6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring Otto Fuel II and its components in environmental media and in biological samples. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify Otto Fuel II and its components. Rather, the intention is to identify wellestablished methods that are used as the standard methods of analysis. Many of the analytical methods used to detect Otto Fuel II and its components 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 may be 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 may be included that refine previously used methods to obtain lower detection limits, and/or to improve accuracy and precision.

6.1 BIOLOGICAL SAMPLES

The chief component (75%) of Otto Fuel II is propylene glycol dinitrate. The balance of Otto Fuel II is composed of 2-nitrodiphenylamine (2%) and dibutyl sebacate (23%). The constituent of Otto Fuel II that presents the major health concern is propylene glycol dinitrate. Therefore, exposure levels to Otto II fuels have been based on measured propylene glycol dinitrate levels and/or its metabolites 1-propylene glycol mononitrate and 2-propylene glycol mononitrate. Methods were located for the analysis of propylene glycol dinitrate or its metabolites in expired air, blood, urine, and hand swabs. However, no methods of detecting the chemical or its metabolites in tissues were found. No information was located concerning the detection or quantification of dibutyl sebacate or 2-nitrodiphenylamine in biological materials. Details of commonly used analytical methods for several types of biological media are presented in Table 6-l.

Two analytical methods exist for detecting propylene glycol dinitrate in expired air. These are gas chromatography (GC) with electron capture detector (ECD) (Horvath et al. 1981; Stewart et al. 1974) and GC with mass spectrometry (MS) (Air Force 1982b). Very little information on these two methods was given. The lower detection limit of the MS method was less than 1 mg/m^3 propylene glycol dinitrate in air, but no information on accuracy and precision was reported. Levels of propylene glycol dinitrate as low as 0.003 mg/m^3 were reported using the ECD method (Horvath et al.

TABLE 6-1. Analytical Methods for Determining Otto Fuel II and Its Components in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath (propylene glycol dinitrate)	Direct injection	GC/ECD	NR	NR	Stewart et al. 1974
Breath and blood gas (propylene glycol dinitrate)	Direct injection	MS	<1 mg/m^3 breath; 1 µg/mL blood	NR	Air Force 1982b
Blood and urine (propylene glycol dinitrate, propylene glycol mononitrate)	Extract with diethyl ether; add anhydrous sodium sulphate; wash with ether	GC/ECD	0.2 µg/mL (blood propylene glycol mononitrate)	83–97% (blood propylene glycol mononitrate)	Litchfield 1968
Blood and urine (propylene glycol dinitrate)	Extract with diethyl ether	Spectrophotometry	0.2 µg/mL (blood)	89–110% (blood)	Litchfield 1968
Blood and urine (propylene glycol dinitrate, propylene glycol mononitrate)	Extract with ether	GC/ECD	10 ng/mL	NR	Erk et al. 1982
Handswab (2-nitrodiphenylamine)	Wipe hand with swab; moistened with methyl *tert*-butyl ether; extract swab in methyl *tert*-butyl ether; centrifuge; decant supernatant and evaporate; redissolve in methyl *tert*-butyl ether in pentane; clean up on Amberlite XAD-7® column, eluting with ethyl acetate; concentrate	HRGC/TEA; HRGC/ECD	pg-low ng	NR	Douse 1985
Explosives and gunshot residues; handswabs (2-nitrodiphenylamine)	Dissolve explosives in acetone; wipe hand with swab moistened with acetone; wash swab with 20% ethanol; filter; inject	HPLC/ECD	4.1 µg/L	NR	Bratin et al. 1981

ECD = electron capture detection; GC = gas chromatography; HPLC = high-performance liquid chromatography; HRGC = high-resolution gas chromatography; MS = mass spectrometry; NR = not reported; TEA = thermal energy analyzer

1981). These limited data suggest that GC/ECD may offer better sensitivity than GC/MS. However, GC/MS generally provides better selectivity.

