7. ANALYTICAL METHODS

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

In January 1997, the California Department of Health Services (DHS) began to test for perchlorate in drinking water wells near the Aerojet production facility outside of Sacramento (EPA 1999a). At that time, the best analytical method available had sensitivities of 400 μg/L. Subsequently, it was improved to 100 μg/L by Aerojet Corporation. Existing data indicated that a 4 μg/L detection limit was required for a comprehensive assessment of the Aerojet site. By March of the same year, the California DHS, in collaboration with an analytical equipment manufacturer, refined the methodology to achieve a method detection limit of approximately 1 μg/L and a reporting limit of 4 μg/L.

With this analytical methodology in place, monitoring studies soon indicated that perchlorate contamination existed far beyond the boundaries of the Aerojet site. Because of concern for potential widespread perchlorate contamination and the importance of ammonium perchlorate in military and aerospace operations there has been a dramatic increase in the research on determining trace quantities of the perchlorate anion, especially in raw and finished drinking water supplies (Urbansky 2000). Extensive effort has also been expended to modify the quantitative techniques developed for water to measure perchlorate in other environmental matrices, such as soil, plants, blood, or sludge.

Four standardized methods are available for quantifying perchlorate in drinking water. These include EPA Method 314.0 (Ion Chromatography), EPA Method 314.1 (Inline Column Concentration/Matrix Elimination Ion Chromatography with Suppressed Conductivity Detection), EPA Method 331.0 (Liquid Chromatography Electrospray Ionization Mass Spectrometry), and EPA Method 332.0 (Ion Chromatography with Suppressed Conductivity and Electrospray Ionization Mass Spectrometry) (EPA

1999c, 2005h, 2005i, 2005j). High salt concentrations can cause interference when using Methods 314.0 and 314.1 (Tikkanen 2006). Method detection limits and minimal reporting levels are 0.5 and 4.0 μg/L, respectively, for Method 314.0 and 0.03 and 0.13–0.14 μg/L, respectively, for Method 314.1 (EPA 1999c, 2005h). EPA Methods 332.0 and 331.0 require isotopically labeled internal standards for quantitation (Tikkanen 2006). Method 332.0 has greater sensitivity than Methods 314.0 and 314.1 with a method detection limit of 0.02 μg/L and a minimum reporting level of 0.10 μg/L (EPA 2005j; Tikkanen 2006). Method 331.0 offers the greatest sensitivity with a method detection limit of 0.005–0.008 μg/L and a minimum reporting level of 0.022–0.056 μg/L (EPA 2005i; Tikkanen 2006).

7.1 BIOLOGICAL MATERIALS

No standardized methods for the detection of perchlorates in biological samples have been reported. Ion chromatography (IC) has been used to detect perchlorate in human breast milk and cow's milk (Kirk et al. 2005). Ells et al. (2000) describe a method for determining perchlorate in urine samples using electrospray ionization mass spectrometry. A method using ion chromatography (IC) and electrospray (ES) tandem mass spectrometry (MSMS) for determining perchlorate in urine (limit of detection 0.025 ng/mL) showed an association between urinary levels and drinking water concentrations of perchlorate (Valentín-Blasini et al. 2005). Similarly, Blount et al. (2006) used IC-ES-MSMS to measure perchlorate in amniotic fluid. This analytical method included measurement of iodide so that the levels of perchlorate relative to iodide could be assessed. Because perchlorate competitively inhibits iodide uptake, the presence of larger quantities of iodide may minimize impact of perchlorate on thyroid function. A method to quantify perchlorate, thiocyanate, nitrate, and iodide in human urine, milk, serum, blood spots, amniotic fluid, and infant formula using IC-ES-MSMS has been published (Blount and Valentín-Blasini 2007).

Methods for detecting perchlorate in food using IC-ES-MSMS have been described (El Aribi et al. 2006; Krynitsky et al. 2004). According to El Aribi et al. (2006), detection of perchlorate at levels as low as 5 ng/L in food is possible. An IC-MSMS method was employed by the FDA for its Total Diet Study, which achieved a detection limit of 1 ppb and a quantification limit of 3 ppb (Murray et al. 2008). Perchlorate has also been measured in plants (Nzengung et al. 1999; Smith et al. 2001; Urbansky et al. 2000c) and mammals, amphibians, fish, and insects (Dodds et al. 2004; Smith et al. 2001) using IC. Narayanan et al. (2003) described a method for measuring perchlorate in biological samples that uses IC coupled with conductivity detection. The detection limits determined for perchlorate in the fluids and tissues of rats were reported to be 3–6 ng/mL and 0.007–0.7 mg/kg, respectively. Perchlorate exposure

was previously assessed by using IC-conductivity to measure perchlorate in urine and serum (Lamm et al. 1999; Lawrence et al. 2000); however, the analytical methods lacked sensitivity (detection limit 500 ng/mL).

