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 2-butoxyethanol, 2-butoxyethanol acetate, their metabolites, and other biomarkers of exposure and effect to these compounds. 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 SAMPLES

Analytical methods for the determination of 2-butoxyethanol, 2-butoxyethanol acetate, and metabolites in biological matrices are shown in Table 6-l. The method of determination in almost all of the methods is gas chromatography (GC) with either electron capture detection (ECD) or flame ionization detection (FID). One of the reported methods used high performance liquid chromatography (HPLC) in conjunction with ultraviolet absorbance detection. 2-Butoxyethanol can be isolated from blood or urine by extraction and analysis (Johanson et al. 1986a; Smallwood et al 1984). The metabolites of 2-butoxyethanol and 2-butoxyethanol acetate include 2-butoxyacetic acid and conjugates of 2-butoxyacetic acid, including the conjugate with glutamine. 2-Butoxyacetic acid and its conjugates are isolated from urine or blood by extraction and are derivatized to increase volatility for GC separation and detection (Begerow et al. 1988; Groeseneken et al. 1989; Johanson 1989; Johanson and Johnsson 1991; Jijnsson and Steen 1978; Sakai et al. 1994) or to enhance detectability for HPLC determinations (Rettenmeier et al. 1993). HPLC has been applied to simultaneously analyze both free 2-butoxyacetic acid and conjugated 2butoxyacetic acid in the urine (Rettenmeier et al. 1993). Following extraction and derivatization to trimethylsilyl derivatives, free 2-butoxyacet acid and 2-butoxyacetylglutamine were determined by HPLC. Measurement of both butoxyacetic acid forms allowed for calculation of total butoxyacetic acid. A single gas chromatographic determination of free urinary 2-butoxyacetic acid and total urinary 2-butoxyacetic acid has been described (Sakai et al. 1994). In this procedure, acid hydrolysis of urine is included. Determination of 2-butoxyacetic acid has been used for the

Table 6-1. Analytical Methods for Determining 2-Butoxyethanol, 2-Butoxyethanol Acetate, and Metabolites in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood, urine (BE)	Addition of sodium sulfate and heptanol internal standard to sample followed by extraction with toluene. Addition of pyridine and pentafluorobenzoyl chloride to form derivative of BE. Evaporation of solvent, redissolution in 90% methanol, extraction into hexane, methanol phase discarded, and hexane phase washed with 90% methanol.	GC/ECD	0.1 µmol/L (11.8 µg/L; 11.8 ppb, w/v)	No data (method reproducibility of 11% RSD)	Johanson et al. 1986a
Urine (BAA)	Addition of 200 µL urine to glass vial containing pentoxyacetic acid (internal standard), 2 mL tetrabutylammonium hydrogen sulfate buffered to pH 6 with potassium phosphate, 2 mL dichloromethane, and 10 µL pentafluorobenzyl bromide. Rotation for 20 hours at room temperature followed by isolation and evaporation of dichloromethane phase, redissolution in hexane and dilution.	GC/ECD	4 µmol/L (528 µg/L; 528 ppb, w/v)	No data (method %RSD = 2)	Johanson 1989
Blood (BAA)	Addition of 200 µL whole blood to glass vial containing pentoxyacetic acid as internal standard, tetrabutylammonium as counter ion, dichloromethane, and pentafluorobenzyl bromide. Rotation for 20 hours at room temperature followed by isolation and evaporation of dichloromethane phase, redissolution in hexane.	GC/ECD	0.04 µmol/L (5.33 µg/L; 5.33 ppb, w/v)	No data (method %RSD = 7.5)	Johanson and Johnsson 1991
Urine (BAA)	Addition of NaCl and HCl to 2 mL of urine followed by 3 extractions with ethyl acetate and solvent exchange to toluene. Formation of trimethylsiliyl ethers.	GC/MS	No data	No data	Jönsson and Steen 1978