Methods exist for detecting propylene glycol dinitrate and its metabolites in blood and urine. The primary method of analysis for propylene glycol dinitrate and its metabolites in blood and urine is gas chromatography with electron capture detection (Litchfield 1968). A disadvantage of this method is that sample preparation is time consuming, taking 2-3 hours. However, a more rapid GC method enables sample preparation to be completed within 5 minutes (Erk et al. 1982). This rapid method reliably detects propylene glycol dinitrate in blood at concentrations ranging from 0.01 μg/mL to 25 μg/mL. Because propylene glycol dinitrate is rapidly broken down in blood to the corresponding mononitrates and inorganic nitrates, extraction from the blood should be carried out immediately after the sample is drawn (Litchfield 1968). An outdated but simple method for determining propylene glycol dinitrate in blood and urine is calorimetric analysis (Litchfield 1968). Propylene glycol dinitrate is determined spectrophotometrically following alkaline hydrolysis and a diazotization and coupling reaction. This procedure has several drawbacks; it is time consuming, nonspecific, and relatively insensitive. The lower detection limit is only 0.2 μg/mL, more than an order of magnitude greater than GCYECD. In addition, the method does not distinguish between individual nitrates in a mixture of esters. The use of GC with ECD overcomes this difficulty, and the identification of nitrate esters can be achieved. MS with a blood gas catheter was used to detect propylene glycol dinitrate in blood; however, the catheter was not useful and had a high retention rate of propylene glycol dinitrate (Air Force 1982b). The limit of detection was only 1 μg/mL.

6.2 ENVIRONMENTAL SAMPLES

Because of the low threshold limit value (TLV) in the United States of 0.05 ppm for propylene glycol dinitrate (ACGIH 1994), effective means of monitoring very low levels of propylene glycol dinitrate vapors are needed to monitor workers, and prevent exposure to toxic levels. Various methods such as polarography, piezoelectric crystals, high-pressure liquid chromatography (HPLC) with flame ionization (FID), electrochemical (EC), or ultraviolet (UV) detector, linear sweep voltammetry (LSV), GC with thermal energy analyzer (TEA), FID, or ECD, and thin-layer chromatography (TLC) have been investigated for detecting propylene glycol dinitrate in environmental samples. Analytical methods were located for monitoring propylene glycol dinitrate in air and water. Details of commonly used analytical methods for air and water are presented in Table 6-2.

TABLE 6-2. Analytical Methods for Determining Otto Fuel II and Its Components in Environmental Samples

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (propylene glycol dinitrate)	Direct measurement	Piezoelectric crystal	<0.05 ppm	NR	Turnham et al. 1985
Air (propylene glycol dinitrate, propylene glycol mononitrate)	Collect sample on Amberlite XAD-2® adsorbent; extract with methanol; concentrate	GC/TEA; HPLC/EC	0.1 µg/mL (propylene glycol dinitrate); 0.5 µg/mL (propylene glycol mononitrate); 0.2 µg/mL (propylene glycol dinitrate); 4 µg/mL (propylene glycol mononitrate)	NR NR	Tomkins et al. 1985
Air (propylene glycol dinitrate)	NR	IR; GC/ECD	NR	NR	Stewart et al. 1974
Air (propylene glycol dinitrate)	NR	FTIR	NR	NR	Akimoto et al. 1980
Water (propylene glycol dinitrate stock solutions)	Prepare standard aqueous solutions	LSV	0.5 mg/L	NR	Fine and Miles 1983
Effluent water (propylene glycol dinitrate)	Extract with benzene	GC	NR	NR	Kessick et al. 1978
Effluent water (propylene glycol dinitrate)	NR	GC/ECD	NR	NR	Navy 1979
Effluent water (propylene glycol dinitrate, propylene glycol mononitrate)	NR	Single sweep polarography	low-ppb	NR	Navy 1979
Effluent water (propylene glycol dinitrate)	Extract with organic solvent; separate on reverse phase HPLC column	HPLC/UV	1 ppm	NR	Hiltz et al. 1986

TABLE 6-2. Analytical Methods for Determining Otto Fuel II and Its Components in Environmental Samples (continued)

