7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring hydrogen sulfide and carbonyl sulfide, their metabolites, and other biomarkers of exposure and effect to hydrogen sulfide and carbonyl sulfide. The intent is not to provide an exhaustive list of analytical methods, but 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

Hydrogen Sulfide. A limited number of analytical techniques have been used for measuring hydrogen sulfide in the breath (expired air) and sulfide in biological tissues and fluids including blood and saliva. These include gas chromatography coupled with flame ionization detection (GC/FID), gas chromatography coupled with flame photometric detection (GC/FPD), iodometric titration, potentiometry with ion-selective electrodes (ISE), spectrophotometry, and high-performance liquid chromatography (HPLC). The measurement of sulfide concentrations in biological materials is difficult due to its volatility, tendency to undergo oxidation, adsorption to glass and rubber, and binding to organic molecules (Richardson et al. 2000). Details of commonly used analytical methods for several types of biological media are presented in Table 7-1.

In air, hydrogen sulfide will exist in its molecular form, and methods are available to measure hydrogen sulfide in air. However, in aqueous solution, hydrogen sulfide is a weak acid, exhibiting two acid dissociation constants. The first dissociation yields bisulfide ion (HS^-) and the second yields sulfide ion (S^{2-}), with pK_a values for each of these dissociations of 7.04 and 11.96, respectively (O'Neil et al. 2001). In biological tissues and fluids, sulfide concentrations typically would be determined. The concentration of the un-ionized hydrogen sulfide can be calculated from the concentration of dissolved sulfide components (e.g., HS^-, S^{2-}), pH of the solution, and acidity constants for hydrogen sulfide using the following equilibrium expressions for the ionization of hydrogen sulfide and bisulfide ion (APHA 1998):

Table 7-1. Analytical Methods for Determining Hydrogen Sulfide, Sulfide, and Thiosulfate in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Blood	Hydrogen sulfide is generated in Kipp's apparatus and trapped in NaOH solution; pH is adjusted to 6.5–6.8, azide and excess iodine are added.	Iodometric method	4 µg/L	98–102	Puacz et al. 1995
Blood	Hydrogen sulfide is generated in Kipp's apparatus and trapped in NaOH solution; sulfide antioxidation buffer is added.	Potentiometry (ISE)	NR	98–102	Puacz et al. 1995
Blood	Liberation of blood sulfide by addition of acid; trapping of hydrogen sulfide gas in NaOH solution.	ISE	10 µg/L	NR	Lindell et al. 1988
Blood and urine	For thiosulfate detection, add 0.2 mL sample to mixture of 0.5 mL of 20 mM PFBBr solution in acetone, 0.05 mL of 5% sodium chloride. Vortex for 1 minute and add 2 mL of 25 mM iodine solution in ethyl acetate and 0.5 mL of internal standard solution (40 µM TBB in ethyl acetate). Vortex for 30 seconds and centrifuge at 2,500 rpm for 15 minutes, allow to stand for 1 hour.	GC/ECD	3 µmol/L	NR	Kage et al. 1997
Blood and urine	For sulfide detection, add 0.2 mL sample to mixture of 0.5 mL of 20 mM PFBBr solution in toluene, 2.0 mL of internal standard solution (10 µM TBB in ethyl acetate), and 0.8 mL of 5 mM tetradecyl-dimethylbenzyl ammonium chloride solution in oxygen-free water saturated with sodium tetraborate. Vortex 1 minute, add 0.1 g potassium dihydrogenphosphate as a buffer. Vortex for 10 seconds, centrifuge at 2,500 rpm for 10 minutes.	GC/ECD	0.3 µmol/L	NR	Kage et al. 1997

Table 7-1. Analytical Methods for Determining Hydrogen Sulfide, Sulfide, and Thiosulfate in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Urine	Freeze and store freshly voided urine samples at -25°C until analysis within 24 hours after exposure. Analyze urinary thiosulfate as its bromobimane product. Correct results for the excreted creatinine analyzed in the same samples.	LC	NR	92, 80	Kangas and Savolainen 1987
Blood and feces	Addition of zinc acetate to trap sulfide, followed by micro-distillation into NaOH solution to trap evolved hydrogen sulfide; analysis by ion chromatography.	IC/ECD	2.5 µmol/L	92–102 (feces) 79–102 (blood)	Richardson et al. 2000
Breath	Connect Teflon sampling probe to analyzer and syringe through sampling valve and loop; insert probe 4 cm into mouth between closed lips; withdraw 20 mL over 6 seconds into syringe; flush and fill the sample loop with 10 mL mouth air; carry sample to analysis in nitrogen gas.	GC/FID	10 µg/m^3 (7 ppb)	NR	Blanchette and Cooper 1976
Breath	Collect air from breathing zone using a midget impinger containing calcium hydroxide-calcium sulfide-arabinogalactan slurry; add solution of N,N-dimethyl-p-phenylene-diamine and ferric chloride.	Spectro-photometry	0.20 µg/m^3 (0.1 ppb)	80	NIOSH 1977a
Saliva	Collect 3 mL aliquot with sterile pipette; introduce into 2-ounce glass container and cap; incubate 24 hours at 37°C; withdraw through cap with gas-tight syringe.	GC/FID, microcoulo-metric titration	NR	NR	Solis and Volpe 1973

