6. ANALYTICAL METHODS

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

6.1 BIOLOGICAL SAMPLES

A limited number of analytical techniques have been used for measuring carbon disulfide and its metabolites in breath (expired air) and biological fluids of humans and animals. These include gas chromatography (GC) and high resolution gas chromatography (HRGC) equipped with an appropriate detector, high-performance liquid chromatography (HPLC), spectrophotometry, thin-layer chromatography (TLC), combined gas chromatography and mass spectroscopy (GC/MS), and iodineazide tests (see Table 6-l).

GC/MS was used in a 1992 study (Phillips 1992) to detect carbon disulfide in human breath and environmental air with a detection sensitivity capable of 7.61×10^{-2} μg/m^3 (2.44×10^{-2} ppb). This highly sensitive technique can be rapidly accomplished by capturing the air sample on solid sorbent like molecular sieves to be later thermally desorbed in the laboratory with subsequent chromatography (Phillips 1992).

Gas chromatography equipped with a flame ionization detector (FID) and quadrupole MS have been employed for measuring carbon disulfide concentrations in the breath of workers following exposure to carbon disulfide (Campbell et al. 1985; Wells and Koves 1974). The MS technique is rapid and requires no sample preparation (Campbell et al. 1985). A detection limit of 1.6 ppb (5 μg/m^3) of

TABLE 6-1. Analytical Methods for Determining Carbon Disulfide and Its Metabolites in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Breath (expired air)	Exhale into a respiratory MS	Quadrupole MS	5 μg/m^3	NR	Campbell et al. 1985
	Bubble sample through a solution of diethylamine in acetone and methyl iodide; acidify and extract sample with butyl chloride	GC/FID	No data	NR	Wells and Koves 1974
	Capture in a sorbent trap containing graphitized carbon and molecular sieve; thermally desorb; concentrate	GC/MS	76 ng/m^3	NR	Phillips 1992
Blood	Acidify blood sample and introduce into a headspace analyzer	GC/FPD	15 μg/L	NR	Campbell et al. 1985
	Add acid and Viles' reagent to sample and warm; purge-and-trap and measure cupric diethyldithiocarbamate at 430 nm	Spectrophotometer	ppm levels	NR	Lam and DiStefano 1982, 1983

TABLE 6-1. Analytical Methods for Determining Carbon Disulfide and Its Metabolites in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	Add internal standard (methyl ethyl ketone in water); agitate; transport from headspace and strip using a cyrogenic trap containing Tenax; thermally desorb	GC/MS	ng/L levels	NR	Brugnone et al. 1993, 1994; Perbellini et al. 1994
Urine (metabolites)	Add urine sample to a solution containing sodium azide, iodine, and potassium iodide	Iodine-azide test	16 ppm	NR	NIOSH 1977
			No data	NR	Baselt 1980
Urine (TTCA)	Acidify urine sample, extract with ether and evaporate; dissolve residue in methanol and analyze	HPLC	82 ng/L	NR	Campbell et al. 1985
	Add internal standard (300 mg sodium sulfate, 100 uL 6M HCl); extract with diethyl ether; dry and resuspend in phosphoric acid	HPLC	25 ng/L	>90%	Lee et al. 1995
	Direct injection of urine after dilution	HPLC	100 ng/mL	95–97%	Simon and Nicot 1993

TABLE 6-1. Analytical Methods for Determining Carbon Disulfide and Its Metabolites in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine (MTZ and thiocarbamide)	Extract sample with ethylacetate and apply extract on TLC plate	GC/MS	No data	NR	Pergal et al. 1972a, 1972b
Milk	Warm sample; purge with helium and trap on Tenax® cartridge; thermal desorption with helium	HRGC/EIMS	NR	NR	Pellizzari et al. 1982
Milk	Warm sample in waterbath at 60°C; purge by aeration and trap in bubbler containing diethylamine-copper solution; make colorimetric determination at 420 nm	Spectrophotometry	µg levels	90%	Cai and Bao 1981

GC/FID = gas chromatography/flame ionization detector; GC/FPD = gas chromatography/flame photometric detector; GC/MS = gas chromatography/mass spectrometry; HPLC = high-performance liquid chromatography; HRGC/EIMS = high-resolution gas chromatography/electron impact mass spectrometry; MS = mass spectrometry; MTZ = 2-mercapto-2-thiazolinone-5; NR = not reported; TLC = thin-layer chromatography; TTCA = 2-thiothiazolidine-4-carboxylic acid

carbon disulfide in air was achieved using this technique. The FID technique is insensitive. Flame photometric, photoionization (11.6 eV) and electron capture detectors, or elemental detectors are sufficiently sensitive to ppb/ppm concentrations.

