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 PAHs, its metabolites, and other biomarkers of exposure and effect to PAHs. 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 SAMPLES

Several analytical techniques have been used to determine trace levels of PAHs in biological tissues and fluids including adipose tissue, lungs, liver, skin, hair, blood, urine, and feces (Table 6-l). These include gas chromatography coupled with flame ionization detection (GC/FID), gas chromatography coupled with a mass spectrometry (GC/MS), high-performance liquid chromatography (HPLC) coupled with an ultraviolet (UV) or fluorescence detector, and thin-layer chromatography (TLC) with fluorescence detection.

Recently, Liao et al. (1988) developed a relatively simple and rapid procedure for purifying human and bovine adipose tissue extracts so that trace levels of complex mixture of target analytes (including PAHs) could be detected and quantified by capillary GC/MS. By employing an activated Florisil column, Liao and co-workers showed that lipid contaminants bind effectively (more than 99.75%) with Florisil, thereby producing a relatively clean sample extract. A detection limit at a low ng/g level and an average sample recovery of 85% were achieved (Gay et al. 1980; Liao et al. 1988; Modica et al. 1982).

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Biological tissues (adipose)	Homogenization in 8% benzene in hexane; clean-up on Florisil column	GC/MS	5–50 ng/g	52–95	Liao et al. 1988
	Extraction into pentane; clean-up on Florisil and silica column	GC/MS	0.05 ng/sample	27–100	Gay et al. 1980
	Extraction into cyclohexane; clean-up on alumina column; concentration	GC/FID	50 ng/sample	83–95	Modica et al. 1982
Lungs	Homogenization in hexane; extraction with 25% DMSO in water (discarding aqueous phase); washing with water; concentration	SF	No data	95	Mitchell 1979
	Extraction into cyclohexane; centrifugation; dry with Na_2SO_4; concentration; analysis in acetonitrile	HPLC/UV	20 ng/g	93.7 (fluoranthene); 65.3 (pyrene); 65 (benzo[a]-anthracene)	Brandys et al. 1989

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Lungs (cont.)	Tissue digestion; extraction and precipitation of DNA with spermine; hydrolysis of DNA in 0.1 M HCl	HPLC/radioisotope counting	13.3–32.7x10^{-15} mol BPDE bound/mg DNA	No data	Weyand and Bevan 1987a
	Tissue digestion; extraction of DNA; isolation of BPDE-DNA adducts (immunoaffinity chromatography); hydrolysis to tetrahydrotetrols	HPLC/fluorescence detector	6 pg B[a]P-tetrol/mL	26–66	Weston and Bowman 1991
Human lymphocytes	Isolation and hydrolysis of DNA to tetrahydrotetral; oxidization to dicarboxylic acid with potassium superoxide; derivatization and clean up on silica	GC/NIEC-MS (BaP adduct)	5 adduct/ 10^7 nucleotide	47	Allan et al. 1993
Liver	Homogenization with DMSO; incubation with S-9 mixture at 37 °C; extraction with ethyl acetate; concentration; analysis for metabolites of indeno[1,2,3-c,d]pyrene	HPLC/UV-VIS	No data	No data	Rice et al. 1985b

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Liver (cont.)	Homogenization with DMSO; incubation with S-9 mixture at 37 °C; extraction with ethyl acetate; concentration and analysis for metabolites of indeno[1,2,3-c,d]pyrene and benzo[b]fluoranthene	HPLC/UV-VIS	No data	No data	Amin et al. 1982
	Homogenization; saponification; extraction into hexane; clean-up on silica or alumina column	HPLC/fluorescence detector	0.006–0.46 ng/g range	No data	Obana et al. 1981
Skin	Digestion and deproteinization of PAH-treated skin tissue; extraction and precipitation of DNA; hydrolysis with 1.2 M HCl	HPLC/fluorescence detector	10^{-15} mol BPDE/sample	No data	Shugart et al. 1983
	Modification of PAH-treated skin DNA *in vitro*; labelling of PAH-DNA adduct by ^{32}P-postlabeling technique	TLC/ autoradiography	90–1,210x10^{-15} mol PAH adduct/ mg DNA	No data	Phillips et al. 1987

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish bile	Hydrolysis of the conjugated PAH metabolite; extraction of the free metabolite into n-hexane; concentration and methylation with methyl iodide; extraction of methylated product	LESS (3-hydroxy-BaP)	0.005 ng/mL	No data	Ariese et al. 1993b
Blood	Hydrolysis of BPDE-DNA adduct with 0.1 M HCl; analysis of hydrolysis products (benzo[a]pyrene-tetrols and triols)	SLS	No data	No data	Haugen et al. 1986
	Extraction into cyclohexane; centrifugation; drying with Na_2SO_4; concentration; analysis in acetonitrile solution	HPLC/UV	20 ng/mL	107 (fluoranthene); 108.6 (pyrene); 101 (benzo[a]-anthracene)	Brandys et al. 1989

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (cont.)	Treatment with 2% horse serum; incubation with rabbit anti-BPDE-DNA antiserum; incubation with alkaline phosphatase-conjugated goat anti-rabbit IgG, PNPP, radiolabeled PNPP, and $MgCl_2$; separation of hydrolyzed radiolabeled PNPP; measurement of radioactivity	USERIA	$0.38–2.2x10^{-15}$ mol/μg DNA	No data	Haugen et al. 1986
	Incubation of equal volumes rabbit anti-serum and sample; wash; incubation with reconstituted biotinylated anti-rabbit IgG; wash; incubation with buffered europium-labeled streptovidin; shaking with enhancement solution at room temperature	Time-related fluorometry (PAH-DNA adduct)	<1 adduct/10^8 nucleotides	No data	Schoket et al. 1993
Blood	Incubation of BPDE-DNA adduct sample with goat antihuman IgG reagent, horseradish peroxidase and substrate solution	ELISA	$0.38–2.2x10^{-15}$ mol BPDE/μg DNA	No data	Haugen et al. 1986

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (cont.)	Separation and isolation of white blood cell DNA by standard RNase and phenol treatment	ELISA	$1x10^{-15}$ mol BPDE/0.001 mg DNA	No data	Perera et al. 1988
			$2–120x10^{-15}$ BPDE/50 µg DNA	No data	Shamsuddin et al. 1985
	Separation of hemoglobin (hb) by lysis and centrifugation; isolation of BPDE-hb adduct by acid hydrolysis; clean-up on Sep-Pak and cellulose column	HPLC/fluorescence detector	$5x10^{-12}$ g BPDE/sample	No data	Shugart 1986
	Isolation of PAH-DNA adduct from white blood cells; digestion of adduct with [gamma ^{32}P] ATP; resolution and quantitation of the ^{32}P-labelled adduct by TLC;	TLC and auto-radiography	$0.3x10^{-15}$ mols adduct/µg DNA	No data	Phillips et al. 1988
	Collection of lymphocyte cells; isolation of BPDE-DNA adduct by standard treatment; assay of BPDE-DNA adduct by immunoassay; analyses by SLS	ELISA/USERIA; SLS	$0.06–0.23x10^{-15}$ mol BPDE/µg DNA	No data	Harris et al. 1985

