6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring fuel oils in environmental media and in biological samples. The intention is not to provide an exhaustive list of analytical methods that could be used to detect and quantify fuel oils. Rather, the intent is to identify well-established methods that are used as the standard methods of analysis. Many of the analytical methods used to detect fuel oils 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

Fuel oils are mixtures of aliphatic and aromatic hydrocarbons (Air Force 1989). Most analytical methods for detecting fuel oils in biological media focus on the detection of kerosene components, as this is a commonly used fuel for residential heaters. Analytical methods for detecting other fuel oils in biological media were not located. See Table 6-l for a summary of the analytical methods most commonly used to measure kerosene in biological materials. For more analytical methods information, see the previous profiles on some of the individual components of fuel oils (e.g., benzene, toluene, total xylenes, and PAHs) (ATSDR 1989, 1990a, 1991a, 1991b).

The primary method for detecting kerosene in biological materials such as blood is gas chromatography (GC). GC may be combined with mass spectroscopy (MS) for peak identification with the gas chromatograph in the electron impact mode (Kimura et al. 1988, 1991). Quantification methods include the use of mass fragmentography (Kimura et al. 1988). Hydrocarbon components of kerosene are determined based on analysis of headspace gas above the sample (Kimura et al. 1991). This method is useful for distinguishing between kerosene intoxication and gasoline intoxication as kerosene gives a high toluene peak and has a pseudocumene-to-toluene ratio only half that of gasoline. Capillary columns are used, with either Porapak, Chromosorb®, or Chemipak®, giving acceptable results (Kimura et al. 1988). The percent recoveries of these methods were not provided. Wide-bore

TABLE 6-1. Analytical Methods for Determining Fuel Oils in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Add internal standard; extract with *n*-pentane; centrifuge; freeze; decant solvent; concentrate; inject to GC	GC/MS	50 pg	NR	Kimura et al. 1988
Blood	Mix sample with internal standard; add salt solution; equilibrate; aspirate headspace vapor and inject to GC	GC/MS	50 pg (toluene)	NR	Kimura et al. 1991
Stomach contents, blood, urine	Extract sample with ethyl acetate; condense; inject to GC	GC/FID/MS	0.2 µg/mL	93–100	Hara et al. 1988

FID = flame ionization detector; GC = gas chromatography; MS = mass spectrometry; NR = not reported

capillary columns have also been used (Hara et al. 1988) for GC/MS analysis combined with flame ionization detectors (FID). This method determined levels of *m*- and *o*-xylene (components of kerosene) in the blood, urine, and stomach contents. The sensitivity and precision of this method was generally good (93-100% recovery).

No analytical methods studies were located for detecting fuel oils in biological samples other than blood, urine, or stomach contents.

6.2 ENVIRONMENTAL SAMPLES

Because fuel oils are composed of a mixture of hydrocarbons, there are few methods for the environmental analysis of fuel oils as a whole, but methods are reported for the analysis of their component hydrocarbons. The methods most commonly used to detect the major hydrocarbon components of fuel oils in environmental samples are GC/FID and GC/MS. See Table 6-2 for a summary of the analytical methods used to determine fuel oils in environmental samples. Several of the components of fuel oils have been discussed in detail in their individual toxicological profiles (e.g., benzene, toluene, total xylenes, and PAHs), which should be consulted for more information on analytical methods (ATSDR 1989, 1990a, 1991a, 1991b).

GC is the most commonly used method to selectively detect, identify, and quantify the volatile hydrocarbon components of fuel oils in air (Andrasko 1983; Baldwin 1977; NIOSH 1994). Air samples may be collected on adsorbent tubes such as charcoal, Florisil®, Tenax®, Porapak®, or Chromosorb®. Active carbons wires have also been used (Andrasko 1983). The hydrocarbons are extracted from the tubes by thermal desorption or with a liquid solvent such as carbon disulfide and analyzed on the gas chromatograph. Precision is good (relative standard deviation = 0.052) using the charcoal tubes (NIOSH 1994); recovery data were not reported for the other types of adsorption tubes, although desorption from the active carbon wires ranged between 90% and 99% recovery, with a detection limit in the ppb range. A Tenax-TA® sorbent trap has been used with subsequent thermal desorption (Andrasko 1983). Combining sample concentration with the headspace method allows for sampling of smaller air volumes and for other environmental samples, such as kerosene combustion debris, that have undergone significant evaporation; the headspace method requires concentrating the sample prior to analysis (Andrasko 1983; Baldwin 1977). An exploratory study indicates that, with

