

## 6. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting and/or measuring and monitoring ethylene glycol or propylene glycol in biological samples or in environmental media. The intent is not to provide an exhaustive list of analytical methods that could be used to detect and quantify ethylene glycol or propylene glycol. 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 ethylene glycol or propylene glycol 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 SAMPLES

Table 6-1 is a summary of some of the most commonly used methods reported in the literature for detecting propylene glycol and ethylene glycol in biological samples. The primary method for measuring ethylene glycol or propylene glycol in biological samples is derivatization followed by gas chromatography (GC) using either a flame ionization detector (FID) or mass spectrometry (MS) for quantification. GC is the preferred analytical method because of the ease of sample preparation and the accuracy of the quantification of sample concentrations. Alkali flame ionization detectors have also been used for ethylene glycol analysis and give a response ratio of 3:1 compared with PID (Bogusz et al. 1986). Capillary gas chromatography with a constant current  $^{63}\text{Ni}$  electron capture detector (ECD) has also been used successfully to detect propylene glycol (Needham et al. 1982).

Sample preparation for GC is important and proceeds through several steps: acidification, esterification, and extraction into an organic solvent. The use of internal standards is necessary for quantification. In clinical cases involving ethylene glycol poisoning, propylene glycol should not be used as an internal standard for quantitation because certain sedatives (Valium and Ativan) may contain propylene glycol (Apple et al. 1993).

**Table 6-1. Analytical Methods for Determining Ethylene Glycol and Propylene Glycol in Biological Samples**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human plasma (EG and PG)	Deproteinization with acetic acid; vortex; centrifugation; supernatant spiked with internal standard; reaction with butylboronic acid; neutralize with $\text{NH}_4\text{OH}$ , extraction with dichloromethane; concentration.	HRGC/MS	5 ppm (EG); 1 ppm (PG)	94–106	Giachetti et al. 1989
Human serum (PG)	Acetonitrile with internal standard added to sample; centrifugation; concentration; extraction with <i>p</i> -bromophenyl boric acid in ethyl acetate.	HRGC/ECD	0.38 ppm	>90	Needham et al. 1982
Human serum (EG)	Internal standard (in acetonitrile) added to sample; centrifugation to remove protein precipitate; esterification with butylboronic acid and 2,2-dimethoxypropane; neutralization with $\text{NH}_4\text{OH}$ in acetonitrile.	HRGC/FID	NR	95	Smith 1984
Human blood (PG)	Deproteinization with $\text{HClO}_4$ ; centrifugation; pH adjustment; centrifugation.	GC/MS	0.6 ppm	NR	Sisfontes et al. 1986
Human serum and urine (EG, PG)	Internal standard added; centrifugation; derivatization with phenylboronate in methanol.	HRGC/FID	1.0 ppm	89–98	Houže et al. 1993
Human blood/tissue (EG)	Anhydrous $\text{Na}_2\text{SO}_4$ ground with sample; derivatization with <i>n</i> -butylboronic acid in acetone containing internal standard; centrifugation or filtration.	GC/FID/AFID	NR	70	Bogusz et al. 1986

**Table 6-1. Analytical Methods for Determining Ethylene Glycol and Propylene Glycol in Biological Samples (continued)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Human serum (glycolic acid)	Colorimetric: precipitation of protein with trichloroacetic acid followed by centrifugation, addition of chromotropic acid, heating, and dilution. Gas chromatographic: addition of internal standard and acetone followed by centrifugation, addition of NaOH, evaporation to dryness, and formation of methyl ester.	Absorbance at 580 nm or GC/FID as appropriate	1.0 mmol/L (60 ppm, w/v) for both methods; 3–6% RSD	NR	Fraser and MacNeil 1993
Humans serum (glycolic acid)	Extraction from salted, acidified serum using methyl ethyl ketone followed by removal of organic phase and evaporation to dryness and derivatization with PNBDI.	HPLC/UV	0.05 mmol/L (3 ppm, w/v); 1% RSD	NR	Hewlett et al. 1986
Urine (EG)	Acidification; extraction with $\text{CHCl}_3$ ; concentration; TLC.	TLC	NR	NR	Riley et al. 1982
Urine (sodium fluorescein) (EG)	Untreated samples read in borosilicate tubes.	Fluorescence (Wood's lamps)	NR	NR	Winter et al. 1990
Dog urine (glycolic acid) (EG)	Dilution; NaCl addition and acidification; extraction in MEK; evaporation; dissolution of residue in ethylacetate; derivatization with PNBDI.	HPLC/UV	1–2 ng	96	Hewlett et al. 1983
Human plasma, urine (oxalate)	Heparinized blood deproteinated by addition of acetonitrile and phosphate buffer (pH = 7), centrifugation, removal of solvent and evaporation to dryness; derivatization as for urine; Urine acidified and derivatized using 1,2-diaminobenzene, adjustment of pH to 5–6, centrifugation.	HPLC/UV	Plasma: 0.15 mg/L (ppm, w/v); 7.5% RSD. Urine: 0.5 mg/L (ppm, w/v); 5% RSD.	85	Brega et al. 1992

