7. ANALYTICAL METHODS

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

Inhaled chlorine gas forms hypochlorous acid and hydrochloric acid upon contact with the moist mucous membranes of the upper respiratory tract (Vetrano 2001; Winder 2001). Since molecular chlorine reacts so quickly inside living systems, it is not found in biological materials. Therefore, analysis of these materials for chlorine is not relevant. Once they have been absorbed into the body, hypochlorous and hydrochloric acid are expected to react with proteins and nucleotides to produce a wide variety of chlorinated organic compounds (EPA 1999; Winder 2001). Based on a study that traced radiolabeled chlorine (as hypochlorite) through metabolism inside rats, it is expected that chlorine is ultimately converted to chloride in the blood and eliminated in the urine and feces of humans and animals primarily as the chloride ion (Abdel-Rahman et al. 1982, 1983; EPA 1999; Suh and Abdel-Rahman 1983). Chloroform has also been detected in the blood of rats exposed to hypochlorous acid (Abdel-Rahman et al. 1984). Since chloride is a natural component of blood, urine, and feces, monitoring chloride concentrations in these materials would not be helpful for assessing exposure to chlorine.

7.2 ENVIRONMENTAL SAMPLES

When chlorine is released into the environment, it reacts very quickly with both organic and inorganic matter forming chloride ion and chlorinated compounds. Therefore, aside from low levels in marine aerosols above the open ocean, and higher concentrations in the areas surrounding recent spills and leaks, molecular chlorine is not found in the environment.

Standardized methods have been established for analyzing chlorinated water for free chlorine (APHA 1998a, 1998b). Free chlorine refers to the combination of the equilibrium species aqueous molecular chlorine, hypochlorous acid, and the hypochlorite ion. These methods do not differentiate between the molecular chlorine and the hypochlorite species. Since molecular chlorine is usually not present in water samples, these tests typically measure the amounts of hypochlorous acid, hypochlorite, and chlorinated derivatives. The most popular of these tests is the DPD (N,N-diethyl-p-phenyldiamine) test (APHA 1998a, 1998b). A small amount of DPD is added to a water sample, which is immediately oxidized by free chlorine to produce a relatively stable free radical and results in a reddish-colored solution. The total chlorine is measured spectrophotometrically at 515 nm (APHA 1998a, 1998b). Some important residuals of water disinfection can also quantified by this method. Since chloramines are slow to react with DPD, they are quantified by the subsequent addition of potassium iodide. The iodide ion acts catalytically causing color production by monochloramine and dichloramine (APHA 1998a, 1998b). The free chlorine and the chloroamines are often referred to as the total chlorine content of the water.

Aside from the DPD test, free chlorine can be measured using the amperometric titration method or the starch-iodide titration method (APHA 1998a). The amperometric titration method involves the titration of the buffered sample with phenylarsine oxide (APHA 1998a). The decrease of free chlorine during the titration is detected by applying an electric potential across two electrodes and measuring the change in current through the solution. The starch-iodide titration method involves addition of potassium iodide and a starch indicator to the sample followed by titration with sodium thiosulfate (APHA 1998a). The end point is reached when the blue color of the solution disappears.

It should be noted that the free chlorine test methods described here work by detecting the presence of oxidizing species and are not actually specific and selective to free chlorine (hypochlorite and hypochlorous acid) (APHA 1998a). Therefore, care must be taken to avoid interference due to non-free chlorine oxidizing or reducing agents. The amperometric titration method is less affected by interference, temperature variations, turbidity, and color; however, this method requires greater operator skill to achieve reliable results (APHA 1998a).

Four standardized methods have been located that describe procedures for measuring molecular chlorine in air (EPA 2000b; NIOSH 1994; OSHA 2007a, 2007b). In EPA Method OAQPS-26, the air sample is passed through a particulate filter followed by a dilute sulfuric acid solution (EPA 2000b). Hydrogen chloride dissolves to form chloride in the acid solution, while chlorine, which is relatively insoluble in the acid, passes through to a dilute sodium hydroxide solution. Chlorine dissolves and disporportionates to

form both chloride and hypochlorous acid. Sodium thiosulfate is then added to the alkaline solution to ensure complete reaction with the hypochlorous acid, freeing the second chloride ion. Analysis is performed using ion chromatography.