A spectrophotometric technique has been used for quantifying μg/rnL levels of free and acid-labile (chemically bound) carbon disulfide in the blood of rats (Lam and DiStefano 1982, 1983). This technique is based on measuring the absorbance at 430 nm of a yellow cupric diethyldithiocarbamate complex that is formed by reacting carbon disulfide in blood with Viles' reagent in the presence of acid and heat. A headspace sampler connected to GC equipped with a sulfur-specific flame photometric detector (FPD) has been developed for measuring low levels of free and acid-labile carbon disulfide in the blood of shift workers exposed to carbon disulfide (Campbell et al. 1985). A detection limit of 15.2 μg of carbon disulfide/L of blood was achieved. Concentrations of free and acid-labile carbon disulfide have also been determined by GS/MS (Brugnone et al. 1993, 1994; Perbellini et al. 1994).

It is also possible to determine the level of metabolites in urine. Urinary 2-thiothiazolidine-4-carboxylic acid is the best available indicator to assess the degree of occupational exposure to carbon disulfide (ACGIH 1994; Theinpont et al. 1990). Theinpont et al. (1990) described the isolation of this compound from urine prior to reverse phase high-performance liquid chromatography. It is based on liquid-liquid extraction with methyl tertbutyl ether, followed by affinity chromatography on organomercurial agarose gel. The detection limit of the procedure was 50 μg of carbon disulfide/L of urine (Theinpont et al. 1990).

The more common method of determining the level of metabolites in urine is HPLC. HPLC has been employed for measuring 2-thiothiazolidine-4-carboxylic acid in the urine of shift-workers following exposure to carbon disulfide (Campbell et al. 1985; Lee et al. 1995; Simon and Nicot 1993; Van Doorn et al. 1985a, 1985b). This technique is sensitive, specific, and noninvasive. A detection limit of 25 μg of carbon disulfide/L of urine was obtained (Lee et al. 1995). The iodine-azide test has been used for the detection of metabolites of carbon disulfide in the urine of humans and animals following exposure to carbon disulfide (Baselt 1980; Djuric 1967; WHO 1979). This method is based on the measurement of the time to decolor iodine, as catalyzed by sulfur-containing metabolites of carbon disulfide most notably thiourea and dithiocarbamates. However, the iodine-azide test is nonspecific and has relatively poor sensitivity for measuring the metabolites of carbon disulfide in the urine

(Baselt 1980; Djuric 1967). TLC has also been employed to detect 2-mercapto-2-thiazolin-5-one and thiocarbamide (metabolites of carbon disulfide) in the urine of workers exposed to carbon disulfide (Pergal et al. 1972a, 1972b; WHO 1979). GC/MS is used to confirm 2-mercapto-2-thiazolin-5-one, thiocarbamide, and TTCA.

Carbon disulfide has also been detected in mother's milk using HRGC/MS and spectrophotometry (Cai and Bao 1981; Pellizzari et al. 1982). Sample preparation for HRGC/MS involves purging the sample with helium and then trapping the analyte on a Tenax cartridge, followed by thermal desorption (Pellizzari et al. 1982). Sensitivity, precision, and recovery were not reported. For the spectrophotometric technique, sample preparation involves purging by aeration and trapping in a bubbler containing diethylamine-copper solution for calorimetric determination at 420 nm (Cai and Bao 1981). Recovery (90%) was excellent and sensitivity is in the μg range. Precision was not reported.

6.2 ENVIRONMENTAL SAMPLES

High-resolution GC equipped with an appropriate detector is the most common analytical technique for measuring the concentrations of carbon disulfide in air and various foods (e.g., grains, grain-based foods, fruits, and beverages). The choice of a particular detector will depend on the nature of the sample matrix, the detection limit, and the cost of the analysis. Because volatile organic compounds in environmental samples may exist as complex mixtures or at very low concentrations, preconcentration of these samples prior to quantification is usually necessary (see Table 6-2 for details).

The primary method of analyzing carbon disulfide in air is by adsorption on an activated charcoal tube followed by solvent elution for subsequent quantification. GC equipped with either an electron capture detector (ECD), photo-ionization detector (PID), or FPD has been used for measuring carbon disulfide after elution from the solid phase. Detection limits of low ppm levels of carbon disulfide in the air sample were achieved with these techniques (McCammon et al. 1975; Peltonen 1989; Smith and Krause 1978; UK/HSE 1983). NIOSH has recommended GC/FPD (method 1600) for determining carbon disulfide in air. The range of quantification is 3-64 ppm for a 5-L air sample (NIOSH 1984b).