Sample Matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water, river sediment (2-nitrodiphenylamine)	Extract with solvent; concentrate; clean up on silica gel; elute with ethyl ether/benzene; concentrate; redissolve in benzene	HPLC/UV	<10 ppm (water)	NR	Army 1976
		GC/FID	NR	91 river sediment	
Sea water (dibutylsebacate)	Extract with XAD-2® resin; fraction on silica gel column	GC/MS/TDMC	NR	NR	Shishido et al. 1984
Propellants (2-nitrodiphenylamine)	NR	GC/FID	NR	NR	Dykes and Alley 1974
Propellants (2-nitrodiphenylamine)	Macerate and homogenize propellant; dilute with methanol; filter	HPLC/UV reverse phase	NR	NR	Army 1986b
Propellants (2-nitrodiphenylamine)	Extract with methylene chloride; concentrate; redissolve in methanol; filter	HPLC/EC reverse phase	4.3 µg/L	NR	Bergens 1987
Smokeless powders (2-nitrodiphenylamine)	Extract with methylene chloride; clean up on silica gel; concentrate	HPLC/UV/TEA	Low-ng	NR	Bender 1983
Gunshot residues	Swab spent casings with swab moistened with ethanol; extract with ethanol; centrifuge; evaporate; dilute with buffer	MECE/UV	NR	NR	Northrop et al. 1991

EC = electrochemical detection; ECD = electron capture detection; FID = flame ionization detector; FTIR = fourier transform infrared spectrometry; GC = gas chromatography; HPLC = high-performance liquid chromatography; IR = infrared spectrometry; LSV = linear sweep voltammetry; MECE = micellar electrokinetic capillary electrophoresis; MS = mass spectrometry; NR = not reported; TDMC = three-dimensional mass chromatography; TEA = thermal energy analyzer; UV = ultraviolet detection

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Various methods have been proposed for detecting propylene glycol dinitrate in air. Coated piezoelectric quartz crystal microbalances have been studied as potential selective and sensitive detectors for the determination of propylene glycol dinitrate vapor levels (Luoma et al. 1987; McCallum 1989; Turnham et al. 1985). A prototype instrument with crystal design containing a trap for propylene glycol dinitrate on the reference crystal eliminated many of the frequency, stability, and selectivity problems associated with these detectors and produced a detection limit of better than 0.05 ppm. Air samples have been analyzed using TLC, GC/TEA, GC/FID, and HPLC/EC in the reductive mode (Tomkins et al. 1985; Wyman et al. 1984). GC/TEA and HPLC/EC have been used to separate and detect mono- and dinitrated propylene glycols, and have been applied to the problem of quantifying trace levels of nitrated propylene glycols (Tomkins et al. 1985). Both GC/TEA and HPLC/EC are capable of rapidly detecting propylene glycol dinitrate at sub-ppm concentrations in a liquid extract. While both methods separated propylene glycol mononitrate from propylene glycol dinitrate, GC/TEA possessed the added advantages of better sensitivity and resolution of the propylene glycol mononitrate isomers. GC/TEA exhibited a linear range of three orders of magnitude and provided detection limits in the μg/mL range or lower for propylene glycol dinitrate and the two propylene glycol mononitrate isomers. IHPLC/EC provided a linear response over two orders of magnitude and was best suited for the determination of propylene glycol dinitrate. GC/FID and TLC could not be compared to other methods because no details on method sensitivity and reliability were reported (Wyman et al. 1984). Infrared spectrometry and GC/ECD have been used in the quantification of propylene glycol dinitrate in an inhalation exposure chamber (Stewart et al. 1974). Propylene glycol dinitrate has also been quantified in a photochemical smog chamber using Fourier transform infrared spectrometry (FTIR), GC/ECD, and GC/MS (Akimoto et al. 1978, 1980; Hoshino et al. 1978). No data on the sensitivity and reliability of these methods were provided.

