicate that diethyl phthalate exposure is associated with negligible toxicological potential, additional studies to identify specific biomarkers of exposure appear unnecessary. Consequently, a data need for identifying biomarkers does not exist. However, additional metabolic and/or comparative toxicokinetic studies, recommended in Section 2.9.2 to elucidate high-dose effects, may incidentally reveal a specific biomarker of exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist for measuring diethyl phthalate in a variety of environmental media, including air (Shields and Weschler 1987), water (EPA 1989; Fatoki and Vernon 1990; Keith et al. 1976; Kopfler et al. 1977; Ray and Giam 1984; Staples et al. 1985), waste water (EPA 1981a), sludge (Zurmtihl 1990), sediment (Peterson and Freeman 1982a; Russell and McDuffie 1983), soil (Russell and McDuffie 1983), and cosmetics (Hancock et al. 1966). Recovery for most methods is over 80%. Furthermore, the failure to identify signs or symptomology of intoxication suggests that increased analytical sensitivity for biological or environmental samples is unnecessary. Consequently, the refinement of current analytical procedures appears unnecessary.

6.3.2 On-going Studies

No on-going studies regarding analytical methods were located for diethyl phthalate.