7. ANALYTICAL METHODS

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

Humans may be exposed to cyanide from dietary, industrial, environmental, and other sources. Inhalation of tobacco smoke is an important source of cyanide, and exposure may occur from smoke due to fires. After absorption, cyanide is rapidly distributed in the body through blood. Some of the common methods available for determining cyanide in biological media are reported in Table 7-1. Since cyanide forms volatile hydrogen cyanide gas, tissue sampling techniques, storage, and cyanide analysis must be done with caution. The choice of tissues and the factors influencing measured cyanide concentrations are also important (Ballantyne 1983c).

The determination of cyanide in body fluids requires the separation of cyanide from thiocyanate, usually by distillation of cyanides or microdiffusion into an absorber solution. The cyanide is measured spectrophotometrically after a colorimetric reaction involving the cyanide ion and chloramines-T plus pyridine-pyrazolone, *p*-benzoquinone, or *p*-phenylene diamine (see Table 7-1). Detection limits are in the low to mid ppb range (µg/L) (Cruz-Landeira et al. 2000; Ganjeloo et al. 1980; Laforge et al. 1994). Most of these techniques are time-consuming, and some lack specificity or sensitivity. Cyanide in blood is almost exclusively localized to the erythrocytes, whereas thiocyanate is confined to plasma (Lundquist and Sorbo 1989); thus, some researchers recommend analysis of erythrocytes (McMillan and Svoboda 1982; Sano et al. 1992). Some interferences can be mitigated. For example, sodium thiosulfate, a

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Separation in a microdiffusion cell; treatment of absorber solution with chloramine T-phosphate and pyridine-pyrazolone reagent	Spectrophotometry (total cyanide)	0.1 ppm	No data	Morgan and Way 1980
Blood	Separation in a microdiffusion cell; treatment of absorber solution with *p*-benzoquinone	Spectrofluorometry (total cyanide)	0.025 ppm	No data	Ganjeloo et al. 1980
Plasma	Deproteinization with trichloroacetic acid; bromination of supernatant and treatment with pyridine-*p*-phenylene diamine	Spectrophotometry (thiocyanate-cyanide determination)	0.07 ppm	96 (thio-cyanate)	Pettigrew and Fell 1972
Erythrocyte suspension	Sample purged; absorption of hydrogen cyanide in sodium hydroxide; conversion of thiocyanate to cyanide by potassium permanganate oxidation	Spectrophotometry (thiocyanate-cyanide determination)	No data	93–97	McMillan and Svoboda 1982
Blood cells	Separation of cells by centrifugation; extraction; derivitization	HPLC with fluorescence detection	2 ng/mL	83	Sano et al. 1992
Blood	Lysis of blood; extraction; derivatization NDA and taurine	Capillary electrophoresis with fluorescence detection	0.1 ng/mL	No data	Chinaka et al. 2001
Blood	Acidification of sample in a sealed vial	Headspace GC/NPD	0.3 µg/mL	No data	Levin et al. 1990
Blood	Acidification of sample; incubation with chloramine-T in sealed vial	Headspace GC/ECD	100 µg/L	No data	Odoul et al. 1994
Blood	Acidification of sample containing $K^{13}C^{15}N$ in a sealed vial; micro-diffusion to NDA derivatization solution	HPLC-MS	5 ng/mL	88.0 (100 ng/mL); 85.7 (500 ng/mL)	Tracqui et al. 2002

