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 used mineral-based crankcase oil, its metabolites, and other biomarkers of exposure and effect to used mineral-based crankcase oil. 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 MATERIALS

Used mineral-based crankcase oil is a complex mixture of hydrocarbons, metals, and additives (Vazquez-Duhalt 1989). It contains high levels of metals, such as lead, zinc, copper, chromium, nickel, and cadmium. It also contains alkanes, cycloalkanes, monoaromatics, diaromatics, PAHs, and aromatic compounds which contain sulfur, nitrogen, or oxygen. Atomic absorption spectrophotometry (AAS) is the most commonly used method for detecting the metal components of used mineral-based crankcase oil in biological materials. Biological materials include the skin, liver, lung, serum, urine, and milk. Analytical methods for measuring both biological materials and environmental samples are described in this chapter. The ^{32}P-postlabeling assay has been used to detect the presence of potentially carcinogenic PAH adducts bound covalently to macromolecules (e.g., DNA) in skin and lung tissues. See Table 6-l for a summary of the analytical methods most commonly used to determine the various components of used mineral-based crankcase oil in biological materials and environmental samples. For more information regarding the analytical methods used for detecting the various components such as PAHs and lead in biological tissues, see the ATSDR profiles for these substances (ATSDR 1990c, 1993b).

TABLE 6-1. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Skin (PAHs)	Isolate epidermal DNA from human and mouse skin treated with used oil; digest DNA enzymatically with micrococcal nuclease, spleen phosphodiesterase, and nuclease P_1; label DNA adduct by ^{32}P-post-labeling technique involving incubation with carrier-free [γ-^{32}P]ATP and polynucleotide kinase	^{32}P-postlabeling followed by TLC and autoradiography	0.20–0.57 fmol adducts/μg DNA	No data	Carmichael et al. 1990, 1991, 1992
Skin (PAHs)	Digest DNA samples with micrococcal nuclease, spleen phosphodieterase, and nuclease P_1; incubate; digest with [γ-^{32}P]ATP and polynucleotide kinase	^{32}P-postlabeling followed by TLC and autoradiography	0.05–0.13 fmol adducts/μg DNA	No data	Phillips et al. 1990

TABLE 6-1. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Skin and lungs (PAHs)	Digest DNA samples with micrococcal nuclease and spleen phosphodiesterase; extract twice with water-saturated butanol; combine organic phases and back extract twice with water; neutralize with Tris and evaporate; label DNA adduct by ^{32}P-postlabeling technique involving incubation with carrier-free [γ-^{32}P]ATP and polynucleotide kinase	^{32}P-postlabeling followed by TLC and autoradiography	40–150 amol total adducts/µg DNA	No data	Schoket et al. 1989
Liver, kidney, and rumen contents (molybdenum)	Dry ash samples; add pyrogallol red and ethyltrimethyl-ammonium bromide dissolved in acetate buffer	UV-VIS spectro-photometer	No data	No data	Sas 1989
Blood (lead)	No data	AAS	No data	No data	Clausen and Rastogi 1977

TABLE 6-1. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (1-pyrenol)	Urine samples immediately frozen at -20°C and kept in the dark until analysis; over-diluted samples (<0.6 g creatinine/liter) were discarded and fresh samples taken	HPLC/fluorescence detector; separation using methanol:water linear gradient	0.02 µg/L (signal/noise ratio >3); mean coefficient of variance and analysis 10%	No data	Granella and Clonfero 1993

AAS = atomic absorption spectrophotometry; amol = attomole; DNA = deoxyribonucleic acid; fmol = femtomole; HPLC = high-performance liquid chromatography; P_i = phosphorus$_i$; ^{32}P = radio-labeled phosphorus; [γ-^{32}P]ATP = [gamma-^{32}phosphorus]adenosine triphosphate; PAHs = polycyclic aromatic hydrocarbons; TLC = thin-layer chromatography; UV-VIS = ultraviolet-visible

AAS has been used to measure lead compounds from used mineral-based crankcase oil in blood (Clausen and Rastogi 1977). AAS is the most common detector used to measure lead compounds in blood, since AAS is a lead-specific detector. The sample preparation procedure, limit of detection, accuracy, and precision of this method were not reported. Molybdenum has been determined in rumen contents and in liver and kidney samples using ultraviolet-visible (UV-VIS) spectrophotometry; however, no information was provided regarding the sample preparation, detection limit, accuracy, or precision of this method (Sas 1989).