OSHA Methods ID-101 and ID-126SGX are based on the reaction between chlorine and iodide to form iodine and chloride (OSHA 2007a, 2007b). In Method ID-101, chlorine is collected in a sulfamic acid solution, which is then reacted with potassium iodide and analyzed using a residual chlorine ion specific electrode. In Method ID-126SGX, chlorine is collected into a neutral solution of potassium iodide, which is then titrated with sodium thiosulfate. A second titration involving chlorine dioxide is performed next. The concentrations of chlorine and chlorine dioxide are determined using stoichiometric calculations. Disadvantages of this method are that it suffers from many interferences and that temperature and strong light affect solution solubility. Both of the OSHA methods recommend using a filter to eliminate particulates that may cause interference.

NIOSH Method 6011 describes a way to measure chlorine in air samples via collection onto a silver membrane filter, desorption into sodium thiosulfite, and subsequent analysis using ion chromatography (NIOSH 1994). This method is subject to positive interference from hydrogen chloride and negative interference from hydrogen sulfide. Also, silver chloride is photosensitive; therefore, the silver filter must be transferred to an amber bottle in the dark. Once the silver chloride has desorbed, it is no longer photosensitive. The detection limit for chlorine listed in this method is 0.007 ppm for a 90L air sample collected at a flow rate of 0.3–1 L per minute; however, Chang et al. (2004) was able to measure chlorine in air to a detection limit of 50 ppt (parts per trillion) using a Dionex DX-120 analyzer and longer sampling times.

Standardized methods for measuring chlorine in air and water including accuracy, detection limits, and additional details are listed in Table 7-1. Methods that analyze soil and sediment for chlorine are not available.

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 is available. Where adequate information is not available, ATSDR, in conjunction with NTP, is required to assure the initiation of a program of research

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Sample is passed through a particulate filter into a dilute sulfuric acid solution followed by a dilute sodium hydroxide solution. Analysis is performed using IC.	EPA OAQPS-26	0.1 ppm	NA	EPA 2000b
Air	Sample is passed through a teflon prefilter and dissolved in a sulfamic acid solution. Potassium iodide is added to the acid solution. Analysis is performed using RCE.	OSHA ID-101	0.14 ppm	NA	OSHA 2007b
Air	Sample is passed through a glass giber pre-filter and collected into a potassium iodide solution. The solution is then titrated with sodium thiosulfate in two steps to determine concentrations of chlorine and chlorine dioxide.	OSHA ID-126SGX	0.3 ppm	NA	OSHA 2007a
Air	Sample is passed through a silver membrane filter. Silver chloride is desorbed from the membrane into a sodium thiosulfate solution, which is then analyzed using IC.	NIOSH 6011	0.007–0.5 ppm for a 90 L air sample (flow rate 0.3–1.0 L/minute); 50 ppt	98.6%	NIOSH 1994; Chang et al. 2004
Water	Acetic acid and potassium iodide are added to the sample to create an acidic solution, which is then titrated using sodium thiosulfate.	APHA 4500-Cl B	40 µg as free chlorine/L	NA	APHA 1998a, 1998b
Water	Acetic acid and potassium iodide are added to the sample to create an acidic solution, which is then titrated using sodium thiosulfate or phenylarsine oxide.	EPA 330.3	250–4,020 µg as free chlorine/L	NA	CAS 1978c

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	Phenylarsine oxide, potassium iodide, and acetate buffer are combined with the sample. For starch iodide determination, a starch indicator is added followed by titration with iodine solution. For amperometric determination, titrate with an iodine solution using an amperometric titrator.	EPA 330.2	250–4,020 µg as free chlorine/L	NA	CAS 1978b
Water	Phosphate buffer is added to the sample, which is then titrated with phenylarsine oxide using a microammeter to observe current changes.	APHA 4500-Cl D	200 µg as free chlorine/L	NA	APHA 1998a
Water	Potassium iodide and acetate buffer are added to the sample, which is then titrated with phenylarsine oxide or sodium thiosulfate using an amperometer to determine the end point.	EPA 330.1	380–3,500 µg as free chlorine/L	NA	CAS 1978a
Water	Add 10 mL of water to 0.5 mL of phosphate buffer solution and 0.5 mL of DPD reagent. Quantify by UV-VIS at 515 nm	APHA 4500-Cl G	10 µg as free chlorine/L	NA	APHA 1998a, 1998b