Analytical methods for detecting propylene glycol dinitrate and its metabolites, propylene glycol 1-mononitrate and propylene glycol 2-mononitrate in effluent water include polarography, GC, and linear sweep voltammetry (LSV). GC has been used to measure the concentration of propylene glycol dinitrate in waste water (Kessick et al. 1978). No information on the detector, or selectivity and sensitivity of this method was provided. GC/ECD has been used to determine propylene glycol dinitrate in effluent water (Navy 1976a, 1976b, 1979). This method detected propylene glycol dinitrate but not propylene glycol mononitrate. Since propylene glycol dinitrate can hydrolyze rapidly to propylene glycol mononitrate in sump water during storage and also during the clean-up operation of the purification system, GC/ECD has limited use in the monitoring of effluent water. Single sweep

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polarographic equipment has been designed for field use to monitor propylene glycol dinitrate and propylene glycol mononitrate in effluent water (Navy 1976a, 1976b, 1977a, 1979). The effluent water monitored by the polarograph was obtained from a carbon adsorption system designed to remove propylene glycol dinitrate from Otto Fuel II waste water to concentration levels of 1.0 mg/L. Sensitivity was in the low ppb range with very good accuracy. LSV has been proposed for use in quantifying propylene glycol dinitrate in effluent water by examining the reduction of propylene glycol dinitrate at a silver wire electrode (Fine and Miles 1983; Navy 1984a, 1984b). Propylene glycol dinitrate was readily detectable at 0.5 mg/L. A procedure using reverse phase HPLC and UV detection has been described for the detection and quantification of propylene glycol dinitrate in waste water down to 1 mg/L (DREA 1986). Further details, including accuracy, precision, and selectivity of the methods, were not provided. TLC and UV spectrophotometry have been used to semiquantitatively monitor propylene glycol dinitrate in aqueous culture media (Wyman et al. 1984). These methods could also be applied to contaminated waste water. Lactic and pyruvic acids, suspected of being metabolites from the photolysis/photooxidation of propylene glycol dinitrate, have been identified using nuclear magnetic resonance (NMR) spectrometry (Wyman et al. 1984), but no details were reported for this method.

Analytical methods have been developed for the determination of aqueous components of Otto Fuel II other than propylene glycol dinitrate. 2-Nitrodiphenylamine has been measured in effluent water and river sediment samples using HPLC/UV and GC/FID (Army 1976). The HPLC-UV method was suitable for measuring 2-nitrodiphenylamine at a detection limit of <l0 ppm (<10 mg/L) in water, but detection limit for the GC-FID method was not provided. Dibutyl sebacate has been detected in seawater and advanced waste treatment effluent water using GC/MS (EPA 1984a; Shishido et al. 1984). No information was provided on the sensitivity and reliability of the method; however, when GC/MS was combined with three-dimensional mass chromatography (TDMC), dibutyl sebacate was easily identified in a contaminated sample for which identification was not possible using GC/MS alone (Shishido et al. 1984).

Limited data were located on the detection of Otto Fuel II components in foods. 2-Nitrodiphenylamine was measured in lettuce using GC/MS (Wickstrom et al. 1986). The sensitivity was in the sub-ppb range. Dibutyl sebacate was detected in packaged foods wrapped in dibutyl sebacate-containing plastics using high-resolution gas chromatography (HRGC)/FID (Castle et al.

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1988). The sensitivity for this method was in the low ppb. No other data on the reliability and usefulness of these methods were reported.