Table 7-1. Analytical Methods for Determining Hydrogen Sulfide, Sulfide, and Thiosulfate in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Brain, lung, and femoral muscle	For sulfide detection, add 0.2 g sample (minced) to mixture of 0.5 mL of 20 mM PFBBr solution in toluene, 2.0 mL of internal standard solution (10 µM TBB in ethyl acetate), and 0.8 mL of 5 mM tetradecyl-dimethylbenzyl ammonium chloride solution in oxygen-free water saturated with sodium tetraborate. Vortex 1 minute, add 0.1 g potassium dihydrogenphosphate as a buffer. Vortex for 10 seconds, centrifuge at 2,500 rpm for 10 minutes.	GC/MS	NR	NR	Kage et al. 1998
Brain, lung, and femoral muscle	For thiosulfate detection, add 0.2 g sample (minced) to mixture of 0.5 mL of 20 mM PFBBr solution in acetone, 0.05 mL of 5% sodium chloride and 0.5 mL of 200 mM L-ascorbic acid. Vortex for 1 minute and add 2 mL of 25 mM iodine solution in ethyl acetate and 0.5 mL of internal standard solution (40 µM TBB in ethyl acetate). Vortex 30 seconds and centrifuge at 2,500 rpm for 15 minutes, allow to stand for 1 hour.	GC/MS	NR	NR	Kage et al. 1998
Brain, liver, and kidney tissue (mouse)	Weigh sample; homogenize in aqueous zinc acetate using a rotostator at 18,000 rpm for 20 seconds; dilute with borate buffer; convert to methylene blue.	Ion-interaction reversed-phase HPLC	nmol/g	NR	Mitchell et al. 1993

Table 7-1. Analytical Methods for Determining Hydrogen Sulfide, Sulfide, and Thiosulfate in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Brain tissue (rat and human)	Homogenization in cold 0.01 M NaOH. Centrifuge and resuspend pellet; add zinc acetate and ascorbic acid; readjust pH; use continuous flow gas dialysis system to separate sulfide.	Gas dialysis/ion chromato-graphy with ECD	0.02 µg/g	95–99 (rat tissue)	Goodwin et al. 1989

[a]Conversion factor: 1 ppm=1.40 mg/m^3

ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; HPLC = high performance liquid chromatography; ISE = ion-selective electrode; LC = liquid chromatography; M = molar; MS = mass spectrometry; NaOH = sodium hydroxide; NR = not reported; PFBBr = pentafluorobenzyl bromide; rpm = revolutions per minute; TBB = 1,3,5-tribromobenzene

7. ANALYTICAL METHODS

$$Ka_1 = \frac{[HS^-(aq)][H^+(aq)]}{[H_2S(aq)]}$$

$$Ka_2 = \frac{[S^{2-}(aq)][H^+(aq)]}{[HS^-(aq)]}$$

Puacz et al. (1995) developed a catalytic method (based on the iodine-azide reaction) for the determination of sulfide in whole human blood. The method involves the generation of hydrogen sulfide in an evolution-absorption apparatus. In addition, the method allows for the determination of sulfide in blood without interference from other sulfur compounds in blood. This method is appropriate for the determination of sulfide in the concentration range of 4–3,000 µg/L. A percent recovery of 98–102% was achieved. Although the accuracy and precision of the catalytic method are comparable to those of the ion-selective electrode method, the catalytic method is simpler, faster, and would be advantageous in serial analysis.

Richardson et al. (2000) developed a method for measuring sulfide in whole blood and feces, which overcomes the problems of viscosity and turbidity that are typical for these types of samples. Turbidity of the sample interferes with colorimetric assays such as methylene blue. In this method, samples are first treated with zinc acetate to trap the sulfide as an insoluble zinc complex. Next, a microdistillation pretreatment is used to release the complexed sulfide into a sodium hydroxide solution. This microdistillation step is coupled to ion chromatography with electron capture detection. A detection limit of 2.5 µmol/L (80 µg/L) and percent recoveries of 92–102% (feces) and 79–102% (blood) were reported.

GC/FPD was employed for measuring hydrogen sulfide in human mouth air with a detection limit of 7 ppb (Blanchette and Cooper 1976) and included improvements such as calibration of the system with permeation tubes, use of a variable beam splitter to produce a wide range of vapor concentrations, and the ability to handle samples of limited volume.

For occupational measurements of airborne concentrations, NIOSH (1977a) recommended the use of a midget impinger for sampling breathing zone air and the methylene blue/spectrophotometric method for the analysis of hydrogen sulfide. The detection limit was 0.14 ppb.

GC/FID has been used for quantifying sulfur volatiles such as hydrogen sulfide in human saliva (Solis and Volpe 1973). This method included microcoulometric titrations and a procedure for incubation of saliva and sampling of headspace sulfur volatile components. The amount of total sulfur volatiles detected in control samples of saliva incubated at 37°C for 24 hours ranged from 4.55 to 13.13 ppm.