DPD = N,N-diethyl-p-phenyldiamine; IC = ion chromatography; NA = not available; RCE = residual chlorine ion specific electrode; UV-VIS = ultraviolet-visible

designed to determine the health effects (and techniques for developing methods to determine such health effects) of chlorine.

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. There are no biomarkers of exposure that are specific to chlorine. Chlorine reacts very quickly inside the body; therefore, analyzing for this substance in biological materials is not relevant. The chlorine reacts to form many chlorinated compounds that are ultimately broken down into chloride ion. Chloride is a natural component of blood, urine, and feces. Monitoring for chloride in biological materials would not be helpful in gauging exposure to chlorine.

Effect. There are no biomarkers of effect that are unique to chlorine exposure. The most obvious effect of exposure to high levels of chlorine is damage to the moist mucous membranes of the lungs and respiratory pathways. Other health effects that have been associated with exposure to chlorine include bronchitis, asthma, pulmonary edema, dermatitis, and conjunctivitis. The odor threshold for chlorine in air is 0.2–0.4 ppm and the lowest concentration in air at which there is perceivable sensory irritation is 1 ppm (EPA 1999; The Chlorine Institute 1998; WHO 1982). Current standardized methods for measuring chlorine in air have detection limits ranging from 0.007 to 0.3 ppm, which are below concentrations at which biological effects occur (EPA 2000b; NIOSH 1994; OSHA 2007a, 2007b).

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods sensitive enough to measure background chlorine concentrations in ocean air are available. Chang et al. (2004) used a more sensitive analyzer and larger sampling volumes with the silver membrane filter method (NIOSH Method 6011) to lower the detection limit from 0.007 ppm to 50 ppt. Background chlorine concentrations measured were <600 ppt. Methods are also available for measuring chlorine in water; however, these methods do not distinguish between the equilibrium species molecular

chlorine, hypochlorous acid, or hypochlorite (APHA 1998a, 1998b). Since hypochlorous acid and hypochlorite are the dominant species at environmental pH, analyzing water for molecular chlorine is not relevant except under very acidic conditions (pH<4) (APHA 1998a, 1998b; Farr et al. 2003). Methods that analyze for chlorine in soil or sediment were not located. Chlorine is not expected to be found in these media since it is so reactive, rapidly oxidizing both organic and inorganic materials that it comes into contact with.

Among the analytical methods referred to in this chapter, only the detection limits reported for NIOSH Method 6011 are sensitive enough to detect chlorine in air at levels at or below the MRL values derived in Chapter 3. For reference, the acute, intermediate, and chronic MRLs derived for chlorine in air are 0.06 ppm, 0.002 ppm, and 50 ppt, respectively. Detection limits reported for NIOSH Method 6011 range from 0.007–0.5 ppm, while a detection limit of 50 ppt was achieved for a modification of this method (NIOSH 1994; Chang et al. 2004). The other detection methods for measuring chlorine levels in air report detection limits of >0.1 ppm (EPA 2000b). Based on the lack of sensitivity of the available methods relative to the derived MRL values, the development of standardized analytical methods that can detect chlorine in air at levels below the MRL values (below 50 ppt) is a data need.

7.3.2 Ongoing Studies

One ongoing study has been located in the Federal Research in Progress Database (FEDRIP 2009) related to the development of analytical methods for chlorine. This study will be led by investigator Eric Saltzman of the University of California-Irvine and is sponsored by the National Science Foundation (NSF). The study will explore the source, presence, and distribution of inorganic dihalogen gases in the marine atmosphere and will include the validation and further development of analytical methods for dihalogens, including chlorine, in air. No other ongoing studies regarding the development of methods for analyzing for chlorine in the environment were located in the available literature.