**Table 6-1. Analytical Methods for Determining Ethylene Glycol and Propylene Glycol in Biological Samples (continued)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Kidney tissue dog (hippurate) (EG)	Tissue ground with acidic methanol; filtration; concentration; spot on 254 nm TLC plate.	TLC	NR	NR	Riley et al. 1982
Human tissue (EG)	Samples extraction in HPLC grade water for 24 hours; filtration of supernatant.	HPLC/RI	5 ppm	98 at 1 mg/mL (1000 ppm)	Wu and Malinin 1987

AFID = alkali flame ionization detector; ATP = adenosine triphosphate;  $\text{CHCl}_3$  = chloroform;  $\text{CH}_3\text{OH}$  = methanol; ECD = electron capture detector; EG = ethylene glycol; FID = flame ionization detector; GC = gas chromatography; HCl = hydrochloric acid;  $\text{HClO}_4$  = chloroform; HPLC = high-performance liquid chromatography; HRGC = high resolution gas chromatography; KOH = potassium hydroxide; MEK = methyl-ethyl ketone;  $\text{MgSO}_4$  = magnesium sulfate; MS = mass spectrometry; NaCl = sodium chloride; NAD = nicotinamide adenine dinucleotide;  $\text{Na}_2\text{SO}_4$  = sodium sulfate;  $\text{NH}_4\text{OH}$  = ammonium hydroxide; NR = not reported; PG = propylene glycol; PNBBI = *O-p*-nitrobenzyl-N,N'-diisopropylisourea; RSD = relative standard deviation; RI = refractive index detector; TLC = thin-layer chromatography; UV = ultraviolet detector  
w/v = weight:volume

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Detection of propylene glycol and ethylene glycol in biological samples using GC with either FID or MS is very sensitive, with detection limits ranging from sub to low ppm. The coefficient of variation (CV) varies with the concentration of glycol used but typically ranges from 0.4% to 27% and is usually less than 10%. In gas chromatographic procedures, the glycols and their acid metabolites are derivatized to form esters in order to facilitate quantitative elution from the chromatographic columns (see Table 6-1). Simple and rapid methods are also available for the quantitation of the glycols in urine, serum, or deproteinated whole blood. These methods use direct sample injection without prior solvent extraction and derivatization (Aarstad et al. 1993; Edinboro et al. 1993; Jonsson et al. 1989). However, such methods, particularly those that use packed columns may misidentify propionic acid (found in patients with methylmalonic acidemia) as ethylene glycol (Shoemaker et al. 1992).

High performance liquid chromatography (HPLC) has also been used to identify ethylene glycol and its metabolites such as glycolate (Hewlett et al. 1983, 1986), hippurate (Riley et al. 1982), and oxalate (Brega et al. 1992) in biological samples, particularly blood and urine. Positive results may be confirmed with GC/MS. This makes GC/MS the preferred method since the HPLC step can be omitted. However, HPLC methods to measure plasma levels of glycolate have been used to aid in diagnosis and treatment of ethylene glycol poisoning (Hewlett et al. 1986; Jacobsen et al. 1988). Gas chromatographic and calorimetric methods for quantification of glycolate have been presented (Fraser and MacNeil 1993).

High-resolution proton nuclear magnetic resonance spectroscopy has potential use in the identification and quantification of propylene glycol and other chemicals in cerebrospinal fluid (CSF) and serum (Petroff et al. 1986). The technique has two advantages: 1) it requires no pretreatment of the specimens prior to analysis and no advance knowledge of possible compounds present in fluids and 2) results are extremely rapid. Propylene glycol was detected at 1 ppm in CSF (Petroff et al. 1986).