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Blood	Separation by diffusion; color development	Spectrophotometry	0.07 µg/mL	No data	Laforge et al. 1994
Blood	Incubation of acidified sample	GC/NPD	1 ng/mL	No data	Seto et al. 1993
Blood	Separation in a microdiffusion cell; absorption in methemoglobin solution	Spectrophotometric (free cyanide determination)	0.4 µg/mL	80	Tomoda and Hashimoto 1991
Blood and liver	Treatment of HCN released from sample digestion with lead acetate and absorption with NaOH	Specific ion electrode (total cyanide)	5 µg/L	100–109 (whole blood, 0.3–130 ppb)	Egekeze and Oehme 1979
Blood and urine	Separation in a Conway microdiffusion cell; treatment of absorber solution with naphthalene-2,3-dialdehyde and taurine	Spectrophotofluoro-metric	0.8 ppb	66–82.6 at 0.0013–0.13 ppm (blood); 75.6–82 at 0.0013–0.13 ppm (urine)	Sano et al. 1989
Urine	Dilution of sample; bromination and treatment with pyridine-*p*-phenylenediamine	Spectrophotometric (thiocyanate-cyanide determination)	0.07 ppm	88 (thiocyanate at 0.6 ppm)	Pettigrew and Fell 1972
Urine	Sample acidified and purged; absorbed and reacted in hydroxycobalamine solution	Spectrophotometry (total cyanide)	28 ng/mL	50	Cruz-Landeira et al. 2000
Saliva	Derivatization	HPLC/UV (thiocyanate)	2 ng (instrumental)	95–99	Liu and Yun 1993

ECD = electron capture detector; GC = gas chromatography; HCN = hydrogen cyanide; HPLC = high performance liquid chromatography; HPLC-MS = high performance liquid chromatography coupled with mass spectrometric detector; NaOH = sodium hydroxide; NDA = naphthalenedialdehyde; NPD = nitrogen-phosphorus detector; UV = ultraviolet detector

common cyanide antagonist that acts as an interference, can be eliminated by using a buffered solution at pH 5.2 as the acidifying agent for cyanide microdiffusion (Sylvester et al. 1982; Way 1984).

Low detection limits (low ng/mL) have been achieved using a headspace/gas chromatographic (GC) technique (Seto et al. 1993) with good precision in the measurements reported (Levin et al. 1990; Seto et al. 1993). The sample is acidified and incubated, and the headspace analyzed by GC with a nitrogen-specific detector (NPD) (Levin et al. 1990; Seto et al. 1993). Cryogenic oven-trapping techniques have been used to trap volatiles from blood, such as cyanide, at the head of the GC column before analysis, thus eliminating problems of sample foaming that are encountered in traditional head-space analysis methods. Detection limits of 2 ng/mL (2 µg/L) have been reported with extraction efficiencies of 3.06% from blood (Watanabe-Suzuki et al. 2002). Blood samples may be treated with chloramine-T prior to incubation to produce a derivative which can be determined by GC with electron capture detection (ECD). Cyanate and thiocyanate do not interfere in this method (Odoul et al. 1994). The detection limit is 5 µg/L (ppb); precision is good (<15% relative standard deviation [RSD]) (Odoul et al. 1994).

Trace amounts of cyanide in blood cells may be determined using a liquid chromatographic technique with fluorescence detection (Felscher and Wulfmeyer 1998; Sano et al. 1992). The blood cells are extracted and the cyanide derivatized prior to chromatography. The detection limit is 2 ng/mL. Recovery is acceptable (>80%), and precision is good (<15% RSD) (Felscher and Wulfmeyer 1998; Sano et al. 1992). Coupling the liquid chromatography technique to a mass spectrometric detector also provides good detection sensitivities (limit of detection=5 ng/mL) and recoveries (>85%) (Tracqui et al. 2002). Using an ion liquid chromatography method coupled with fluorescence detection, Chinaka et al. (1998) were able to achieve detection limits down to 3.8 pmoles/mL (0.10 ng/mL) of cyanide and 86 pmoles/mL (5.1 ng/mL) of thiocyanate in blood.

Cyanide in biological tissue and fluids can be measured spectrophotometrically after reaction with methemoglobin (Tomoda and Hashimoto 1991). The detection limit is 0.4 µg/mL. Other performance data were not reported (Tomoda and Hashimoto 1991). Cyanide in urine has been determined using microdiffusion separation and fluorimetric determination (Sano et al. 1989). The detection limit of the assay is reported to be 30 ng/L with recoveries of 75.6–82.6% (Sano et al. 1989).

