7. ANALYTICAL METHODS

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

Analytical methods to quantify glutaraldehyde in biological materials are not readily available since the determination of glutaraldehyde in biological matrices is confounded by its reactivity with amine and other functional groups of proteins and enzymes. It is this property that has led to its widespread use as a cross-linking agent for proteins. Furthermore, *in vivo* and *in vitro* studies using laboratory animals suggest that glutaraldehyde is rapidly metabolized in the body. The proposed metabolic pathway of glutaraldehyde has been summarized in Section 3.4.3 (Metabolism) of this profile. Glutaraldehyde can be detected in urine samples by reaction with diethyl thiobarbituric acid in a potassium phosphate buffer solution and measuring the fluorescence of the resultant complex (Wu et al. 1994). This method was discussed in the context of glutaraldehyde's use as a masking agent adulterant added to urine samples in order to cause false negatives during enzyme immunoassay drug testing rather than a screening method for exposure to gluteraldehyde.

Stable-isotope dilution assays involving conventional electron-impact ionization and gas chromatography-mass spectrometry (GS-MS) have been developed to detect and quantify levels of glutaric acid (a metabolite of glutaraldehyde) and other substances in body fluids (Baric et al. 1999; Shigematsu et al. 2005). In the method described by Shigematsu et al. (2005), ethylacetate was used to extract glutaric acid from a mixture containing serum or cerebrospinal fluid, sodium chloride, hydrochloric acid, and known amounts of radiolabeled glutaric acid and 3-hydroxyglutaric acid. Glutaric acid was extracted from urine samples (mixed with creatinine and radiolabeled glutaric acid and 3-hydroxyglutaric acid) by incubation with urease and deproteinization using methanol. The limit of

quantification for glutaric acid in aqueous solution was 0.0019 nmol/mL using QP5050 GC-MS and 0.057 nmol/mL using SSQ710 GC-MS; percent recovery was in the range of 83–86%.

7.2 ENVIRONMENTAL SAMPLES

Atmospheric glutaraldehyde concentrations can be determined by a number of methods. Some methods include thermal desorption/gas chromatographic analysis; OSHA method 64: high performance liquid chromatographic (HPLC) analysis; NIOSH method 2531; silica gel adsorption/gas chromatographic (GC) analysis; alumina adsorption/gas chromatographic analysis; colorimetric determination using 3-methyl-2-benzothiazolinone hydrazone (MBTH); and direct-reading instruments (NICNAS 1994). These methods are summarized in Table 7-1.

In the thermal desorption/GC method, air sampled by a pump is passed through an adsorption tube containing Tenax-GC. The tube is connected to the GC, which is fitted with a flame ionization detector (FID), and the sample is thermally desorbed and separated with temperature programming over approximately 15 minutes (NICNAS 1994).

With the OSHA method 64, samples collected by a pump on 37-mm glass fiber filters are treated with a 5% solution of dinitrophenyl-hydrazine hydrochloride (DNPH), followed by desorption using acetonitrile. The solution is injected into the HPLC, which uses a ultra-violet (UV) absorption detector. For this method, the detection limit has been reported as approximately 0.1 μg and 18 $\mu g/m^3$ (NICNAS 1994).

A similar HPLC method was also utilized by Sekine et al. (2005) to determine glutaraldehyde concentrations. A passive sampler packed with DNPH was used to collect glutaraldehyde samples in air, eluted with 10 mL of acetonitrile, and concentrations of glutaraldehyde were determined using HPLC. The HPLC utilized a Shimadzu LC-6A pump with an SPD-6A UV-visible detector and the following conditions; a 4.6x150 mm, 5 μm, Inertsil ODS-80A column; a 60/40 acetonitrile/distilled water eluent at 1.5 mL/minute; detection at 360 nm; and an injection volume of 20 μL. The limit of detection was 1.2 ppb of glutaraldehyde for 8-hour sampling (3 times the HPLC baseline noise); the limit of quantitation was 3.9 ppb of glutaraldehyde (10 times the HPLC baseline noise).