Microscopy can be used to identify metabolic products of ethylene glycol. Scanning electron microscopy (SEM) at 20 kilovolts will detect crystals of calcite, calcium oxalate monohydrate, and calcium oxalate dihydrate in kidney tissue (Siew et al. 1975b). Phase-contrast polarization and light microscopy X-ray powder diffraction may be used to identify hippuric acid crystals in urine (Foit et al. 1985).

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The use of ethylene oxide to sterilize tissue for transplantation may result in the formation of ethylene glycol when ethylene oxide is in prolonged contact with tissue. To quantify the formation of ethylene glycol in tissue, an HPLC method using a differential refractive index detector has been developed. The HPLC system can be used to detect ppm levels of ethylene glycol with a sensitivity of  $2 \times 10^{-6}$  refractive index unit full scale. This procedure has three advantages: 1) requires only 4 minutes for analysis, 2) simple sample preparation, and 3) good reproducibility (Wu and Malinin 1987).

Techniques using gas chromatography and various detection systems to detect and quantify ethylene glycol in human blood have been developed for use in hospital laboratories to assist in the diagnosis of ethylene glycol poisoning (caused by drinking antifreeze containing ethylene glycol). These techniques are quite rapid, usually less than 30 minutes, and do not require elaborate sample preparation. Some may also be used for detection of propylene glycol. The specific techniques used for each analytical method are listed in the table if that information was provided by the author(s).

An alternative method, developed in a hospital, for detecting ethylene glycol in blood is the use of the DuPont *Automated Clinical Analyzer* triglyceride assay pack. This enzymatic method, while relatively simple, cannot be used when the triglyceride concentration of the serum exceeds 12 g/L and requires that positive results for ethylene glycol be confirmed using another method (Ochs et al. 1988; Ryder et al. 1986). The enzymatic method has been modified to eliminate some of the interference problems present in the earlier methods (Blandford and Desjardins 1994).

Thin-layer chromatography (TLC) with a chloroform solvent has been used to detect ethylene glycol and its metabolites in urine or renal tissue (Riley et al. 1982). Metabolites of ethylene glycol in the blood may be detected by analytical isotachopheresis using a system equipped with both a conductivity detector and an ultraviolet detector. Blood and serum samples should not have been previously treated with oxalate, citrate, or ethylene diamine tetracetic acid. This technique may be of value when ethylene glycol poisoning is suspected but sufficient time has elapsed for metabolism of the compound to have occurred (Ovrebo et al. 1987). A simple and rapid calorimetric method that uses chromatropic acid has been proposed for the quantitation of glycolic acid, the major toxic metabolite of ethylene glycol (Fraser and MacNeil 1993).

No information was located on detecting ethylene glycol or propylene glycol in feces, adipose tissue, or human milk.

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**6.2 ENVIRONMENTAL SAMPLES**

As with biological samples, GC is the major technique used to determine ethylene or propylene glycol concentrations in environmental samples whether in air, water, food, drugs, or other substances. Capillary gas chromatography with FTD or ECD, possibly followed by MS, generally gives good quantitative results down to the ppm range with recovery usually greater than 80%. The determination of ethylene glycol or propylene glycol in air requires adsorption onto a surface and subsequent extraction. Water samples may be analyzed without preparation (EPA 1995a, 1995b). Detection of ethylene glycol or propylene glycol in foods and drugs may be accomplished by chromatography of the sample; for substances with a high fat content, extraction with hexane may be used to remove the fat. Table 6-2 is a summary of some of the most commonly used methods reported in the literature for detecting ethylene glycol or propylene glycol in environmental samples. The specific techniques used for each analytical method are listed in the table if that information was provided by the author(s).

Air sampling for ethylene glycol is performed by adsorption onto a resin column such as Amberlite XAD-2. Although activated charcoal filters have some utility, recovery is greater with the Amberlite, and it is the preferred adsorption medium. Ethylene glycol is then solvent-extracted with recovery of 98%. If activated charcoal is used for adsorption, 5% methanol in dichloromethane is the best solvent with maximum recovery of 84% (Andersson et al. 1982, 1984). An alternative method for sampling ethylene glycol involves passage of air through a glass fiber filter with a silica gel tube. Ethylene glycol is then extracted in a 2-propanol:water solvent mixture and injected into the gas chromatograph (Tucker and Deye 1981). A slightly modified version of this method is the NIOSH-approved method for the determination of ethylene glycol in occupational air (see Table 6-2). The sensitivity of this method can be increased by use of a hot on-column injection technique using methanol as the solvent (Lang 1986).

