7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring chlorine dioxide and chlorite, its metabolites, and other biomarkers of exposure and effect to chlorine dioxide and chlorite. 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 methods for determining chlorine dioxide in biological materials were located. Most studies concerning human health effects measure the concentrations of chlorine dioxide in the air or in water. The measurement of chlorine dioxide in biological materials is not commonly used because of the rapid conversion of chlorine dioxide to chlorine-containing metabolites, such as chlorite and chloride ions.

Abdel-Rahman et al. (1980b) developed a method to quantitatively and qualitatively measure the metabolites of chlorine dioxide (e.g., ClO_2^-, and ClO^-) in biological fluids. These biomarkers can be used to indirectly measure chlorine dioxide exposure.

The concentration of residual chlorite ion in vegetables and eggs treated with sodium chlorite was determined by UV-ion chromatography (Suzuki et al. 1997). Sodium chlorite was extracted with water and cleaned-up using C18 cartridge. The detection limit of sodium chlorite in vegetables and eggs was 1 mg/kg with recoveries of 90–100%.

7.2 ENVIRONMENTAL SAMPLES

Chlorine dioxide has been measured in air and water. Methods for determining levels in the air include spectrophotometry and ion chromatography. Environmental analyses of chlorine dioxide in water are

performed using electrochemical, chromatographic, or spectrophotometric methods. Analytical methods for the determination of chlorine dioxide in environmental samples are given in Table 7-1. Ion chromatography may also be used to analyze the inorganic disinfection by-products of chlorine dioxide (i.e., chlorite ions) in an analogous manner using EPA Method 300.0 (Pfaff and Brockhoff 1990).

Atmospheric chlorine dioxide may be sampled by pulling a given volume of air through a toxic gas vapor detector tube. The tube contains chemicals that react only with chlorine dioxide. If chlorine dioxide is present, the indicator chemical in the tube will change color. The concentration of the gas or vapor may be estimated by either the length-of-stain compared to a calibration chart or the intensity of the color change compared to a set of standards (EMMI 1997). Diffusive samplers have been used to monitor chlorine dioxide and chlorine in workplace air. In this technique, workplace air is diffused into an absorbing solution of neutrally buffered potassium iodide. In the absorbing solution, chlorine dioxide and chlorine are reduced by iodide ions to chlorite and chloride ions, respectively. The formed ions are then separated and quantified by ion chromatography. The analytical detection limits have been found to be 0.02 and 0.07 ppm for chloride and chlorite ions, respectively (Björkholm et al. 1990).

Spectrophotometry (or colorimetry) has been used to measure chlorine dioxide in water using indicators that change colors when oxidized by chlorine dioxide. Spectrophotometric analyzers determine the concentration of chlorine dioxide by measuring the optical absorbance of the indicator in the sample solution. The absorbance is proportional to the concentration of the chlorine dioxide in water. Indicators used for this technique include *N,N*-diethyl-*p*-phenylenediamine, chlorophenol red, and methylene blue (APHA 1998; Fletcher and Hemming 1985; Quentel et al. 1994; Sweetin et al. 1996). For example, chlorophenol red selectively reacts with chlorine dioxide at pH 7 with a detection limit of 0.12 mg/L. The interferences from chlorine may be reduced by the addition of oxalic acid, sodium cyclamate, or thioacetamide (Sweetin et al. 1996).

APHA Method 4500-CLO2-B, iodometric titration analysis, measures the concentration of chlorine dioxide in water by titration with iodide, which is reduced to form iodine. Iodine is then measured colorimetrically when a blue color forms from the production of a starch-iodine complex. The detection limit for this method is 20 µg/L (APHA 1998).

Table 7-1. Analytical Methods for Determining Chlorine Dioxide and Chlorite in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	None.	Toxic gas vapor detector tube	0.05 ppm	No data	EPA 1997
Work-place air	Diffusion of air into potas-sium iodide solution at pH 7.	Ion chromato-graphy (of chlorite ion in solution)	0.02 ppm of chlorine dioxide	No data	Björkholm et al. 1990; Hekmat et al. 1994 (OSHA Method 202)
Water	None.	Ion chromato-graphy with conductivity detector	0.01 mg/L (as chlorite ion)	No data	Hoehn et al. 2000 (EPA Method 300.0)
			0.03 mg/L (as chlorite ion)		Pfaff and Brockhoff 1990
Water	To 100 mL sample, add 2 mL glycine solution and mix. In a separate flask, place 5 mL buffer reagent and N,N-diethyl-p-phenylene-diamine indicator solution and mix. Add 200 mg EDTA, disodium salt, and then add glycine-treated sample and mix.	UV/VIS spectrometry	>0.1 mg/L[a]	No data	APHA 1998 (Method 4500-CLO2-D)
Water	Add buffer and indicator.	UV/VIS spectrometry	Indicator (detection limit); acid chrome violet K (0.02 mg/L); amaranth (0.005 mg/L); lissamine green B (0.03 mg/L); methylene blue (0.02 mg/L); chlorophenol red (0.12 mg/L)	No data	Fletcher and Hemmings 1985; Hofmann et al. 1998; Hui et al. 1997; Sweetin et al. 1996
Water/ waste water	Measure initial temperature and pH and protect sample from light throughout the procedure. Phenylarsine oxide is used as standard titrant.	Amperometric titration	0.5 mg/L[a]	No data	APHA 1998 (Method 2350-C), (Method 4500-CLO2-C) (Method 4500-CLO2-E)
Water	None.	Flow injection using redox electrode detector	3.4 ppb (as chlorite ion)	No data	Ohura et al. 1999