Cyanide in the body is biotransformed to thiocyanate quickly. People may also be exposed to thiocyanate from dietary, industrial, and medical sources. The plasma concentration of thiocyanate has also been used as an index of long-term exposure to cigarette smoke (Liu and Yun 1993). Some authors have determined

thiocyanate in body fluids as a measure of cyanide exposure, while others measure cyanide concentrations in body fluids directly.

Serum levels of thiocyanate are usually determined spectrophotometrically after a colorimetric reaction of thiocyanate with ferric nitrate or barbituric acid and pyridine (Li et al. 1993; Olea et al. 1992). Ion exchange resin chromatography has been used to isolate thiocyanate from serum (Olea et al. 1992). Detection limits are in the ppb range (Li et al. 1993; Olea et al. 1992). Recovery and precision, where reported, are good (recovery >90%; precision <15% RSD) (Li et al. 1993). Methods are available for measuring thiocyanate in saliva by high performance liquid chromatography (HPLC) (Liu and Yun 1993) and in saliva and blood spectrophotometrically (Tominaga et al. 1991; Yamanaka et al. 1991). Table 7-2 lists representative analytical methods for determining thiocyanides in biological matrices.

7.2 ENVIRONMENTAL SAMPLES

Hydrogen cyanide and cyanide salts are important environmental contaminants, and there are numerous reports dealing with the identification and quantitation of cyanide in air, water, and other environmental media. Representative examples of monitoring methods for cyanide are included in Table 7-3.

Hydrogen cyanide in environmental or workplace air is usually collected in sodium hydroxide solution, then measured spectrophotometrically after color development (Agrawal et al. 1991; NIOSH 1989b). One of the most significant problems in cyanide monitoring is the instability of the collected samples (NIOSH 1986b). The collection solution is pH ≥11 to avoid volatilization loss of molecular hydrogen cyanide. However, carbon dioxide from air may react with the solution during storage, thereby lowering the pH and releasing hydrogen cyanide gas, making the reading for cyanide lower than actually presented. Oxidizing agents in solution may transform cyanide during storage and handling. Ferrocyanide and ferricyanide complexes of cyanide undergo photodecomposition with ultraviolet light. Particulate cyanides are known to decompose in moist air with the liberation of hydrogen cyanide and cyanic acid (HOCN). The recommended method for the storage of cyanide samples is to collect the samples at pH 12–12.5 in closed, dark bottles and store them in a cool, dark place. It is also recommended that the samples be analyzed immediately upon collection. The sample handling and preservation methods have been discussed (NIOSH 1986b; Egekeze and Oehme 1979). Cyanide determination in air usually distinguishes between two forms of cyanides: hydrogen cyanide gas and particulate cyanides. Filters are usually used to collect particulate cyanides, and the hydrogen cyanide gas that passes through the

Table 7-2. Analytical Methods for Determining Biomarkers for Cyanide

Analyte	Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Thiocyanate	Human serum, urine, saliva	Extraction of buffered (pH 7) 2-benzoyl pyridine thiosemi-carbazone and sample with isoamyl acetate	Flame atomic absorption spectrometry	4 ng/mL	96–102	Chattaraj and Das 1992
Thiocyanate	Serum	Addition of acetonitrile, centrifugation, separation	Spectro-photometry	0.3 µg/mL	94	Li et al. 1993
Thiocyanate	Human urine, saliva	Derivatization of basic pH sample with pentafluorobenzyl bromide in the presence of Kryptofix 222 B polymer and extraction into methylene chloride then back extraction into isooctane	GC with ECD	0.0115 nmol (in 0.2 mL)	83–106	Chen et al. 1994
Thiocyanate	Human urine, saliva	Dilution with water then filtration (0.45 µm)	Ion chromatography utilizing ODS column coated with cetyl-dimethylamine and with UV absorbance (210 nm) detection	20 ng/mL	95–101	Michigami et al. 1992
Thiocyanate	Urine	Ion chromatography using weakly basic resin; acidification of eluate with HCl; addition of bromine water, arseneous oxide and pyridine-*p*-phenylene diamine	Spectro-photometry	2.5 µmol/L (lowest reported)	No data	Tominaga and Midio 1991
Thiocyanate	Human urine	Dilution with water then passage through disposable Toyo pack ODS and IC-SP columns	Suppressed ion chromatography with conductivity detection	200 nM	No data	Miura and Koh 1991