A portable, automated, photoionization gas chromatograph has been used to detect ethylene glycol in air samples in industrial facilities at levels as low as 0.05 ppm (Adams and Collins 1988).

Ethylene glycol may be detected by a calorimetric reaction with 3-methyl-2-benzothiazolinone hydrazone hydrochloride after oxidation of the glycols to the corresponding aldehydes with acidified permanganate. The solution is read at 630 nm in a spectrophotometer. This method may be used for

**Table 6-2. Analytical Methods for Determining Ethylene Glycol and Propylene Glycol in Environmental Samples**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air <sup>a</sup>	Sample collection on glass fiber filters and silica gel; extraction with 2-propanol:water.	GC/FID	2 ppm	81–111	NIOSH 1984
Air	Sample adsorbed on Amberlite <sup>®</sup> XAD-2 with personal sampling pump; extraction with diethyl ether.	GC/FID	NR	75–98	Anderson et al. 1982
Water	Direct injection (Method 8015b)	GC/FID	NR	NR	EPA 1995a
Water	Direct injection (Method 8430)	GC/FTIR	120 mg/L (ppm, w/v)	NR	EPA 1995b
Plastics	Sample extraction from plastic with carbon disulfide	GC/FID	16.5 ng	58–61	Muzeni 1985
Plastics	Sample extraction with solvent of ethylacetate-water-methanol	GC/FID	2 ppm	NR	DeRudder et al. 1986
Cosmetics (PG)	Co-distillation with isooctane.	GC/FID	NR	NR	Helrich 1990a
Ground tobacco	Extraction with anhydrous methanol.	GC/FID	NR	NR	Helrich 1990b
Aqueous solution	Sample concentration, then dilution with water; concentration with helium gas; redilution.	GC/FID	50 ppb	97–103	Kashtock and Breder 1980
Beer	Addition of ammonium sulfate and extract with ethyl acetate.	HRGC/FID	0.73 ppm	88	Williamson and Iverson 1993
Vanilla extract (PG)	Refluxing with heptane and addition of KIO <sub>4</sub> , NaHCO <sub>3</sub> , KI, and starch to aqueous phase followed by titration with K <sub>2</sub> Cr <sub>2</sub> O <sub>7</sub> .	Titration	NR	NR	Helrich 1990c

**TABLE 6-2. Analytical Methods for Determining Ethylene Glycol and Propylene Glycol in Environmental Samples (continued)**

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food	Addition of hot water to sample to obtain slurry; extraction with hexane; precipitation of sugars with calcium hydroxide; concentration; derivatization with BSTFA.	HRGC/FID GC/MS	10 ppm	78–107	Castle et al. 1988b
Anchovies	Extraction with methanol and concentration.	HRGC/MS/MS (PICI)	12.5 ppb	NR	Matusik et al. 1993

<sup>a</sup>For ethylene glycol only

BSTFA = bis(trimethylsilyl)trifluoroacetamide; FID = flame ionization; GC = gas chromatography; HRGC = high resolution gas chromatography; MS = mass spectrometry; MS/MS = tandem mass spectrometry; PG = propylene glycol; PICI = positive ion chemical ionization

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ethylene glycol in water (Evans and Dennis 1973) or to detect ethylene oxide in air (Kring et al. 1984); however, this method is not quantitative and is relatively insensitive compared with GCMS.

The migration of ethylene glycol from plastics into solution can be studied with GC. Sample preparation methods include extraction in hydrochloric acid (Ball 1984) distilled water (Spitz and Weinberger 1971), carbon disulfide (Muzeni 1985), dimethylformamide (Danielson et al. 1990), and a mixture of ethyl acetate, water, and methanol (DeRudder et al. 1986). Other methods for detecting ethylene glycol in industrial products include HPLC (Aboul-Enein and Islam 1989) and a periodate flow-through ion-selective electrode (Diamandis et al. 1980).

