6. ANALYTICAL METHODS

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

Gasoline is a complex mixture of hydrocarbons and additives. The major hydrocarbon component categories in gasoline include alkanes, isoalkanes, cycloalkanes, alkenes, and aromatics (MacFarland et al. 1984). The methods most commonly used to detect the major hydrocarbon components in gasoline in biological materials include gas chromatography (GC) and high resolution gas chromatography (HRGC) combined with flame ionization detection (FID). GC combined with mass spectrometry (MS) has been used for both identification and quantification of the hydrocarbon components in gasoline and increases the reliability of the technique. GC or HRGC combined with atomic absorption spectrometry (AAS) are the most commonly used methods for detecting lead or alkyllead compounds.. Highperformance liquid chromatography (HPLC) combined with electron capture detector (ECD) has also been used to detect alkyllead compounds. See Table 6-l for a summary of the analytical methods most commonly used to determine gasoline in biological materials. For more analytical methods information, see the ATSDR toxicological profiles on some of the individual components of gasolines (e.g., benzene, toluene, xylene, cyclohexane, ethane, ethylene, and lead) (ATSDR 1989, 1990, 1991).

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

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Alveolar air (benzene)	Expire into glass tube; transfer headspace gas to cryogenic trap; desorb by heating	HRGC/FID HRGC/MS	NR	NR	Brugnone et al. 1986
Lung gas (isopentane, *n*-pentane, 2-methyl-2-butene, 2-methyl-pentane, 3-methyl-pentane, *n*-hexane)	Collect sample in vial; heat in water bath; inject headspace gas	GC/FID GC/MS	NR	NR	Ikebuchi et al. 1986
Blood (benzene)	Collect venous blood sample into glass tube; transfer headspace gas to cryogenic trap; desorb by heating	HRGC/FID HRGC/MS	NR	NR	Brugnone et al. 1986
Blood (isopentane, *n*-pentane, 2-methyl-pentane, benzene, 2-methylhexane, 3-methylhexane, toluene)	Add internal standard to blood; heat; inject headspace gas	GC/FID GC/MS	NR	81-125%	Matsubara et al. 1988

TABLE 6-1. Analytical Methods for Determining Gasoline in Biological Materials (*continued*)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (aromatic and aliphatic hydrocarbons)	Add internal standard to blood; heat; inject headspace gas	GC/MS	0.01 µg	NR	Kimura et al. 1988
Blood (tetramethyl lead)	Hemolyze blood samples by freezing; extract alkyllead compound with *n*-heptane in ultrasonic bath	HRGC/AAS	0.01 µg/mL	90-95%	Andersson et al. 1984
Blood, urine (lead)	Add ^{206}Pb to sample and digest with acid; coprecipitate lead by adding barium nitrate, followed by electro-deposition on platinum wire	IDMS	NR	98-99%	Manton and Cook 1984
Urine (benzene)	Steam distill urine specimens in sulphuric acid	Spectrophotometry	NR	NR	Pandya et al. 1975; Buchwald 1966
Urine (benzene metabolites)	Hydrolyze sample with perchloric acid; extract phenol and cresol with diisopropyl ether	GC/FID	NR	NR	NIOSH 1974

TABLE 6-1. Analytical Methods for Determining Gasoline in Biological Materials (*continued*)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (benzene metabolites)	Digest sample enzymatically and with acid; extract phenol, phenyl sulfate, and phenyl glucuronide with ethyl ether	GC/FID	1 mg/L	92-98%	Bochet 1988 (IARC Method 6)
Urine (benzene metabolites)	Centrifuge sample; analyze supernatant; elute with potassium phosphate-acetonitrile	HPLC/UV	4-5 mg/L	100-102%	Ogata and Taguchi 1987
Urine (toluene metabolite)	React sample with benzenesulfonyl chloride to form colored hippuric acid product	Spectrophotometry	2 mg/L	NR	NIOSH 1984a
Urine (toluene metabolite)	Extract sample with ethyl acetate; evaporate; redissolve in water (hippuric acid)	HPLC/UV	30 mg/L	NR	NIOSH 1984b
Urine (lead)	Wet ash sample with acid mixture; dissolve in diluted perchloric acid	ASV	4 µg/L	90-110%	NIOSH 1977 (Method P&CAM 200)

TABLE 6-1. Analytical Methods for Determining Gasoline in Biological Materials (*continued*)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Tissues (blood, brain, and lung)	Place tissue samples in water bath; heat; inject headspace gas	HRGC/FID	NR	NR	Shankles et al. 1982

AAS = atomic absorption spectrometry; ASV = anodic stripping voltammetry; FID = flame ionization detector; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; IDMS = isotopedilution mass spectrometry; MS = mass spectrometry; NR = not reported; UV = ultraviolet detection