TABLE 6-2. Analytical Methods for Determining Fuel Oils in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Adsorb to solid sorbent tube (e.g., charcoal); desorb in CS_2; equilibrate; inject aliquot to GC	GC/FID	0.1 mg/ 5–10 mL sample	96–106	NIOSH 1994
Air	Adsorb to quartz filter	Evolved Gas Analysis	10 µg	NR	Daisey and Gundel 1991
Air	Adsorb to Florisil filter; elute with CS_2; evaporate under vacuum	GC	NR	NR	Baldwin 1977
Water	Strip sample in sparger with helium; adsorb effluent gas to adsorption tube; thermally desorb to GC	GC/FID/MS	10 µg/L	89.7–95.7	Bianchi et al. 1991
Water	Acidify sample; extract with hexane; dry solvent phase; inject to GC	GC/FID	0.25µL/L	NR	Dell'Acqua and Bush 1973
Water (purgeable aromatics)	Purge sample with inert gas; adsorb vapor in trap; heat trap; backflush to GC	GC/PID	0.2 µg/L	92–96	EPA 1991c

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Purge sample with helium; collect vapor on adsorption tube; thermally desorb; concentrate; backflush to GC	GC/FID	10 µg/L	99–114 (fuel oil no. 2); 91–112 (kerosene)	Belkin and Esposito 1986
Water	Purge sample with ambient air, adsorb to charcoal filter; extract filter with CS_2; inject to GC	GC/MS	5 ng/L	0.4–89 (75% average)	Coleman et al. 1981
Water	Extract aqueous sample with pentane; equilibrate; inject to GC	GC/MS	NR	NR	Coleman et al. 1984
Water (base/neutral and acids)	Adjust sample pH to >11; extract sample with CH_2Cl_2 solvent; adjust pH to <2; reextract; dry; concentrate; inject to GC	GC/MS	1.5–7.8 µg/L (varies with actual compound)	NR	EPA 1991c
Seawater	Extract aqueous phase of sample with pentane; evaporate; inject to GC	GC/MS	NR	NR	Boylan and Tripp 1971
Soil (other solid materials)	Extract sample with CCl_4; inject extract	GLC	NR	NR	Midkiff and Washington 1972

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil	Extract sample with CCl_4; centrifuge; remove water and humic materials with Na_2SO_4 and Al_2O_3; inject extract	GC/FID	NR	NR	Galin et al. 1990a
Soil	Purge at elevated temperatures; heat trap to desorb material into GC column	GC	NR	NR	Chang et al. 1990
Soil	Sample added to substrate and placed in cup mounted on optical fiber	Fluorescence Spectroscopy	NR	NR	Apitz et al. 1993
Soil	Sample extracted using water and cyclohexane	Synchronous Scanning Fluorescence Spectroscopy	NR	NR	Pharr et al. 1992
Sediment	Add internal sample to sample; extract with KOH in methanol; partition into petroleum ether; concentrate; purify and isolate hydrocarbon fractions using TLC or column chromatography	GLC/FID	NR	NR	Gearing et al. 1980
Shellfish tissue (mussel)	Extract with NaOH; isolate fractions with column chromatography; inject fractions to GC	GC or GC/MS	NR	34–120 (GC); 36–87 (GC/MS)	Farrington et al. 1982a

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Shellfish tissue	Extract with methanol then pentane; dry; concentrate; inject to GC	GC	NR	NR	Blumer et al. 1970

Al_2O_3 = aluminum oxide; CCl_4 = carbon tetrachloride; CH_2Cl_2 = dichloromethane (methylene chloride); CS_2 = carbon disulfide; FID = flame ionization detector; GC = gas chromatography; GLC = gas liquid chromatography; KOH = potassium hydroxide; MS = mass spectrometry; Na_2SO_4 = sodium sulfate; NaOH = sodium hydroxide; NR = not reported; PID = photoionization detector; TLC = thin-layer chromatography

additional research, the microanalytical evolved gas analysis technique (EGA) can be further developed to measure kerosene soot in indoor aerosols (Daisey and Gundel 1991).

GC/FID and GC/MS have been used to measure the water-soluble components of fuel oils, particularly kerosene, in industrial effluents and estuarine water (Bianchi et al. 1991), sea water (Boyland and Tripp 1971), drinking water (Coleman et al. 1984; Dell'Acqua and Bush 1973), and groundwater (Thomas and Delfino 1991a). Purge-and-trap sample preparation methods have been used to determine purgeable (volatile) aromatic components of fuel oils. This method requires a trap with a Tenax®/Chromosorb® absorbent and the use of a gas chromatograph with a photoionization detector (PID) (EPA 1991c), an ion trap detector (ITD), or FID (Thomas and Delfino 1991a). A modification of the purge-and-trap method uses ambient temperatures, has the advantage of being applicable to a variety of waters, requires virtually no sample preparation (no solvents are required), and has an analysis time of approximately 30 minutes (Bianchi et al. 1991). While this method may be used for determining the presence of petroleum contaminants in water, it cannot distinguish between various sources of this contamination, e.g., between gasoline, kerosene, and diesel oil.