Fresh and frozen mouse tissue samples obtained from brain, liver, and kidney have been analyzed for hydrogen sulfide levels by sulfide-derived methylene blue determination using ion-interaction reversed-phase HPLC (Mitchell et al. 1993). This method can quantify nmol/g levels of sulfide. Gas dialysis/ion chromatography with ECD has been utilized for measurement of sulfide in rat brain tissue with 95–99% recovery (Goodwin et al. 1989). Goodwin et al. (1989) also applied this method to human brain tissue samples from two suspected hydrogen sulfide fatalities.

Carbonyl Sulfide. As carbonyl sulfide is believed to metabolize to hydrogen sulfide (Chengelis and Neal 1979), a limited number of tests were found for measuring carbonyl sulfide in biological samples. The following describes potential methods of identifying carbonyl sulfide in blood and breath samples. Analytical methods for biological media are presented in Table 7-2.

In blood samples, carbonyl sulfide can be detected using GC. Blood samples were obtained in heparinized or ethylenediaminetetraacetic acid (EDTA)-impregnated blood tubes, which were used to prevent clotting of the blood. Volatiles were then captured by cryogenic trapping and preconcentration on Tenax GC columns and analyzed by GC fitted with a Chrompack flame-photometric detector (Tangerman 1995).

Wang and Sahay (2009) reviewed several optical spectroscopic techniques that could be employed to detect carbonyl sulfide and other trace gasses in human breath. Cavity leak-out spectroscopy (CALOS) was used in the mid-infrared (MIR) region with a continuous wave CO_2 laser operating at 4.9 µm to detect carbonyl sulfide in breath and the ambient atmosphere. Detection limits of 9 and 7 ppt were reported for breath and atmosphere samples, respectively. Carbonyl sulfide was identified in breath samples using infrared spectroscopy and a Herriott cell with an effective path-length of 36 m. A Herriott cell is a multipass absorption cell that is commonly employed to measure low concentration components or samples that have low absorption in a spectral region. In the spectral range of 4.85–4.87 µm, a detection limit of 1.2 ppb was attained for carbonyl sulfide. A compact sensor which used a thermo-electrically cooled quantum cascade diode laser (QCDL) source operating at 4.86 µm was shown to be a

Table 7-2. Analytical Methods for Determining Carbonyl Sulfide in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Blood	Blood sampled in heparinized or EDTA-impregnated blood tubes followed by cryogenic trapping of volatiles on Tenax GC column	GC with flame-photometric detector		No data	Tangerman 1995
Breath	Direct sampling	Laser spectroscopy; continuous-wave CO_2 laser in MIR region	9 ppt	No data	Wang and Sahay 2009
Breath	Direct sampling	Laser spectroscopy using Herriott cell; spectral range of 4.85–4.87 µm	1.2 ppb	No data	Wang and Sahay 2009
Breath	Direct sampling	TDLAS-based compact absorption spectrometer with thermo-electrically cooled MIR tunable QCDL	30 ppb	No data	Wang and Sahay 2009

[a]Conversion factor: 1 ppm = 2.44 mg/m^3.

EDTA = ethylenediaminetetraacetic acid; GC = gas chromatography; MIR = mid-infrared; QCDL = quantum cascade diode laser; TDLAS = tunable diode laser absorption spectroscopy.

potentially useful sensor for measuring trace gases in breath samples. A 30 ppb detection limit was reported for carbonyl sulfide.

7.2   ENVIRONMENTAL SAMPLES

The methods most commonly used to detect hydrogen sulfide in environmental samples include GC/FPD, gas chromatography with electron capture detection (GC/ECD), iodometric methods, the methylene blue colorimetric or spectrophotometric method, the spot method using paper or tiles impregnated with lead acetate or mercuric chloride, ion chromatography with conductivity, and potentiometric titration with a sulfide ion-selective electrode. Details of commonly used analytical methods for several types of environmental samples are presented in Table 7-3.

Hydrogen Sulfide. Several methods for determining hydrogen sulfide in air have been investigated. GC/FPD has been widely used for analyses of hydrogen sulfide at levels ranging from 10^{-11} to 10^{-8} g/ 0.56 mL (EPA 1978; Stetter et al. 1977) and for hydrogen sulfide in emissions from tail gas controls units of sulfur recovery plants to a sensitivity of 0.5 ppmv (EPA 2000b). Sampling of a standard reference (0.055 ppm hydrogen sulfide) with this method resulted in a relative standard deviation of <3% (WHO 1981). The sensitivity of hydrogen sulfide detection in air was improved with GC/ECD (Stetter et al. 1977). The detector operation is based upon the measurement of the current when hydrogen sulfide is electrochemically oxidized at a diffusion electrode. Use of this method resulted in a lower detection limit of $3x10^{-12}$ g hydrogen sulfide and a precision of 0.5%. Analyses were achieved within 2 minutes. GC/FPD has been used to measure hydrogen sulfide that has been removed from air by activated carbon fiber (Choi et al. 1991). Activated carbon fiber (made from coal tar) effectively oxidized hydrogen sulfide (200 ppm) to sulfate.