6. ANALYTICAL METHODS

GC/FID, HRGC/FID, GC/MS, and HRGC/MS have been used for quantification and identification of the hydrocarbon components of gasoline (aromatics, isoalkanes, alkanes, and alkenes) in alveolar air and lung gas (Brugnone et al. 1986; Ikebuchi et al. 1986). Since many of the components are volatile, analysis of the headspace gas is the most commonly used technique. Although the limit of detection for each component was not reported, sensitivity for the method, based on data reported, is in the ppb to sub-ppm range. Precision was very good (3.9-7% coefficient of variation [CV]) for measuring the components in lung gas (Ikebuchi et al. 1986). Precision data were not reported for alveolar air. Recovery data were not reported for either alveolar air or lung gas.

HRGC/FID, HRGC/MS, GC/FID and GC/MS have been used for quantification and identification of the hydrocarbon components of gasoline (aromatics, isoalkanes, alkanes, alkenes) in blood (Brugnone et al. 1986; Matsubara et al. 1988; Kimura et al. 1988). The hydrocarbon components were measured by analyzing headspace gas (Brugnone et al. 1986; Kimura et al. 1988; Matsubara et al. 1988). The headspace technique combined with GC/MS is rapid and makes for reliable qualitative and quantitative estimations of small amounts of volatile fuel components (Kimura et al. 1988). The limit of detection for GC/MS was 0.01 μg (Kimura et al. 1988). GC/FID is also a rapid and simple method for determining gasoline in blood (Matsubara et al. 1988). Accuracy is generally good (81-125% recovery) and precision (4.8-24% CV) is adequate (Matsubara et al. 1988). Although the limit of detection for various components was not reported, the sensitivity of the method, based on data reported, is in the ppm-range (Matsubara et al. 1988).

GC and HRGC combined with AAS have been used to measure lead and alkyllead compounds of gasoline, such as tetramethyl lead, in blood and urine (Andersson et al. 1984; Harman et al. 1981; Moore et al. 1976). AAS is the most common detector used to measure lead or alkyllead compounds in blood and urine since AAS is a lead-specific detector (Andersson et al. 1984; Harms et al. 1981; Moore et al. 1976). The alkyllead compounds are solvent extracted (Andersson et al. 1984; Harman et al. 1981). For blood samples, recovery was excellent (>90%) and precision was adequate (<10% relative standard deviation [RSD]) (Andersson et al. 1984). The detection limit was in the ppm-range (Andersson et al. 1984). No recovery, precision, or sensitivity data were reported for measuring lead in urine (Harman et al. 1981; Moore et al. 1976). Another method for determining alkyllead compounds (tetraethyl lead and tetramethyl lead) in gasoline (no matrix reported) has been investigated

(Bond and McLachlan 1986). This method includes HPLC coupled with ECD at both solid and mercury electrodes (Bond and McLachlan 1986). This method is more specific for alkyllead compounds in gasoline than atomic absorption spectrometric detection since the mercury electrode acts as a very specific detector for alkyllead compounds (Bond and McLachlan 1986). The limit of detection is in the low ppm range (≈ 2 mg/L) for both tetramethyllead and tetraethyllead (Bond and McLachlan 1986). Precision is excellent (±3% CV) (Bond and McLachlan 1986). Spectrophotometric detection of phenol in urine has been used for determining benzene (a component of gasoline) in urine (Pandya et al. 1975; Buchwald 1966). No details were given regarding recovery, precision, or detection limits.

A single method of analyzing the hydrocarbon components of gasoline in tissue samples was located (Shankles et al. 1982). This method utilized HRGCFID and involved injection of headspace gas. The limit of detection, accuracy, and precision of this method were not reported.

6.2 ENVIRONMENTAL SAMPLES

The methods most commonly used to detect the major hydrocarbon components of gasoline in environmental samples include GC/FID, GC/MS, HRGC/FID, HRGCMS, and HRGC/photoionization detector (PID)/FID. GC combined with photoionization-ion mobility spectrometry (PI-IMS) has been used and is selective to aromatic hydrocarbons. See Table 6-2 for a summary of the analytical methods used to determine gasoline in environmental samples. Several of the gasoline components have been discussed in detail in their individual toxicological profiles (e.g., benzene, toluene, xylene, cyclohexane, ethane, ethylene, and lead), which should be constituted for more information on analytical methods.