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Feces	Extraction with benzene:MeOH (4:1); add MeOH:H_2O (4:1); clean-up on silica gel column	HPLC/UV	0.05 µg/g	No data	Hecht et al. 1979
Urine	Acidify to pH 3 with HCl; clean-up on activated Sep-Pak C_{18} cartridge column; reduction with hydriodic acid	HPLC/fluorescence detector	<1 µg PAH/mmol creatinine	10–85	Becher and Bjorseth 1983
	Extraction into cyclohexane; concentration; reduction with hot acid	GC/FID	1.2–6.48 µg PAH/mmol creatinine	No data	Becher and Bjorseth 1985
	Hydrolysis; isolation of tetrol by Sep-Pak chromatography; clean up by immunoaffinity chromatography (anti BP-tetrol-modified guanosine column)	HPLC/SFS (7,8,9,10-BaP tetrol)	0.01 pmol/mL	>30	Weston et al. 1993a
	Isolation on a Sep-Pak column, washing with water followed by 10% MeOH; elution with 100% MeOH; concentration; addition of 0.1 M HCl with heating	SLS	25 pg metabolite/mL	No data	Uziel et al. 1987

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (cont.)	Collection of radiolabeled benzo[a]pyrene urine sample; addition of MeOH; isolation on C_{18} Sep-Pak column; elution with aqueous MeOH	HPLC/UV	$5x10^{-12}$ mol 7-BPDE-Gua/10 µg of labelled benzo[a]-pyrene	No data	Autrup and Seremet 1986
	Buffer to pH=5.5; enzymatic hydrolysis with β-glucuronidase/sulfatase (4 hours at 37.5 °C); clean-up using Sep-Pak C_{18} cartridge; isolation of 1-pyrenol	HPLC/fluorescence detector	0.45 nmol/L (1-pyrenol)	No data	Tolos et al. 1990
	Dilution; extraction into $CHCl_3$; precipitation of protein; wash extract with CH_3OH; evaporation and analysis of residue	HPLC/UV; FLNS	~1 fmol (BP tetrol)	No data	Rogan et al. 1990
Feces	Homogenization and drying; extraction with $CHCl_3$; evaporation and analysis of residue	HPLC/UV; FLNS	~1 fmol (BP tetrol)	No data	Rogan et al. 1990
Hair	Incubation of hair follicle with (-)-B[a]P-7,8-diol for 24 hours; addition of acetone; centrifugation; analysis of supernatant	HPLC/fluorescence detector	~0.3 fmol of tetrols	No data	Alexandrov et al. 1990

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Thymus and liver	Preparation of fluoranthene-modified DNA *in vitro*; digestion with enzyme; isolation of adducts using disposable C_{18} cartridge; nuclease P1 pretreatment to remove residual unmodified nucleotides; labelling of fluoranthene-DNA adduct by ^{32}P-postlabelling technique; nuclease P1 digestion	HPLC/radioisotope counting	0.1 fmol adduct (3 adducts/10^8 nucleotides in 1 µg DNA)	10–15	Gorelick and Wogan 1989
Thymus	Preparation of B[a]P-DNA adduct; digestion; labelling of adduct by ^{35}S-postlabelling technique	HPLC/radioisotope counting	1 adduct/10^8 nucleotides for 60 µg DNA	20	Lau and Baird 1991
Embryo and thymus	Preparation of PAH-DNA adduct; digestion; labelling of adduct by ^{35}P-postlabelling; separation of steroisomers by immobilized boronate chromatography	IP-RP-HPLC/radioisotope flow detects (+) and (–) enantiomers of anti- and syn- PAH-DE-DNA adduct	No data	No data	Baird et al. 1993

TABLE 6-1. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Biological Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Placenta	Hydrolysis of DNA; addition of phosphate-buffered saline; neutralization with NaOH; incubation of sensor in sample	FIS	$14x10^{-18}$ mol BPT	No data	Vo-Dinh et al. 1991

ATP = adenosine triphosphate; B[a]P = benzo[a]pyrene; BPDE = 7_{β},8α-dihydroxy-[9α, 10α]-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene; BPT = benzo[a]pyrene tetrol; $CHCl_3$ = chloroform; CH_3OH = methanol; DMSO = dimethyl sulfoxide; DNA = deoxyribonucleic acid; ELISA = enzyme linked immunosorbent assay; FIS = fluoroimmunosensor; FLNS = fluorescence line narrowing spectrometry; fmol = femtomole; GC/FID = gas chromatography/flame ionization detector; GC/MS = gas chromatography/mass spectrometry; Gua = Guanosine; H_2O = water; HCl = hydrogen chloride; HPLC = high performance liquid chromatography; IgG = immunoglobin; IP-RP-FPLC = ion-paired reverse phase high pressure liquid chromatography; KOH = potassium hydroxide; LESS = laser-excited Stepol'skii spectroscopy; M = molar; MeOH = methanol; $MgCl_2$ = magnesium chloride; mmol = millimole; $NADP^+$ = oxidized nicotinamide adenosine dinucleotide; NaOH = sodium hydroxide; Na_2SO_4 = sodium sulfate; ng = nanogram; NIEC-MS = negative ionization electron capture mass spectrometry; nmol = nanomole; PAHs = polycyclic aromatic hydrocarbons; pg = picogram; pmol = picomole; PNPP = para nitrophenyl phosphate; SF = spectrofluorometry; SFS = synchronous fluorescence spectroscopy; SLS = synchronous luminescence spectroscopy; TLC = thin-layer chromatography; USERIA = ultra sensitive enzyme radioimmuno assay; UV = ultraviolet; UV-VIS = UV-visible detector.

Obana et al. (1981) reported the identification and quantification of six PAHs on EPA's priority pollutant list: anthracene, pyrene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, and benzo[g,h,i]perylene using the HPLC/fluorescence detector technique. Levels measured in human. tissue ranged from 0.006 to 0.460 ng/g. Following extraction of the PAHs from the sample matrices by saponification with KOH, the extract was cleaned on alumina and silica gel columns, prior to quantitation. The known carcinogens, benz[a]anthracene and dibenz[a,h]anthracene, were not detected (detection limit <0.005 ng/g). The HPLC/UV detection technique has also been used to simultaneously determine fluoranthene, benz[a]anthracene, and pyrene in blood and lung tissues (Brandys et al. 1989). A detection limit of ppb (ng/g or ng/mL), satisfactory recoveries (65-109%), and adequate precision (119% relative standard deviation [RSD]) were achieved (Brandys et al. 1989).

In addition to direct measurement of PAHs in biological tissues, it is also possible to determine the concentration of metabolites in biological fluids. Pyrene is predominantly excreted as a 1-hydroxypyrene conjugate (glucoronate and sulfate), although 1,2-dihydroxy- 1,2-dihydropyrene conjugates are .also excreted in urine (Grimmer et al. 1993). Phenanthrene, on the other hand, is mainly excreted as dihydrodiol conjugates. The metabolites of phenanthrene that have been detected in human urine are 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 4-hydroxyphenanthrene, 9-hydroxyphenanthrene, 1,2-dihydroxy-1,2-dihydrophenanthrene, 3,4-dihydroxy-3,4-dihydro-phenanthrene, and 9,10-dihydroxy-9,10-dihydrophenanthrene (Grimmer et al. 1993). There are apparently individual variations in the phenanthrol (hydroxyphenanthrene) and phenanthrene dihydrodiol conjugates excreted in the 24-hour urine sample (Grimmer et al. 1993). The major metabolite of benzo[a]pyrene in human tissue and body fluid is 7,8,9,10-tetrahydroxy-7,8,9,10-tetrahydrobenzo[a]pyrene (Weston et al. 1993a, 1993b).