Table 6-1 (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (BAA)	Adjustment of urine to pH 7; addition of 3-chloropropionic acid (internal standard) and lyophilization overnight. Dissolution of residue in methanol containing pentafluorobenzyl bromide followed by equilibration at 90 °C for 3 hours. Addition of water and extraction of esters with dichloromethane.	GC/FID	0.03 mg/L (0.03 ppm, w/v)	95.1% (average of 3.5 %RSD)	Groeseneken et al. 1989
Blood (BE)	Extraction of whole blood with dichloromethane followed by filtration of organic layer and volume reduction.	GC/FID	4.0 µg/g (4 ppm, w/w)	81% (range 64–105%; 20% RSD)	Smallwood et al. 1984
Urine (BAA)	Addition of external standard to 50 mL urine, adjustment of pH to 1–2 and removal of cationic impurities by cation-exchange. Isolation of BAA by adsorption onto XAD-4 resin and elution using diethyl ether. Evaporation of solvent followed by derivatization with diazomethane.	GC/FID	0.02 mg/L (0.02 ppm, w/v)	100.9% (%RSD ranged from 4.8 to 12.6)	Begerow et al. 1988
Urine (BAA)	Addition of saturated aqueous sodium chloride, acidification with HCl, extraction three times with ethyl acetate. Evaporation of solvent, redissolution in toluene followed by removal of water. Addition of methoxylamine-hydrochloride followed by N,O-bis-(trimethylsilyl)-trifluoroacetamide.	GC/MS	No data	No data	Jönsson and Steen 1978
Blood (BE)	Addition of sodium heparin and an ion-pairing reagent (sodium hydroxide and tetrabutylammonium hydrogen sulfate). Addition of pentafluorobenzyl chloride followed by heating.	GC/MS	16–18 ng/g	Range from 92.3–103.4%	Bormett et al. 1995

Table 6-1 (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood (BAA)	Addition of sodium heparin and an ion-pairing reagent (sodium hydroxide and tetrabutylammonium hydrogen sulfate). Extractive alkylation performed by adding pentafluorobenzyl bromide and methylene chloride. Mixture was vortex-mixed and separated by centrifuge. The methylene chloride layer was analyzed by GC.	GC/MS	16–18 ng/g	Range from 102.1–107.8%	Bormett et al. 1995
Urine (BAA, N-butoxyacetyl-glutamine)	Adjustment of pH of urine sample to pH 2–3 after addition of pentoxyacetic acid and 2-ethylhexyloxyacetic acid-glutamine conjugate as internal standards. Extraction with ethyl acetate, isolation of organic layer and solvent evaporation. Derivatization with 4-nitrobenzylbromide at 80 °C for 30 minutes.	HPLC/UV	BAA: <0.08 µmol/mL (10.6 ppm, w/v); BAA-glutamine: <0.09 µmol/mL (24.9 ppm, w/v)	No data	Rettenmeier et al. 1993
Urine (total BAA, free plus conjugated)	Addition of internal standard, acidification and boiling for 1 hour. Extraction with dichloromethane: isopropanol (2:1, v/v) and formation of trimethylsilyl esters.	GC/FID	0.05 mg/L (0.05 ppm, w/v)	100.8% (3.3% RSD)	Sakai et al. 1994

BAA = 2-butoxyacetic acid; BE = 2-butoxyethanol; BEA = 2-butoxyethanol acetate; ECD = electron capture detector; FID = flame ionization detection; öGC = gas chromatography; HCl = hydrochloric acid; HPLC = high performance liquid chromatography; MS = mass spectrometry; NaCl = Sodium chloride; RSD = relative standard deviation; UV = ultraviolet absorbance detection; v/v = volume/volume; w/v = weight/volume

biological monitoring of 2-butoxyethanol exposure (refer to sections 2.3 “Toxicokinetics” and 2.6 “Biomarkers of Exposure and Effect”). It is noteworthy, however, that biological monitoring studies typically measure only free 2-butoxyacetic acid. Since a substantial fraction of 2-butoxyacetic acid is eliminated as the amino acid conjugate, exposure levels to 2-butoxyethanol would be underestimated in these typical biological monitoring studies.

