

7. ANALYTICAL METHODS

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

Carbon monoxide forms a strong coordination bond with the iron atom complex of Hb forming COHb, which is a unique biomarker for carbon monoxide exposure for humans. COHb absorbs radiation in the blue wavelength region of the visible spectrum known as the Soret region (390–440 nm) and COHb levels in blood can be analyzed spectrophotometrically (Rodkey et al. 1979). Carbon monoxide-oximeters are specialized medical instruments that are employed in clinical and hospital settings to rapidly determine COHb, O₂Hb, and methemoglobin (MetHb) levels in blood by taking the difference between the light absorption at each of these Hb derivatives and the total light absorption for all Hb derivatives that are present in the blood sample (Mahoney et al. 1993). This instrument is particularly useful for measuring high levels of COHb. Fetal Hb and bilirubin have the potential to interfere with spectrophotometric determination of COHb if present in sufficient quantities. Methylene blue cardiac dye and sulfhemoglobin can also adversely affect these measurements. In addition, some carbon monoxide-oximeters have been shown to overestimate low concentrations of COHb as compared to gas chromatography (GC) methods (Mahoney et al. 1993).

GC with reduction gas detectors is a selective and sensitive method to measure the amount of carbon monoxide bound to Hb in the blood and is considered a highly reliable method for measuring exposure to carbon monoxide, especially at low levels. The addition of potassium ferricyanide to Hb-bound carbon monoxide oxidizes Hb to MetHb and releases carbon monoxide, which is injected in the gas chromatograph (Vreman et al. 1984). Carbon monoxide eluted from the GC column reacts with mercuric

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oxide (HgO) to form gaseous mercury vapor; the mercury released and measured spectrophotometrically at 254 nm is proportional to the amount of carbon monoxide gas released. The detection limits for this method were reported as 0.05 nL of carbon monoxide per μL of blood, corresponding to a saturation of approximately 0.005% COHb in blood (Vreman et al. 1984).

Carbon monoxide levels in humans can be estimated by the analysis of exhaled breath using GC and electrochemical devices. In many measurement techniques, the subject performs an inhalation-breathhold maneuver and exhales through a mouthpiece into the instrument inlet. The alveolar air is retained for analysis, and the reading in ppm of carbon monoxide can be converted to COHb using a calibration curve (EPA 2000). A rapid and sensitive technique for the analysis of carbon monoxide in a single exhaled breath sample has been described (Fritsch et al. 2007). This analysis requires the subject to exhale into a mouthpiece that is attached to a Nafion[®] dryer and cooling trap to remove water, CO₂, and other possible interfering species. Detection is by high resolution infrared cavity ring-down laser spectroscopy in a multipath absorption cell. Amperometric electrochemical sensors have also been used for the analysis of carbon monoxide in exhaled breath and ambient air (Vreman et al. 1994). These sensors typically consist of a series of electrodes (working, reference, and counter electrodes) immersed in an electrolyte solution. Carbon monoxide diffuses through a porous Teflon membrane and is oxidized at the working electrode. The transfer of electrons that accompanies the redox reaction yields the output signal of the sensor. A reference electrode measures the potential of the electrolyte. The current flowing through the working electrode is compensated by the third electrode, a counterelectrode through which an equal and opposite current flows (Vreman et al. 1994).

The analytical methods used for the detection of carbon monoxide in biological samples are summarized in [Table 7-1](#).

7.2 ENVIRONMENTAL SAMPLES

Analytical methods for the detection of carbon monoxide in environmental samples have been thoroughly discussed in several review articles (EPA 1991, 2000; Novelli 1999). [Table 7-2](#) provides a summary of many of these analytical methods that are commonly employed in environmental monitoring studies of carbon monoxide. Analytical methods used to measure carbon monoxide in environmental samples require the use of high purity standard reference gas in order to frequently calibrate the instrument and ensure consistent measurements between different laboratories.

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Table 7-1. Analytical Methods for Determining Carbon Monoxide in Biological Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Collect sample and transfer to a 5 mL heparinized syringe and shake for 1 minute before analysis.	Spectrophotometric determination using CO-oximeters	<5% COHb		Mahoney et al. 1993
Blood	Blood placed into 2 mL CO-free GC vial containing 100 g/L $K_3Fe(CN)_6$ in 0.1 mol/L potassium phosphate buffer, pH 6.0, containing 10 g of saponin/L.	GC with mercury reduction gas detector	0.005% COHb		Mahoney et al. 1993
Blood	Obtain blood by heel stick (infant) or fingerstick (adult) and venipuncture. Mix with $K_3Fe(CN)_6$ containing solution to liberate carbon monoxide to a headspace.	GC with mercury reduction gas detector	0.005% COHb		Vreman et al. 1984
Breath	Breath samples collected with a reverse syringe pump and analyzed within 30 minutes or kept on ice until analysis within 3 hours.	EC sensor or GC with mercury reduction gas detector	EC: 0.1 μ L carbon monoxide/L air GC: 50% of the noise level of 0.01 μ L carbon monoxide/L air		Vreman et al. 1994