Becher and Bjorseth (1983, 1985) and Becher (1986) developed an HPLC method for biological monitoring of PAHs and PAH metabolites in the urine of humans following occupational exposure to PAHs. Using the HPLC/fluorescence detector technique, recoveries of the individual PAH compounds varied between 10 and 85% with the more volatile 3-ring PAHs having the lowest recoveries. A detection limit of less than 1 µg of PAHs per mmol of creatinine was obtained. HPLC equipped with a fluorescence detector has also been used to measure 1-pyrenol (1-hydroxypyrene, a pyrene metabolite) in urine of workers exposed to PAHs in coal tar pitch with a detection limit of

0.45 nmol/L (Tolos et al. 1990). Recovery and precision data were not reported. A strong correlation was observed between the concentrations of urinary 1-hydroxypyrene in workers and environmental PAHs, indicating that pyrene may be used as a biomarker of exposure for assessing worker exposure to coal tar pitch containing pyrene (Tolos et al. 1990). Since 1-Hydroxypyrene glucuronide is approximately 5 times more fluorescent than 1-hydroxypyrene, the former may be a more sensitive biomarker for PAH exposure (Strickland et al. 1994). A sensitive HPLC/synchronous fluorescence spectroscopic method is available for the determination of 1-hydroxypyrene glucuronide (Strickland et al. 1994). Hecht et al. (1979) employed an HPLC analytical technique for determining the concentrations of benzo[a]pyrene and its metabolites in the feces of humans and rats following consumption of charcoal-broiled beef. A detection limit of 0.05 μg of benzo[a]pyrene metabolites per gram of sample was noted with HPLC/UV detection.

There is considerable evidence that PAHs are enzymatically converted to highly reactive metabolites that bind covalently to macromolecules such as DNA, thereby causing mutagenesis and carcinogenesis in experimental animals. Thus, benzo[ajpyrene, a prototype of the carcinogenic PAHs and the most thoroughly studied PAH, is activated by microsomal enzymes to 7β, 8α-dihydroxy-(9α,10α)-epoxy-7,8,9,10-tetrahydrobenzo[a]pyrene (BPDE) and binds covalently to DNA, resulting in formation of BPDE-DNA adducts (Harris et al. 1985; Haugen et al. 1986; Uziel et al. 1987). Sensitive methods are available to detect PAH-DNA adducts in the blood and tissues of humans and animals. These include immunoassays, i.e., enzyme-linked immunosorbent assays (ELISA), radioimmunoassay (RIA), dissociation-enhanced lanthanide fluoroimmunoassay (DELFIA), and ultrasensitive enzyme radioimmunoassay (USERIA); ^{32}P- and ^{35}S-postlabelling with radioactivity counting; surface-enhanced Raman spectroscopy; and synchronous luminescence spectroscopy (SLS) (Gorelick and Wogan 1989; Gorelick and Reeder 1993; Harris et al. 1985; Haugen et al. 1986; Helmenstine et al. 1993; Herikstad et al. 1993; Lau and Baird 1991; Perera et al. 1988; Phillips et al. 1987; Schoket et al. 1993).

The ELISA technique is used for detection of antibodies in serum bound to BPDE-DNA adducts. The USERIA method involves measuring the immunological response of BPDE-DNA in the presence of rabbit anti-serum. Several researchers have employed the immunoassay techniques for detecting PAH-DNA adducts at 10^{-15} mol levels in the blood and tissues of humans occupationally exposed to

PAHs (Harris et al. 1985; Haugen et al. 1986; Newman et al. i988; Perera et al. 1988; Shamsuddin et al. 1985; Weston et al. 1988).

^{32}P-postlabelling is a highly sensitive and specific method for detecting PAH-DNA adducts in the blood and tissues of humans and animals (Gorelick and Wogan 1989; Phillips et al. 1988, 1987; Willems et al. 1991). Detection limits ranging from $0.3x10^{-15}$ mol of PAH adduct per µg of DNA (<1 adduct in 10^7 nucleotides) to $<10^{-18}$ mol of adduct per ug of DNA have been achieved (Phillips et al. 1988, 1987; Willems et al. 1991). Further advantages of the ^{32}P-postlabelling technique are that adducts do not need to be fully characterized in order to be detected, and that the method is particularly suited to occupational exposure to a complex mixture of PAHs. Coupling ^{32}P-postlabelling methodology with HPLC analysis has improved the resolution of the labeled nucleotides and can be used to identify and quantify specific PAH-DNA adducts such as fluoranthene-DNA adducts (Gorelick and Wogan 1989). A detection limit of 0.1 femtomole (fmol) of adduct (3 adducts per 10^8 nucleotides in 1 µg DNA) has been achieved. The advantage of this method is that it is not limited with respect to the amount of DNA that can be analyzed; therefore, sensitivity can be enhanced by analyzing larger quantities of DNA. Average recovery was 10-15% at 3 adducts per 10^6 nucleotides. Recovery was greater (30-40%) from DNA containing higher levels of adducts (Gorelick and Wogan 1989). The ^{32}P-postlabelling assay and a combination of thin-layer and reverse-phase HPLC was also used to separate DNA adducts of 6 nitrated PAHs (King et al. 1994). PAH-DNA adducts have also been detected and identified using [^{35}S]phosphorothioate postlabelling combined with HPLC analysis (Lau and Baird 1991). The sensitivity of this assay is 1 adduct per 10^8 nucleotides for a 60-µg DNA sample with an overall adduct recovery of 20%. An advantage of ^{35}S-postlabelling over ^{32}P-postlabelling is that ^{35}S has a longer half-life (87 days) than ^{32}P (14 days). This allows longer storage times between labeling and adduct analysis with minimal loss in sensitivity. ^{35}S also has a lower radioactive decay energy than ^{32}P, which reduces the risk of human radiation exposure and eliminates the need for the radioisotope-shielding equipment that is required for studies with high specific radioactivity. On the other hand, ^{35}S is also less sensitive than the ^{32}P-postlabelling analysis because of the lower specific activity of [^{35}S]adenosine triphosphatase (ATP) compared to [^{32}P]ATP and because of the requirement for more radioactivity per adduct for accurate HPLC analysis. However, if large samples of DNA are available, the sensitivity of ^{35}S-postlabelling/HPLC can be increased substantially (Lau and Baird 1991).