Distinctions between water-soluble fractions of mixed hydrocarbons may be made by using solvent extraction of the water-soluble base/neutral and acid fractions with methylene chloride (EPA 1991c; Thomas and Delfino 1991a). This separation of base/neutral and acid fractions will permit the GC resolution of the type of water soluble hydrocarbons present in the aqueous phase. Hexane has also been used as a solvent (Dell'Acqua and Bush 1973), as has pentane (Coleman et al. 1984).

A dynamic thermal stripper has also been used to detect low levels (ppb range) of fuel oil no. 2 and kerosene present in water samples (Belkin and Esposito 1986). This method traps the fuels on an adsorption tube using helium gas for purging. The fuel is then thermally desorbed and backflushed to a gas chromatograph with FID. This method also does not require any solvent and needs only a 15 mL sample. Recovery for this method is good (91-114%) with precision ranging from 6.4 to 14.3% relative standard deviation. A modified Grob closed-loop-stripping method, which uses a wall-coated open tubular glass capillary column combined with GC/MS, has been used to extract and quantify low levels (ppt) of hydrocarbons in water samples. The method continually recirculates an ambient air stream through the 3.8-liter water sample for approximately 2 hours and collects the vapor on an activated carbon filter, followed by extraction with carbon disulfide and analysis (Coleman et al. 1981). An optical fiber fluorescence spectroscopy system has been used for real-time *in situ*

measurements of low levels (at ppb of diesel fuel marine equivalent) of petroleum hydrocarbons in seawater, showing temporal and spatial variability (Lieberman et al 1993).

A qualitative method for determining diesel oil in water has been proposed that is based on changes in the internal reflection of an optical fiber coated with an organophilic compound caused by the presence of hydrocarbons. The method does not require any sample preparation but is limited to relatively high concentrations of contaminants, e.g., 17 mg/L for diesel oil (Kawahara et al. 1983). An alternative method uses a Fourier transform infrared spectrometer (FTIR). This method has the advantage of no sample preparation, a short analysis time (20-30 seconds), and good accuracy (±20%). A detection limit of 0.5 ppb has been determined for a l-liter sample of sea water; 10 mL is sufficient if a detection limit of 0.05 ppm is acceptable. The FTIR may be coupled with a GC or liquid chromatography for the analysis of complex mixtures (Mille et al. 1985).

GC/FID (Galin et al. 1990a), gas liquid chromatography (GLC) with FID (Midkiff and Washington 1972), and elevated temperature purge and trap with GC (Chang et al. 1990) have been used to measure fuel oils in soils. An enzyme immunoassay has been developed using a monoclonal antibody reagent that detects gasoline and diesel fuel in soil; commercialization of this assay will offer significant advantages over current testing methods of gasoline and fuel contamination levels in soil (Allen et al. 1992b). GLC has also been used to determine fuel oils in marine sediments (Gearing et al. 1980) and other environmental samples such as paper, cloth, and wood (Midkiff and Washington 1972). Extraction is used to concentrate the sample because fuel oils do not provide sufficient vapors to allow the use of a headspace sampling method. Carbon tetrachloride is the recommended solvent as it causes less interference with the chromatographic peaks of the fuel oils (Galin et al. 1990a; Midkiff and Washington 1972). Quantification of fuel oil hydrocarbons from sediments is a more elaborate process. Following extraction, the saturated and olefinic hydrocarbon fraction is separated from the aromatic hydrocarbon fraction using thin-layer chromatography or column chromatography. Fractions are subsequently analyzed by GLC (Gearing et al. 1980). Recovery, sensitivity, and levels of detection data were not reported. Quantification of oils and grease, by gross weight only, in soils and sludges may be accomplished by extraction with a Soxhlet apparatus using either trichlorotrifluoroethane (APHA 1985) or methylene chloride (Martin et al. 1991) as the solvent, although this method may not be used to identify the specific type of oil or grease present in the soil sample. Synchronous scanning fluorescence spectroscopy can be used to identify kerosene, fuel oil number 2, fuel oil number 5, and other aromatic-containing products in groundwater and soil samples. This analytical method is more

efficient than chromatographic methods, and its spectra are easier to interpret for identification purposes (Pharr et al. 1992). Fluorescence spectroscopy has been used for *in situ* detection of petroleum hydrocarbon plumes in soil; this technique allows for measurements in soils before monitoring wells are drilled and is thus independent of the fractionation and transport problems inherent when sampling well fluids (Apitz et al. 1992).

The age of diesel oil in the subsurface soil environment can be determined by utilizing the fact that the composition of the diesel oil (the ratio between *n*-alkanes and isoprenoids) changes due to biodegradation. In one study, the ratio of C_{17} to pristane was highly correlated with the residence time of diesel fuel at 12 test locations (Christensen et al 1993).