The ^{32}P-postlabelling assay is a highly sensitive and specific method for detecting PAH-DNA adducts in the skin and lungs of humans and animals (Carmichael et al. 1990, 1991, 1992; Phillips et al. 1990; Schoket et al. 1989). The technique generally involves isolating epidermal DNA from human and mouse skin treated with used oil, and then digesting the DNA enzymatically with micrococcal nuclease, spleen phosphodiesterase, and nuclease Pl before labeling the DNA adduct by ^{32}P-postlabelling. Detection limits ranging from 0.05 to 0.57 femtomole (fmol) adducts/μg DNA (Carmichael et al. 1990, 1991, 1992; Phillips et al. 1990) and from 40 to 150 attomole (amol) total adducts/μg DNA (Schoket et al. 1989) have been achieved. Recovery and precision data were not reported.

The urinary concentration of 1-pyrenol, a pyrene metabolite, can also be used as a biological indicator of exposure to PAHs in automotive repair workers. Granella and Clonfero (1993) evaluated the skin absorption of PAHs in automotive repair workers whose skin was exposed to used engine oils from cars and trucks and compared the results to a control group. For each worker, data were obtained on smoking habits, use of medicines, and hobbies. Subjects were also instructed not to eat foods with high concentrations of PAHs, such as grilled or barbecued meat, 48 hours before the urine samplings. The control group was placed on control diets and not occupationally exposed to PAHs. This group had a higher percentage of smokers, but among smokers in both the control and the exposed groups, there was no difference in the daily consumption of cigarettes (Granella and Clonfero 1993). The levels of 1-pyrenol were determined using high-performance liquid chromatography (HPLC) with a fluorescence detector. Urinary excretion of 1-pyrenol in this group of workers compared to occupationally exposed subjects (e.g., creosote, coke oven, and graphite electrode workers) indicated that exposure to PAHs present in used engine oil through the skin during automotive repair work is very low (Granella and Clonfero 1993). The urinary 1-pyrenol values were higher in both smoking (0.259 ± 0.201 umol/mol creatinine) and nonsmoking workers (0.154 ± 0.105 pmol/mol creatinine, as

compared to 0.083 ± 0.042 pmol/mol creatinine for the nonsmoking controls) (Granella and Clonfero 1993). However, according to the study authors, tobacco smoking and PAH-rich diets are confounding factors when monitoring this type of exposure, as they influence the urinary concentration of 1-pyrenol in the general population. Hence, smoking habits and diets should be verified accurately, including exposure to air pollution, in order to evaluate the specific effect of low-level PAH exposure, especially among repair workers. In used engine oils, the concentration of 1-pyrenol ranges from 32% (petrol engines) to 3% (diesel engines) (Granella and Clonfero 1993).

6.2 ENVIRONMENTAL SAMPLES

AAS is the most commonly used method for detecting the metal components of used mineral-based crankcase oil found in environmental samples. The methods most commonly used to detect or identify the major hydrocarbon components of used mineral-based crankcase oil in environmental samples include gas chromatography equipped with a flame ionization detector (GC/FID), flame photometric detector (GC/FPD), or mass spectrometer (GC/MS), and high-performance liquid chromatography with ultraviolet detection (HPLC/UV) or a fluorescence detector. Volatile organics have been detected using an ion trap mass spectrometer (EMS). Infrared spectroscopy (IR) is commonly used to characterize the major components found in used crankcase oil. See Table 6-2 for a summary of the analytical methods used to determine used mineral-based crankcase oil in environmental samples. For further information regarding the analytical methods for detecting the various components such as PAHs, lead, copper, nickel, and zinc in environmental samples see the ATSDR profiles for these substances (ATSDR 1989b, 1990b, 1990c, 1992e, 1993b).

