

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 hydraulic fluids, their metabolites, and other biomarkers of exposure and effect to hydraulic fluids. 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.

Mineral oil, water-in-oil emulsion, and polyalphaolefin hydraulic fluids all have components in common with a very large number of other products that are based on mineral oil and synthetic mineral oils (polyalphaolefins); these products include motor oils, fuel oils, and petroleum distillates. No analytical method can distinguish the source of mineral oil components; that is, mineral oil components that result from hydraulic fluids are indistinguishable from those resulting from other forms of mineral oil taken from the same distillation and processing cut. The analytical methods presented here are capable of identifying long chain alkanes (the components present in mineral oils and polyalphaolefins used in hydraulic fluids) and will be most useful when something is known about the source of contamination.

Similarly, organophosphate esters are used in a wide variety of applications including hydraulic fluids, plasticizers, and antiwear additives to hydraulic fluids and engine oils. All of these uses have the potential to contaminate the environment, and all of the organophosphate ester components present in hydraulic fluids also are present in plasticizers and antiwear additives. Therefore, detection of a particular organophosphate ester in the environment or in biological media cannot identify the source of the contamination (i.e., hydraulic fluids, plasticizers, antiwear additives).

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6.1 BIOLOGICAL SAMPLES

Mineral Oil Hydraulic Fluids. Several methods are available for analysis of the components of mineral oil hydraulic fluids (straight and branched chain alkanes) in biological samples. They are summarized in Table 6-1. Several methods involve a preparation step followed by purification procedures, then analysis (Ferrario et al. 1985a, 1985b; Hesselberg and Seelye 1982). Briefly, sample preparation includes homogenization and extraction. A low boiling hydrocarbon extraction solvent, alone or in combination with a more polar solvent, probably is best since all of the components of the mineral oil will be soluble in it. If lipids are present, they should be removed by gel permeation chromatography and the alkanes separated from other more polar compounds by silica gel chromatography. The purge-and-trap method involves homogenizing the sample, and then purging it with nitrogen (or other inert gas) while warming the sample. The purged gases are trapped on a Tenax/silica gel column maintained at a low temperature. The trap is then heated to desorb the trapped material onto a GC column. All of the methods presented in this chapter for analyzing mineral oil hydraulic fluids in biological samples use gas chromatography (GC) for peak separation, coupled with mass spectrometry (MS) for peak identification.

Organophosphate Ester Hydraulic Fluids. Few methods are available for determining organophosphate esters in biological samples. A summary is shown in Table 6-2. The only biological matrices with well established analytical procedures for organophosphate esters are fish (Muir et al. 1981) and human fat (LeBel and Williams, 1983; 1986). Preparation of tissue includes extraction and clean-up steps; determination is usually accomplished using GC with nitrogen/phosphorus detection (NPD). Selective detectors, such as the NPD, are preferred since biological matrices are usually complex.

Fish tissue is homogenized with a Polytron apparatus using methanol as a solvent, or extracted in a ball mill with hexane. The extracts are evaporated to dryness, dissolved in ethyl acetate toluene, and cleaned up on a gel permeation column followed by an alumina column. Analysis is performed by GC/NPD (Muir et al. 1981). Recovery is acceptable (79-97%) and limit of detection is 10 mg/g (Muir et al. 1981).

Human adipose tissue samples are analyzed by homogenizing the sample with benzene or 15:85 acetonehexane and then centrifuging the sample and filtering the supernatant through a sodium sulfate (Na_2SO_4) column. The organophosphate esters in the benzene extract are then separated from the fat by gel permeation chromatography, and the eluate is cleaned up using a Florisil column. Analysis is performed using GC/NPD.

Table 6-1. Analytical Methods for Determining Mineral Oil and Polyalphaolefin Hydraulic Fluids in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish	Extraction by column chromatography; GPC fractionation; solvent exchange; fractionation of alkanes on silica gel column chromatography	GC/MS	Not specified	Not specified	Hesselberg and Seelye 1982
Oysters and clams	Homogenization; purged with nitrogen for 2 hours at 25 °C, then 2 hours at 70 °C onto Tenax/silica gel trap; thermal desorption	Capillary GC/MS	Not specified	Not specified	Ferrario et al. 1985a
Human coronary plaque	Plaque gruel is solvent extracted and concentrated; fractionation on silica gel columns	Capillary GC/MS	Not specified	Not specified	Ferrario et al. 1985a

GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry

Table 6-2. Analytical Methods for Determining Organophosphate Ester Hydraulic Fluids in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Fish	Polytron (methanol) or ball mill (hexane) extraction; clean-up by GPC and alumina column chromatography	GC/NPD	0.01 µg/g	79–97	Muir et al. 1981
Human adipose tissue	Homogenization; solvent extraction; GPC and Florisil column clean-up	Capillary GC/NPD; confirmation by GC/MS	Low ng/g	69–104%	LeBel and Williams 1983

GC = gas chromatography; GPC = gel permeation chromatography; MS = mass spectrometry; NPD = nitrogen/phosphorus detection

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Confirmation by GUMS is recommended. Recovery is acceptable (82%). Tributyl phosphate and triphenyl phosphate contamination was reported in procedural blanks and may prevent determination of phosphate esters at levels near the detection limit (1 ng/g) (LeBel and Williams 1983; 1986).

Polyalphaolefin Hydraulic Fluids. The methods for analyzing polyalphaolefin hydraulic fluids are identical to those for the mineral oil hydraulic fluids (see Table 6-1). Polyalphaolefin oils can be distinguished from mineral oils because they will be present in combinations of the alphaolefin from which they were synthesized (Shubkin 1993). Thus, polyalphaolefins obtained from 1 -decene will be present as dimers (C_{20} alkanes), trimers (C_{30} alkanes), tetramers (C_{40} alkanes), pentamers (C_{50} alkanes), etc., with no alkanes between these isomers (e.g., there will be no C_{21} alkanes present in the oil). This method of identification will only be possible if the polyalphaolefin hydraulic fluids contain no mineral oils or if the samples being analyzed were not exposed to mineral oils.

6.2 ENVIRONMENTAL SAMPLES

Mineral Oil Hydraulic Fluids. Methods are available for analysis of the hydrocarbon components of mineral oil hydraulic fluids (predominantly straight and branched chain alkanes) in environmental samples. Some of these methods are summarized in Table 6-3. In general, water and sediment samples are extracted with a suitable solvent in a Soxhlet extractor (for solid samples) or in separatory funnel or shake flask (for liquid samples) (Bates et al. 1984; Peterman et al. 1980). The extract is cleaned up on silica gel or Florisil columns using a nonpolar solvent to elute the nonpolar alkanes. Analysis is usually performed by GC/MS (Bates et al. 1984; Kawamura and Kaplan 1983; Peterman et al. 1980). Method performance has not been reported, although 82% recovery of aliphatic hydrocarbons was reported for rainwater (Kawamura and Kaplan 1983).

Air samples can be analyzed by passing a known volume of air through a Teflon filter to catch air particulates followed by an activated charcoal filter to trap any gas-phase materials. The Teflon filters are extracted with hexane, concentrated, and analyzed by GC/MS. The charcoal traps are desorbed with carbondisulfide, concentrated, and analyzed by GC/MS. No performance data were reported (Dannecker et al. 1990).

Table 6-3. Analytical Methods for Determining Mineral Oil and Polyalphaolefin Hydraulic Fluids in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water/wastewater	pH adjustment to ≥ 11 ; solvent extraction; fractionation on Florisil columns	GC/MS	Not specified	Not specified	Peterman et al. 1980
Rainwater	Solvent extraction; liquid-liquid partition clean-up; fractionation on silica gel plates	Capillary GC/FID and GC/MS	Not specified	82	Kawamura and Kaplan 1983
Sediment	Soxhlet extraction; liquid-liquid partition cleanup; fractionation on silica gel columns	GC/MS	Not specified	Not specified	Bates et al. 1984
Air (particulates and gas)	Sequential collection on Teflon filters and charcoal tubes; solvent extraction with hexane (filters) and carbon disulfide (charcoal tubes)	GC/MS	Not specified	Not specified	Dannecker et al. 1990

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

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Organophosphate Ester Hydraulic Fluids. Few analytical methods are available for analysis of organophosphate esters in environmental samples. A summary of methods is shown in Table 6-4. Care must be taken in the laboratory to assure that all labware, solvents and reagents are free of interfering contaminants. Organophosphate esters have widespread use and have been reported as sources of contamination (Muir 1984; LeBel and Williams 1986).