Methylene blue techniques have been widely utilized for continuous, quantitative monitoring of hydrogen sulfide in air and are sensitive to hydrogen sulfide concentrations down to approximately 1–3 ppb (NIOSH 1977a). This method provides adequate specificity with good accuracy and precision (WHO 1981). The amount of sulfide is determined by spectrophotometric or colorimetric measurement of methylene blue. The method has been improved to eliminate the formation of the precipitate cadmium sulfide, which can result in the obstruction of the sampling impinger (Van Den Berge et al. 1985). The simplified method can also be used to measure hydrogen sulfide levels in the viscose rayon industry because it is not as sensitive to carbon disulfide. Limitations of the methylene blue method include potential interferences from light, mercaptans, sulfides, nitrogen dioxide, and sulfur dioxide, and that the

Table 7-3. Analytical Methods for Determining Hydrogen Sulfide and Sulfide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Air	Filter through a 0.5 µm Zefluor; absorb on a solid sorbent tube containing shell charcoal; desorb with ammonia hydroxide and hydrogen peroxide; dilute.	Ion chromatography, conductivity	11 µg/sample; working range 0.6–14 ppm for a 20-L air sample	NR	NIOSH 1994 (Method 6013)
Air	Aspirate through cadmium hydroxide; precipitate as cadmium sulfide; add STRactan 10®; react with N,N-dimethyl-p-phenylenediamine and ferric chloride to yield methylene blue.	Spectrophotometry	0.20 µg/m^3	80	Adams et al. 1975; EPA 1978; NIOSH 1977a
Air	Aspirate through sodium hydroxide and ethanol; react with N,N-dimethyl-p-phenylenediamine and ferric chloride to yield methylene blue.	Spectrophotometry	No data	NR	Van den Berge et al. 1985
Air	Absorb onto cadmium(II)-exchange zeolite; precipitate as cadmium sulfide; convert to methylene blue; measure at 750 nm.	PAS	0.01 µg	NR	NIOSH 1979
Air	Electrochemically oxidize sample at potential-controlled Teflon-bonded diffusion electrode.	GC/ECD	3 pg	NR	Stetter et al. 1977
Air	Introduce sampled air and carrier gas onto column.	GC/FPD	5–13 µg/m^3	NR	EPA 1978
Air	Introduce sample onto column packed with activated carbon filter.	GC/FPD	No data	NR	Choi et al. 1991
Air	Absorb in an impinger containing a standardized solution of iodine and potassium iodide; titrate with standard sodium thiosulfate solution.	Iodometric titration	No data	NR	EPA 1978
Air	Trap H_2S in aqueous NaOH and ascorbic acid in a midget impinger; titrate resulting sulfide ion with $CdSO_4$ solution.	Potentiometry	ppb levels	NR	Ehman 1976

Table 7-3. Analytical Methods for Determining Hydrogen Sulfide and Sulfide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Air	Aspirate through ammoniacal cadmium chloride; strip sulfur dioxide by aeration; dissolve cadmium sulfide; precipitate in concentrated HCl; titrate with iodine using a starch indicator.	Iodometric titration	0.7 µg/L	NR	EPA 1978
Air	Filter measured volume of air through lead-acetate-impregnated filter paper tape; compare optical density with unexposed impregnated spot of similar area.	Lead-acetate-impregnated filter paper tape	No data	NR	EPA 1978
Air	Filter measured volume of air through mercuric chloride-impregnated filter paper tape; compare optical density with unexposed impregnated spot of similar area.	Mercuric chloride-impregnated filter paper tape	0.7 µg/m^3 (0.5 ppb)	NR	EPA 1978
Air	Pass air through silver membrane filter.	Silver membrane filters/optical density measurements	No data	NR	EPA 1978
Air (fuel gas streams in petroleum refineries)	Hydrogen sulfide is absorbed on impingers containing cadmium sulfate, forming cadmium sulfide, which is measured iodometrically.	Iodometric titration	8–740 mg/m^3 (6–520 ppm)	NR	EPA 2000a
Air (emissions from stationary sources)	A gas sample is extracted from the emission source and diluted with clean dry air.	GC/FPD	0.5 ppmv	NR	EPA 2000b
Water	Add an amine-sulfuric acid reagent and a ferric chloride solution to the sample, mix gently; after 3–5 minutes add (NH$_4$)$_2$HPO$_4$ solution; analyze after 3–15 minutes.	Colorimetry	Applicable to sulfide concentrations ranging from 0.1 to 20.0 mg/L	89–92	APHA 1998 (Methylene Blue Method)
Water	For unpreserved samples, add solutions of zinc acetate, sodium hydroxide, and ascorbic acid, shake and let stand for 30 minutes; for preserved samples, omit the zinc acetate step.	Spectro-photometry	Applicable at sulfide concentrations from 0.002 to 0.100 mg/L	97.6–104.2	APHA 1998 (Gas Dialysis, Automated Methylene Blue Method)