Table 7-1. Analytical Methods for Determining Chlorine Dioxide and Chlorite in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Transfer 5 mL acetic acid, or enough to adjust sample pH between 3 and 4, and 1 g KI, and 1 mL starch solution; pour in sample and mix.	Iodometric titration	20 µg/L	No data	APHA 1998 (Method 4500-CLO2-B)
Water, drinking	Add sample to 1,2-di-hydroxy-anthra-quinone-3-sulphonic acid in phosphate buffer.	Polarographic analyser	2 µg/L	No data	Quentel et al. 1994

[a]Hofmann et al. (1998)

APHA = American Public Health Association; EDTA = ethylene diamine tetraacetic acid; EPA = Environmental Protection Agency; KI = potassium iodide; OSHA = Occupational Safety and Health Administration; UV/VIS = ultraviolet/visible light

For APHA Methods 2350-C and 4500-CLO2-E, amperometric analyzers are used to measure chlorine dioxide in water. Amperometric analyzers measure the current that is necessary to maintain a constant concentration of titrant as chlorine dioxide reduces the titrant (e.g., phenylarsine oxide). This method is limited by interference from compounds that might react with the titrant (e.g., chlorine and chloroamine) (APHA 1998).

Because of its sensitivity and precision, ion chromatography (EPA Method 300.0) is a good technique for analyzing chlorine dioxide in water. Ion chromatography utilizes the ability of certain ion exchange resins to separate a mixture of anionic species. A liquid mobile phase (e.g., eluant) is used to carry the sample through the system either by isocratic (using same eluant) or gradient (varying concentration or flow rate) elution. After separation is achieved, the separated anions are measured using a detector (e.g., conductometric, ultraviolet/visible, or fluorescence). Typically, chlorine dioxide is indirectly analyzed as chlorite ions (Hoehn et al. 2000). Detection limits for chlorite ions range from 0.01 to 0.03 mg/L (Hoehn et al. 2000; Pfaff and Brockhoff 1990). Other detection methods used with ion chromatography, such as ion-spray mass spectrometry, have been developed and offer greater ion selectivity and sensitivity (Charles and Pépin 1998). Precolumn sample treatments using tetraborate/boric acid to separate analytes from common interfering ions (e.g., chloride, carbonate, and nitrate) result in lower detection limits on the order of 10 µg/L for chlorite ions (Hautman and Bolyard 1992a). With postcolumn derivatization of chlorite ions to tribromate ions, detection limits on the order of 0.4 µg/L have been achieved for chlorite ions (Weinberg and Yamada 1998).

Gas-diffusion flow injection analysis is capable of detecting very low concentrations of chlorine dioxide in water (i.e., detection limit is 5 µg/L). A chemiluminescence flow-through detector cell is used to measure the concentration chlorine dioxide as a function of chemiluminescence intensity. A gas diffusion membrane separates the donor stream from the detecting stream and removes ionic interferences from iron and manganese compounds, as well as from other oxychlorinated compounds, such as chlorate and chlorite (Hollowell et al. 1986; Saksa and Smart 1985).

A rapid potentiometric flow inject technique for the simultaneous determination of oxychlorine species (e.g., ClO_2^-) was developed by Ohura et al. (1999). The analytical method is based on the detection of a large transient potential change of the redox electrode due to chlorine generated via the reaction of the oxychlorine species (e.g., ClO_2^-). The detection limit for ClO_2^- is 3.4 ppb.

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 chlorine dioxide and chlorite are available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (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 chlorine dioxide and chlorite.

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.

Exposure. Metabolites of chlorinated phenoloic compounds in fish bile have been found to be sensitive biomarkers of bleach pulp mill effluent exposure (Brumley et al. 1996). Analysis of metabolites of chlorinated syringaldehydyes in fish bile can provide a biomarker of effluent exposure that is sensitive to low levels of exposure and correlates well with exposure concentrations. Methods for determining biomarkers of exposure in human were not located. Abdel-Rahman et al. (1980b) developed a method to quantitatively and qualitatively measure the metabolites of chlorine dioxide (e.g., ClO_2^-, and ClO^-) in biological fluids. These biomarkers may be used to indirectly measure chlorine dioxide exposure. Methods for determining biomarkers of exposure in humans are needed to determine background levels in the population and levels at which biological effects occur.

Effect. Methods for determining biomarkers of effect in human were not located. Methods for determining biomarkers of effect in humans are needed to determine background levels in the population and levels at which biological effects occur.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods for determining chlorine dioxide and chlorite in air and water, the media of most concern for human exposure, are reliable, but may not be sensitive enough to measure background levels in the environment.

No data are available on methods for determining chlorine dioxide and chlorite in soil and other solid media. In addition, there is insufficient information on the methods for determining chlorine dioxide and chlorite in media such as shellfish, fish, and plants. Some exposure to chlorine dioxide and chlorite may occur via ingestion of food, and thus, standardized methods for foods are needed. Methods with sufficient sensitivity for measuring background levels in foods are needed as well.

7.3.2 Ongoing Studies

No ongoing studies were located as a result of a search of Federal Research in Progress (FEDRIP 2003).