7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring Jet A, JP-5, and JP-8, their metabolites, and other biomarkers of exposure and effect to Jet A, JP-5, and JP-8. 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.

7.1 BIOLOGICAL MATERIALS

No analytical methods were located for detecting JP-5, JP-8, and Jet A fuels in biological materials. However, analytical methods for detection in biological samples do exist for kerosene, which has a similar chemical composition as jet fuels (Air Force 1989a; Army 1988; DOD 1992). See Table 7-1 for a summary of the analytical methods most commonly used to measure kerosene in biological samples. Analytical methods are available for a number of the components of jet fuels; the analytical methods for some of the individual hydrocarbon components of JP-5, JP-8, and Jet A fuels (e.g., benzene, toluene, xylenes, and PAHs) are discussed in the toxicological profile for the component (ATSDR 1995, 2007a, 2007b, 2015b). The toxicological profile for total petroleum hydrocarbons (ATSDR 1999) provides additional information on analytical methods.

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 to distinguish between kerosene intoxication and gasoline intoxication since kerosene gives a high toluene peak and has a pseudocumene-to-toluene ratio only half that of gasoline. Capillary columns were used, with either Porapak, ChromosorbB, or ChemipakB, giving acceptable results (Kimura et al. 1988).

Table 7-1. Analytical Methods for Determining Kerosene 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 gas chromatograph	GC/MS	50 pg	Not reported	Kimura et al. 1988
Blood	Mix sample with internal standard; add salt solution; equilibrate; aspirate headspace vapor and inject to gas chromatograph	GC/MS	50 pg (toluene)	Not reported	Kimura et al. 1991
Stomach contents, blood, urine	Extract sample with ethyl acetate; condense; inject to gas chromatograph	GC/FID/MS	0.2 µg/mL	93–100	Hara et al. 1988

FID = flame ionization detection; GC = gas chromatography; MS = mass spectrometry

The percent recoveries of these methods were not provided. Wide-bore 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).

B'Hymer et al. (2005, 2012b) discussed an analytical method to detect 2-methoxyethoxy acetic acid (MEAA) in urine samples using GC with a MS detector (detection limit 0.1 μg/mL). MEAA is a metabolite of 2-(2-methoxyethoxy) ethanol, a glycol ether that is used as an anti-icing agent in JP-8. In a study of Air Force personnel exposed to JP-8, mean post-shift urinary MEAA levels in personnel assigned to a high exposure group (aircraft fuel system maintenance workers) were approximately 10 times greater than personnel assigned to a low exposure group, and the frequency of detection (n>the limit of detection) of MEAA in post-shift urine samples was 94% for the high exposure group and only 3% for the low exposure group (B'Hymer et al. 2012b).

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

7.2 ENVIRONMENTAL SAMPLES

Because JP-5, JP-8, and Jet A fuels are composed of a complex mixture of hydrocarbons, there are few methods for the environmental analysis of the actual mixtures (IARC 1989). However, methods are reported for the analysis of the component hydrocarbons of kerosene. The methods most commonly used to detect the major hydrocarbon components of kerosene in environmental samples are GC/FID and GC/MS. See Table 7-2 for a summary of the analytical methods used to determine hydrocarbon components in environmental samples. Environmental levels of JP-5, JP-8, and Jet A fuels are often characterized by measuring the total hydrocarbons and other important constituents typically found in the jet fuels (benzene, toluene, ethylbenzene, xylene, and naphthalene) and reporting these levels. NIOSH method 1550 provides a general description of an analytical procedure for characterizing various types of hydrocarbon mixtures (NIOSH 1994). Several of the components of kerosene and jet fuels have been discussed in detail in their individual toxicological profiles (e.g., benzene, toluene, xylenes, and PAHs), which should be consulted for more information on analytical methods (ATSDR 1995, 2007a, 2007b, 2015b). The toxicological profile for total petroleum hydrocarbons (ATSDR 1999) provides additional information on analytical methods for environmental samples.