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Table 7-3. Analytical Methods for Determining Hydrogen Sulfide and Sulfide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit[a]	Percent recovery	Reference
Water	To the sample, add an excess of standard iodine solution; back titrate with a sodium thiosulfate solution.	Iodometric titration	Accurate method for determining sulfide concentrations >1 mg/L	NR	APHA 1998 (Iodometric Method)
Water	Add an AAR and zinc acetate to the sample; measure the potential and compare to a calibration curve.	ISE	Applicable for sulfide concentrations >0.03 mg/L	NR	APHA 1998 (ISE Method)
Water	Collect water sample; acidify; strip sample with helium; collect gas in nitrogen-cooled trap.	GC/FPD	0.6 pmol/L	NR	Radford-Knoery and Cutter 1993
Water and sludge	Acidify sample to convert sulfide ion to hydrogen sulfide; measure hydrogen sulfide absorption at 196 nm using the selenium atomic line.	AAS	0.25 µg (1–10 mL sample volume)	NR	Parvinen and Lajunen 1994
Sediment	Acidify sample to convert sulfide ion to hydrogen sulfide; trap hydrogen sulfide in sodium hydroxide; sulfide reacts with N,N-dimethyl-p-phenylene-diamine to form methylene blue.	Colorimetry	0.01 µmol/g	NR	Allen et al. 1994
Sediment	Trap in silver nitrate solution as insoluble silver sulfide.	Gravimetry	10 µmol/g	NR	Allen et al. 1994
Sediment	Trap in a sulfide antioxidant buffer.	Potentiometry/ISE	No data	NR	Allen et al. 1994

[a]Conversion factor: 1 ppm=1.40 mg/m^3

AAR = alkaline antioxidant reagent; AAS = atomic absorption spectroscopy; CdSO$_4$ = cadmium sulfate; ECD = electron capture detector; FPD = flame photometric detection; GC = gas chromatography; HCl = hydrochloric acid; H$_2$S = hydrogen sulfide; ISE = ion-selective electrode; NaOH = sodium hydroxide; NR = not reported; PAS = photoacoustic spectroscopy

system is not portable (NIOSH 1977a). Photoacoustic spectroscopy of hydrogen sulfide converted to methylene blue has been demonstrated to yield greater sensitivity than standard spectrophotometric methods (NIOSH 1979). By maximizing instrument response to the 750-nm peak, it was possible to achieve a detection limit of 0.01 μg when collected at 2.0 L/minute for a 1-hour period.

NIOSH (Method 6013) describes the measurement of hydrogen sulfide in the air by ion chromatography (NIOSH 1994). This method has a working range of 0.6–14 ppm for a 20-L air sample and an estimated limit of detection of 11 μg per sample. However, sulfur dioxide may interfere with the measurement of hydrogen sulfide.

The iodometric method has been utilized in analyzing hydrogen sulfide in the air (EPA 1978). The method is based on the oxidation of hydrogen sulfide by absorption of the gas sample in an impinger containing a standardized solution of iodine and potassium iodide. This solution will also oxidize sulfur dioxide. The iodometric method is suitable for occupational settings. The accuracy of the method is approximately 0.50 ppm hydrogen sulfide for a 30-L air sample (EPA 1978). Another application of the iodometric method is for the determination of hydrogen sulfide in fuel gas emissions in petroleum refineries (EPA 2000a). In this method, the sample is extracted from a source and passed through a series of impingers containing cadmium sulfate. The hydrogen sulfide is absorbed, forming cadmium sulfide, which is then measured iodometrically. The sensitivity range of this method is 8–740 mg/m^3 (6–520 ppm) (EPA 2000a).

Paper tapes impregnated with lead acetate have been widely used for air sample measurements of hydrogen sulfide in the field (EPA 1978; WHO 1981). The presence of other substances capable of oxidizing lead sulfide can lead to errors. This method has been improved by impregnating the paper with mercuric chloride or silver nitrate (EPA 1978; WHO 1981). Mercuric chloride paper tape is sensitive and reliable for measurement of hydrogen sulfide in air with a sensitivity of 0.7 μg/L (EPA 1978). Tapes impregnated with silver nitrate are suitable for determination of hydrogen sulfide concentrations in the range of 0.001–50 ppm (WHO 1981).

Potentiometric titration with a sulfide ion-selective electrode as an indicator has been used to measure hydrogen sulfide in the air at ppb levels (Ehman 1976). This method has been shown to have very good accuracy and precision. No interference could be found from nitrogen dioxide, sulfur dioxide, or ozone.

Passive card monitors can be used to detect hydrogen sulfide in workplace environments (Saunders et al. 2002). These monitors can be categorized as quantitative, semiquantitative, and indicator cards. Quantitative cards use an optical reader to assess exposure and calculate a hydrogen sulfide concentration in air; the results are digitally displayed. Semiquantitative cards are read by comparing the exposed card to a chart or by observing a progressive color development in windows on the card that represent differing exposure concentration ranges. The indicator cards change color above a certain threshold concentration of hydrogen sulfide. Saunders et al. (2002) reported detection limits of 4–8 and 0.8–4 ppm-hours for two commercial quantitative card monitors, 1 and 0.1 ppm-hours for two commercial semiquantitative card monitors, and 0.05 and 0.4 ppm-hour for two commercial indicator card monitors.

