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 oxide 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 ethylene oxide. 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 oxide in environmental samples are the methods approved by federal agencies 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 a trade association such as the Association of Official Analytical Chemists (AOAC) and by 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 MATERIALS

Ethylene oxide is relatively reactive in biological systems and undergoes chemisorption to biological materials to form addition products with compounds that contain hydroxyl, phenolic, carbonyl, amino, or sulfhydryl groups. Therefore, it is usually necessary in biological samples to determine these addition products. Examples of such products that are determined to measure in vivo exposure to ethylene oxide are N-(2-hydroxyethyl)histidine and N-(2-hydroxyethyl)valine (Bailey et al. 1987; Farmer et al. 1986). Methods have been published for the determination of ethylene oxide in blood and alveolar air (Brugnone et al. 1986).

As with other materials in biological samples, samples containing ethylene oxide, its reaction products, and its metabolites must undergo some form of sample cleanup prior to analysis. Cleanup is a separation procedure that ideally isolates the analyte in a mixture, concentrates it, and eliminates most of the sample matrix. The chemical and biochemical reactivity of ethylene oxide complicates the cleanup of the biological samples in which it is contained.

Methods for the determination of ethylene oxide and its reaction products in biological samples are summarized in Table 6-l.

6.2 ENVIRONMENTAL SAMPLES

Ethylene oxide in environmental samples is most commonly determined after derivatization to stable, volatile halogenated species, particularly 2-bromoethanol (Cummins et al. 1987), followed by gas chromatography with an electron capture detector (GC/ECD) for

halogenated derivatives, or by gas chromatography/mass spectrometry (GC/MS) (Farmer et al. 1986). Infrared spectrometry may also be used (APHA 1985). A sensitive method for ethylene oxide determination has been published in which the brominated compound is formed in a standard solution of propylene oxide and the chromatographic peak ratios for the brominated ethylene oxide and propylene oxide derivatives are compared (Kikuchi et al. 1988).

The most straightforward means of determining ethylene oxide in air is direct analysis of air samples without analyte collection. This has been done with a portable gas chromatograph using clean air as a carrier gas and a photoionization detector (PID) for detection (Bond and Dumas 1982; Collins and Barker 1983). Ethylene oxide can be concentrated from air samples with a solid sorbent, desorbed with carbon disulfide, and measured by gas chromatography (NIOSH 1977). A major problem with this approach is the reaction of ethylene oxide with moisture or with halides, resulting in loss of the analyte. However, this reaction tendency can be used to advantage by derivatization of ethylene oxide to 2-bromoethanol on a collection column treated with hydrobromic acid, followed by elution of the product with benzene/carbon disulfide and measurement by GC/ECD. In the analysis of ethylene oxide in air by direct GC/ECD determination of 2-bromoethanol formed by reaction of ethylene oxide with HBr on HBr-coated charcoal, reproducibility problems have been encountered as a consequence of interference by unreacted HBr (Cummins et al. 1987). This interference has been overcome by forming a derivative of 2-bromoethanol by reaction with heptafluorobutyrylimidazole and measuring the product with GC/ECD (Cummins et al. 1987).

A method for the determination of ethylene oxide in water and in soil by partition infrared spectrophotometry has been reported (APHA 1985; Environment Canada 1985).

Methods for the determination of ethylene oxide in environmental samples are summarized in Table 6-2.

6.3 ADEQUACY OF THE DATABASE

Section 104(i)(5) of CERCLA, 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 oxide 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 ethylene oxide.

TABLE 6-1. Analytical Methods for Determining Ethylene Oxide in Biological Materials

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Blood	No data	GC	No data	No data	Brugnone et al. 1986
Alveolar air	No data	GC	No data	No data	Brugnone et al. 1986
Hemoglobin adducts from blood	Separation of erythrocytes, derivatization of N-(2-hydroxy ethyl)histidine or N-(2-hydroxyethyl)valine	GC/MS	2 ng/mL	No data	Farmer et al. 1986
Hemoglobin	Separation of erythrocytes, derivatization of N-(2-hydroxy-ethyl)histidine	HRGC/MS	No data	No Data	Bailey et al. 1987

GC = gas chromatography; MS = mass spectrometry; HRGC = High Resolution Gas Chromatography.