2-Nitrodiphenylamine has also been detected in other environmental samples including propellants, smokeless powders, explosives, and gunshot residues. GC/FID was used to determine the presence of 2-nitrodiphenylamine in nitrocellulose propellants (Dykes and Alley 1974). This method had an estimated relative standard deviation (RSD) of 0.44% which is excellent repeatability for this type of analysis. However, GC methods may not be desirable for the analysis of 2-nitrodiphenylamine as N-nitrosodiphenylamine (aging product of nitrodipheylamine) is denitrosated to diphenylamine in the heated zone of the chromatograph and remains undetected (Via and Taylor 1992). HPLC/UV using reverse phase columns has also been used to determine the presence of 2-nitrodiphenylamine in propellants (Army 1986b). The method accurately identified and quantified 2-nitrodiphenylamine in the complex propellant mixture. 2-Nitrodiphenylamine in propellants has also been detected using reverse phase HPLC/EC (Bergens 1987). The author stated that HPLC/EC is more selective and sensitive than HPLC/UV and that it provides a more uniform response compared with UV detection. The detection limit was 4.3 μg/L, and the RSD was 1.O-2.0%. Methods for detecting 2-nitrodiphenylamine in smokeless powders and in gunshot residues have been proposed. For smokeless powders, use of HPLC/UV/TEA was investigated (Bender 1983). By using UV/TEA detectors in tandem, compounds could be analyzed in the low nanogram range and selectivity was increased. HRGC combined with TEA or ECD has been used for analysis of explosives in spiked hand swabs (Douse 1985). The author stated that TEA was shown to approach the sensitivity of ECD but was more selective, enabling low nanogram levels of explosives in hand swabs to be detected. Methods of analysis that were located for explosives and gunshot residues include HPLC/ECD, HPLC/UV (Bratin et al. 1981; Dahl and Lott 1987), and micellar electrokinetic capillary electrophoresis with UV spectrophotometry (MECE/UV) (Northrop et al. 1991). HPLC/ECD had a lower detection limit (4.1 μg/L) than HPLC/UV (45.5 μg/L) (Bratin et al. 1981). MECE/JV is a proposed qualitative method for providing rapid and efficient separation and detection of organic gunshot and explosive constituents. MECE is an adaptation of capillary electrophoresis where the addition of a charged micellar agent to the electrolyte provides a separation of neutral molecules. Because each component has a characteristic UV absorption profile, analysis at several different wavelengths can provide additional identification of the components. No information on accuracy, sensitivity, or specificity was given so the method cannot be compared to other located methods. Perhaps the best method available for the analysis of Otto Fuel II components in complex solid

matrices is supercritical fluid extraction, supercritical fluid chromatography and MS detection and quantification. This method assures sample integrity, affords faster analysis time compared to conventional methods and enhances the sensitivity of detection (Via and Taylor 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 Otto Fuel II and its components 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 Otto Fuel II and its components.

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

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. Methods exist for measuring propylene glycol dinitrate in breath, blood, and urine (Air Force 1982b; Erk et al. 1982; Litchfield 1968; Stewart et al. 1974). Most of the studies did not report enough relevant information on sensitivity, accuracy, precision, and selectivity to determine their reliability for monitoring exposure to Otto Fuel II and its components. No methods of measuring 2-nitrodiphenylamine or dibutyl sebacate in biological media were located. More thorough studies of possible methods for monitoring exposure are needed to determine the best biomarkers for monitoring exposure to Otto Fuel II and its components.

The biomarkers of effect for Otto Fuel II and its components are very general and include headache, mild metbemoglobinemia, and dizziness (Horvath et al. 1981). Since these effects are nonspecific and

cannot be quantified, they are not useful biomarkers of effect for Otto Fuel II and its components. A specific biomarker of exposure is needed so that monitoring studies of occupationally exposed workers and other potentially exposed populations can be conducted.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist for detecting propylene glycol in air and water (Akimoto et al. 1980; DREA 1986; McCallum 1989; Navy 1979, 1984a, 1984b; Tomkins et al. 1985; Wyman et al. 1984), 2nitrodiphenylamine in water, sediment, lettuce, and munitions compounds (Army 1976, 1986a; Wickstrom et al. 1986; Wyman et al. 1984), and dibutyl sebacate in water and food (Castle et al. 1988; EPA 1984a; Shishido et al. 1984). Very few details on the sensitivity, accuracy, precision, and selectivity of the methods were provided. More detailed analyses of these methods are needed in order to determine their reliability for measuring background and greater levels of Otto Fuel II and its components in environmental media.

6.3.2 Ongoing Studies

No ongoing studies were located regarding analytical methods for Otto Fuel II or its components,