6.2 ENVIRONMENTAL SAMPLES

Routine analyses for the glycol ethers in water, including 2-butoxyethanol and its acetate are often not completed because frequently used general-purpose GC S methods designed to measure priority pollutants do not readily detect these compounds (Eckel et al. 1996). For example, recovery of 2-butoxyethanol was 6.7% by EPA method 625 for semivolatiles.

Analytical methods specific for the determination of 2-butoxyethanol and 2-butoxyethanol acetate in environmental matrices are shown in Table 6-2. The method of determination in all of the methods is GC in conjunction with either flame ionization detection (FI) or mass spectrometric (MS) detection. Sample preparation methods for air samples are based on the adsorption of the target compounds onto a sorbent such as charcoal (Guest et al. 1985; NIOSH 1994a; OSHA 1990) or Amberlite XAD-7 (Andersson et al. 1982) followed by elution of the compounds with a solvent. Carbon disulfide has been used to recover non-polar analytes from charcoal but fails to recover the more polar 2-butoxyethanol and 2-butoxyethanol acetate (Fracchia et al. 1977; Mueller and Miller 1979). As a result, solvents such as 5% methanol in dichloromethane are used and provide good recoveries of both target compounds (NIOSH 1994a; OSHA 1990). Although thermal desorption is frequently used to recover volatile analytes from sorbents, this technique often fails with charcoal because the temperature required for volatilization of strongly adsorbed compounds results in the decomposition of the analytes, especially in the presence of water (Cocheo et al. 1987). Adsorbed samples generally appear to be stable9 but N OSH (1994a) recommends freezer storage of samples until elution to minimize any decomposition. High concentrations of less volatile analytes and water can impact the retention of 2-butoxyethanol and 2-butoxyethanol acetate on charcoal, so the analyst must be aware that premature breakthrough could result under these conditions and when large air volumes are sampled (NIOSH 1994a; Posner and Okenfuss 198 1). The analytic methods for determining 2-butoxyethanol in air samples allow for the detection of 2-butoxyethanol at concentrations that include the inhalation MRLs.

Table 6-2. Analytical Methods for Determining 2-Butoxyethanol and 2-Butoxyethanol Acetate in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (validated for BE; should be applicable to BEA)	Sorption of analytes onto charcoal followed by elution with 5% (v/v) methanol in dichloromethane.	GC/FID (NIOSH Method 1403, Issue 2)	1 mg/m^3 (0.21 ppm, v/v) with 10 L air sample	92% (6.5% RSD)	NIOSH 1994
Air (BE, BEA)	Sorption of analytes onto charcoal followed by elution with 5% (v/v) methanol in dichloromethane.	GC/FID (OSHA Method 83)	BE: 150 µg/m^3 (31 ppb, w/v) with 48 L sample; BEA: 116 µg/m^3 (24 ppb, w/v) with 48 L sample	BE: 99% (5.2% RSD); BEA: 101.5% (5.5% RSD).	OSHA 1990
Air (BE)	Sorption of analytes onto XAD-7 sorbent followed by elution with diethyl ether.	GC/FID	<50 mg/m^3 (10.5 ppm, v/v, with 5 L sample)	95% (4% RSD) at 50.8 mg/m^3 (10.5 ppm , v/v)	Andersson et al. 1982
Groundwater (BE)	Addition of deuterated internal standard and direct injection.	GC/MS	0.4 mg/L (0.4 ppm, w/v)	88.2% at 5 mg/L	Beihoffer and Ferguson 1994
River water (BE)	Vacuum distillation followed by continuous liquid-liquid extraction (24-hour) with ether and concentration of dried extract using Kuderna-Danish device.	GC/MS	No data	No data	Yasuhara et al. 1981
Waste water (BE)	Purge and trap of VOCs from water containing sodium sulfate onto Tenax-GC. Water removal using dry purge followed by thermal desorption.	GC/MS	No data	No data	Michael et al. 1991
Waste water (BE)	Extraction of acidified water with dichloromethane for 6 hours followed by drying and concentration of extract.	GC/MS	No data	No data	Jungclaus et al. 1976

