7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring 1,3-butadiene, its metabolites, and other biomarkers of exposure and effect to 1,3-butadiene. 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.

7.1 BIOLOGICAL MATERIALS

No standardized method to test for the presence of 1,3-butadiene in biological materials presently exists. Only a limited number of techniques have been employed to measure this compound in biological materials.

Perbellini et al. (2003) have developed a method to measure unmetabolized 1,3-butadiene concentrations in human blood, urine, and exhaled air. Breath samples were collected by expiration into headspace vials. Venous blood samples with EDTA added as an anticoagulant or urine samples were injected into a glass tube. Samples were analyzed using gas chromatography-mass spectrometry (GC-MS). Reported detection limits for 1,3-butadiene were 0.5 ng/L in blood, 1 ng/L in urine, and 0.8 ng/L in alveolar air.

A technique for the determination of 1,3-butadiene in margarine samples was reported by Startin and Gilbert (1984). The margarine sample is placed in a vial, sealed, and heated to 70 °C where it is allowed to equilibrate for 1 hour. The amount of 1,3-butadiene in the sample is determined by withdrawing a headspace sample, and injecting it directly into a GC equipped with a MS detection system. Quantitation is obtained by comparison of the peak height to that of a standard of known concentration. The sensitivity of this method allows quantitation down to 0.001 mg/kg (1 ppb). A similar headspace technique was used to test for the presence of butadiene in olive oil, vegetable oil, and yogurt samples (McNeal and Breder 1987).

7.2 ENVIRONMENTAL SAMPLES

Standardized methods for determining 1,3-butadiene in environmental samples are limited to air samples, as no methodology has been described for analyzing this compound in water or soil samples (EPA 1982, 1986). A representative list of the methods available for the determination of 1,3-butadiene in air samples can be found in Table 7-1. The determination of 1,3-butadiene in personal air can be obtained using the procedures outlined in NIOSH Method 1024 (NIOSH 1994) and OSHA Method 56 (OSHA 2009a) or EPA Methods TO-14A and TO-15 (EPA 1999a, 1999b).

For NIOSH Method 1024, the air sample is obtained by passing a known volume of air (5–25 L) through a set of tandem coconut charcoal tubes, which adsorb 1,3-butadiene and remove it from the air stream (NIOSH 1994). The collected 1,3-butadiene is then removed from the adsorption tube by extraction with methylene chloride. Injection of the methylene chloride solution into a GC equipped with a flame ionization detector (FID) separates 1,3-butadiene from any interfering compounds that may be present. The choice of chromatography column for this determination is not crucial, as long as it cleanly separates 1,3-butadiene from other compounds.

The estimated quantitation limit (LOQ) of this method is 0.02 ppm, with an applicable range of 0.04–220 μg per sample (approximately 0.04–100 ppm) for a 25 L sample. The precision of this method appears to change as a function of the concentration being measured, due to desorption efficiencies changing as a function of sample concentration. With increasing concentration, the preparation of a standard becomes more difficult. A limitation of this study is the relatively high LOQ (20 ppb), since concentrations observed in environmental settings are often <1 ppb.

In NIOSH Method 1024, quantitation of 1,3-butadiene is accomplished by comparing the area under the sample's response signal to that of a known amount of 1,3-butadiene. The preparation and injection of a gaseous 1,3-butadiene standard is a difficult procedure; it must be performed carefully or erroneous results will occur. Sample storage appears to dramatically affect the results of the measurement. Samples stored at -4 °C displayed an average recovery of 93–98% over a 21-day period, while samples stored at room temperature ranged from 61 to 95%.

OSHA Method 56 for analyzing 1,3-butadiene in air samples is similar to the NIOSH method described above. Air is drawn through sampling tubes containing charcoal absorbent coated with 4-*tert*-butyl-catechol. The samples are then desorbed with carbon disulfide and analyzed using gas chromatograph-

Table 7-1. Analytical Methods For Determining 1,3-Butadiene in Environmental Samples

Sample air	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Personal air (Method 1024)	Pass air through charcoal tube followed by desorption with methylene chloride	GC/FID	0.04–220 µg/sample (25L sample)	61–98	NIOSH 1994
Personal air (Method 56)	Pass air through charcoal tube coated with 4-*tert*-butylcatechol followed by desorption with carbon disulfide	GC/FID	200 µg/m^3 (90 ppb)	77–94	OSHA 2009a
Air (Method TO-14A)	Pressurized container sampling followed by cryogenic concentration	GC/MS/SIM or SCAN	No data	No data	EPA 1999a
Air (Method TO-15)	Pressurized container sampling followed by multisorbent concentration and thermal desorption	GC/MS/SIM or SCAN	No data	No data	EPA 1999b
Air	Collect air in Tedlar bag concentrate on Tenax cartridge, thermal desorption	GC/FID	No data	No data	Stump and Dropkin 1985
Air	Pass air through charcoal tube solvent desorption	GC/MS	No data	No data	Texax Air Control Board 1990
Air (real time)	Draw air into 12-foot sampling loop, direct injection	GS/MS	No data	No data	Texax Air Control Board 1990
Air	Pass air through sorbent tubes containing Carbopack B/Carbosieve SIII followed by thermal desorption	GC/MS	0.11–0.16 µg/m^3	>95%	Kim et al. 1999

FID = flame ionization detector; GC = gas chromatography; MS = mass spectrometry; SCAN = wide range of mass to charge ratio scanning; SIM = select ion monitoring

flame ionization detector (GC-FID). The recommended air volume and sampling rate is 3 L at 0.05 L/minute. The detection limit is 200 μg/m^3 and the quantitation limit is 343 μg/m^3. As with NIOSH Method 1024, the usefulness of OSHA Method 56 for analysis of 1,3-butadiene in environmental media is limited since the reported detection and quantitation limits are much higher than levels often observed in environmental settings.