The presence of ethylene glycol or propylene glycol in foods packaged with plastic films containing the compounds has been studied, as have ethylene glycol levels in drugs sterilized with ethylene oxide. Sample preparation is important because procedures vary depending on the fat content of the food sample. Foods with low fat content can be extracted with ethyl acetate, derivatized to a trimethylsilyl ether, and then injected into the gas chromatograph. For foods with a high fat content, hexane is used as the defatting agent prior to derivatization. Quantifying ethylene glycol or propylene glycol in wines requires no preparation of the samples prior to analysis (Kaiser and Rieder 1987; Klaus and Fischer 1987). Drugs in aqueous solutions may be analyzed directly, water insoluble drugs should be extracted in water, and ointments may be dissolved in hexane and then extracted with water. Recovery is between 80 and 114%, with detection limits in the low-ppm range (Hartman and Bowman 1977; Manius 1979). The use of ion exchange chromatography with sulfuric acid as the mobile phase has also given good recovery (98-101%) with a detection limit of 5 µg/mL propylene glycol from pharmaceuticals (Iwinski and Jenke 1987). Although the use of TLC (Ballarin 1980) has been recommended, it has been superseded by GC.

Propylene glycol in cigarette smoke has been detected using electrostatic precipitation or filter pad, with extraction and separation with capillary gas chromatography (Borgerding et al. 1990).

No information was located on techniques for detecting and analyzing ethylene glycol and propylene glycol in soil.

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### 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 ethylene glycol and propylene glycol 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 ethylene glycol and propylene glycol.

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.** Methods are available for the determination of ethylene glycol in blood, tissue, and urine (Bogusz et al. 1986; Giachetti et al. 1989; Houze et al. 1993; Riley et al. 1982; Smith 1984; Wu and Malinin 1987) with sensitivities in the low and sub-ppm range. Methods for the determination of propylene glycol in blood and urine are also available (Giachetti et al. 1989; Houze et al. 1993; Needham et al. 1982; Sifontes et al. 1986) with sensitivities in the sub-ppm range. Methods are also available for metabolites of ethylene glycol (glycolic acid, oxalic acid) in blood and urine (Brega et al. 1992; Fraser and MacNeil 1993; Hewlett et al. 1986) with sensitivities as low as 3 ppm for glycolic acid and of 0.15 ppm for oxalic acid. Glycolic acid was identified in Chapter 2 as a sensitive biomarker of exposure to ethylene glycol. These methods seem to be adequate for the measurement of propylene glycol and ethylene glycol and its metabolites in the human population.

Serum concentrations of blood urea nitrogen (BUN) or creatinine can serve as indicators of renal toxicity (biomarkers of effect) induced by exposure to ethylene glycol but these are not specific for ethylene glycol intoxication (Grauer et al. 1987).

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### **Methods for Determining Parent Compounds and Degradation Products in**

**Environmental Media.** Methods for the determination of ethylene glycol and propylene glycol have been reported for air (Anderson et al. 1982; NIOSH 1984), water or aqueous solutions (EPA 1995a, 1995b; Kashtock and Breder 1980), and foods (Castle et al. 1988b; Matusik et al. 1993; Williamson and Iverson 1993). Methods have also been developed for the determination of glycols that leach from plastics (DeRudder et al. 1986; Muzeni 1985) and that can end up in foods stored in containers made from the plastics.

The MRL for inhalation exposure to ethylene glycol is 0.5 ppm and thus requires a method level of detection (LOD) of 0.5 ppm. This value is below the LODs of the methods reported. Although it should be possible to increase the sampling volumes and increase the sensitivities of the methods, this would need to be shown to be free of problems. Oral MRLs have been established to be 2 mg/kg/day for acute exposure and 0.2 mg/kg/day for chronic exposure. Assuming a 70-kg individual and oral intakes of either 2 L/day of water or 2 kg/day of food, analytical methods would need sensitivities below 70 ppm in water or food. The methods reported for aqueous solutions (LOD = 50 ppb, Kashtock and Breder 1980), beer (LOD = 0.73 ppm, Williamson and Iverson 1993), and foods (LOD = 10 ppm, Castle et al. 1988b; LOD = 12.5 ppb, Matusik et al. 1993) should be adequate to measure acute exposure. However, chronic exposure requires method LODs of approximately 7 ppm and only the methods of Kashtock and Breder (1980) for aqueous solutions, Williamson and Iverson (1993) for beer, and Matusik et al. (1993) for anchovies can meet this requirement. The applicability of these methods to other beverages and foods has not been demonstrated. Thus, additional methods should be developed and validated for ethylene glycol in other beverages and foods at concentrations relevant to the chronic oral MRL.

An MRL of 0.009 ppm for intermediate inhalation exposure to propylene glycol has been defined and none of the methods reported would be adequate without modification. It is likely that the LODs of some of the methods could be reduced but this remains to be shown.

### **6.3.2 Ongoing Studies**

No ongoing research on analytical methods for the determination of ethylene or propylene glycol was found.