TABLE 6-2. Analytical Methods for Determining Ethylene Oxide in Environmental Samples

Sample Matrix	Sample Preparation	Analytical Method	Sample Detection Limit	Accuracy	Reference
Air	Direct injection of air sample	GC/FID	<1 ppb by volume	No data	Bond and Dumas 1982
Air	Direct injection of air sample	GC/FID	No data	No data	Collins and Barker 1983
Air	Collect on HBr-coated charcoal tube forming 2-bromoethanol, desorb with dimethylformamide to produce volatile derivative	GC/ECD	0.1 ppm (volume)	97% Rec. of 2 ppm	Cummins et al. 1987
Air	Collect on charcoal, desorb with carbon disulfide	GC	No data	No data	NIOSH 1977
Air	Derivatize to 2-bromoethanol	GC/ECD	0.45 μg/ sample	No data	NIOSH 1987
Soil	No data	Partition infrared	>40 ppm	No data	APHA 1985
Water	No data	Partition infrared	40-400 ppm	NR	APHA 1985

GC = gas chromatography; FID = flame ionization detector; ECD = electron captive detector; Rec. = recovery; NR = not reported.

6. ANALYTICAL METHODS

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. 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. Ethylene oxide is rapidly metabolized in biological systems and tends to form addition products such as N-(2-hydroxyethyl)histidine and N-(2-hydroxyethyl)valine. Although existing methodology is adequate to provide qualitative evidence of exposure, it would be useful to have the means to determine corresponding levels of exposure to ethylene oxide from the levels of these substances in biological media.

There are currently no available methods that can be used to associate the levels of ethylene oxide in biological media with the onset of adverse health effects. Further information in this area would be useful. It is not known if existing methods are sensitive enough to measure background levels of these compounds in the blood, urine or other biological media of the general population.

Supercritical fluid extraction coupled with supercritical fluid chromatography and immunoassay analysis are two areas of intense current activity from which substantial advances in the determination of ethylene oxide metabolites in biological samples can be anticipated. The two techniques are complementary in that supercritical fluid extraction is especially promising for the removal of analytes from sample materials and immunoassay analysis is very analyte selective and sensitive (Vanderlaan 1988). This combination has been described for the determination of sulfonylurea herbicides and their metabolites in complex media including soil, plant materials, and a cell culture medium (McNally and Wheeler 1988). This technique should be applicable to many other toxicologically and environmentally significant analytes including ethylene oxide metabolites.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available for the determination of ethylene oxide in a clean, dry, gas-phase matrix. However, because of ethylene oxide's reactivity, its determination in air, water and soil matrices is difficult. The development of methods for analysis of ethylene oxide that have improved sensitivity and selectivity would be useful.

6. ANALYTICAL METHODS

There is an ongoing effort to develop a "Master Analytical Scheme" for organic compounds in water (Michael et al. 1988). The overall goal is the development of technology capable of detecting and measuring organic compounds present at levels of 0.1 µg/L in drinking water, 1 µg/L in surface water, and 10 µg/L in effluent waters. In addition to volatile compounds (bp < 150° C), analytes are to include numerous semivolatile compounds and some compounds that are sparingly soluble in water.

Determination of the degradation products of ethylene oxide in environmental media is difficult, not because of analytical problems, but because the fundamental environmental chemistry of these compounds in water, soil, air, and biological systems is not known.

The development of analytical methods to measure ethylene oxide in situ in water and other environmental media could contribute to environmental studies of this compound.

6.3.2 On-going Studies

Studies designed to improve methods for the determination of environmental contaminants may provide refinements and improvements in the determination of ethylene oxide. The current high level of activity in supercritical fluid extraction of solid and semisolid samples should yield improved recoveries and sensitivities for the determination of ethylene oxide and its environmental degradation products in solid wastes, and these compounds should be amenable to supercritical fluid chromatographic analysis. Immunoassay analysis (Vanderlaan 1988) is an area of intense current activity from which substantial advances in the determination of ethylene oxide in environmental samples can be anticipated.