Pyrene levels in cloths used by automotive repair workers were determined by HPLC with a UV-VIS detector. Pyrene contents in oily material taken from cloths used to clean the different types of engines ranged from 2.8 ± 0.4 ppm (mean ± SD) for matter from diesel truck engines to 9.3 ± 8.2 ppm for matter from petrol car engines. The recovery rate was 96%. These values were much lower than that found in coal tar, creosote, petroleum, coke, and quenching oils. However, petrol engines produced five times more pyrene than diesel engines (Granella and Clonfero 1993).

AAS is the most commonly used method to selectively detect and identify the metal components (lead, cadmium, manganese, copper, nickel, iron, and zinc) of used mineral-based crankcase oil in air (Clausen and Rastogi 1977), street or stormwater runoff (Latimer et al. 1990; Newton et al. 1974), soil

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (lead)	Collect air sample through Whatman Glass filter paper	AAS	No data	No data	Clausen and Rastogi 1977
Air (engine oil)	Collect airborne mist of engine oil on membrane filter; extract with $C_2Cl_3F_3$	IR spectrophotometry	0.5 mg/m^3	98	NIOSH 1984
Air (oil mist)	Collect airborne mist of oil on membrane filter; extract with carbon tetrachloride	IR spectrophotometry	0.3 mg/m^3	No data	NIOSH 1978
Air (oil mist)	Collect airborne mist of oil on membrane filter; extract with chloroform	Fluorescence spectrophotometry	No data	97.7	NIOSH 1977
Air (volatile organics)	Direct injection with no sample preparation (direct air sampling)	ITMS	1 ppb	No data	Buchanan et al. 1990
Air (volatile organics)	Preconcentrate on resin trap followed by direct thermal desorption	ITMS	ppt levels	No data	Buchanan et al. 1990
Crankcase oil	Heat sample to 300°C and use hydrogen as the carrier gas and nitrogen as the make-up gas	PID; FID	No data	≥97	Bemgård and Colmsjö 1992

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Waste incineration by-products (crankcase oil; smelter particulates; composite waste particulates)	No preparation for samples on filter; solid samples are ground and mounted in bulk specimen. If there are insufficient quantities for bulk handling, samples are cast in a collodion film on glass slide	XRD; samples scanned with a diffractometer and the XRD data is reduced using JCPDS files	No data	No data	Briden 1984
Waste incineration by-products (crankcase oil; smelter particulates; composite waste particulates)	No preparation for samples on filter; solid samples are ground, mixed 1:3 with organic binder, pressed into a pellet	XRF; samples qualitatively scanned on sequential wave-length spectrometer	No data	No data	Briden 1984
Water (volatile organics)	Direct purge of sample with helium into the mass spectrometer sample interface	ITMS	ppb or lower	No data	Buchanan et al. 1990
Surface water	Add sulfuric acid and (CCl_4) to water sample; jet-air evaporate CCl_4 extract; concentrate; bring up to volume with hexane	GC/FID; UV fluorescence spectroscopy; GC/MS	No data	No data	Tanacredi 1977

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water (PAHs)	Extract water samples with methylene chloride in separatory funnels; evaporate; clean up on deactivated silica/alumina gel column; elute with benzene-methanol	GC/FID; GC/MS	No data	No data	Tanacredi and Cardenas 1991
Stormwater runoff (hydrocarbon analysis)	Filter runoff samples through glass fiber filter; add internal standards; extract filtrate with methylene chloride; solvent exchange to hexane; evaporate to 1 mL; clean up by adsorption column chromatography using silica gel; elute saturated hydrocarbons (normal, branched, and cyclic alkanes) with hexane; elute unsaturated hydrocarbons (PAHs) with hexane/methylene chloride	No data	No data	No data	Latimer et al. 1990

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Stormwater runoff (hydrocarbons)	Fractionate water sample to separate aqueous and particulate phases; pass aqueous portion through activated carbon column, then dry; centrifuge particulate phase and dessicate; extract both fractions successively with hexane, benzene, and chloroform using Soxhlet extraction; clean up using silica gel column; elute with hexane (aliphatic hydrocarbons), benzene (aromatic hydrocarbons), and chloroform/methanol (oxy-polar compounds); evaporate to dryness and record weight	IR; GC/FID/FPD	No data	85–95	Hunter et al. 1979
Street runoff (lead)	Acid digest sample	AAS	No data	No data	Newton et al. 1974
Stormwater runoff and sediment	Extract and saponify by Soxhlet extraction using a benzene/potassium solvent system	GC/FID	No data	No data	Brown et al. 1985