HPLC/UV detection and HPLC/fluorescence detection have been used for determining concentrations of PAH-DNA adduct and hydrolyzed PAH-DNA adducts in biological tissues and fluids (Alexandrov et al. 1990; Autrup and Seremet 1986; Jongeneelen et al. 1986; Rice et al. 1985b; Rogan et al. 1990; Salhab et al. 1987; Shugart 1986; Shugart et al. 1983; Weston and Bowman 1991; Weston et al. 1988). A detection limit of l0-l5 mol of tetrols per sample was achieved (Haugen et al. 1986; Shugart et al. 1983; Weyand and Bevan 1987a). HPLC with a fluorescence detector has been used to measure the stereospecific formation of benzo[a]pyrene tetrols from cytochrome P-450-dependent metabolism of (-)-7,8-dihydroxy-7,8-dihydrobenzo[a]pyrene to BPDE in human hair (Alexandrov et al. 1990). This assay is simple, requiring only three human hair follicles and a low (0.5-2 μmol) substrate concentration. The limit of detection is ≈0.3 fmol of tetrols (Alexandrov et al. 1990). This is a rapid and noninvasive method that could be used to determine an individual's capacity to activate carcinogens to DNA-binding intermediates (Alexandrov et al. 1990). HPLC with fluorescence detection has also been used to detect BPDE-DNA adducts in human lung tissues (Weston and Bowman 1991). A detection limit of 6 pg benzo[a]pyrene-tetrol/mL (1 adduct in 10^8 nucleotides) was achieved (Weston and Bowman 1991). Recoveries ranged from 26 to 66% for the procedure. HPLC/UV has been used to identify and quantify a benzo[a]pyrene-DNA adduct, specifically 7-(benzo[a]pyrene-6-yl)guanine (BP-N7Gua) in urine and feces in the femtomole range (Rogan et al. 1990). The structure of the adduct was established by fluorescence line narrowing spectrometry (FLNS). Recovery and precision data were not reported (Rogan et al. 1990).

Using benzo[a]pyrene as a model carcinogen, Vahakangas et al. (1985), Haugen et al. (1986), and Harris et al. (1985) have developed an synchronous luminescence spectroscopy (SLS) technique for detecting trace levels of PAH-DNA adducts in the blood of humans occupationally exposed to high levels of PAHs. Vahakangas et al. (1985) detected less than 1 benzo[a]pyrene moiety per 10^7 DNA molecules by SLS technique following *in vitro* acid hydrolysis of BPDE-DNA adduct. Fiber-optic antibody-based fluoroimmunosensor (FIS) has been used to measure DNA adducts of benzo[a]pyrene in biological samples such as human placenta (Tromberg et al. 1988; Vo-Dinh et al. 1991). The FIS is used to detect the highly fluorescent benzo[a]pyrene 7,8,9,10-tetrol (BPT) after release from the weakly fluorescent BPDE-DNA by mild hydrolysis. The FIS is highly specific because of the antigen-antibody reaction. This assay is highly sensitive, achieving a detection limit of 14×10^{-18} mol

of BPT (Vo-Dinh et al. 1991). FIS precision is adequate (6.2-15% RSD) (Tromberg et al. 1988). Recovery data were not reported.

6.2 ENVIRONMENTAL SAMPLES

One of the difficulties associated with determination of PAHs in environmental samples is the complexity of PAH mixture in these samples. Even after extensive and rigorous clean-up, the PAH fraction may contain hundreds of compounds. Analytical methods that offer combinations of good chromatographic resolving power and detector selectivity are usually required to quantify selected compounds in such mixtures. There is essentially a three-step procedure for the analysis and determination of PAHs in environmental samples: (1) extraction and isolation of PAHs from the sample matrix; (2) clean-up of the PAH mixtures from impurities and fractionation of PAH into subgroups; and (3) identification and quantitative determination of the individual components in each of these subgroups.

The collection of PAHs from air for quantification requires special considerations. Some of the PAHs, especially those with lower molecular weights, exist primarily in the vapor phase while PAHs with higher molecular weights exist primarily in the particulate phase (Santodonato et al. 1981). Therefore, a combination of a particulate filter (usually glass-fiber filter) and an adsorbent cartridge (usually XAD-2 or polyurethane foam) is used for the collection of PAHs (Andersson et al. 1983; Harvath 1983; Hawthorne et al. 1993). Therefore, collection methods that use either a filtration system or an adsorbent alone may be incapable of collecting both particulate and vapor phase PAHs. In addition, a few PAHs are known to be susceptible to oxidation by ozone and other oxidants present in the air during the collection process (Santodonato et al. 1981).

The commonly used methods for the extraction of PAHs from sample matrices are Soxhlet extraction, sonication, or partitioning with a suitable solvent or a solvent mixture. Dichloromethane, cyclohexane, benzene, and methanol have been widely used as solvents (see Table 6-2). Supercritical fluid extraction (SFE) of heterogeneous environmental samples with carbon dioxide in the presence of a modifier, such as 5-10%. methanol or dichloromethane is preferable to the conventional extraction method because SFE is much less time consuming and has comparable or better PAH extraction

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Collection on XAD and glass-fiber filters with 2-D labeled PAH internal standards; extraction with toluene; fractionation and clean-up on activated silica and alumina	HRGC/MS	0.001–0.03 ng/m^3	No data	Hippelein et al. 1993
	Collection on glass-fiber filter; extraction with methylene chloride; clean-up on silica gel column; analysis at 254 nm	GC/DAD	0.2–4.8 ng/sample	75–100	Desilets et al. 1984
	Collection of on glass-fiber filter; extraction with benzene:MeOH (4:1); concentration; fractionation into acid/neutral/base fractions; clean-up neutral fraction by column chromatography; concentration	GC/FID; HPLC/fluor-escence detector	0.05 ng/m^3	No data	Matsumoto and Kashimoto 1985
	Collection on a glass-fiber filter; thermal desorbtion of filter onto GC column	GC/LIMF	1–15 µg/sample	No data	Galle and Grennfelt 1983
	Collection on a glass-fiber filter; extraction with 35% methylene chloride in cyclohexane	HPLC/fluorometric detector	<0.01 ng/sample	No data	Golden and Sawicki 1978
	Collection on filter; extraction with organic solvent	TLC; GC/MS	<1 pg/sample	No data	Majer et al. 1970

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (cont.)	Collection on fiber-glass filter; extraction with cyclohexane; concentration	GC/GPFD	$1x10^{-6}$ ppm	100.5	Mulik et al. 1975
	Collection on filter; extraction with toluene; acid/base fractionation; drying and concentration	HPLC; GC/MS	0.11 ppm	>85	Naikwadi et al. 1987
	Collection on glass-fiber filter; ultrasonic extraction with benzene; concentration; fractionation by HPLC	HRGC/FID	low ng/m^3	8–100	Tomkins et al. 1982
	Collection on filter; extraction with cyclohexane; clean-up on silica column	GC/MS	0.001–0.002 ppm	No data	Oehme 1983
	Collection on glass-fiber filter (particulates) and XAD-2 resin (vapor); extraction with benzene or methylene chloride	HPLC; GC/MS	0.001–0.1 ppm	No data	Harvath 1983
	Collection on glass-fiber filter; extraction with benzene; concentration	HPLC/fluor-escence detector	0.000025 ppm	92–100	Fox and Staley 1976
	Collection through filter onto XAD-2 resin; extract with benzene, cyclohexane or methylene chloride (NIOSH Methods 5506 and 5515)	HPLC; GC/FID	<1 ppm	No data	NIOSH 1984