COHb = carboxyhemoglobin; EC = electrochemical; GC = gas chromatography; $K_3Fe(CN)_6$ = potassium ferricyanide

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Table 7-2. Analytical Methods for Determining Carbon Monoxide in Environmental Samples

Sample matrix	Preparation method ^a	Analytical method	Sample detection limit ^b	Percent recovery	Reference
Air	Atmospheric constituents separated using two-column isothermic chromatography in a temperature-controlled oven, followed by analysis using FID, HgO/UV absorbance, or ECD.	GC-FID GC-HgO/UV GC-ECD	25–50 ppbv GC-FID 10 ppb GC-ECD 1–2 ppb GC-HgO/UV	NR	Novelli 1999
Air	Air samples passed through reference and measurement optical cells. An optical filter is used to transmit only a small band width of infrared radiation.	NDIR-GFC	22–70 ppbv	NR	Novelli 1999
Air	Air samples introduced into commercial instruments over a wide spectral bandwidth or the use of optical filters can be employed to narrow the spectral wavelength.	FTIR	1 ppbv	NR	Novelli 1999
Air	Tune laser to 4.7 μm and measure absorption by carbon monoxide gas.	TDLS	<1 ppbv	NR	Novelli 1999
Air	Measure in fluorescence chambers operated at low pressure to avoid O ₂ absorption.	RF	5 ppbv	NR	Novelli 1999
Workplace air	Each sample collected by drawing a known volume of air into a five-layer aluminized gas sampling bag. A portion of the gas sample is introduced into a gas sampling loop, injected into a gas chromatograph, and analyzed using a discharge ionization detector.	GC-DID	0.12 ppmv; (0.400 ppmv quantitative)		OSHA 1991 (Method 210)

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Table 7-2. Analytical Methods for Determining Carbon Monoxide in Environmental Samples

Sample matrix	Preparation method ^a	Analytical method	Sample detection limit ^b	Percent recovery	Reference
Sea water	Water samples siphoned into a glass stopcock and a portion of the water replaced with carbon monoxide free synthetic air to create a headspace. The flask was shaken vigorously by hand for 1 minute and kept standing at room temperature for 5 minutes in the dark.	GC-FID	6 nL of carbon monoxide/L of water		Ohta 1997
Soil gas	Samples collected using a 1-cm ³ syringe fitted with a 50-mm-long 26-gauge side port needle. A 1-cm ³ sample was obtained (sampling time, about 60 seconds); the syringe was then raised, and its contents were rapidly analyzed in the field by using GC.	GC-HgO/UV	<5 ppbv	NR	King 1999b

^aHydrogen-rich carrier gas is used to reduce carbon monoxide to methane prior to analysis by FID and N₂O is added to a nitrogen rich carrier gas for analysis by ECD. Helium gas is used as the carrier gas for analysis by DID.

^bMeasurements of atmospheric carbon monoxide levels are relative to high purity reference gas used to calibrate the instruments.

DID = discharge ionization detector; ECD = electron capture detector; FID = flame ionization detector; GC = gas chromatography; FTIR = Fourier transform spectroscopy; GFC = gas filter correlation; HgO = mercuric oxide; NDIR = non-dispersive infrared; NR = not reported; RF = resonance fluorescence; TDLS = tunable diode laser spectroscopy; UV = ultraviolet

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Most techniques used for the measurement of carbon monoxide in the environment employ spectroscopic methods or GC. Non-dispersive infrared (NDIR) spectroscopy and GC using flame ionization detection (FID) are the two most common methods used to analyze carbon monoxide in environmental samples. Commercial instruments are capable of detection limits in the ppb range. For analysis by NDIR spectroscopy, air is pumped into sample cells and the absorption is measured at a carbon monoxide vibrational band at $2,174\text{ cm}^{-1}$ ($4.7\text{ }\mu\text{m}$). An optical filter is employed before the sample to remove light of other frequencies. Most NDIR spectrometers use gas filter correlation (GFC) methodology to improve sensitivity and reduce interferences by other gasses. In this method, the sample is simultaneously passed through both a reference cell containing a high concentration of carbon monoxide and a sample cell containing nitrogen gas. Gas species other than carbon monoxide will attenuate radiation equally in both cells; however, when carbon monoxide is present, the amount of light passing through the reference cell is unchanged, but that passing through the sample cell is absorbed at the characteristic carbon monoxide frequency. This difference in absorption between the light passing through the cells is linearly related to carbon monoxide concentrations in the air sample (EPA 2000).