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil (volatile organics)	Add water to soil sample; purge	ITMS	ppb or lower	No data	Buchanan et al. 1990
Street dust, surface soil, vegetation, atmospheric deposition (hydrocarbon analysis)	Collect dust and particulates on filter; air dry and weigh all samples; reflux in KOH mixture in methanol; water; partition hydrocarbons against petroleum ether; evaporate extracts to 1 mL; clean up with adsorption column chromatography with silica gel; elute saturated hydrocarbons with hexane; elute unsaturated hydrocarbons including PAHs with hexane:methylene chloride	GC/FID	No data	No data	Latimer et al. 1990
Stormwater runoff, dust, soil, vegetation, atmospheric deposition (metals analysis [Pb, Cd, Mn, Cu, Fe, and Zn])	Leach samples for one week in 5% HNO_3 solution	AAS	No data	No data	Latimer et al. 1990

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Stormwater runoff (aromatic hydrocarbons and sulfur compounds)	Separate aqueous and particulate phase by centrifugation; filter particulates and dry in oven; adsorb supernatant onto activated carbon column; Soxhlet extract both phases using hexane, benzene, and chloroform; extracts adsorbed to silica gel; elute with hexane to obtain aliphatic hydrocarbons; elute with benzene to obtain aromatic hydrocarbon and sulfur compounds; evaporate to dryness; weigh; resolubilize in methylene chloride; pass through copper column to eliminate free sulfur contamination	GC/FID/FPD; GC/MS	No data	≈70 (dibenzo-thiophene)	MacKenzie and Hunter 1979

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Used engine oil (pyrene)	Carefully preweighed cloth left in workshops which a worker cleaned with his hands; cloth then weighed and oily matter in the cloth extracted with acetonitrile in ultrasonic bath thermostated at 40°C for 15 minutes; this operation repeated three times; concentration of pyrene in extracts then determined	HPLC/UV-VIS detector at 350 mm wavelength; 20 µL of extract eluated with acetonitrile water gradient for 15 minutes at 40°C, flow rate 1.0 mL/minute	No data	96	Cranella and Clonfero 1993
Wastewater (1-nitropyrene and 1,6-dinitropyrene)	Fractionate waste water into diethyl ether-soluble neutral, acidic, and basic fractions	HPLC/UV detector/ fluorescence detector; GC/MS	1.1×10^{-2} pmol (1-NP); 1.3×10^{-2} pmol (1,6-diNP)	No data	Manabe et al. 1984
Waste water (hydrocarbons)	Clean up sample on alumina or silica gel column; elute hydrocarbons with petroleum ether; concentrate	GC; IR; TLC	0.5 mg/L	No data	Farrington and Quinn 1973

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil and vegetation (Cd, Ni, Pb, and Zn)	Agitate soil samples with HCl and centrifuge; digest grass samples with hot, concentrated HNO_3 and $HClO_4$	AAS	No data	No data	Lagerwerff and Specht 1970
Soil (heavy metals)	Dry soil samples; add alcoholic magnesium nitrate; heat to dryness; ash; add concentrated HCl to cooled sample; heat to dryness; add concentrated HCl and water to dried material; bring solution to boil; bring cooled solution up to volume; shake and filter	AAS	No data	99.4 (Cd); 102.5 (Ni); 98.8 (Pb); 95.25 (Zn)	Gish and Christensen 1973