Badges that can be worn in a worker's breathing zone that change color based on exposure to toxic gases (including hydrogen sulfide) are available from American Gas & Chemical Co. The sensitivity for the hydrogen sulfide badges is 10 ppm/10 minutes with a color change from white to brown (American Gas & Chemical Co. 2005; Ho et al. 2001). Other colorimetric methods for monitoring of hydrogen sulfide include handheld colorimetric tubes. Air is drawn through the tube and a color change indicates the presence of hydrogen sulfide by reaction with a chemical reagent in the glass tube. Tubes for hydrogen sulfide are available from Draeger Safety, Inc. in various measuring ranges from 0–5 to 100–2,000 ppm (Draeger Safety 2005).

Electrochemical sensors are the most commonly used sensors for toxic gases (including hydrogen sulfide) and are the best sensor for ambient toxic gas monitoring. These sensors are specific to a particular gas, are very accurate, do not get poisoned, and monitor at the ppm level. However, they have a narrow temperature range and a short shelf life, particularly in very hot and dry areas. When sensitivity to low concentrations of hydrogen sulfide (ppm levels) is needed, semiconductor sensors are one of the best sensors. Some advantages of semiconductor sensors for hydrogen sulfide include small size, ruggedness, and sensitivity to ppm concentrations. Disadvantages include slow response on aged sensors, requirement of a temperature controlled heater, and cost (Delphian Corporation 2005).

The Iowa Department of Natural Resources (DNR) monitors airborne levels of ammonia, hydrogen sulfide, and odor concentrations near animal feeding operations. Approved monitoring methods and equipment for the hydrogen sulfide must incorporate a thermal oxidizer and an EPA reference method analyzer that is designed for sulfur dioxide. There are several instruments that meet the requirements for the Iowa DNR, all of which detect hydrogen sulfide by first oxidizing it to sulfur dioxide, which is then measured using a fluorescence detector. The hydrogen sulfide and total reduced sulfide analyzer (Model

101A) from Advanced Pollution Instrumentation, Inc. has a range of 0–50 ppb to 0–2 ppm for hydrogen sulfide. In addition, three hydrogen sulfide analyzers from Thermo Electron Corporation are also approved by the Iowa DNR. Minimum detection limits of 0.5 ppb can be achieved for models 45C and 450C and 0.06 ppb can be achieved for model 450C-TL (API 2005; Iowa DNR 2004, 2005; Thermo Electron Corp. 2005a, 2005b, 2005c).

The APHA (1998) defines three categories of sulfides that must be taken into account for analytical methods measuring sulfides in water: total sulfide, dissolved sulfide, and un-ionized hydrogen sulfide. Total sulfide includes all sulfide containing species, dissolved hydrogen sulfide, bisulfide ion, and acid-soluble metal sulfides in suspended matter. Dissolved sulfide includes sulfide-containing components that remain after suspended solids have been removed. The concentration of the un-ionized hydrogen sulfide can be calculated from the concentration of dissolved sulfide components, pH of the solution, and the acidity constants for hydrogen sulfide using the equilibrium expressions for the ionization of hydrogen sulfide and bisulfide ion (APHA 1998).

Samples that contain sulfide species can be either analyzed immediately after collection, or preserved with a zinc acetate solution for later analysis (APHA 1998). The addition of zinc ion (Zn^{2+}) to the sample will precipitate any sulfides as zinc sulfide. A qualitative sulfide test (such as a precipitation test using potassium antimony tartrate or testing for hydrogen sulfide vapors using lead acetate paper or silver foil) can be useful and are advisable when testing industrial wastes that may contain substances that interfere with certain test methods, such as the methylene blue method (APHA 1998).

The total sulfide concentration in a water sample can be determined using an iodometric titration. In this method, sulfide is reacted with a measured excess of iodine in an acidic solution; the remaining unreacted iodine is then determined by titration with a thiosulfate solution. This method is an accurate method for determining sulfide concentrations of >1 mg/L, if interferences are absent and the loss of hydrogen sulfide from the solution is avoided. The iodometric method is best suited for the analysis of samples freshly taken (i.e., from wells and springs) (APHA 1998).

The methylene blue method is applicable to sulfide concentrations ranging from 0.1 to 20.0 mg/L. In this method, an amine-sulfuric acid reagent and a ferric chloride solution are added to the sample to produce methylene blue, which is then quantified colorimetrically. In the automated methylene blue method, a gas dialysis technique separates the sulfide from the sample matrix, which removes most inferences (i.e.,

turbidity and color). Addition of ascorbic acid, an antioxidant, improves sulfide recovery. The automated methylene blue method is applicable at sulfide concentrations from 0.002 to 0.100 mg/L (APHA 1998).