Table 7-1. Analytical Methods for Determining Glutaraldehyde in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Drawing air through sampling tubes containing Amberlite XAD-2 with DNPH	UV spectrophotometer	0.02 mg/m^3 for 3-L air sample		Rietz 1985
Air	Passive sampler containing DNPH; elution with acetonitrile	HPLC	1.2 ppb for 8-hour sample		Sekine et al. 2005
Air	Cassette with DNPH; desorbed with acetonitrile	HPLC with UV detector at 355 nm	0.27 µg/ sample; 0.004 ppm for 15-minute sample		Wellons et al. 1998
Air	Passive badge sampler with DNPH; desorbed with acetonitrile	HPLC with UV detector at 355 nm	0.006 µg/ sample; 0.016 ppm for 15-minute sample		Wellons et al. 1998
Air	Air sampled by pump passed through adsorption tube containing Tenax-GC	GC with FID			NICNAS 1994
Air	Sample on 37 mm glass fiber filter treated with 5% DNPH hydrochloride; desorbed in acetonitrile	HPLC with UV absorption	0.1 µg or 18 µg/m^3		NICNAS 1994
Air	Sample on XAD-2 tube treated with DNPH hydrochloride	GC with FID	0.3 and 1 µg		NICNAS 1994
Air	Samples on adsorption tubes filled with silica gel; desorbed in acetone	GC with FID	0.02 ppm		NICNAS 1994
Air	Samples in adsorption tubes packed with silica gel; desorbed in acetone	GC with FID	0.29 µg/ sample; 0.005 ppm for 15-L sample		Wellons et al. 1998
Air	Samples on adsorption tubes packed with alumina; desorbed with phosphate buffer	GC with Tenax-GC column, FID			NICNAS 1994
Air	Samples drawn through impingers containing distilled water	Colorimetric analysis with MBTH			NICNAS 1994
Air	Direct reading instrument	Fuel cell sensor; glutaraldehyde catalytically oxidized to produce electrical response	0.05–5 ppm v/v or 0.03–4 ppm		NICNAS 1994; Wellons et al. 1998
Air	Diffusive sampler filter with DNPH; washed with acetonitrile	HPLC	0.03 mg/m^3		Lindahl and Levin 1995

Table 7-1. Analytical Methods for Determining Glutaraldehyde in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Diluted 25% glutaraldehyde solutions prepared in methanol	Spectrophotometer with absorbance at 480 nm	1 mg/L		Jolibois et al. 2002
Water	Diluted 25% glutaraldehyde solutions prepared in methanol	GC/MS			Jolibois et al. 2002
Aqueous solutions		Titration with 0.5 N hydroxylamine hydrochloride	10–50% glutaraldehyde solutions		NICNAS 1994
Aqueous solutions	Water sample added to MBTH	Absorbance at 605 or 610 nm	0.5–10 ppm glutaraldehyde solutions		NICNAS 1994
Aqueous solutions	Sodium bisulfide added to water sample	Titrated with standardized sulfuric acid	25–5,000 ppm glutaraldehyde solutions		NICNAS 1994
Aqueous solutions	Water sample	GC with Tenax-GC or Porapak PS column, FID	1–2,500 ppm w/v glutaraldehyde solutions		NICNAS 1994

DNPH = 2,4-dinitrophenylhydrazine; FID = flame ionization detector; GC = gas chromatography; HPLC = high performance liquid chromatography; MBTH = 3-methyl-2-benzothiazolinone hydrazine; UV = ultraviolet

Wellons et al. (1998) utilized a method based on OSHA method 64, whereby glutaraldehyde was also measured as a DNPH derivative. A 37 mm filter cassette with DNPH pre-coated AE glass fiber filters was used with high-volume personal sample pumps at 1 L/minute to collect 15-minute air samples. The filter cassettes were capped and frozen until analysis, whereby each section was desorbed in 2 mL acetonitrile, with a desorption efficiency of nearly 100%. Solutions were analyzed with HPLC using a UV detector at 355 nm. A lower limit of quantitation of 0.27 µg/sample, or 0.004 ppm for a 15-minute sample was attained, with percent recoveries of 105 and 96% after 17 days ambient temperature and 16 days -20 ºC storage, respectively (Wellons et al. 1998). Similarly, Wellons et al. (1998) also tested a passive badge sampler, containing a filter impregnated with DNPH that has a sampling rate of 5.88 mL/minute. Badges were exposed to air for 15 minutes, then capped. Glutaraldehyde was desorbed with 1 mL acetonitrile and the same HPLC method was utilized. The method was found to have a lower limit of quantification of 0.006 µg/sample, or 0.016 ppm for 15 minutes (Wellons et al. 1998).