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil and plants	Air dry and grind samples; boil soil samples for 2 hours in perchloric acid; digest dried plant samples in 4:1 mixture of nitric and perchloric acids	AAS	No data	No data	Motto 1970
Soil (motor oil)	Dry soil sample; extract with methylene chloride and agitate; concentrate extract using low heat	TLC	100 ppb	70	Newborn and Preston 1991
Sediments (hydrocarbons)	Extract lipids from sediments by Soxhlet extraction with benzene and methanol; saponify to separate hydrocarbons from fatty acid esters; isolate hydrocarbons by column chromatography on alumina packed over silica beds; elute aliphatic hydrocarbons with pentane	GC/FID	No data	>75	Wakeham and Carpenter 1976
Clams (PAHs)	Homogenize tissue; Soxhlet extract in hexane for 8 hours	HPLC/UV detector	No data	No data	Tanacredi and Cardenas 1991

TABLE 6-2. Analytical Methods for Determining Used Mineral-based Crankcase Oil in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Earthworms	Blend sample; mix with alcohol magnesium nitrate; dry, char, and ash sample; wet cooled ash with nitric acid; dry; add concentrated HCl and heat to dryness; add concentrated HCl and water and bring to a boil; bring cool solution up to volume	AAS	No data	89.25 (Cd); 106.5 (Ni); 93.25 (Pb); 89.15 (Zn)	Gish and Christensen 1973

AAS = atomic absorption spectrophotometry; CCl_4 = carbon tetrachloride; $C_2Cl_3F_3$ = trichlorotrifluoroethane; Cd = cadmium; Cu = copper; Fe = iron; FID = flame ionization detector; FPD = flame photometric detector; GC = gas chromatography; HCl = hydrochloric acid; $HClO_4$ = perchloric acid; HNO_3 = nitric acid; HPLC = high-performance liquid chromatography; IR = infrared; ITMS = ion trap mass spectrometer; JCPDS = Joint Committee on Powder Diffraction Standards; KOH = potassium hydroxide; Mn = manganese; MS = mass spectrometry; Ni = nickel; PAHs - polycyclic aromatic hydrocarbons; Pb = lead; PID = photoionization detector; TLC = thin-layer chromatography; UV = ultraviolet; VIS = visible; XRF = x-ray fluorescence; XRD = x-ray diffraction; Zn = zinc

(Gish and Christensen 1973; Lagerwerff and Specht 1970; Motto 1970), dust, vegetation, atmospheric deposition, and earthworms (Gish and Christensen 1973; Lagerwerff and Specht 1970; Latimer et al. 1990). Air samples are collected on filter paper (Clausen and Rastogi 1977). The other matrices (runoff, soil, vegetation, and earthworm samples) are usually subjected to an acid digestion procedure. Although sensitivity data were not reported, ppm-to-ppb levels can be detected based on the measured samples. Recovery data for measuring metals in air and runoff samples were not reported. Recoveries for soil samples (>95%) and earthworms (>89%) were very good (Gish and Christensen 1973). Precision data were not reported.

GC/FID and GUMS are methods used for detecting the hydrocarbon components of used crankcase oil in surface water (Tanacredi 1977), stormwater runoff and waste water (Brown et al. 1985; Farrington and Quinn 1973; Latimer et al. 1990), and sediments (Wakeham and Carpenter 1976). Sample preparation methods generally include solvent extraction followed by a cleanup step using adsorption chromatography. Although detection limits were not reported for these methods, sensitivity is in the ppm-to-ppb range based on data reported. Recovery for hydrocarbons in sediment using GC/FID was >75% (Wakeham and Carpenter 1976). Precision data were not reported.

Petroleum-derived aromatic and aliphatic hydrocarbons and associated sulfur compounds in urban stormwater runoff and sediment samples were characterized using GC equipped with a flame ionization/sulfur specific flame photometric detector system (Hunter et al. 1979; MacKenzie and Hunter 1979). These detectors allowed simultaneous detection of both hydrocarbon and sulfur-containing materials. Although sulfur compounds represent only about 4% of a petroleum oil, sulfur fingerprints have been shown to be a valuable aid in oil characterization and source correlation (MacKenzie and Hunter 1979). The sulfur aromatics in petroleum oil are predominantly thiophenes (benzothiophene, dibenzothiophene, naphthobenzothiophene, and their alkyl derivatives). Aromatic sulfur compounds have been detected in petroleum oils from stormwater runoff. Dibenzothiophene and phenanthrene and/or anthracene were present in all samples (both used crankcase oil and stormwater particulates). An average of approximately 70% of the dibenzothiophene was recovered in stormwater by the analytical method and separation scheme. Recoveries for the aliphatic and aromatic fractions ranged from 85% to 95% (Hunter et al. 1979). Although detection limits were not reported, ppm-to-ppt concentrations can be measured based on levels reported (Hunter et al. 1979; MacKenzie and Hunter 1979). Precision data were not reported.