Table 7-2. Analytical Methods for Determining Kerosene and Hydrocarbons in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery[a]	Reference
Air	Samples are collected by drawing a known volume of air through glass sampling tubes containing coconut shell charcoal; samples are extracted with 99:1 carbon disulfide (CS_2):N,N-dimethyl-formamide (DMF)	GC/FID	0.1 mg/5–10 mL sample	96–106	NIOSH 1994 (Method 1550)
Air	Adsorb to Florisil filter; elute with CS_2; evaporate under vacuum	GC	Not reported	Not reported	Baldwin 1977
Water	Strip sample in sparger with helium; adsorb effluent gas to adsorption tube; thermally desorb to gas chromatograph	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 gas chromatograph	GC/FID	0.25 mcl/L	Not reported	Dell'Acqua and Bush 1973
Water (purgeable aromatics)	Purge sample with inert gas; adsorb vapor in trap; heat trap; backflush to gas chromatograph	GC/FID	0.2 µg/L	92–96	EPA 1991b (Method 602 and 610)
Water	Purge sample with helium; collect vapor on adsorption tube; thermally desorb; concentrate; backflush to gas chromatograph	GC/FID	10 µg/L	91–112	Belkin and Esposito 1986
Water	Purge sample with ambient air; adsorb to charcoal filter with CS_2; inject to gas chromatograph	GC/MS	5 ng/L	0.4–89 (75% average)	Coleman et al. 1981
Water	Extract aqueous sample with pentane; equilibrate; inject to gas chromatograph	GC/MS	Not reported	Not reported	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 gas chromatograph	GC/MS	1.5–7.8 µg/L (varies with actual compound)	Not reported	EPA 1991b (Method 602 and 610)

Table 7-2. Analytical Methods for Determining Kerosene and Hydrocarbons in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery[a]	Reference
Seawater	Extract aqueous phase of sample with pentane; evaporate; inject to gas chromatograph	GC/MS	Not reported	Not reported	Boylan and Tripp 1971
Soil (other solid materials)	Extract sample with CCl_4; inject extract	GLC	Not reported	Not reported	Midkiff and Washington 1972
Solid waste matrices	Solvent extraction followed by purge-and-trap or direct injection	GC/MS	0.01–0.50 µg/L	84–109	EPA 2006 (Method 8260 C)
Soil	Extract sample with CCl_4; centrifuge; remove water and humic materials with NA_2SO_4 and Al_2O_3; inject extract	GC/FID	Not reported	Not reported	Galin et al. 1990
Soil	Purge at elevated temperatures; heat trap to desorb material into gas chromatograph column	GC	Not reported	Not reported	Chang and Lopez. 1992
Soil	Sample extracted using water and cyclohexane	Synchronous scanning fluorescence spectroscopy	Not reported	Not reported	Phaff et al. 1992
Sediment	Sample dried, ground, and extracted with *n*-pentane	GC/FID	Not reported	Not reported	Guiney et al. 1987b
Fish tissue	Extract with KOH in methanol; partition into *n*-pentane; concentrate; analyze using gas chromatograph	GC/FID	Not reported	95	Guiney et al. 1987b

[a]The sample detection limit and percent recovery will vary for each of the components of these mixtures. The reported values in these tables are for the specific components analyzed in each method.

Al_2O_3 = aluminum oxide; CCl_4 = carbon tetrachloride; CH_2Cl_2 = dichloromethane (methylene chloride); FID = flame ionization detection; GC = gas chromatography; GLC = gas liquid chromatography; KOH = potassium hydroxide; MS = mass spectrometry; Na_2SO_4 = sodium sulfate

GC is the most commonly used method to selectively detect, identify, and quantify the volatile hydrocarbon components of kerosene in air (Andrasko 1983; Baldwin 1977; NIOSH 1994). Air samples may be collected on adsorbent tubes such as charcoal, Plorisil®, Tenax®, Porapak®, or Chromosorb®. Active carbon 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 SD = 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).

GC/FID and GC/MS have been used to measure the water-soluble components of kerosene in industrial effluents and estuarine water (Bianchi et al. 1991), sea water (Boylan and Tripp 1971), drinking water (Coleman et al. 1984; Dell'Acqua and Bush 1973), and groundwater (Thomas and Delfino 1991). Purge-and-trap sample preparation methods have been used to determine purgeable (volatile) aromatic compounds in stream water contaminated by an "aviation kerosene" spill (Guiney et al. 1987b). This method requires a trap with a Tenax®/ChromosorbB absorbent and the use of a gas chromatograph with a photoionization detector (PID) (EPA 1991b), an ion trap detector (ITD), or FID (Guiney et al. 1987b; Thomas and Delfino 1991). 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.

EPA Method 8260C is a GC/MS method that is used to quantify volatile organic compounds in various solid waste matrices and is applicable for the components of JP-5, JP-8, and Jet A fuels. This method is appropriate for nearly all types of environmental sample matrices, regardless of water content. Sample types that can be analyzed include air sampling trapping media, groundwater, surface water, aqueous sludges, caustic liquors, acid liquors, waste solvents, oily wastes, mousses, tars, fibrous wastes, polymeric emulsions, filter cakes, spent carbons, spent catalysts, soils, and sediments (EPA 2006).

Distinctions between WSFs of mixed hydrocarbons may be made by using solvent extraction of the water-soluble base/neutral and acid fractions with methylene chloride (EPA 1991b; Thomas and Delfino 1991). This separation of base/neutral and acid fractions till permit 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 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-L 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).