GC/FID, HRGC/FID and HRGC/MS are the most commonly used methods to selectively detect and identify the hydrocarbon components of gasoline in air (Berglund and Petersson 1990; Brown 1988; Brugnone et al. 1986; Esposito and Jacobs 1977; Russo et al. 1987). Air samples are usually collected on an adsorbent tube, examples of which include charcoal, Tenax@, and Chromosorb@. Analytes are then extracted by heat or liquid solvent and analyzed. Collection efficiency (>90% recovery) was very good using charcoal tubes (Esposito and Jacobs 1977; Russo et al. 1987). No recovery data were

TABLE 6-2. Analytical Methods for Determining Gasoline in Envrionmental Samples

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (benzene)	Collect air sample from breathing zone of individual; transfer headspace gas to cryogenic trap; desorb by heating	HRGC/FID HRGC/MS	NR	NR	Brugnone et al. 1986
Air (benzene)	Collect sample in Tedlar bag; inject	GC/PID	50 ppb	NR	NIOSH 1987 (Method 3700)
Air (toluene)	Collect sample on activated carbon; extract with carbondisulfide	GC/FID	0.01 mg	NR	NIOSH 1987
Air (benzene, ethylbenzene)	Collect sample on charcoal sorbent; desorb with carbon disulfide	GC/FID	10-100 ppb	NR	NIOSH 1984c (Methods 1500 and 1501)
Air (aliphatic and aromatic components)	Collect air sample onto Chromosorb®-106 and charcoal tubes; desorb by heat	HRGC/FID	NR	NR	Brown 1988
Air (hydrocarbons)	Collect air sample onto adsorbent tube, Tenax®-GC; desorb by heat	HRGC/FID	NR	NR	Berglund and Petersson 1990

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

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (isobutane, *n*-butane, isopentane, *n*-hexane)	Collect air onto two charcoal tubes; desorb with carbon disulfide	GC/FID	NR	>90%	Russo et al. 1987
Air (aromatics)	Collect air samples on charcoal tube; extract aromatics with carbon disulfide and H_2SO_4	GC/FID	NR	92-100%	Esposito and Jacobs 1977
Water (volatile hydrocarbons)	Sparge (purge and trap) water sample; collect volatile analytes on glass tube containing Tenax®/ Ambersorb®/charcoal; thermally desorb; concentrate	HRGC/FID	NR	95-104%	Belkin and Hable 1988
Groundwater (hydrocarbons)	Collect water sample; acidify; extract with hexadecane	GC/FID	NR	NR	Dell'Acqua et al. 1976

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

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater (BTEX)	Collect sample in glass vial; add mercuric chloride to prevent biodegradation; place in water bath; manually withdraw headspace gas with syringe; inject gas	HRGC/PID/FID	NR	NR	Roe et al. 1989
Sea water (hydrocarbons)	Extract sample with methylene chloride; column cleanup; concentrate; solvent exchange to hexane	HRGC/MS	NR	≈60%	Dimock et al. 1980
Soil (volatile aromatics)	Analyze headspace gas vapors of soil sample	GC/PI-IMS	0.18 mg/Kg	NR	Eiceman et al. 1987
Soil (BTEX)	Collect soil sample in glass vial; extract with distilled water; add mercuric chloride to sample to prevent biodegradation; place in water bath; manually withdraw headspace gas with syringe	HRGC/PID/FID	NR	NR	Roe et al. 1989

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

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Bivalve mollusks (hydrocarbons)	Digest tissue; extract with methylene chloride; column cleanup; solvent exchange to hexane	HRGC/MS	NR	≈60%	Dimock et al. 1980

BTEX = benzene, toluene, ethylbenzene, and three xylene isomers; FID = flame ionization detection; GC = gas chromatography; H_2SO_4 = sulfuric acid; HRGC = high-resolution gas chromatography; MS = mass spectrometry; NR = not reported; PID = photoionization detection; PI-IMS = photoionization-ion mobility spectrometry

reported for other types of adsorption tubes for comparison purposes. Both GC and HRGC combined with FID have adequate reproducibility (precision ranging from 5-12% CV) (Brown 1988; Russo et al. 1987). Although detection limits were not reported for these methods, based on data reported, sensitivity is in the low- to sub-ppm range.