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (cont.)	Collection on a glass-fiber filter; extraction with cyclohexane; clean-up by partitioning to DMSO and pentane	GC/MS	0.00001 ppm	No data	Karlesky et al. 1987
	Collection of air particulates with high-volume sampler; ultrasonic extraction with acetonitrile	HPLC/fluor-escence detector	10–50 pg	No data	Miguel and DeAndrade 1989
Sea water	Extraction with hexane or carbon tetrachloride; acid-base fractionation; and clean-up on silica and alumina column	GC/FID	0.024–0.045 µg/L	44–85	Desiderie et al. 1984
Water	Collection on a column containing XAD-4:XAD-8 (1:1) resin; elution with acetone followed by chloroform	HPLC/UV; GC/MS	0.01–3 µg/L	No data	Thruston 1978
	Extraction with cyclohexane	HPLC with time-resolved fluorescence detection	180×10^{-15} g/sample	89–100	Furuta and Otsuki 1983
	Filtration into flotation vessel; adjustment to pH 3; addition of Triton X-100; bubbling nitrogen through mixture; collection of foam, and extraction with methylene chloride; evaporation and dissolution of residue in methanol	HPLC/fluor-escence detector	low ng/L	86–107	Xu and Fang 1988

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water (cont.)	Extraction at neutral pH with methylene chloride (EPA Methods 8100, 8250,and 8310)	HPLC/fluor-escence detector GC/FID GC/MS	0.64–0.013 µg/L 2.0–45.1 µg/L 1.9–7.8 µg/L	No data	EPA 1986
	Collection through sampling cartridges containing XAD-2 resin; elution with acetone:hexane (15:85)	GC/MS	0.00005 µg/L	57–100	Beniot et al. 1979
Municipal and industrial waste water	Extraction with methylene chloride; reconstitution in cyclohexane; clean-up on silica gel column (EPA Method 610)	HPLC/UV fluorescence detector	0.013–2.3 µg/L	78–116	EPA 1982
	Adjustment to pH >11.0; extraction with methylene chloride; drying with sodium sulfate; concentration (EPA Method 625)	GC/MS	1.6–7.8 µg/L	41–83	EPA 1982
Sediments	Extraction with methylene chloride; clean-up on alumina column	TLC; GC/MS	0.2–2.7 µg/g	86–89	John and Nickless 1977
	Freeze drying, sieving and homogenization; extraction with methylene chloride; clean-up on silica gel followed by sephadex column	HPLC/DAD/MS	pg range	No data	Quilliam and Sim 1988

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediments (cont.)	Extraction of dry sample with methylene chloride; injection into supercritical fluid extracting system	GC/MS	2.8–7.3 µg/g	91–97	Hawthorne and Miller 1987a, 1987b
	Extraction of dried sample with benzene; clean-up on silica gel and alumina column	GC/FID; GC/MS	0.014–0.093 µg/g	76–110	Szepesy et al. 1981
Sediments	Direct sampling of sediment in sample insert of SSJ/LIF	SSJ/LIF	1.8 ppm (B[a]P); 0.4 ppm (pyrene)	No data	Lai et al. 1990
	Extraction by sonication; clean-up on silica mini-columns	Spectrofluoro-metry	0.008–4.5 ng/mL	80–95	Saber et al. 1991
Waste water and sediments	Freeze drying; extraction with chloroform:MeOH (2:1); concentration of crude extract; clean-up by TLC followed by HPLC	GC/FID	0.12–0.46 µg/g	51–100	Readman et al. 1986
Water and sediments	Extraction in organic solvent	GC/FT-IR	0.01–0.06 µg/g	No data	Gurka et al. 1987
Soil	Extraction in organic solvent; concentration	GC/FT-IR	0.025–0.25 µg/sample	0.998–0.85 correlation coefficient	Gurka and Pyle 1988
	Ultrasonic extraction of sieved sample with acetonitrile; filtration through teflon filter	HPLC/SF	0.017 µg/g	No data	Tanaka and Saito 1988

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Sediments/ suspended matter (river), airborne particulate, dust and soil	Extraction of dried sample with methylene chloride; clean-up on activated copper column followed by sephadex	GC/FID; GC/MS	0.03–0.09 µg/g	99–113	Giger and Shaffner 1978
Diesel exhaust particulate and dust	Collection on fiber filter; extraction with hexane; concentration; partitioning with DMSO; concentration of organic extract	HPTLC; FSD	1–50 pg/sample	No data	Butler et al. 1984a
Cigarette smoke	Collection in trap of smoking machine; dissolution in benzene:MeOH:H_2O (2:1:2); clean-up on silicic acid and gel filtration column	GC/FID	No data	92–95	Severson et al. 1976
	Collection on filter pad; extraction with cyclohexane	HPLC/fluore-scence detector	3 pg/sample	89–108	Risner 1988
Cooking oil fume	Collection on glass-fiber filter; extraction with acetone; concentration, then dissolution in cyclohexane; clean-up by partitioning in DMF and reconstitution in cyclohexane	TLC/FSD	0.11–0.41 ng	96–99	Shuguang et al. 1994
Coal-fly ash	Drying at 150 °C, cooling in desiccator; ultrasonic extraction with methylene chloride; concentration	GC/MS	No data	No data	Low et al. 1986

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Highly refined coal- and petroleum-derived fuels	Dissolution in methylene chloride	HPLC/HPLC/fluorescence detector	3.5–46 µg/L	56–100	Tomkins and Griest 1987
	Collection in brown Winchester bag; addition of 20% aqueous MeOH with shaking; clean-up on Sep-Pak C_{18} cartridge column	HPLC/UV and fluorescence detector	0.1–7.1 µg/L	45–95	Symons and Crick 1983
	Dissolution in hexane; clean-up on silica and alumina gel column	TLC; SPF	µg/L range	No data	Monarca and Fagioli 1981
Highly refined coal- and petroleum-derived fuels (con.)	Dissolution in methylene chloride	HPLC/UV-VIS; GC/MS	2000 µg/L	No data	Tomkins et al. 1986
Solvent refined coal	Crushing into fine particles; dissolution in benzene; filtration	N-SSL R-SSL	$7x10^{-7}$ M $7x10^{-5}$ M	No data	Lin et al. 1991
Shale and fuel oil	Dissolution in cyclohexane; fractionation into acid and base/neutral fractions; clean-up base/neutral fraction on alumina followed by alumina-silica column	XEOL	10 ng/sample	No data	Woo et al. 1980
	Dilution in ethanol	RTP SLS	No data No data	±15 RSD ±4 RSD	Vo-Dinh et al. 1984
Sun tan oil	Extraction with hexane; clean-up on silica gel column	TLC; SPF	Low µg/L	79–93	Monarca et al. 1982

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
White petroleum products	Collection on silica gel column; elution with pentane:ether (1.5:1); concentration; clean-up on basic and acidic alumina column	FPS	µg/L range	No data	Popl et al. 1975
Sewage sludge	Homogenization; extraction with cyclohexane; centrifugation; separation and concentration of organic phase	2-Dimensional TLC/fluorescence detector	<1 µg/g	80–100	McIntyre et al. 1981
Smoked foods (e.g., fish and meat)	Saponification; extraction with cyclohexane; clean-up on Florisil column	HPLC/fluorescence detector GC/FID GC/MS	2–27 pg/sample 10 pg/sample 1,000 pg/kg	28–142	Lawrence and Weber 1984
	Soxhlet extraction of homogenized sample with acetone; saponification with ethanolic KOH; extraction with cyclohexane; drying and concentration; clean-up on alumina column; concentration, then dilution in methanol	HPLC/fluorescence detector	0.1–µg/kg	75–90	Moll et al. 1993
Charcoal-broiled beef	Extraction of ground sample with benzene:MeOH (4:1); evaporation to dryness; dissolution of residue in MeOH:H_2O (4:1); clean-up on silica gel column	HPLC/fluorescence detector	20–50 ng/g	No data	Hecht et al. 1979