Water samples are acidified and extracted with solvent (Kawamura and Kaplan 1983; Muir et al. 1981). Clean-up steps may be used (Kawamura and Kaplan 1983). Methylene chloride is often used as the extracting solvent, and it may interfere with the nitrogen-phosphorus detector. In this case, a solvent exchange step is used (Muir et al. 1981). Analysis by GC/NPD or GC/MS provides specificity (Kawamura and Kaplan 1983; Muir et al. 1981). Accuracy is acceptable (>80%), but precision has not been reported. Detection limits were not reported, but are estimated to be 0.05-0.1 µg/L (Muir et al. 1981). Detection limits at the low ppt level (ng/L) were achieved by concentrating organophosphate esters on XAD-2 resin. The analytes were solvent extracted from the resin and analyzed by GC/NPD and GC/MS. Recovery was acceptable (>70%) and precision was good (<10% RSD) (LeBel et al. 1981).

A method for sediment involves Soxhlet extraction followed by filtration, and concentration to 5 mL. The residue is diluted with water, acidified, extracted with methylene chloride, and then the extracts are dried and evaporated to dryness. The residue is cleaned up on an alumina column. Analysis is performed by GC/NPD. Good recovery (81-97%) and precision (>15% RSD) were reported; detection limits were not reported (Muir et al. 1981).

A method for food (fish) has been reported. The sample is ground with sodium sulfate and extracted with petroleum ether. The extract is cleaned up by liquid-liquid partition, followed by Florisil column chromatography. Analysis is performed by GC/NPD. Detection limits are 0.1 ppm; recovery was not reported (Lombard0 and Egry 1979).

Polyalphaolefin Hydraulic Fluids. The methods for analyzing polyalphaolefin hydraulic fluids are identical to those for the mineral oil hydraulic fluids (see Table 6-3). Polyalphaolefin oil can be distinguished from mineral oils because they will be present in combinations of the alphaolefin from which they were synthesized (Shubkin 1993). Thus, polyalphaolefins obtained from 1-decene will be present as dimers (C₂₀ alkanes), trimers (C₃₀ alkanes), tetramers (C₄₀ alkanes), pentamers (C₅₀ alkanes), etc., with no alkanes between these isomers (e.g., there will be no C₂₁ alkanes present in the oil). This method of identification will

Table 6-4. Analytical Methods for Determining Organophosphate Ester Hydraulic Fluids in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Occupational air (NIOSH Method 7905)	Sample collection on Tenax sorbent tube	GC/FPD(P)	0.005 µg/sample	94	NIOSH 1989a
Occupational air (NIOSH Method 7300)	Sample collection on cellulose filter	ICAP	1 µg/sample		NIOSH 1989b
Occupational air (NIOSH Method 5037)	Sample collection on a filter; solvent extraction		Not specified		NIOSH 1989c
Drinking water	Sample collection on XAD-2 resin; solvent extraction; solvent exchange	GC/NPD; confirmation by GC/MS	Low ppt	>70	LeBel et al. 1981
Water	Solvent extraction of acidified water (pH 3); solvent exchange	GC/NPD	0.05–0.1 µg/L (est.)	91–118	Muir et al. 1981
Rainwater	Solvent extraction; clean-up by liquid-liquid partition; fractionation on silica gel plates	Capillary GC/FID and GC/MS	Not specified	>80	Kawamura and Kaplan 1983
Sediment	Soxhlet extraction; clean-up by liquid-liquid partition, alumina column chromatography	GC/NPD		81–97	Muir et al. 1981
Food (fish)	Grinding with petroleum ether; solvent extraction; clean-up by liquid-liquid partition, Florisil column chromatography	GC/NPD	0.1 ppm	Not specified	Lombardo and Egry 1979

est. = estimated; FID = flame ionization detector; FPD(P) = flame photometric detector operated in phosphorus mode; GC = gas chromatography; ICAP = inductively coupled argon plasma spectrometry; MS = mass spectrometry; NIOSH = National Institute for Occupational Safety and Health; NPD = nitrogen-phosphorus detector

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only be possible if the polyalphaolefin hydraulic fluids contain no mineral oils or if the samples being analyzed were not exposed to mineral oils.

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 hydraulic fluids 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 hydraulic fluids.

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.