GC/FID (Galin et al. 1990), gas liquid chromatography (GLC) with FID (Midkiff and Washington 1972), and elevated temperature purge and trap with GC (Chang et al. 1992) have been used to measure jet fuels in soils. Sediments of a trout stream contaminated with "aviation kerosene" were analyzed for hydrocarbon residues using GC/FID (Guiney et al. 1987b). Carbon tetrachloride is the recommended solvent because it causes less interference with the chromatographic peaks of the jet fuels (Galin et al. 1990; Midkiff and Washington 1972). Synchronous scanning fluorescence spectroscopy can be used to identify kerosene 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).

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 has the advantage of not requiring any sample preparation. 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 C18 columns that offer these advantages: rapid separation (approximately 15 minutes for a run); good recovery of hydrocarbons (85% for the C18 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.

Tissues of fish from a trout stream contaminated with "aviation kerosene" were analyzed for kerosene-range hydrocarbon residues using standard GC/FID techniques (Guiney et al. 1987b). GC analyses of the fish samples revealed >95% recovery.

7.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, as amended, directs the Administrator of ATSDR (in consultation with the Administrator of EPA and agencies and programs of the Public Health Service) to assess whether adequate information on the health effects of JP-5, JP-8, and Jet A fuels 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 JP-5, JP-8, and Jet A fuels.

The following categories of possible data needs have been identified by a joint team of scientists from ATSDR, NTP, and EPA. They are defined as substance-specific informational needs that if met would reduce the uncertainties of human health assessment. This definition should not be interpreted to mean that all data needs discussed in this section must be filled. In the future, the identified data needs will be evaluated and prioritized, and a substance-specific research agenda will be proposed.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect.

Exposure. While standard procedures exist for identifying or quantifying exposure to volatile compounds 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 jet fuels. 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 JP-5, JP-8, and Jet A fuels and to other types of hydrocarbon mixtures. Egeghy et al. (2003) noted a correlation to the levels of naphthalene in air and breath of Air Force personnel who were highly exposed to JP-8, but noted that benzene levels in breath could not be correlated solely to exposure from JP-8. In a similar study, Serder et al. (2003) concluded that naphthalene and napthols (1- and 2-hydroxynaphthalene) may

be useful urinary biomarkers of exposure to populations routinely exposed to JP-8, such as aircraft maintenance workers. Smith et al. (2012) also concluded that elevated urinary naphthol levels could be used as a surrogate for short-term occupational exposure to JP-8. The sum concentration of nonane, decane, undecane, and dodecane was used as a composite fingerprint of JP-8 short term exposure for Air Force personnel regularly exposed to this fuel (Air Force 2001).

MEAA was shown to be a urinary metabolite for exposure to 2-(2-methoxyethoxy)ethanol, which is an additive to JP-8 (B'Hymer et al. 2005, 2012a). Because this substance has limited industrial uses, its addition to jet fuels makes its metabolite a possible biomarker for exposure to these fuels.

Effect. No specific biomarkers of effect were identified for JP-5, JP-8, and Jet A fuels because the effects associated with exposure to jet fuels are not unique for them (i.e., the effects may be caused by other chemicals or hydrocarbon mixtures). General neurologic effects such as loss of coordination, headache, fatigue, intoxication, dizziness, difficulty concentrating, moodiness, and sleep disturbances were observed in people exposed to general "jet fuel" and JP-5 vapors (Knave et al. 1978; Porter 1990). These effects are not used as biomarkers of effect because they are nonspecific and could also indicate exposure to other chemicals or hydrocarbons. No standard procedures exist for identifying and quantifying specific biomarkers of effect for JP-5 or JP-8.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist to detect major hydrocarbon components of JP-5, JP-8, and Jet A fuels in air (Andrasko 1983; Baldwin 1977; NIOSH 1994), water (Bianchi et al. 1991; Boylan and Tripp 1971; Dell'Acqua and Bush 1973; EPA 1991b; Guiney et al. 1987b), sediment (Guiney et al. 1987b), soil (Galin et al. 1990; Midkiff and Washington 1972), and biological media (Guiney et al. 1987b). 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 jet fuels found in the environment and the levels at which health effects occur.

7.3.2 Ongoing Studies

No ongoing studies for JP-5, JP-8, and Jet A fuels were identified using the NIH RePORTER version 6.1.0 or the DTIC online database. Analytical methods are continuously being developed and updated for individual constituents that may be contained in JP-5, JP-8, and Jet A fuels. For additional information,

see toxicological profiles for substances such as benzene, toluene, xylenes, and PAHs (ATSDR 1995, 2007a, 2007b, 2015b).