1,3-Butadiene, along with other volatile hydrocarbons, has been found in ambient air samples by a technique that uses cryogenic concentration before GC analysis. This technique is performed by collecting a large volume of air in a specially designed bag or other sampling container and concentrating the volatile components by condensation at low temperatures. The sample is separated into its components by GC and quantified with an internal standard. Numerous variations of this method were found in the literature (Curren 2006; Graham et al. 2004; Lonneman et al. 1979; Neligan 1962; Stephens and Burleson 1967, 1969; Stump and Dropkin 1985).

EPA Methods TO-14A and TO-15 describe procedures for the analysis of volatile organic compounds (VOCs) in air (EPA 1999a, 1999b). Method TO-14A calls for cryogenic concentration of the air sample as described above. Method TO-15 calls for pressurized air sampling using a stainless steel canister. The sample is then passed through a solid multisorbent concentrator and the concentrator is finally dry-purged with helium. The sample is thermally desorbed prior to analysis. For both of these methods, analysis is performed using GC followed by either a specific or nonspecific detector. However, the use of a specific detector is recommended, such as linear quadrupole mass spectrometer operating in either select ion monitoring (SIM) mode or a mode that scans a wide range of mass to charge ratios (SCAN). Precision and recovery data for 1,3-butadiene are not specified in these methods. Kim et al. (1999) developed an improved method by using a combination Carbopack B/CarbosieveSIII sorbent as a collection material. Sample collection was followed by thermal desorption and GC/MS analysis. These authors reported a precision of 2.4–13%, recoveries of >95%, and a detection limit of 0.11–0.16 μg/m^3 for this method. Therefore, this method is useful for measuring 1,3-butadiene concentrations in the low ppb (μg/m^3) range in environmental air samples.

7.3 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 1,3-butadiene 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 1,3-butadiene.

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.

7.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. No standardized method for the determination of biomarkers of exposure and effect for 1,3-butadiene was located.

Exposure. Biomarkers that have been proposed as indicators of exposure to 1,3-butadiene include the urinary metabolites 1,2-dihydroxybutyl mercapturic acid (DHBMA or M1) and 1- and 2-monohydroxy-3-butenyl mercapturic acid (MHBMA or M2) and the hemoglobin adducts 1- and 2-hydroxy-3-butenyl valine (MHB-Val) and N-(2,3,4-trihydroxy-butyl)valine (THB-Val) (Albertini et al. 2001; Boogaard et al. 2001a; Carrieri et al. 2009; McDonald et al. 2004; Sapkota et al. 2006; Schettgen et al. 2009; Shen et al. 2009). Measurement of unmetabolized 1,3-butadiene in human blood and urine may be preferable for assessing very low levels of exposure to this substance (0.4 $\mu g/m^3$ median concentration in personal air) (Fustinoni et al. 2004; Perbellini et al. 2003; Schettgen et al. 2009).

Both the urinary metabolites and the hemoglobin adducts have been well correlated with exposure to 1,3-butadiene (Albertini et al. 2001; Preston 2007). Methods developed to measure these biomarkers have utilized either HPLC or GC followed by tandem MS (Boogaard et al. 2001a; Carrieri et al. 2009; Sapkota et al. 2006; Schettgen et al. 2009). Shen et al. (2009) has developed a method based on liquid chromatography/electrospray ionization-mass spectrometry to measure 3-butene-1,2-diol, a 1,3-butadiene urinary metabolite intermediate.

Effect. Biomarkers of effect resulting from 1,3-butadiene exposure, such as gene mutation and chromosomal changes, have been explored; however, no clear associations have been observed (Albertini et al. 2001; Preston 2007).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Data on the determination of 1,3-butadiene in environmental media were limited. 1,3-Butadiene in air samples has been detected by techniques routinely used for detecting volatile hydrocarbons (Kim et al. 1999; Stump and Dropkin 1985; Texas Air Control Board 1990; EPA 1999a, 1999b). Improvements to these methods have made possible the detection of 1,3 butadiene concentrations in the low ppb ($\mu g/m^3$) range in environmental air samples (Kim et al. 1999). Procedures accepted for the determination of volatile hydrocarbons in other environmental media (soil, water, sediment, plants, etc.) may also be suitable for 1,3-butadiene. This question can be answered only by the data obtained from properly designed experiments. The information will assist in determining the prevalence of this compound in the environment and aid in a quantitative determination of human exposure to 1,3-butadiene.

7.3.2 Ongoing Studies

Ongoing studies related to the development of analytical methods for 1,3-butadiene were not located.