ECD = electron capture detector; GC = gas chromatography; IC/SP = ion chromatography/sulfopropyl type column; ODS = octadecyl silane; nM = nanomolar

Table 7-3. Analytical Methods for Determining Cyanide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Occupational air (NIOSH Method 7904)	Filtered air collected in midget impinger containing NaOH; extraction of filter with NaOH; sulfide removed	Specific ion electrode (HCN cyanide salts)	2.5 µg CN^{-}[a]	96.7 at 5–21 mg/m^3	NIOSH 1989a
Occupational air (NIOSH Method 6010)	Collection of breathing zone air samples on adsorbent; extraction with water; treatment with barbituric acid/ pyridine reagent	Spectrophotometry (HCN)	1 µg CN^{-}[b]	~100	NIOSH 1989b
Occupational air	Passage of filtered air through midget impinger containing NaOH; conversion of NaCN to sodium formate; optional ion exchange clean-up	Ion-chromatography/ amperometric detector (HCN)	5–10 ppm	100–109 at 5–20 ppm	Dolzine et al. 1982
Air	Filtered air collected in midget impinger	Ion-chromatography/ amperometric detection (HCN only)	0.04 ppm (for 2.6 L of air)	91 at air flow rate of 0.171 L/minute	NIOSH 1986b
Water (drinking, surface, saline, domestic, and industrial waste) (EPA Method 335.1)	Chlorination of sample at pH 11–12 and ClCN driven off; reflux-distillation of residual sample; absorption of released HCN in NaOH; treatment with chloramine-T and pyridine-pyrazolone or pyridine-barbituric acid	Spectrophotometry (cyanide amenable to chlorination)	No data	No data	EPA 1983a
Water (drinking, surface, saline, domestic, and industrial waste) (EPA Method 335.2)	Reflux-distillation of sample; absorption of released HCN in NaOH scrubber; treatment of absorbing solution with chloramine-T and pyridine-pyrazolone or pyridine barbituric acid	Spectrophotometry (total cyanide)	0.02 ppm	85–102 at 0.28–0.62 ppm	EPA 1983a

Table 7-3. Analytical Methods for Determining Cyanide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water (drinking, surface, saline, domestic, and industrial waste) (EPA Method 335.2) (EPA Method 335.3)	Reflux-distillation of sample; absorption of released HCN in NaOH; titration of absorbing solution with $AgNO_3$ in presence of *p*-dimethylamino-benzalrhodanine indicator	Titrimetric (total cyanide)	1 ppm	No data	EPA 1983a
Water (drinking, surface, saline, domestic, and industrial waste) (EPA Method 335.4)	Reflux-distillation of sample; absorption of released HCN in NaOH; treatment with chloramine-T, pyridine barbituric acid	Semi-automated spectrophotometry (total cyanide)	~0.02 ppm	95 (ave.)	EPA 1993h
Water	None	Ion-chromatography/ amperometric detection (free and a few complexed cyanides)	2 ppb	100–112	Rocklin and Johnson 1983
Water	Separation of acidified sample in a microdiffusion cell; absorption in NaOH	Potentiometric (free cyanide)	0.018 mg/L CN^-	96.5–103.9 at 0.037–3.49 mg/L	Rubio et al. 1987
Water (ASTM Method D2036A)	Separation of acidified sample in a microdiffusion cell; absorption in NaOH	Potentiometric (total cyanides)	0.03 mg/L	99.0–111	ASTM 1999
Water	None	FIA; spectrophotometric detection (free cyanide)	20 ng/mL	88–107	Ma and Liu 1992
Water	None	FIA; amperometric detection (free cyanide)	2.6 ng/mL	99–103	Nikolić et al. 1992
Water	Sample shaken in presence of quinoline and benzoyl chloride at pH 7	HPLC with spectrophotometric detection (free CN^-)	26 pg/mL	No data	Madungwe et al. 1991