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Seafoods	Saponification of homogenized sample with ethanolic KOH; extraction with 1,1,2-trichlorotrifluoroethane; concentration; clean-up by silica, alumina and C_{18} cartridge (modified FDA method)	GC-MS	1–5 µg/kg	73–144	Nyman et al. 1993
	Saponification of homogenized sample with ethanolic KOH; extraction with 1,1,2-trichlorotrifluoroethane; concentration; clean-up by silica alumina and gel permeation HPLC (NMFS method)	GC/MS	1–5 µg/kg	63–106	Nyman et al. 1993
Cooked beef	Saponification of ground sample with methanolic KOH; extraction with cyclohexane, DMF, and n-hexane; concentration	LT-MLS SLS HPLC/ fluorescence detector	0.9 ppb 0.2 ppb 1.0 ppb	75–85 (extraction efficiency)	Jones et al. 1988
Food (meat/fish, dried dairy products, cereals, leafy vegetables, and oils)	Digestion with alcoholic KOH; partitioning into cyclohexane or isooctane; removal of lipids by solvent partitioning with dimethylformamide or dimethylsulfoxide/water; clean-up on silica gel, Florisil, or Sephadex	HPLC/fluor-escence detector; GC-MS/SIM	2–90 ng/kg	20.6–92.5 (ocean perch); 34.2–62.7 (bran cereal); 98 (powdered milk)	Lawrence and Das 1986

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Cereal products	Saponification; extraction with cyclohexane; reextraction with 15% caffeine in formic acid; dilution in sodium chloride solution; reextraction with cyclohexane; clean-up on silica gel; concentration	GC/MS	20 pg/inj	40–100	Tuominen et al. 1988
Vegetable oil	Dilution with *n*-pentane	LC-GC/MS	1 pg/sample	No data	Vreuls et al. 1991
Fat products	Dissolution in light petroleum; extraction with caffeine in formic acid; dilution in sodium chloride solution; reextraction with light petroleum; clean-up on silica gel column	HPLC/fluor-escence detector	0.1–0.5 ppb	76–85	Van Heddeghem et al. 1980
Barley malt	Homogenization; ultrasonic extraction with cyclohexane; centrifugation; clean-up of supernatant on silica gel-alumina column	HPLC/UV and fluorescence detector	2.5–5 ng/g	78–97	Joe et al. 1982
Alcoholic beverage	Continuous extraction with cyclohexane for 20 hours	HPLC/UV; GC/FID	1 µg/L	60	Toussaint and Walker 1979
Tea	Saponification; extraction with hexane; addition of DMSO with shaking; clean-up on silica gel	TLC; FSD	2–12 ng/g	92–95	Poole et al. 1987

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish tissue	Homogenization with 1.15% KCl solution; isolation on extraction cartridges packed with a styrene-divinylbenzene copolymer resin; washing with water; extraction with acetone:MeOH (1:1); extraction with methylene chloride:2-propanol (75:25)	HPLC/fluorescence detector; GC/MS	50–1,100 ng/g	>90	Krahn and Malins 1982
	Homogenization with distilled water and KOH pellets; reflux, then extraction with methylene chloride; clean-up on basic alumina column	GC/MS	<0.2 ng/g	72	Vassilaros et al. 1982
	Homogenization in methylene chloride; centrifugation; clean-up on alumina column	HPLC/UV; GC/MS	No data	89–98	Krahn et al. 1988
B[a]P metabolite formulation	Dissolution in MeOH	HPLC/MS	low ng/sample	No data	Bieri and Greaves 1987
PAH formulation	Dissolution in methylene chloride	GC/MS with laser multiphoton ionization detection	200×10^{-15} g sample	No data	Rhodes et al. 1983
	Dissolution in acetonitrile	UV-RRS	<1 ppb	No data	Asher 1984
	Dissolution in ethanol	HPLC/fluorescence detector	500–16,000 ppb	No data	Su et al. 1982

TABLE 6-2. Analytical Methods for Determining Polycyclic Aromatic Hydrocarbons in Environmental Samples (continued)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
PAH formulation (cont.)	Conversion to nitroaromatic compound (packing sample in glass tube between glass-wool plugs, passing reagent gas through tube for 3 to 5 seconds); analysis for nitroaromatic compound	TQMS	100–500 ppb	No data	Hunt et al. 1983

B[a]P = benzo[*a*]pyrene; DMF = dimethylformamide; DMSO = dimethyl sulfoxide; EPA = Environmental Protection Agency; EtOH = ethanol; FPS = fluorescence and phosphorescence spectrometry; FSD = fluorescence scanning densitometry; GC = gas chromatography; GC/FID = gas chromatography/flame ionization detector; GC/DAD = gas chromatography/diode array detector; GC/LIMF = gas chromatography/laser induced molecular fluorescence; GC/GPFD = gas chromatography/gas phase fluorescence detector; GC/MS = gas chromatography/mass spectrometry; GC/FT-IR = gas chromatography/fourier transform-infra-red spectrometry; HPLC = high performance liquid chromatography; HCl = hydrochloric acid; HPLC/HPLC = high performance liquid chromatography/high performance liquid chromatography; HPLC/UV = high performance liquid chromatography/ultraviolet; HPLC/DAD/MS = high performance liquid chromatography/diode array detector/mass spectrometry; HPLC/SF = high performance liquid chromatography/spectrofluoremetry; HPTLC = high performance thin-layer chromatography; HPTLC/UV = high performance thin-layer chromatography/ultraviolet; H_2O = water; H_2SO_4 = sulfuric acid; inj = injection; KOH = potassium hydroxide; LC = liquid chromatography; LT-MLS = low temperature-molecular luminescence spectrometry; LC-GC/MS = liquid chromatography-gas chromatography/mass spectrometry; M = molar; MeOH = methanol; ng = nanogram; NMFS = National Marine Fisheries Service; PAHs = polycyclic aromatic hydrocarbons; R/N-SSL = resonant/nonresonant-synchronous scan luminescence; RSD = relative standard deviation; RTP = room temperature phosphorescence; SF = spectrofluorometry; SLS = synchronous luminescence spectroscopy; SSJ/LIF = supersonic jet/laser induced fluorescence; SIM = selected ion monitoring; SJS/SFC = supersonic jet spectroscopy/supercritical fluid chromatography; SP = spectrophotometer; SPF = spectrophotofluorometer; TCTFE = 1,1,2-trichloro-1,2,2-trifluoroethane; TLC = thin-layer chromatography; TQMS = triple quadruple mass spectrometer; UV-RRS = ultraviolet-resonance raman spectrometer; UV-VIS = UV-visible detector; XEOL = x-ray excited optical luminescence

recovery than the conventional methods (Burford et al. 1993; Dankers et al. 1993; Hawthorne et al. 1993; Hill and Hill 1993).