TABLE 6-2. Analytical Methods for Determining Carbon Disulfide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air	Adsorb sample on charcoal tube and desorb with toluene	GC/ECD	1.5 μg/m^3	NR	Peltonen 1989
	Adsorb sample on charcoal tube and desorb with acetonitrile	GC/PID	9.5 μg/m^3	100%	Smith and Krause 1979
	Adsorb sample on charcoal tube and desorb with toluene	GC/FPD	200 mg/sample; working range 3–64 ppm for a 5-L air sample	NR	NIOSH 1984 (Method 1600)
	Desorb sample from charcoal tube and react with pyrrolidine; react the resultant dithiocarbamate with copper to form a chelate; extract chelate with isoamyl acetate	AAS	0.7 μg/m^3	70–101%	Kneebone and Freiser 1975
	Adsorb sample on charcoal tube and desorb with benzene	GC/FPD	Low μg/m^3 levels	94%	McCammon et al. 1975
	Cryogenic preconcentration of sample (catalytic fluorination after GC to form SF_6)	GC/ECD	6.25 ng/m^3	NR	Johnson and Bates 1993

TABLE 6-2. Analytical Methods for Determining Carbon Disulfide in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
	Prepare with isotopically labelled internal standard (ILS); cryogenic preconcentration	GC/MS	0.625 ng/m^3	NR	Bandy et al. 1985, 1993a
	Scrub and dry air sample; cryogenic preconcentration	GC/SCD 355	10 pg	NR	Ivey and Swan 1995
Water, soil, and solid waste	Purge-and-trap sample; thermally desorb	GC/MS	low ppb level (water, solid waste); low ppm level (soil)	NR	EPA 1984; Haile and Lopez-Avila 1984; Hewitt et al. 1991 (EPA Methods 624, 8015, and 8240)
Water, soil, oil, and fish tissue	Vacuum distillation; trap vapors in a cryotrap	GC/MS	0.008 mg/m^3 in air; 0.01 mg/m^3 in soil; 0.6 mg/m^3 in oil; 0.017 mg/m^3 in tissue	110% in air; 50% in soil; 98% in oil; 79% in tissue	Hiatt et al. 1994

TABLE 6-2. Analytical Methods for Determining Carbon Disulfide in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Various foods (e.g., grains, grain-based foods, fruits, and beverages)	Ground sample in solution containing isooctane, phosphoric acid, and acetone	GC/ECD, GC/HECD	ng/g levels	NR	Daft 1988a, 1988b
	Steam-distill sample in toluene	GC/ECD	ng/sample	40–50%	Bielorai and Alumot 1966
	Reflux sample in isooctane and acid	GC/ECD	Low µg/g levels	59–105%	Malone 1970
	Extract sample by soaking in acetone:water (5:1)	GC/ECD	0.2 ng/sample	98–99% recovery	Heuser and Scudamore 1968
	Extract sample with acetone:water (5:1)	GC/ECD	No data	NR	AOAC 1984
	Dilute with acetone; static headspace technique	GC/SCD 355	ug/L levels	NR	Nedjma and Maujean 1995

AAS = atomic absorption spectrophotometry; EPA = Environmental Protection Agency; GC/ECD = gas chromatography/electron capture detector; GC/FPD = gas chromatography/flame photometric detector; GC/HECD = gas chromatography/ Hall's electrolytic conductivity detector; GC/MS = gas chromatography/mass spectrometry; GC/PID = gas chromatography/photoionization detector; ILS = isotopically labelled standard; NR = not reported; SCD = Sievers chemiluminescence detector

Gas chromatography with ECD has also been used to detect carbon disulfide in air (Johnson and Bates 1993). In this system, carbon disulfide is separated by chromatography, converted to sulfur hexafluoride (SF_6), and then detected by ECD. The ECD detector is 10-100 times more sensitive than the FPD detector and requires smaller sample volumes (Johnson and Bates 1993).

A.R. Bandy, D.C. Thornton, and A.R. Driedger (Drexel University, Philadelphia) have developed a GC/MS technique with isotopically labelled internal standards capable of real-time measurement of carbon disulfide and other volatile sulfur compounds in the air with a detection limit of 0.2 ppt. This technique is approximately 10 times more sensitive than GC/FPD for measuring carbon disulfide in the air and has improved precision and accuracy (Bandy et al. 1993a).