Table 7-3. Analytical Methods for Determining Cyanide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Water	None	Ion chromatography with postcolumn derivatization and fluorimetric detection (free cyanide)	0.1 ng/mL	94–96	Gamoh and Imamichi 1991
Water	Treatment of sample with NaOH and hypophosphite; passage through silver filter (free cyanide); treatment in photo cell prior to filter for total cyanide and selective oxidation for cyanides not amenable to chlorination (CNATC)	Flame AAS or graphite furnace AAS	2 ng/mL (flame AAS); 0.06 ng/mL (graphite furnace AAS	107 (free cyanide), 90.4 (CNATC), 98.1 (total cyanide)	Rosentreter and Skogerboe 1992
Water	Samples sealed in vials with nitrogen	Headspace GC/ECD (cyanogen chloride)	0.04 ng/mL	91 average	Xie and Reckhow 1993
Water	Absorption on to SPME fiber; thermal desorption in GC	GC/ECD (cyanogen chloride or bromide)	77 ng/L (cyanogen chloride) 41 ng/L (cyanogen bromide)	No data	Cancho et al. 2000
Water (APHA Method 4500-CN^- J)	Adjustment of sample pH to 8.0–8.5 using phosphate buffer; addition of pyridine-barbituric acid	Spectrophotometry (cyanogen chloride)	0.02 µg/mL (as CN^-) (lowest calibration)	No data	APHA 1992
Water and waste water (APHA Method 4500-CN^- M)	Filtration of sample; optional treatment with resin; treatment with ferric nitrate solution	Colorimetric detection (thiocyanate)	No data	71–99, 0.07–1.42 mg/L	APHA 1992

Table 7-3. Analytical Methods for Determining Cyanide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Waste or leachate (EPA Method 9012A)	Reflux-distillation of acidified sample; absorption of released HCN in NaOH; treatment with $AgNO_3$ and an indicator (titrimetric) or chloramine-T/pyridine-barbituric acid (colorimetric)	Titrimetric or colorimetric detection (total and amenable cyanide)	0.1–0.2 mg/L (titrimetric) 0.02 mg/L (colorimetric)	(titrimetric) 94–99 (total cyanide), 87–97 (amenable cyanide)	EPA 1996
Waste water	Addition of sample to buffered methemoglobin	Spectrophotometry (free cyanide)	0.2 µg/mL	No data	Tomoda and Hashimoto 1991
Waste water	None	FIA with spectrophotometric detection	3 ng/mL	98	Kubáň 1992
Waste water	Sample acidified and irradiated with UV; microdiffusion and absorption in NaOH solution	FIA with amperiometric detection (total cyanide)	0.5 µg/L	99.5 (2 µg/L) 99 (30 µg/L)	Weinberg and Cook 2002
Waste water	Complexation of sample with 2-benzoyl-pyridine thiosemi-carbazone; solvent extraction	Flame AAS (free cyanide)	4.8 ng/mL	97–101	Chattaraj and Das 1991
Solid waste or oil waste (EPA Method 9013)	Extraction of solid component with water at pH 10 and hexane	Titrimetric or colorimetric detection (soluble cyanides)	No data	60–90 (solid) 88–92 (oil)	EPA 1992e
Soils/ sediments (USGS Method I6302)	Reflux-distillation of sample; absorption of released HCN in NaOH; treatment with chloramine-T, pyridine barbituric acid	Colorimetric detection (recoverable simple cyanides)	0.5 mg/kg	No data	USGS 1985
Food (cereal and other foodstuffs)	Extraction of sample with water/ acetonitrile, dried	GC/ECD at low detection voltage (free cyanide)	0.1 ppm	90	Heuser and Scudmore 1969