GC/FID, HRGC/FID, HRGU/PID/FID, and HRG/MS have been used to measure the hydrocarbon components of gasoline in water, groundwater, and sea water (Belkin and Hable 1988; Dell'Acqua et al. 1976; Dimock et al. 1980; Kanai et al. 1991; Roe et al. 1989). Sample preparation methods include solvent extraction, purge-and-trap (dynamic headspace), and static headspace techniques. With the purge-and-trap technique, the multicomponent tube (Tenax@/Ambersorb@/charcoal) was effective in the collection and adsorption of a wide range of compounds found in gasoline (Belkin and Hable 1988). A disadvantage associated with the use of the purge-and-trap method is that it is subject to the inherent problems associated with the use of adsorbents, such as overloading, carryover, and breakdown with repeated heating and purging cycles (Roe et al. 1989). The static headspace method, however, is an attractive method due to its rapid turn around times and its simplicity, i.e., no sample workup is required outside the vial (Roe et al. 1989). The static headspace method is less expensive than the purge-and-trap because of the lack of expensive purging equipment (Roe et al. 1989). With the headspace method, multiple runs can be performed on a single sample vial, whereas the purge-andtrap method is essentially destructive; the sample may only be purged and analyzed once (Roe et al. 1989). Poor extraction efficiency (≈60% recovery) was obtained using a solvent extraction technique (Dimock et al. 1980). Good recovery (95-104%) and adequate precision (9.4-10.6% CV) were obtained using the purge-and-trap technique with HRGUFID (Belkin and Hable 1988). No recovery data were reported using the static headspace preparation method with HRGC/PID/FID; however, precision was good (2-8% RSD) (Roe et al. 1989). The use of serial detectors (PID/FID) with HRGC enhanced selectivity by providing an additional means of discrimination for the complex-gasoline mixture. Although detection limits were not reported for any method, based on data reported, sensitivity is in the ppm-to-ppb range.

HRGC/PID/FID and GC/PI-IMS are methods that have been used to measure the volatile aromatic components of gasoline in soil (Eiceman et al. 1987; Roe et al. 1989). Sample preparation is simple because the static headspace method is used. The use of serial detectors (PID/FID) with HRGC

enhanced selectivity. No recovery data or detection limits were reported for HRGC/PID/FID; however, precision was good (2-8% RSD) (Roe et al. 1989). PID has a high selectivity to aromatic hydrocarbons (Eiceman et al. 1987). The combination of PI with IMS (PI-IMS) provided a second basis for resolution of chemical information and thus enhanced selectivity (Eiceman et al. 1987). Reproducibility for the GCM-IMS method ranged from 10 to 60% with a detection limit of 18 mg/kg (Eiceman et al. 1987).

Limited data were located regarding methods for detecting the hydrocarbon components of gasoline in biota (bivalve mollusks) (Dimock et al. 1980). The methods used were GC (detector not reported) and GUMS. Sample preparation included tissue digestion, extraction and clean-up, and solvent exchange to hexane. Recovery was poor (≈60%). Sensitivity and precision were not reported.

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 gasoline 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 gasoline.

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 will be proposed.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods exist to measure lead (organic and inorganic) levels in blood and urine (Andersson et al. 1984; Bond and McLachlan 1986; Harman et al. 1981; Moore et al. 1976). Elevated urinary and blood lead levels may be indicators of exposure to leaded gasoline, but are not specific for gasoline. Methods also exist for measuring the hydrocarbon components of gasoline in alveolar air and lung gas (Brugnone et al. 1986; Ikebuchi et al. 1986), blood (Brugnone et al. 1986; Kimura et al. 1988; Matsubara et al. 1988), and urine (Buchwald 1966; Pandya et la. 1975) as biomarkers of exposure to gasoline, but again, are not specific for gasoline. The existing methods are sensitive enough to measure background levels in the population and levels at which biological effects occur. Recovery, precision, and accuracy data are needed for measuring urinary lead levels. Recovery and detection limit data are needed for measuring the hydrocarbon components in alveolar air, lung gas, blood, and urine. These data will help to improve the reliability and reproducibility of the methods and will be useful in monitoring populations exposed to gasoline.

There do not appear to be any biomarkers of effect that are specific for gasoline.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist to detect the major hydrocarbon components of gasoline in air (Berglund and Petersson 1990; Brown 1988; Brugnone et al. 1986; Esposito and Jacobs 1977; Russo et al. 1987), water (Belkin and Hable 1988; Dell'Acqua et al. 1976; Dimock et al. 1980; Kanai et al. 1991; Roe et al. 1989), and soil (Eiceman et al. 1987; Roe et al. 1989). The most commonly used methods are GC/FID, HRGC/FID, GC/MS, HRGC/MS, HRGC/PID/FID, and GC/PI-IMS. These methods are relatively sensitive, selective, and reliable and can be used to detect the levels of the gasoline components found in the environment and levels at which health effects occur. Recovery data and detection limits are needed for measuring components found in all media. Recovery data will help to assess and improve reproducibility of the methods. Detection limit data will aid in comparison of sensitivity between methods and indicate where improvements in sensitivity are needed. This information will be useful in monitoring gasoline contamination in the environment.

6.3.2 On-going Studies

No on-going analytical methods studies were located.