BE = 2-butoxyethanol; BEA = 2-butoxyethanol acetate; GC = gas chromatography; MS = mass spectrometry; NIOSH = National Institute for Occupational Safety and Health; RSD = relative standard deviation; v/v = volume/volume; w/v = weight/volume

Preparation methods for water samples are based on purge and trap (Michael et al. 1991), solvent extractions (Jungclaus et al. 1976; Nguyen et al. 1994; Yasuhara et al. 1981), or direct injection (Beihoffer and Ferguson 1994). High concentrations of dissolved solids in sample extracts can contaminate the GC injector or column after only a few analyses (Beihoffer and Ferguson 1994), so adequate quality control procedures should be in place. Yasuhara et al. (198 1) included a vacuum distillation step of highly polluted river water prior to solvent extraction to avoid the contamination of the extract with high molecular weight, non-volatile compounds. The method of determination steps of all of the methods found for water were based on GC/FID or GC/MS.

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 2-butoxyethanol or 2-butoxyethanol acetate 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 2-butoxyethanol and 2-butoxyethanol acetate.

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 2-butoxyethanol in blood and urine (Johanson et al. 1986a; Smallwood et al. 1984). The utility of 2-butoxyethanol as a biomarker of exposure is limited because of its short elimination half-time in blood (40 minutes) and the small fraction of the absorbed dose (less than 0.03%) excreted unchanged in urine (Johanson 1988; Johanson et al. 1986a). In contrast, the half-time of urinary 2-butoxyacetic acid was reported to be 5.77 hours after a controlled, 2-hour exposure to 2-butoxyethanol (Johanson et al. 1986a). The amount of 2-butoxyacetic acid excreted in urine corresponded to 17-55% of the absorbed dose of 2-butoxyethanol and will thus serve as a sensitive marker of exposure (Johanson et al. 1986a). Sensitive and reproducible methods

for 2-butoxyacetic acid are available. No methods were found for the determination of 2-butoxyethanol acetate in physiological matrices, but this ester is rapidly metabolized to 2-butoxyethanol in the body resulting in 2-butoxyacetic acid in urine (Johanson 1988; Johanson et al. 1989). Thus, 2-butoxyacetic acid will provide measures of exposure to both 2-butoxyethanol and its acetate. Conjugates of 2-butoxyacetic acid with glutamine have been measured in urine and methods exist to determine the 2-butoxyacetic acid glutamine conjugate (Rettenmeier et al. 1993) or 2-butoxyacetic acid after hydrolysis of the conjugate (Sakai et al. 1994). The method of Sakai et al. (1994) permits the calculation of 2-butoxyacetic acid conjugate concentrations based on determinations of 2-butoxyacetic acid with and without hydrolysis; this method provides measurements of total (2-butoxyacetic acid and 2-butoxyacetic acid conjugates) and free 2-butoxyacetic acid, respectively. No new methods are needed.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods exist for the determination of 2-butoxyethanol in air (Andersson et al. 1982; NJOSH 1994a; OSHA 1990), groundwater (Beihoffer and Ferguson 1994), river water (Yasuhara et al. 198 l), and waste water (Jungclaus et al. 1976; Michael et al. 1991; Nguyen et al. 1994). Methods also exist for the determination of 2-butoxyethanol acetate in air (NJOSH 1994a; OSHA 1990). Limits of detection (LOD) for these compounds in air are in the sub-ppm range. Only one reported method for 2-butoxyethanol in water appeared to be characterized, and an LOD of less than 0.4 ppm (1.9 mg/m^3) was claimed (Beihoffer and Ferguson 1994). Additional information is needed about the method LODs for 2-butoxyethanol in water and waste water; additional methods are needed for other matrices (i.e., wastes, soils). No method was found for 2-butoxyethanol acetate in a matrix other than ah-; additional methods for other matrices are needed. No studies were found in which the concentrations of these two compounds were determined in foods. Methods are needed if contamination of foodstuffs and the resultant human exposure via ingestion is of concern.

6.3.2 Ongoing Studies

No ongoing method development studies were found.