Table 7-3. Analytical Methods for Determining Cyanide in Environmental Samples

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Food (soybean and soybean products)	Sample mixed with water, lead nitrate, tartaric acid, and anti-forming agent; acidification and distillation; treatment of distillate with pyridine-barbituric acid	Spectrophoto-metry (total cyanide)	No data	32–80	Honig et al. 1983

[a]Method detection limits depend upon the volume of air sampled; the working range is 5–20 mg/m^3 for a 10-L air sample.
[b]Method detection limits depend upon the volume of air sampled; the working range is 1–333 mg/m^3 for a 3-L sample.

AAS = atomic absorption spectroscopy; $AgNO_3$ = silver nitrate; ASTM = American Society for Testing and Materials; ClCN = cyanogen chloride; CN^- = cyanide ion; CNATC = cyanides not amenable to chlorination (Rosentreter and Skogerboe 1992); EPA = Environmental Protection Agency; FIA = flow injection analysis; GC/ECD = gas chromatograph/electron capture detector; HCN = hydrogen cyanide; NaOH = sodium hydroxide; NIOSH = National Institute for Occupational Safety and Health; USGS = United States Geological Survey; UV = ultraviolet

membrane is trapped in sodium hydroxide. The collected particulate cyanides can be quantified separately after acid distillation. Detection limits are in the ppm range for occupational air (Dolzine et al. 1982; NIOSH 1989a, 1989b) and sub-ppm range for ambient air (NIOSH 1986b). Reported recovery is good (>90%) (Dolzine et al. 1982; NIOSH 1986b, 1989a, 1989b).

Inorganic cyanides in water can be present both as complexed and free cyanide. Cyanide in water is usually determined in three different forms: free cyanide, cyanide amenable to chlorination, and total cyanide. Free cyanides such as sodium cyanide, potassium cyanide, and hydrogen cyanide are readily ionized to the cyanide ion under the conditions used in most common analytical techniques. Methods for determining cyanide amenable to chlorination measure simple metal cyanides and most complex cyanides with the exception of iron cyanides. Total cyanide is a measure of all cyanides including iron cyanide complexes. Table 7-3 lists representative analytical methods for determining cyanides that may be present in various forms. A number of standard methods are available (APHA 1992; ASTM 1999; EPA 1983a, 1996; NIOSH 1989a, 1989b). An ion-exchange chromatography method has been developed to separate and quantify cyano complexes of gold, silver, iron, nickel, cobalt, and copper using a UV detection technique (Karmarkar 2002). Minimum detection limits of 0.07–0.83 mg/L and recoveries of 99.8–118.8% were reported, both of which varied considerably depending on the cyano metal complex.

Procedures for extracting cyanide from aqueous matrices usually involve acidifying the sample followed by heating and refluxing to evolve hydrogen cyanide, which is then trapped in an impinger containing absorption media. Complex cyanides and metal cyanide complexes can be degraded to free cyanide through UV irradiation (314 nm) of an acidified sample (Weinberg and Cook 2002). Cyanide is usually measured by colorimetric, titrimetric, or electrochemical methods (for example, APHA 1992). All are subject to interference problems. Sulfide, certain oxidizing agents, nitrate or nitrite, thiocyanate, aldehydes, and ketones may interfere under acid distillation conditions, thus producing erroneous results from both colorimetric and titrimetric methods. In addition, fatty acids in samples may distill over and form soaps under alkaline titration conditions, thus causing interference in the titrimetric method (EPA 1983a, 1996). Colorimetric methods may be based on pyridine with chloramine-T as the oxidizing agent and barbituric acid as the coupling component (EPA 1996) or pyrazolone as the coupling agent (EPA 1983a). Low detection limits are attained (10–20 µg/L), but sulfide and thiocyanate are common interferents (Csikal and Barnard 1983; Drikas and Routley 1988). Titrimetric methods usually employ silver nitrate (EPA 1983a, 1996); however, the detection limits are in the low mg/L range. Methods using specific ion electrodes (electrochemical) respond to numerous interferences (sulfur, chlorine, iodine, bromine, cadmium, silver, zinc, copper, nickel, and mercury) (NIOSH 1986b).