In Manabe et al. (1984), mutagenic studies (using *S. typhimurium* strains) of crankcase oil from a gasoline engine, fractionated by Soxhlet extraction, indicated that used engine oil contained 1-nitropyrene (1-NP) and dinitropyrenes (diNP). Crankcase oils from gasoline and diesel engines contained 138 and 349 ng of 1-NP and 2 and 31 ng 1,6-diNP, respectively, per mL of oil. In this study, the 1-NP fraction was collected, analyzed, and quantified by GC/MS and HPLC to prove that waste water contained 1-NP. Since many unknown compounds in the waste water showed the same retention time as 1-NP using HPLC, 1-NP was not quantitated precisely by use of an ultraviolet detector and HPLC. Since the reduced product of 1-NP, 1-aminopyrine (l-AP), is fluorescent and easily detectable with the fluorescence detector of HPLC, 1-NP was reduced with nitroreductase and l-AP was then measured. The reaction mixture was incubated at 37°C for 5 or 15 hours without shaking. An ethyl acetate extract of the mixture was then evaporated, redissolved in methanol, and injected into the chromatograph. Detection limits for 1-NP and diNP were $1.1x10^{-2}$ and $1.3x10^{-2}$ pmol, respectively. Recovery and precision data were not reported. 1,6-diNP (31 ng) accounted for 12% of the total mutagenicity of the neutral fraction in the assay system (Manabe et al. 1984). According to the study authors, diNPs showed higher mutagenic activity than 1-NP, and 1,6-diNP was shown to be carcinogenic in mice.

X-ray diffraction (XRD) phase analysis in conjunction with x-ray florescence (XRF) elemental analysis is an extremely effective method for characterizing waste incineration by-products, particularly crankcase oil incineration residues. XRD is a useful technique for determining the phase of the compounds in a sample. The technique involves scanning the sample with a diffractometer and interpreting the data according to the Joint Committee on Powder Diffraction Standard (JCPDS) files. To determine which elements are present in the sample, XRF is conducted using minimal sample preparation and analysis time. This method analyzes the samples for elements ranging from sodium to uranium by quantitatively scanning with a sequential wavelength dispersive spectrometer. In the past, using XRD phase analysis, together with XRF elemental analysis techniques, has produced accurate percentages of the elements present in the emissions from used mineral-based crankcase oil commercial waste incinerators and from lead and copper smelters (Briden 1984).

Volatile organics found in used mineral-based crankcase oil have been measured in air, water, and soil using an ITMS (Buchanan et al. 1990). The ITMS operates at a higher pressure than conventional mass spectrometers, making it amenable to accommodating higher gas loads, thereby allowing the direct introduction of gaseous analytes into the ITMS with little or no sample preparation. Because no

chromatography is used, sample turn around is less than 5 minutes. This method yields excellent detection limits (ppb-to-ppt levels) and is reproducible for all media. Recovery and precision data were not reported.

Thin-layer chromatography (TLC) has been used as a screening tool for on-site environmental analysis of used mineral-based crankcase oil in soil (Newborn and Preston 1991). A detection limit of 100 ppm and a recovery of 70% were achieved for motor oil. TLC is a cost-effective tool for field screening of samples, especially soils, when low detection limits (i.e., the sensitivity of gas chromatography) are not required.

IR and fluorescence spectrophotometry have been used to detect mineral oil in air (NIOSH 1977, 1978, 1984). For IR, sensitivity is in the low-ppm range (NIOSH 1978, 1984). For both methods, recovery (98%) and precision (5% relative standard deviation; 6.5% coefficient of variation) are good (NIOSH 1977, 1978, 1984). Sensitivity was not reported for fluorescence spectrophotometry. IR, fluorescence spectrophotometry, and ultraviolet spectroscopy have been used to detect and identify the main components of oils in sea water (Adler et al. 1990; Zieba 1985).