A less commonly used technique for quantifying carbon disulfide in air is atomic absorption spectrophotometry (AAS) for copper in a copper chelate whose concentration is related directly to carbon disulfide concentration (Kneebone and Freiser 1975). Analysis of carbon disulfide in air for AAS quantification involves desorbing the carbon disulfide from an activated charcoal tube with isoamylacetate containing pyrolidine. The eluate is shaken with acidified copper sulfate solution to form a copper chelate, which is quantified by AAS (Kneebone and Freiser 1975). A detection limit of 0.7 μg of carbon disulfide/m^3 of air was obtained. Carbon disulfide is usually isolated from various foods by liquid-liquid extraction and steam distillation (AOAC 1984; Bielorai and Alumot 1966; Clower et al. 1986; Daft 1988a, 1988b; Heuser and Scudamore 1968; Malone 1970; McMahon 1971). GC equipped with ECD or the Hall electrolyte conductivity detector (HECD) is the method of choice for measuring ppb levels of carbon disulfide in foodstuffs (Bielorai and Alumot 1966; Daft 1988a, 1988b; Heuser and Scudamore 1968).

6.3 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 disulfide is available. Where adequate information is not available, ATSDR, in conjunction with the 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 disulfide.

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.

6.3.1 Identification of Data Needs

Methods for Determining Biomarkers of Exposure and Effect. GC (equipped with either FID, FPD, or MS), HPLC, spectrophotometry, and the iodine-azide test have been used for measuring carbon disulfide and its metabolites in the breath, urine, blood, and milk of humans and animals (Baselt 1980; Cai and Bao 1981; Campbell et al. 1985; Djuric 1967; Lam and DiStefano 1982, 1983; Pellizzari et al. 1982; Pergal et al. 1972a, 1972b; Wells and Koves 1974; WHO 1979). GC and HPLC are sensitive enough to measure background levels in the population, as well as levels at which biological effects might occur. Analysis of the parent compound is complicated by the chemical's high volatility and short half-life in biological materials, making detection of urinary metabolites a more reliable approach. Sensitive and selective methods of measuring urinary metabolites of carbon disulfide exist, but further studies might be useful in correlating measured levels of metabolites with carbon disulfide exposure levels and levels at which biological effects might occur.

No specific biomarkers of effect have been exclusively associated with carbon disulfide exposure. Some biological parameters, e.g., decreased nerve conduction velocity and changes in lipid metabolism, have been tentatively linked to carbon disulfide exposure, but there are insufficient data with which to assess the analytical methods associated with measurement of these potential biomarkers. Further investigations into these potential biomarkers, in conjunction with improvements in their detection methods might aid in establishing reliable biomarkers of effect for carbon disulfide.

At present, no specific biomarkers of exposure or effect other than the parent compound or its metabolites are available for carbon disulfide. However, the covalent cross-linking of erythrocyte spectrin by carbon disulfide may serve as a potential biomarker (Valentine 1993). There are no data to indicate whether a biomarker, if available, would be preferred over chemical analysis for monitoring exposure to carbon disulfide.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. A GC/MS method that uses an isotopically labelled variant of the analyte as an internal standard has been developed (Bandy 1985, 1993b). It is an accurate technique for the determination of ppt levels of carbon disulfide in the atmosphere. GC in combination with an electron capture sulfur detector is also sensitive enough to measure ppt levels in the atmosphere. GC equipped with either ECD, HECD, FPD, or PID is also used for measuring low ppm to ppb levels of carbon disulfide in air (Kneebone and Freiser 1975; McCammon et al. 1975; Peltonen 1989; Smith and Krause 1978) and in various foodstuffs such as grains, fruits, and beverages (AOAC 1984; Bieloral and Alumot 1966; Clower et al. 1986; Daft 1988a, 1988b; Heuser and Scudamore 1968; Malone 1970; McMahon 1971; NIOSH 1985; UK/HSE 1983). GC/MS is the analytical method used to measure low ppm to ppb levels of volatile organic compounds (VOCs) in water, soil, and solid waste (DOD 1991; EPA 1984b, 1984c). The air and foodstuffs are the media of most concern for potential human exposure to carbon disulfide. GC techniques are sensitive for measuring background levels of carbon disulfide in these media and levels of carbon disulfide at which health effects might begin to occur. NIOSH has recommended GC/FPD as the method (Method 1600) for measuring low levels of carbon disulfide in air. GC equipped with either ECD or HECD is the method of choice for measuring ppb levels of carbon disulfide in various foodstuffs. No additional analytical methods for measuring carbon disulfide in these environmental media appear to be necessary at this time. Although carbon disulfide was not one of the analytes mentioned in EPA methods for measuring VOCs in water, soil, and solid waste, the GC/MS method would be appropriate for measuring carbon disulfide in these media since it is a volatile compound.

6.3.2 On-going Studies

The Environmental Health Laboratory Sciences Division of the National Center for Environmental Health, Centers for Disease Control and Prevention, is developing methods for the analysis of carbon disulfide and other volatile organic compounds in blood. These methods use purge and trap methodology, high-resolution gas chromatography, and magnetic sector mass spectrometry, which gives detection limits in the low parts per trillion (ppt) range.