Mineral Oil Hydraulic Fluids. No methods were identified for determining biomarkers of exposure to or effect of mineral oil hydraulic fluids. Nonetheless, the available analytical methods for identifying C₁₅₋₅₀ alkanes in biological tissues could be used to determine exposure levels (Ferrario et al. 1985a, 1985b; Hesselberg and Seelye 1982). The methods appear to be sensitive; however, since many compounds are being detected, the exact sensitivity depends on the alkane under consideration. The development of a method to identify metabolites of mineral oil hydraulic fluids in humans would aid in determining exposures.

Organophosphate Ester Hydraulic Fluids. The measurement of organophosphate esters in fish and human adipose tissue has been used to assess environmental contamination in several studies (Mayer et al. 1981). The methods are able to detect concentrations of 0.1 mg/kg in fish and 2.5 µg/kg in human adipose tissue. At concentrations below this level, interferences from organophosphate esters used as plasticizers appear in

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procedural blanks (Muir 1984). Several organophosphate esters have been detected in distilled water, pesticide grade solvents, and o-rings used in metering valves (Muir 1984). The methods appear to be adequate to identify background levels and can identify concentrations below known effect levels.

Polyalphaolefin Hydraulic Fluids. No methods were identified for determining biomarkers of exposure or effect. Nonetheless, the available analytical methods for identifying C₁₅₋₅₀ alkanes in biological tissues could be used to determine exposure levels (Ferrario et al. 1985a, 1985b; Hesselberg and Seelye 1982). The methods appear to be sensitive; however, since many compounds are being detected, the exact sensitivity depends on the alkane under consideration. The development of a method to identify metabolites of polyalphaolefin hydraulic fluids in humans would aid in determining exposures.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media.

Mineral Oil Hydraulic Fluids. Analytical methods similar to those described above have been used to analyze straight and branch chained alkanes in the environment (Ferrario et al. 1985a, 1985b; Hesselberg and Seelye 1982). The methods appear to be sensitive, although the sensitivity depends on the alkane being analyzed. Determining the presence of a specific mineral oil hydraulic fluid (e.g., Sunsafe F hydraulic fluid) in the environment may be possible if all the components are known, but this may or may not work for other brands of the fluid. The mineral oil in a hydraulic fluid cannot be distinguished from other mineral oils derived from the same distillation cut/processing stream. If more information on the exact alkane isomer distribution present in representative common hydraulic fluids was available, it might be possible to distinguish, for example, a hydraulic fluid from a motor oil.

Organophosphate Ester Hydraulic Fluids. Analytical methods similar to those described above have been used in many monitoring studies (Deleon et al. 1986; EPA 1978, 1979; FMC 1977c, 1979, 1980; Konasewich et al. 1978; Mayer et al. 1981; Monsanto 1981; Sheldon and Hites 1978, 1979; Strachan 1974; Weber and Ernst 1983). The methods are sensitive to ≈ 0.05 mg/kg for sediments and ≈ 0.5 μ g/L for water. Interferences from organophosphate esters used as plasticizers appear in procedural blanks, and the potential for sources of contamination must be examined at sub-nanogram per gram levels (Muir 1984). Several organophosphate esters have been detected in distilled water, pesticide grade solvents, and o-rings used in metering valves (Muir 1984). The methods appear to be sensitive enough to determine background levels. While the major exposure medium for organophosphate ester hydraulic fluids is from the neat fluid, the

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environmental media of concern are water and sediments since organophosphate ester hydraulic fluids appear to be discharged most often to water where they partition to sediments.

Polyalphaolefin Hydraulic Fluids. Analytical methods similar to those described above have been used to analyze straight and branch chained alkanes present in polyalphaolefin hydraulic fluids in the environment (Ferrario et al. 1985a, 1985b; Hesselberg and Seelye 1982). The methods appear to be sensitive, although the sensitivity depends on the alkane being analyzed. Determining the presence of a specific polyalphaolefin hydraulic fluid (e.g., MLH-83282) in the environment may be possible if all the components are known, but this may or may not work for other types of polyalphaolefin fluids. The polyalphaolefin oil in a hydraulic fluid cannot be distinguished from other polyalphaolefin oils derived from the same catalyst/operating parameters/processing. If more information on the exact alkane isomer distribution present in representative common polyalphaolefin hydraulic fluids was available, it might be possible to distinguish, for example, a polyalphaolefin hydraulic fluid from a polyalphaolefin motor oil.

6.3.2 Ongoing Studies

No ongoing studies on the analytical chemistry of mineral oils, organophosphate esters, or polyalphaolefins were located in the available literature.