Potentiometric methods using a silver electrode are also suitable for determination of sulfide concentrations in water and are unaffected by sample color or turbidly. In this method, an alkaline antioxidant reagent (AAR) and zinc acetate are added to the sample. The potential of the sample is measured using an ISE and the measurement is compared to a calibration curve. This method is applicable for sulfide concentrations >0.03 mg/L (APHA 1998).

Three methods for quantifying acid volatile sulfides in sediment have been described (Allen et al. 1994). These include methylene blue/colorimetric methods, gravimetry, and potentiometry with an ion-selective electrode. Prior to measurement, the acid volatile sulfide in the sample is converted to hydrogen sulfide by acidification. The hydrogen sulfide is then purged from the sample and trapped in aqueous solution for the colorimetric and potentiometric methods. In the gravimetric method, hydrogen sulfide is trapped with silver nitrate ($AgNO_3$), and the mass of the insoluble silver sulfide (Ag_2S) that is formed is determined. The methylene blue/colorimetric method is generally preferred and is capable of determining acid volatile sulfide concentrations in sediment as low as 0.01 μmol/g (0.3 μg/g) dry weight. The gravimetric method can be used for samples with moderate or high acid volatile sulfides. However, below concentrations of acid volatile sulfides in dry sediment of 10 μmol/g (320 μg/g), accuracy may be affected by incomplete recovery of precipitate or by weighing errors. The limit of detection of the ion-selective electrode method as applied to measuring hydrogen disulfide in sediment was not reported.

GC/FPD has been used to measure hydrogen sulfide, free (uncomplexed) sulfide, and dissolved metal sulfide complexes in water (Radford-Knoery and Cutter 1993). Hydrogen sulfide was measured in the headspace of the sample (100 mL) with a detection limit of 0.6 pmol/L (20 pg/L). A detection limit of 0.2 pmol/L (6 pg/L) was obtained for total dissolved sulfide. This method allows for the determination of the concentration of free sulfide that is in equilibrium with hydrogen sulfide. Complexed sulfide can be estimated from the difference between total dissolved sulfide and free sulfide.

A molecular absorption spectrophotometry method using a sharp-line irradiation source has been developed for the determination of sulfide (as hydrogen sulfide) in water and sludge samples. The method was tested with measurements of real waste water samples. The limit of detection was 0.25 μg (1–10 mL sample volume) (Parvinen and Lajunen 1994).

Carbonyl Sulfide. Carbonyl sulfide can be detected in environmental samples using various gas chromatography methods, as well as cell-based sensors. Details of commonly used analytical methods to detect carbonyl sulfide in several types of environmental samples are presented in Table 7-4. Air concentrations of carbonyl sulfide can be determined using gas chromatography. Sulfur compounds are collected in a small glass tube that is packed with Tenax GC, and concentrated at -196°C. Water is removed from the air samples using calcium chloride. The Tenax tube is placed in the GC, where sulfur compounds are released at 200°C into a carrier gas stream and analyzed using a flame photometric detector. After 1 week of storage at -196°C, 91% of carbonyl sulfide was recovered (Tangerman 1986).

Blake et al. (2004) also determined carbonyl sulfide concentrations in the atmosphere utilizing a mass spectrometer method. From an aircraft, whole air samples were collected using an external air intake to fill 2 L stainless steel canisters to approximately 4 atmospheres of pressure. Samples were pre-concentrated at liquid nitrogen temperatures (-196°C) on a stainless steel loop filled with glass beads, which was then put in hot water to evaporate the sample. The sample was flushed to a splitter, where it broke into five streams and went to one of five column-detector combinations. Concentrations were determined using a DB-5ms column with an HP-5973 quadrupole Mass Spectrometric Detector, which was used in single ion monitoring (SIM) mode. Carbonyl sulfide was measured with a detection limit of 20 pptv (Blake et al. 2004).

The NASA Chemical Instrumentation Test and Evaluation 3 (CITE 3) method evaluates carbonyl sulfide concentrations in air. The method utilizes a gas chromatograph/mass spectrometer (GC/MS) and an isotopically labeled compound as an internal standard (GC/MS/ILS). GC/MS/ILS uses an isotopomer of the analyte to calibrate every sample. The detection limit of this method has been reported as 4 fmol/s (Bandy et al. 1993).

Oceanic air samples have been analyzed for carbonyl sulfide. Samples were collected in 6- and 800-mL stainless steel bottles using a Metal Bellows MB-155 pump to fill to an internal pressure of 30 psi. Samples in 35- and 1.6-L bottles were collected cryogenically (which increases stability) by putting the canister on liquid nitrogen and utilizing the internal vacuum to collect the sample. The samples were analyzed using a Finnigan 4021 GC/MS data system (Rasmussen et al. 1982a).