A photoionization detector (PID) in conjunction with capillary chromatography has been used to detect and identify PAHs. This is done by comparing the PID response with that of a flame ionization detector (FID) which gives a substance-specific response ratio. This method is limited in its usefulness because the obtained ratios are specific to the particular system being used. Ratios for the same compound can be off by as much as 20% for the same compound in different systems (Berngird and Colmsjo 1992).

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 used mineral-based crankcase oil 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 used mineral-based crankcase oil.

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

Exposure. Although specific biomarkers for exposure to used mineral-based crankcase oil have not been identified, methods exist to measure the PAH and metal components of used mineral-based crankcase oil in tissues (skin, lung, liver, kidney) and blood (Carmichael et al. 1990, 1991, 1992; Phillips et al. 1990; Sas 1989; Schoket et al. 1989). Biomarkers such as metal content of the blood (Clausen and Rastogi 1977; Sas 1989) or DNA adduct formation (Carmichael et al. 1990, 1991, 1992; Kurelec and Gupta 1993; Schoket et al. 1989) have been identified as possible indicators of exposure to chemicals found in used mineral-based crankcase oil. These biomarkers are not specific for exposure to used mineral-based crankcase oil but may be specific for chemicals found in the oil. Caution should be used to avoid misinterpretation when using DNA adducts as biomarkers in lower invertebrates such as sea urchins and sponges due to their inability to form DNA adducts when exposed to certain compounds such as PAHs. Another consideration with respect to using DNA adducts as biomarkers is that DNA modifications are found in varying levels in certain aquatic organisms depending on the season (Kurelec and Gupta 1993). Biomarkers of exposure to metals and aromatic hydrocarbons are discussed in more detail in other ATSDR profiles (ATSDR 1989b, 1990b, 1990c, 1992e, 1993b). The existing methods are sensitive enough to measure background levels in the population and levels at which biological effects occur. Recovery and precision data are needed for measuring PAH-DNA adduct levels in tissues and levels of metals in blood and tissue. Detection limit data are also needed for measuring metals in blood and tissues. These data will help to improve the reliability and reproducibility of the methods and will be useful in monitoring populations exposed to used mineral-based crankcase oil.

Effect. No information was located regarding biomarkers of effect that were specific for used mineral-based crankcase oil. The biomarkers of effect are similar to the biomarkers of exposure listed above.

The methods used to measure biomarkers of exposure are the same as those used to measure biomarkers of effect and data needs for biomarkers of effect are also similar to those mentioned for biomarkers of exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist to detect the various components of used mineral-based crankcase oil, such as metals (Clausen and Rastogi 1977; Gish and Christensen 1973; Lagerwerff and Specht 1970; Latimer et al. 1990; Motto 1970; Newton et al. 1974), as well as hydrocarbons and additives (Adler et al. 1990; Brown et al. 1985; Buchanan et al. 1990; Farrington and Quinn 1973; Hunter et al. 1979; Latimer et al. 1990; MacKenzie and Hunter 1979; Manabe et al. 1984; NIOSH 1977, 1978, 1984; Tanacredi 1977; Wakeham and Carpenter 1976; Zieba 1985). These methods detect components in air, water (including runoff), soil, sediments, and some aquatic and terrestrial organisms. They include AAS, GC/FID, GC/MS, GC/FID/FPD, ITMS, HPLC/UV, HPLC/fluorescence detector, TLC, IR, fluorescence spectrophotometry, and ultraviolet spectroscopy. These methods are relatively sensitive and selective and can be used to detect the levels of used mineral-based crankcase oil components found in the environment and the levels at which health effects could occur. However, recovery, sensitivity, and detection limit data are needed for measuring the components found in all media. Recovery and precision data will help to assess and improve the 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 used mineral-based crankcase oil contamination in the environment. So far, degradation products of used mineral-based crankcase oil have not been detected.

6.3.2 On-going Studies

No on-going analytical methods studies were located.