A set of neural networks has been trained to identify seven classes of petroleum hydrocarbon based fuels from their fluorescence emission spectra; this technique correctly identified at least 90% of the test spectra (Andrews and Lieberman 1994).

High-performance liquid chromatography (HPLC), followed by GC/MS, has been used to fractionate and then quantitate the aliphatic and aromatic hydrocarbons present in liquid fuel precursors in order to determine the fuel potential of the compounds. Kerosene had the advantage of not requiring any sample preparation. Other light fuel oils may require the use of methylene chloride as a solvent prior to HPLC analysis (Lamey et al. 1991). The sensitivity, precision, and recovery of this method were not reported. An alternative method for fractionating and purifying petroleum hydrocarbons prior to GC or HPLC separation has been developed (Theobald 1988). The method uses small, prepacked, silica or C_{18} columns that offer the advantage of rapid separation (approximately I5 minutes for a run); good recovery of hydrocarbons (85% for the C_{18} column and 92% for the silica column); reusability of the columns; and for the silica column in particular, good separation of hydrocarbon from nonhydrocarbon matrices as may occur with environmental samples. Infrared analysis and ultraviolet spectroscopy were used to analyze the aromatic content in diesel fuels; these methods are relatively inexpensive and faster than other available methods, such as mass spectrometry, supercritical fluid chromatography, and nuclear magnetic resonance (Bailey and Kohl 1991).

Due to the tendency of hydrocarbons in the soil to undergo subsurface oxidation, measuring CO_2 levels in the soil gas could be used as a cost-effective field screening tool. In one soil-gas survey, CO_2 levels in soil gas correlated well with petroleum hydrocarbons in the soil (Diem et al. 1988).

A two-dimensional supercritical fluid chromatography (SFC) system has been developed for the determination of saturates, alkenes, and mono-, di-, and tri-aromatics in diesel fuel. This technique results in a short analysis time (less than 8 minutes) and good relative standard deviations at low alkene content (Andersson et al. 1992).

The principal method for detecting kerosene or its components in biota is GC (Blamer et al. 1970; Farrington et al. 1982a; Newton et al. 1991). Aliphatic and aromatic hydrocarbon components of fuel oils taken up by shellfish (whole mussels without shells) were isolated by column chromatography following extraction. Both the alka.ne/cycloalkane and alkene/aromatic fractions were analyzed by GC with recoveries in the range of 67-100% for alkanes and 71-78% for some aromatics; these aromatics were also analyzed using GC/MS with recoveries between 49% and 74% (Farrington et al. 1982a). Determination of hydrocarbons may also be accomplished by fractionating the hydrocarbon components. Extraction of hydrocarbons from contaminated shellfish may be accomplished using Soxhlet extraction with methanol followed by reextraction with pentane. The extracts are then dried and concentrated prior to injection into the GC (Blumer et al. 1970). Other data on detection limits and precision were not provided.

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 fuel oils 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 fuel oils.

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.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. No biomarkers of exposure were identified for fuel oils because, while standard procedures exist for identifying or quantifying exposure to fuel oils based on hydrocarbon components in blood, urine, and stomach contents (Hara et al. 1988; Kimura et al. 1988, 1991), none of these are applicable solely to fuel oils. These methods are sensitive enough to measure the levels at which health effects occur and may be adequate for determining background levels in the population; however, they cannot distinguish between exposure to different fuel oils or other types of hydrocarbon mixtures. Analytical methods are needed for measuring the hydrocarbon components of fuel oils in lungs. Biomonitoring studies are needed to adequately assess exposure to fuel oils.

No biomarkers of effects were identified for fuel oils because the effects associated with exposure to fuel oils are not unique for them, i.e., the effects may be caused by other chemicals or hydrocarbon mixtures. Analytical methods do exist for determining angiotensin-converting enzyme activity in the lungs. This enzyme may be used to determine the lung damage caused by a fuel oil. Analytical methods are needed to determine whether the tissue damage is specific to fuel oils and the target organs.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist to detect major hydrocarbon components of fuel oils in air (Andrasko 1983; Baldwin 1977; NIOSH 1994), water (Bianchi et al. 1991; Boyland and Tripp 1971; Dell'Acqua and Bush 1973; EPA 1991c), sediment (Gearing et al. 1980), and soil (Galin et al. 1990a; Midkiff and Washington 1972). The most commonly used methods are GC/FID and GC/MS. These methods are relatively sensitive, selective, and reliable, and can be used to detect the levels of the various components of fuel oils found in the environment and levels at which health effects occur.

6.3.2 On-going Studies

No on-going analytical methods studies were located.