Column chromatography on silica, alumina, Sephadex or Florisil has been used most often for the clean-up and fractionation of PAHs in the sample extract (Desiderie et al. 1984; Desilets et al. 1984; Oehme 1983; Quilliam and Sim 1988). HPLC can also be used for the clean-up and fractionation of PAHs in sample extract (Readman et al. 1986). A disposable Sep-Pak cartridge with an amino stationary phase was used for the clean-up of benzo[a]pyrene in cigarette smoke condensate (Dumont et al. 1993). Some soil and sediment samples containing high amounts of sulfur may require clean-up on an activated copper column (Giger and Schaffner 1978).

A variety of analytical methods has been used for determining trace concentrations of PAHs in environmental samples (Table 6-2). These include GC with various detectors, HPLC with various detectors, and TLC with fluorimetric detectors. Various detection devices used for GC quantification include FID, MS, Fourier transform infrared spectrometer (FT-IR), laser induced molecular fluorescence detector (LIMF), diode array detector (DAD), and gas phase fluorescence detector (GPFDA). GC/MS and HPLC with UV or spectrofluorimetric detectors are perhaps the most prevalent analytical methods for determining concentrations of PAHs in environmental samples.

Oehme (1983) and Low et al. (1986) employed capillary GC coupled with negative ion chemical ionization MS for detecting and differentiating isomeric PAHs (including PAHs on EPA's priority pollutant list). This procedure was successfully used to differentiate the isomers benzolj]fluoranthene and benzo[b]fluoranthene at low ppb levels in complex matrices, such as air particulate matter and coal fly ash. An alternative method for the elucidation of PAH isomers is GC coupled with a charge-exchange and chemical ionization MS (Simonsick and Hites 1985). Simonsick and Hites (1985) demonstrated that the structural isomers pyrene, fluoranthene, aceanthrylene and acephenanthrylene can be identified on the basis of their first ionization potential and $(M+l)^+/M^+$ mass ion ratio.

HPLC has been one of the most widely used analytical methods for determining PAHs in complex environmental samples. The development of a chemically nonpolar stationary phase for HPLC has

provided a unique selectivity for separation of PAH isomers that are often difficult to separate by GC columns. For example, chrysene, benz[a]anthracene, and triphenylene are baseline resolved with a C-18 reverse phase column packing. A detection limit of subpicogram to picogram levels of PAHs per sample has been achieved by HPLC with fluorescence detector (Fox and Staley 1976; Furuta and Otsuki 1983; Futoma et al. 1981; Golden and Sawicki 1978; Lawrence and Weber 1984; Marcomini et al. 1987; Miguel and De Andrade 1989; Nielsen 1979; Risner 1988; Ton&ins et al. 1982). HPLC equipped with a fluorescence detector has selectively measured 10 PAHs (phenanthrene, fluoranthene, pyrene, benz[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, and indeno[l,2,3-c,d]pyrene) in ambient air (Miguel and De Andrade 1989). Detection limits for the 10 PAHs were in the range of 10-50 g and RSD was <10%. Recovery data were not reported. PAH levels in the ng/L range have also been successfully determined in water using flotation enrichment and HPLC/fluorescence detection (Xu and Fang 1988). Good recoveries (86-107%) were achieved, and RSD was 2.7-13.6% RSD. A quenchofluorometric detection system provides an inexpensive method to achieve selective detection for the fluoranthenic PAHs as a group (Konash et al. 1981). UV detectors have been used to measure PAHs in fats and oil samples; however, these detectors lacked the sensitivity and specificity of the fluorescence detectors for determining PAHs at low levels (ppb and lower) (Van Heddeghem et al. 1980).

A number of less commonly used analytical techniques are available for determining PAHs. These include synchronous luminescence spectroscopy (SLS), resonant (R)/nonresonant (NR)-synchronous scan luminescence (SSL) spectrometry, room temperature phosphorescence (RTP), ultravioletresonance Raman spectroscopy (UV-RRS), x-ray excited optical luminescence spectroscopy (XEOL), laser-induced molecular fluorescence (LIMF), supersonic jet/laser induced fluorescence (SSJ/LlF), low-temperature fluorescence spectroscopy (LTFS), high-resolution low-temperature spectrofluorometry, low-temperature molecular luminescence spectrometry (LT-MLS), and supersonic jet spectroscopy/capillary supercritical fluid chromatography (SJS/SFC) (Asher 1984; Garrigues and Ewald 1987; Goates et al. 1989; Jones et al. 1988; Lai et al. 1990; Lamotte et al. 1985; Lin et al. 1991; Popl et al. 1975; Richardson and Ando 1977; Saber et al. 1991; Vo-Dinh et al. 1984; Vo-Dinh and Abbott 1984; Vo-Dinh 1981; Woo et al. 1980). More recent methods for the determination of PAHs in environmental samples include GC-MS with stable isotope dilution calibration (Bushby et al. 1993), capillary electrophoresis with UV-laser excited fluorescence detection (Nie et al. 1993), and

laser desorption laser photoionization time-of-flight mass spectrometry of direct determination of PAH in solid waste matrices (Dale et al. 1993).

Among the less commonly used spectroscopic methods, SLS and room temperature phosphorescence (RTP) are used for determining trace levels of PAHs in environmental media. Vo-Dinh (1981), Vo-Dinh and Abbott (1984), and Vo-Dinh et al. (1984) reported a cost-effective and relatively simple SLS and RTP technique for determining trace amounts of PAHs (less than $1x10^{-9}$ g per sample) in air particulate extracts collected at a wood-burning area. Improved selectivity is the main advantage of SLS and RTP over conventional luminescence or fluorescence spectroscopy. Additionally, R/N-SSL spectrometry has been applied to determine trace amounts of anthracene and its derivatives in solvent-refined coal (Lin et al. 1991). The sensitivity of N-SSL ($7x10^{-7}$ M) is about two orders of magnitude better than that of R-SSL spectrometry (7x10-5 M). The detection limit for N-SSL is several times better than that of conventional fluorescence spectrometry ($3x10^{-6}$ M). The better sensitivity comes from a higher efficiency in fluorescence collection (Lin et al. 1991). The combination of R- and N-SSL spectrometries provides a sensitive and selective analytical method because of the spectral simplicity of R-SSL and the high sensitivity of N-SSL spectrometry (Lin et al. 1991). This spectrometric method is also applicable to other PAHs in the environment, such as benzo[a]pyrene in airborne particulates.

Low temperature-molecular luminescence spectrometry (LT-MLS), SLS, and HPLC/fluorescence detection have been used to measure pyrene in broiled hamburger (Jones et al. 1988). A comparison of the three methods showed that sensitivity for all three methods was in the low-ppb range and that all methods were comparably reproducible (6 9% RSD). Adequate recovery (75-85%) was obtained from the extraction procedure for all three methods. While HPLC is the least expensive and easiest to operate, it has the longest analysis time (30 minutes), and it provides the least resolution of components. LT-MLS is the fastest technique (5 minutes), and it gives mores spectral information than the other two methods. SLS, with an analysis time of 15 minutes, offers no real advantages over LT-MLS other than cost of equipment.