The NIOSH method 2531 utilizes a sample collected on washed XAD-2 tubes that are then treated with dinitrophenyl-hydrazine hydrochloride. Samples are analyzed with GC using FID. The detection limit of this method has been reported at both 0.3 and 1 µg (NICNAS 1994).

Similarly, Rietz (1985) determined glutaraldehyde concentrations in air by passing known volumes of air through sampling tubes that contained Amberlite XAD-2 that were coated with DNPH as the adsorption material. As the experiment was designed to sample glutaraldehyde as well as formaldehyde and acrolein, acetonitrile was used to elute the resulting hydrazones and the compounds were then separated using an RP C-18 column. The chemicals were identified using a UV spectrophotometer at a wavelength of 365 nm. For a 3-L air sample, the limit of detection for glutaraldehyde was determined to be 0.02 mg/m^3 of air with a 95% confidence level. This method was determined to be acceptable for glutaraldehyde concentrations ranging from 0.04 to 2.1 mg/m^3 of air (Rietz 1985).

A diffusive sampler was used to determine the glutaraldehyde concentrations in air by Lindahl and Levin (1995). The sampler utilized a 20x45 mm filter coated impregnated with DNPH, placed in a 60x30x5-mm polypropylene housing unit along with a 2.9-mm thick screen. The samples were analyzed by eluting the glutaraldehyde-DNPH from the filter with 2.0 or 3.0 mL acetonitrile by shaking for 1 minute and injecting 10 µL samples into the HPLC. The HPLC consisted of two Waters M-6000 A pumps with a Waters M-710 B autosampler and Shimadzu absorbance detector. The detection limit was determined to be approximately 0.03 mg/m^3 for 15-minute samples (Lindahl and Levin 1995).

In the silica gel adsorption/GC analysis method, samples are obtained by a pump on adsorption tubes that are filled with silica gel. The sample is then desorbed with acetone and the solution is injected into a GC using FID. For 15-minute exposure samples of a 30-L air sample, the detection limit has been reported as 0.02 ppm (NICNAS 1994).

This method of direct measurement of glutaraldehyde in air was evaluated by Wellons et al. (1998). A 70x6 mm (OD) glass tube was packed with silica gel. High-volume personal sample pumps drew air through the tubes at 1 L/minute for 15 minutes. Tubes were then capped and frozen until analysis, where the glutaraldehyde was desorbed with 1 mL of acetone. Glutaraldehyde concentrations were determined by GC using an FID. Losses of 4 and 7% glutaraldehyde occurred from freezing for 14 and 21 days, respectively. A detection limit of 0.29 µg/sample, or 0.005 ppm for a 15-L sample, was attained (Wellons et al. 1998).

Alumina adsorption/GC analysis is accomplished using air samples obtained with a pump. The samples are collected on adsorption tubes packed with alumina. Samples are then desorbed with a phosphate buffer solution and injected into a GC that has a Tenax-GC column and FID installed (NICNAS 1994).

Glutaraldehyde concentrations in air can also be evaluated by colorimetric determination using 3-methyl-2-benzothiazolinone hydrazone (MBTH). Air samples containing glutaraldehyde are drawn through impingers that contain distilled water, such as with a reciprocating air pump with flow rates up to 1 L/minute. Glutaraldehyde absorbs into the water, due to its soluble nature, and concentrations can then be determined using colorimetric analysis with MBTH solution. This method may be problematic if other aldehydes and ketones are present (NICNAS 1994).