Continuous monitoring methods based on amperometric (Nikolić et al. 1992) or spectrophotometric (Kubáň 1992; Ma and Liu 1992) techniques for the quantification of free cyanide are also available. Ion chromatography with amperometric determination provides good sensitivity (2 ppb) and selectivity for free cyanide and for the weak complexes of cadmium and zinc (Rocklin and Johnson 1983). Postcolumn derivatization and fluorescence detection provides low detection limits as well (0.1 ppb) (Gamoh and Imamichi 1991).

Methods were identified in the available literature for the determination of the concentrations of cyanides and thiocyanates present in soils at low levels. A colorimetric method (USGS 1985) is used to quantify simple cyanides in soils and sediments (see Table 7-3). A reflux-distillation of soil or sediment samples converts CN^- to hydrogen cyanide, which is released from the sample and then absorbed in a 1 M NaOH solution. The absorbed cyanide is chlorinated with Chloramine-T and then reacted with pyridine-barbituric acid to form the chromophore. Detection limits of >0.5 mg/kg are reported; no recovery data were given. The method will decompose thiocyanate in the samples to cyanide and sulfide, resulting in a concentration of cyanide that is higher than was originally present in the sample. Sulfide concentrations of >10 mg/kg will greatly interfere with the quantification of cyanide in the assay. In addition to simple cyanides, complex cyanides can also be measured in soil and sediment samples utilizing a modified sample preparation procedure that uses an ultraviolet digestion-distillation method to break down the complex cyanides to the cyanide ion. Brown and Morra (1991) extracted simple cyanides from soils using a calcium chloride solution followed by analysis by ion-chromatography (see Table 7-4). The assay provided a detection limit of 0.02 µg/L with a recovery averaging 94%, depending on soil type.

Recent method development strategies for quantifying cyanide in water are using biological sensing elements (i.e., organisms, enzyme, receptor, antibody, etc.) coupled to an electronic signal-transducing element. With respect to organisms, the assay takes advantage of the effect that cyanide has on respiration and metabolism. For example, Karube et al. (1998) use yeast in a flow-through reactor setup where river water containing cyanide is pumped through the reactor and the oxygen concentration in the effluent stream is measured using an oxygen electrode. When cyanide passes through the reactor, it inhibits yeast respiration and therefore decreases oxygen consumption, resulting in an increase in the current at the oxygen electrode. A detection limit of 0.2 ppm (3µM) was reported for the assay with a linear response to 1 ppm. In another approach, Nikolelis et al. (1997) use a lipid bilayer embedded with

Table 7-4. Analytical Methods for Determining Environmental Degradation Products of Cyanide

Analyte	Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
SCN^-	Water	Filtration (0.45 µm)	Reversed-phase ion-pair chromatography with amperometric detection	104 ng/mL	No data	Xu et al. 1993
SCN^-	Water/waste water	Filtration; acidification; reaction with ferric chloride	Colorimetric	0.1 mg/L	No data	ASTM 1994a
SCN^-	Soil	Extraction of soil with calcium chloride solution	Ion chromatography with conductivity detection	0.02 µg/g	83; avg. 94, depending on soil type	Brown and Morra 1991

SCN^- = thiocyanate ion

methemoglobin to detect and quantify cyanide in water samples. The binding of cyanide to the methemoglobin causes a change in the electrochemical potential across the membrane that is measured with an electrometer. A detection limit of 4.9 nM (0.0032 ppm) was reported with a relative standard deviation (RSD) of 5.2% at cyanide concentration of 150 nM.