Methods 8100, 8250, and 8310 are the test methods recommended by EPA (1986) for determining PAHs in a variety of matrices at solid waste sites. EPA Methods 610 and 625, recommended for

municipal and industrial waste water have been used to measure PAHs in groundwater contaminated by petroleum hydrocarbons at detection limits in the low-ppb range (Thomas and Delfino 1991). Recovery and precision data were not reported. NIOSH (1985) has recommended methods 5506 and 5515 as the analytical methods for determining PAHs in air samples at concentrations below ppm evel.

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 PAHs 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 PAHs.

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. Adequate methods are available to separate and quantify PAHs in biological materials such as adipose tissue (Gay et al. 1980; Liao et al. 1988; Modica et al. 1982), lungs (Brandys et al. 1989; Mitchell 1979; Tomingas et al. 1976; Weston and Bowman 1991; Weyand and Bevan 1987a), liver (Amin et al. 1982; Obana et al. 1981; Rice et al. 1985b), skin (Phillips et al. 1987; Shugart et al. 1983), hair (Alexandrov et al. 1990), blood (Brandys et al. 1989; Harris et al. 1985; Haugen et al. 1986; Perera et al. 1988; Phillips et al. 1988; Shamsuddin et al. 1985; Shugart 1986), urine (Au&up and Seremet 1986; Becher and Bjorseth 1985; Rogan et al. 1990; Tolos et al. 1990; Uziel et al. 1987), and feces (Hecht et al. 1979; Rogan et

al. 1990). These methods include GC/FID, GCMS, HPLC, TLC, and spectrofluorometry (SF). The difficulties involved in recovering bound benzo[a]pyrene from feces hinder studies on absorption and bioavailability in humans after exposure to benzo[a]pyrene. Therefore, there is a need to develop a satisfactory analytical method for the determination of benzo[a]pyrene in feces. Immunoassays (i.e., ELISA and USERIA, ^{32}P-arid ^{35}S-postlabelling, SLS, and FIS) are methods currently being developed to detect the presence of carcinogenic PAH adducts bound covalently to macromolecules (e.g., DNA). The parent compound is generally measured in biological tissues, but both the parent compound and its metabolites can be measured in biological fluids, particularly urine. However, improved methods for identifying and characterizing conjugated PAH metabolites from various biological fluids would be useful. PAH-DNA adducts can be measured in blood, serum, and other tissues. These methods are accurate, precise, and sensitive enough to measure background levels in the population and levels at which biological effects occur. Additional quantitative information regarding the relationships between body and environmental levels of PAHs for both short- and long-term exposures might allow investigators to predict environmental exposure levels from measured body levels.

The urinary level of 1-Hydroxypyrene has the potential to be used as a biomarker for exposure to PAHs, and analytical methods for the detection of the hydroxy metabolite in urine of exposed and non-exposed control persons are available (Ariese et al. 1993a; Jongeneelen et al. 1988; Kanoh et al. 1993; Mercado Calderon 1993; Van Hummelen et al. 1993). The correlation coefficient between total PAHs in air of a coke production plant and hydroxypyrene in urine of workers was 0.77 ($p<0.0001$) (Mercado Calderon 1993). A study attempted to use benzo[a]pyrene metabolite 3-hydroxy-benzo[a]pyrene in urine as a biomarker for occupational exposure to PAH (Ariese et al. 1993a). Since the level of 3-hydroxybenzo[a]pyrene is about 3 orders of magnitude lower than 1-hydroxypyrene, a sensitive method was developed to estimate levels of 3-hydroxybenzo[a]pyrene in occupational groups (Ariese et al. 1993a). However, no significant correlation between the metabolite and levels of airborne benzo[a]pyrene was found.

The available biomarkers of effect for PAHs are not specific for effects induced by PAHs other than cancer or genotoxicity. PAHs form DNA adducts that can be measured in body tissues or blood following exposure to PAHs and mixtures that contain PAHs. The formation of benzo[a]pyrene-DNA adducts has been demonstrated, and this may serve as a biomarker of PAH-induced carcinogenicity.

HPLC and immunoassays, (i.e., ELISA and USERIA, ^{32}P-a d ^{35}S-postlabelling, SLS, and FIS) are sensitive, selective, and reproducible methods being developed to detect the presence of carcinogenic PAH adducts bound covalently to macromolecules (e.g., DNA) (Gorelick and Wogan 1989; Haugen et al. 1986; Lau and Baird 1991; Phillips et al. 1988; Weston and Bowman 1991). Chromosomal aberration and sister chromatid exchange methods were used to show that several types of cultured human tissue cells demonstrated positive results for benzo[a]pyrene-induced genotoxicity (Abe et al. 1983a, 1983b; Huh et al. 1982; Lo Jacono et al. 1992; Van Hummelen et al. 1993; Weinstein et al. 1977; Wienke et al. 1990). However, statistically significant correlation between the cytogenetic markers and airborne occupational PAH levels was not found (Van Hummelen et al. 1993).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Standardized methods are available that are reliable, reproducible, and sensitive enough to separate and quantify PAHs in air (Andersson et al. 1983; Fox and Staley 1976; Golden and Sawicki 1978; Harvath 1983; Karlesky et al. 1987; Majer et al. 1970; Miguel and De Andrade 1989; Naikwadi et al. 1987; NIOSH 1984; Oehme 1983; Tomkins et al. 1982; Matsumoto and Kashimoto 1985), water (Beniot et al. 1979; Desiderie et al. 1984; EPA 1986; Furuta and Otsuki 1983; Thomas and.Delfino 1991; Thruston 1978; Xu and Fang 1988), soil and sediment (Hawthorne and Miller 1987a, 1987b; John and Nickless 1977; Saber et al. 1991; Szepesy et al. 1981; Tanaka and Saito 1988), and other media, such as food (Hecht et al. 1979; Joe et al. 1984; Jones et al. 1988; Krahn and Malins 1982; Krahn et al. 1988; Lawrence and Das 1986; Lawrence and Weber 1984; Poole et al. 1987; Toussaint and Walker 1979; Tuominen et al. 1988; Van Heddeghem et al. 1980; Vassilaros et al. 1982; Vreuls et al. 1991), cigarette smoke (Risner 1988; Severson et al. 1976), coal tar (Alben 1980; Goates et al. 1989; Low et al. 1986), and fuels (Lin et al. 1991; Monarca and Fagioli 1981; Symons and Crick 1983.; Ton&ins and Griest 1987; Vo-Dinh et al. 1984; Woo et al. 1980). These methods include GC, HPLC, TLC, and others. Various detection devices used for GC quantification include FID, MS, FT-IR, LIMF, DAD, or GPFDA. GC/MS and HPLC are perhaps the most prevalent analytical methods for determining concentrations of PAHs in environmental samples. These methods are adequate to measure environmental levels that may be associated with adverse human effects. All of the available analytical methods for PAHs in soil and food items are sensitive down to levels of <1 ppb.

6.3.2 Ongoing Studies

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health and Injury Control, Centers for Disease Control and Prevention, is developing methods for the analysis of PAHs and other volatile organic compounds in blood. These methods use purge and trap methodology, high resolution gas chromatography, and magnetic sector mass spectrometry, which gives detection limits in the low parts per trillion (ppt) range.