In GC analysis of carbon monoxide, air samples are pumped through a silica gel or alumina pre-column followed by an analytical column composed of a molecular sieve. The pre-column captures CO_2 , non-methane hydrocarbons, and water, and the molecular sieve effectively separates carbon monoxide from hydrogen gas and methane. Since the sensitivity for carbon monoxide by FID is low, a hydrogen-rich carrier gas is used to reduce carbon monoxide to methane using heated nickel as a catalyst prior to FID analysis. Minimum detection limits of 0.05 ppmv or better have been achieved (Novelli 1999). With the development of the discharge ionization detector (DID) for use with GC analysis, it is possible to measure carbon monoxide concentrations directly at low levels in occupational settings (OSHA 1991). Helium is generally used as the sample carrier gas and as the ionized species. Helium passes through a chamber where a glow discharge is generated and high-energy photons are produced. The high-energy photons pass through an aperture to a second chamber and ionize the carbon monoxide in the sample stream. The resulting ions produce an electrical current, which is measured with a standard electrometer (OSHA 1991). The greater the concentration of carbon monoxide in the sample, the more ions will be produced, resulting in a greater current. OSHA method 210 discusses the GC-DID analysis for carbon monoxide and reports a detection limit of 0.12 ppmv and quantification limit of 0.40 ppmv.

As discussed in Novelli (1999), electron capture detection (ECD) can also be used in conjunction with GC to measure atmospheric levels of carbon monoxide. Carbon monoxide does not elicit a strong

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response from the ECD because it does not easily capture electrons; however, the addition of N_2O to a N_2 carrier gas improves sensitivity. In addition, GC/mass spectrometry, GC with mercury reduction gas detection, Fourier transform infrared (FTIR) spectroscopy, tunable diode laser spectroscopy (TDLS), and resonance fluorescence spectroscopy have also been used to measure carbon monoxide levels in air (Novelli 1999).

Commercially available electrochemical carbon monoxide detectors for home use can be purchased at most hardware or home improvement stores. These detectors use electrodes that reside in an electrolyte solution such as dilute sulfuric acid. Carbon monoxide is oxidized at one electrode to CO_2 and O_2 is consumed at the other electrode (Vreman et al. 1994). Measurement of the current in the cell provides a measure of the concentration of carbon monoxide in the atmosphere. Metal oxide semiconductor detectors such as tin oxide sensors operate on the principle that the resistance of a tin oxide layer decreases as carbon monoxide adsorbs to the surface of the sensor and is oxidized to CO_2 (Dobos and Zimmer 1985). When the conductance exceeds a preset threshold level, an 85 dB alarm is sounded (the same loudness as a smoke alarm). Biomimetic detectors use a gel-coated disc containing synthetic Hb that turns black when carbon monoxide is present at high levels. Reduction in the amount of transmitted light through the disc alerts the sensor to possible carbon monoxide contamination and sets off the alarm. Early carbon monoxide detectors were designed to provide an audible warning at relatively low levels of exposure and were often difficult to reset (Leikin 1996). Furthermore, the general public's knowledge regarding the use and placement of these alarms in the residence was limited. Recent changes to the design and standards of these alarms have made them more user friendly to the general population and helped to eliminate low-level activations, which can be caused by a variety of common occurrences. The use and effectiveness of these home-based alarms, including guideline standards from Underwriters Laboratory, have been reviewed by Rhee and Leikin (2007).

Ohta (1997) discussed a GC-FID method for the detection of carbon monoxide in seawater. Water samples were collected in a glass stopcock and a portion of the water was replaced with carbon monoxide free synthetic air to create a headspace. The flask was shaken vigorously by hand for 1 minute and kept standing at room temperature for 5 minutes in the absence of sunlight in order to complete the equilibration between aqueous and gaseous phases. A small portion of air (1–2 mL) was injected into the GC for analysis. Carbon monoxide concentrations were corrected based on equilibrium constants of carbon monoxide between the aqueous and gaseous phases in the flask for headspace preparation. The concentrations were further corrected for water vapor contained in the headspace gas at concentrations of

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3.8–4.1% in volume at 26–27 °C. The detection limit was reported as 6 nanoliters of carbon monoxide per liter of water.

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 carbon monoxide 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 carbon monoxide.

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. COHb is a unique biomarker of exposure for humans to carbon monoxide and accurate, sensitive methods exist for its measurement (Mahoney et al. 1993). GC methods are also available for measuring Hb-bound carbon monoxide (Vreman et al. 1984).

Effect. No specific biomarkers of effect for carbon monoxide were identified. Effects and symptoms of carbon monoxide poisoning are generally related to tissue hypoxia and are not specific for carbon monoxide exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental

Media. Sensitive analytical methods are available for determining the levels of carbon monoxide in air samples. Thorough reviews on this subject are available from the EPA (1991, 2000) and Novelli (1999). Analytical methods are also available for the determination of carbon monoxide in water samples (Ohta 1997).

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7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2009) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified. Platypus Technologies LLC located in Madison, Wisconsin is developing a liquid crystal-based sensor for real-time detection of ozone, carbon monoxide, and CO₂. Kestrel Labs Inc. located in Boulder, Colorado is developing a new carbon monoxide-oximeter that can use laser light sources for accurate photoplethysmographic measurements of COHb.