A direct-reading instrument known as the Lion Glutaraldemeter is commercially available for the determination of glutaraldehyde concentrations in air. Air samples of 10 mL are drawn in with a self-contained sample pump, and response times are approximately 60 seconds. A fuel cell sensor in the meter causes glutaraldehyde to go through catalytic oxidation, producing an electrical response, which is proportional to the amount of glutaraldehyde in the air. This method has a reported detection limit of 0.05–5 ppm v/v, although this was also reported as 0.03–4 ppm. The instrument may give erroneous readings if alcohols and other aldehydes are present, although the manufacturer offers an optional filter which removes phenol interference and corrects for alcohol interference (NICNAS 1994; Wellons et al. 1998).

Methods have also been developed to determine glutaraldehyde concentrations in water and aqueous solutions. For high levels of glutaraldehyde in aqueous solutions (i.e., solutions of 10–50%), the standard method involves titration with 0.5 N hydroxylamine hydrochloride. To determine glutaraldehyde concentrations at lower levels, the following methods may be used: colorimetric determination using MBTH; titration after reaction with sodium bisulfite, and GC analysis. These methods are summarized in Table 7-1.

Colorimetric determination of glutaraldehyde in water is performed by adding the sample to a solution of MBTH and measuring the absorbance at 605 or 610 nm. While the presence of other ketones or aldehydes may interfere, this method is usable for glutaraldehyde concentrations of 0.5–10 ppm in water (NICNAS 1994).

Jolibois et al. (2002) examined glutaraldehyde concentrations in hospital waste water. The study utilized a variation on the spectrophotometric method, whereby standard solutions of a diluted 25% commercial glutaraldehyde solution were prepared in methanol. A coloring reagent mixture of 40 µL of 5% aqueous phenol solution and 10 mL of 70% perchloric acid was prepared. The experiment was performed by adding 100 µL of the methanolic sample to 1 mL of the reagent. After a 15-minute time period for color development, the absorbance was measured on a spectrophotometer at 480 nm. A detection limit of 1 mg/L was obtained. The method was verified by analyzing the methanolic eluates by GC/mass spectrometry (MS) using a Varian 3900 GC with a Saturn 2100 detector, splitless injector (230°C), and CP-SIL 8CB-MS fused silica column (30 m by 0.25 mm ID) with a 1 µL injection volume and helium as the carrier gas (Jolibois et al. 2002).

Glutaraldehyde concentrations in water can also be determined using a titration technique. Sodium bisulfide is added to the water sample to react with the carbonyl groups. The solution is then titrated with standardized sulfuric acid. This method may be used for glutaraldehyde solutions ranging from 25 to 5,000 ppm, but interference by ketones and other aldehydes, as well as acids and bases in the sample, may be an issue (NICNAS 1994).

In GC analysis of glutaraldehyde in water, the sample is injected into a GC equipped with a Tenax-GC or Porapak PS column and a flame ionization detector. This method can detect glutaraldehyde concentrations ranging from 1 to 2,500 ppm w/v (NICNAS 1994).

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 glutaraldehyde 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 glutaraldehyde.

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.

Methods are available to detect glutaric acid (a metabolite of glutaraldehyde) in bodily fluids. Such methods could be applied to detect and quantify glutaraldehyde metabolites in urine and/or serum of glutaraldehyde-exposed workers, although environmental exposure to low levels of glutaraldehyde might not warrant this type of biomonitoring.

Exposure. Additional information concerning procedures to determine glutaraldehyde exposures in biological materials is needed.

Effect. Information concerning procedures to determine the effect of glutaraldehyde in biological materials is needed.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media.

Methods are available to detect glutaraldehyde in air and water at levels that may be a concern for health. No methods for determining glutaraldehyde concentrations in soil were located, but it is unlikely that this

would be an important environmental medium for glutaraldehyde as it is rapidly degraded in soil, possibly by bacteria.

7.3.2 Ongoing Studies

No ongoing analytical studies for glutaraldehyde were identified in the NIH Research Portfolio Online Reporting Tools (RePORTER 2014).