Carbonyl sulfide can also be monitored in air using a pyrolyzer-electrochemical cell-based sensor, such as the MIDAS gas detector. The system utilizes an internal pump to sample the air, which is then fed to a pyrolyzer. It converts carbonyl sulfide to hydrogen sulfide by pyrolysis at high temperatures and in

Table 7-4. Analytical Methods for Determining Carbonyl Sulfide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Whole air samples from aircraft; preconcentrated at -196°C, followed by hot water evaporation and split into five streams.	MS in SIM mode	20 pptv		Blake et al. 2004
Air	Collected in glass tube with Tenax GC; concentrated at -196°C; water removed with calcium chloride.	GC with flame photometric detector		91%	Tangerman 1986
Air	Air sample.	GC/MS/ILS	4 fmol/s		Bandy et al. 1993
Air	Internal pump to sample air; pyrolyze carbonyl sulfide to hydrogen sulfide at ambient moisture.	Pyrolyzer-electrochemical cell-based sensor	20 ppm		Chase et al. 2010
Air	Collected in steel bottles with pump or cryogenically.	GC/MS data system			Rasmussen et al. 1982a
Seawater	Air samples equilibrated with seawater; separated on chromatographic column.	Flame photometric sulfur detector			Meinrat et al. 1992
Seawater	Collected in vacuum extraction flasks; air removed.	GC/MS data system			Rasmussen et al. 1982a

GC = gas chromatography; GC/MS/ILS = gas chromatography/mass spectrometry with isotopically labeled compound as internal standard; MS = mass spectrometry; SIM = single ion monitoring

ambient moisture. An electrochemical sensor cartridge is then used to determine the hydrogen sulfide content. The limit of detection for carbonyl sulfide was reported as 20 ppm (Chase et al. 2010).

The optical sensors reviewed by Wang and Sahay (2009) and discussed in Section 7.1 for potential use in exhaled breath analysis could also be employed to analyze for carbonyl sulfide in ambient air.

The concentration of dissolved carbonyl sulfide in seawater can be determined by equilibrating air samples with seawater. The equilibrated air samples are then sent through a cold trap that is submerged in liquid nitrogen. Trace gases are then separated on a chromatographic column and the carbonyl sulfide saturation ratio is determined using a flame photometric sulfur detector. The dissolved carbonyl sulfide concentration is calculated from the saturation ratio using Henry's law (Meinrat et al. 1992).

In another study, samples of seawater were obtained using 1,000 mL vacuum extraction flasks, constructed of Erlenmeyer flasks with a Kovar-to-glass seal on the top connected to a valve. Bottles were filled such that the headspace air and water sample were in equal volumes. The flask was placed in a heated chamber at 100°C and the air was pumped out with a vacuum for one hour to keep a pressure of 0.1 torr. The sample was then collected by submerging the flask and releasing the valve or by removing the sample under vacuum by putting the water sample in a funnel and opening the valve. The samples were analyzed using a Finnigan 4021 GC/MS data system and the concentration of carbonyl sulfide was calculated using Henry's law and the headspace concentration (Rasmussen et al. 1982a).

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 hydrogen sulfide and carbonyl sulfide 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 hydrogen sulfide and carbonyl sulfide.

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. Methods are available for measuring hydrogen sulfide in expired air (Blanchette and Cooper 1976; NIOSH 1977a). Methods are available for measuring sulfide in blood (Puacz et al. 1995; Richardson et al. 2000) and brain tissue (Goodwin et al. 1989) and for measuring sulfur volatiles in saliva (Solis and Volpe 1973). Methods are available for measuring thiosulfate levels in urine (Kage et al. 1992; Kangas and Savolainen 1987; Milby and Baselt 1999). Analytical methods with satisfactory sensitivity and precision are available to determine levels of hydrogen sulfide and thiosulfate in human tissues and body fluids. Methods that can quantitatively correlate levels in biological fluids and tissues with environmental exposure levels would be useful in estimating exposure to hydrogen sulfide. Limited methods are available for determining carbonyl sulfide concentrations in biological samples (Tangerman 1995; Wang and Sahay 2009) since it is likely that carbonyl sulfide metabolizes to hydrogen sulfide in biological organisms.

Effect. No methods have been identified that can be used to directly associate levels of hydrogen sulfide in biological samples with the onset of adverse health effects.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media.   Methods are available for measuring hydrogen sulfide in air (Ehman 1976; EPA 1978, 2000a, 2000b; NIOSH 1977a, 1979, 1994; Stetter et al. 1977; Van Den Berge et al. 1985; WHO 1981). Methods are available for measuring sulfide in sediment (Allen et al. 1994), water (APHA 1998; Radford-Knoery and Cutter 1993), and sludge (Parvinen and Lajunen 1994). Ho et al. (2001) reviewed sensors and technologies that can be used to monitor various chemicals (including hydrogen sulfide) with particular attention to sensors that have the potential to be used for long-term monitoring applications. Since hydrogen sulfide is part of the natural environment, dissociates in aqueous solution, and can bind to various metal ions in environmental media, in most cases, it would not be possible to distinguish the specific source of sulfide ions in environmental media. In the event of a release of hydrogen sulfide, increased sulfide concentrations in surrounding environmental media would likely be due to the release.

7.3.2 Ongoing Studies

No ongoing studies for examining analytical methods to detect hydrogen sulfide or carbonyl sulfide levels were identified.