7.2 ENVIRONMENTAL SAMPLES

EPA method 314.0 (EPA 1999c) was developed for the analysis of perchlorate in drinking water samples by IC. This method reports a minimum detection limit of 0.53 μg/L and a minimum reporting limit of 4 μg/L. Separation of anions is accomplished on a Dionex IonPac AS5 ion chromatography column (or equivalent) using a 50 mM sodium hydroxide eluent. Sample detection is accomplished using a suppressed conductivity detector (Dionex CD20). Large concentrations of other anions, such as chloride, sulfate, or carbonate may interfere with the analysis. Perchlorate identification is based on retention time. Other variations of the IC method for determining perchlorate in water samples have also been described (Ellington and Evans 2000; Jackson et al. 1999, 2000; Liu and Mou 2003; Liu et al. 2002; Okamoto et al. 1999; Polesello et al. 2001; Tian et al. 2003). According to the Department of Defense Perchlorate Handbook, EPA Method 331.0 (Liquid Chromatography Electrospray Ionization Mass Spectrometry), and EPA Method 332.0 (Ion Chromatography with Suppressed Conductivity and Electrospray Ionization Mass Spectrometry) (EPA 1999c, 2005h, 2005i, 2005j) are the preferred methods for drinking water analysis at Department of Defense sites (DOD 2006a).

Another technique that is used to determine perchlorate in water samples is electrospray ionization mass spectrometry, which provides better analytical selectivity compared with conductivity detection (Urbansky 2000). This technique has been used to determine perchlorate in a variety of water samples (Ells et al. 2000; Koester et al. 2000; Magnuson et al. 2000). The detection limit of this technique is approximately 0.030 μg/L if microextraction using an organic solvent was employed before analysis (Urbansky 2000). Winkler et al. (2004) describe a method for detecting perchlorate in water and soil by ES liquid chromatography/MSMS. The method detection limits were 0.05 μg/L for water and 0.5 μg/kg for soil. U.S. Army Corp of Engineers (2004) described a calorimetric method for the field screening of water and soil samples. Detection limits were 1 μg/L for water and 0.3 μg/g for soil.

IC has also been used to analyze fertilizer samples for perchlorate (Collette et al. 2003; De Borba and Urbansky 2002; Urbansky and Collette 2001). In addition to IC, Collette et al. (2003) analyzed fertilizer for perchlorate using complexation electrospray ionization mass spectrometry, and high field asymmetric waveform mass spectrometry in addition to IC. These authors reported that using these techniques in

concert offers a more powerful approach since each method depends on a different property of perchlorate for detection.

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 perchlorates 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 perchlorates.

The following categories of possible data needs have been identified. 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. Perchlorate levels in human urine, milk, blood, or other tissue are biomarkers of exposure. In humans, perchlorate is primarily excreted in the urine; however, lactating mothers also excrete perchlorate in milk (Anbar et al. 1959; Kirk et al. 2005). IC-conductivity and IC-MS have been used to measure perchlorate in human milk as a biomarker of exposure (Kirk et al. 2005). Human exposure to perchlorate has also been assessed by using IC-ES-MSMS to measure perchlorate in urine (Valentín-Blasini et al. 2005). A similar analytical approach was used to measure perchlorate in amniotic fluid as a biomarker of exposure of the developing fetus (Blount et al. 2006). Serum levels of free iodine, T4, T3, and TSH hold potential as biomarkers of effect if they can be correlated with environmental exposures. These methods for measuring biomarkers of exposure and effect should improve the assessment of human exposure to perchlorate and potential health effects.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Surface water, groundwater, and drinking water have been monitored using EPA Method 314.0. Derivations of this ion chromatography method have been used to determine perchlorate in a

variety of different environmental media. Although further work to develop methods that can quantify perchlorate in a wider variety of matrices is required, this is currently a highly active area of research. These methods involve electrospray ionization mass spectrometry.

7.3.2 Ongoing Studies

No ongoing analytical methodology studies were located as a result of a search of Federal Research in Progress (FEDRIP 2008). It should be noted that new techniques are continually being applied to the IC method to allow a variety of different sample matrices to be analyzed. It should also be noted that additional information on the accuracy of other quantitative techniques that can be used to measure perchlorate is continually appearing in the scientific literature. Much of this work is being performed by both private and Governmental laboratories and, therefore, would not be cited in FEDRIP. Interested readers that require the latest information on analytical techniques that can be used to quantify perchlorate are urged to consult the scientific literature.