Few methods are available for the determination of cyanogen and cyanogen chloride in environmental matrices. Methods available include gas chromatographic-flame ionization detection of cyanogen chloride and cyanogen (Brown et al. 1986), headspace gas chromatography with electron capture detection (GC/ECD) (Cancho et al. 2000; Xie and Reckhow 1993) and colorimetric detection (APHA 1992). Detection limits are in the low ppb range for the colorimetric method (APHA 1992) and in the sub-ppb range for the GC/ECD method (Xie and Reckhow 1993).

Standard methods are available for measuring thiocyanate in aqueous matrices (APHA 1992; ASTM 1994a). These are colorimetric methods and are subject to interferences. In addition, thiocyanate is biodegradable; thus, care must be exercised in sample collection, preservation, and storage. The detection limit is 100 ppb (ASTM 1994a). An automated method with good sensitivity (0.5 ppb) is available for determining thiocyanate in water and waste water (ASTM 1994b). Various methods have been reported for determination of thiocyanate in soils; however, ion chromatographic determination provides good selectivity and sensitivity (20 ppb) (Brown and Morra 1991). Representative examples of monitoring methods for thiocyanate are included in Table 7-4.

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 cyanide 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 cyanide.

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. Besides environmental exposure, exposure to cyanide can also occur from consumption of cyanide-containing food and smoking cigarettes. Since so many factors can influence cyanide exposure, the exact correlation between cyanide concentrations in the body and its level in the environment has not been made. Therefore, measuring cyanide and/or thiocyanate levels in blood and urine cannot be used as a biomarker for exposure to low cyanide concentrations. Reliable analytical methods are available for the detection of cyanide and thiocyanate in blood, plasma, and urine of both unexposed and exposed persons are available (see Tables 7-1 and 7-2). Further studies determining biomarkers for exposure to low cyanide concentrations would be useful since current methods that are used to measure cyanide and/or thiocyanate in blood and urine are not effective at assessing exposures to low concentrations of cyanide.

Effect. Although certain effects, such as cyanosis and endemic goiter, have been associated with cyanide exposure (see Section 3.8.2), a positive correlation between cyanide exposure and one of its effects has not yet been established. Additional studies establishing a correlation between cyanide exposure and one of its effects will be useful in diagnosing cyanide exposure.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. The concentration of hydrogen cyanide in most ambient air is so low that it is beyond the detection limit of the standard analytical methods. An infrared absorption method of a large vertical tropospheric column was used to measure the hydrogen cyanide concentration in the troposphere (Cicerone and Zellner 1983). Similarly, ground-based millimeter wave emission spectroscopy was used to measure stratospheric concentration of hydrogen cyanide (Jaramillo et al. 1989). Similarly, the level of cyanogen chloride in drinking water ranges from 0.45 to 0.80 ppb (Krasner et al. 1989), which is beyond the detection limit of the standard analytical methods without concentration and trapping procedures. Cyanogen chloride in water was determined by a purge and trap GC/MS method (Krasner et al. 1989), a method that is not available to many laboratories. There is, therefore, a need to develop standard

analytical methods capable of quantifying hydrogen cyanide in air and cyanogen chloride in water at levels that are generally found in these media.

Cyanide metabolizes in the human body to thiocyanate, and its biodegradation products include ammonia, carbon dioxide, nitrate, or nitrogen (Richards and Shieh 1989). The detection of thiocyanate in body fluids may indicate cyanide exposure. Similarly, the amounts of cyanide degradation products formed in an environmental medium could be used to measure cyanide's biodegradation rate. A summary of methods for determining environmental degradation products is shown in Table 7-4. Suitable analytical methods are available to detect all of these compounds (Pettigrew and Fell 1973; Richards and Shieh 1989).

7.3.2 Ongoing Studies

The Federal Research in Progress (FEDRIP 2004) database provides additional information obtainable from a few ongoing studies that may fill in some of the data needs identified in Section 7.3.1 and provides examples of new methods used in the detection and quantification of cyanide or thiocyanide. These studies are summarized in Table 7-5.

Table 7-5. Ongoing Studies on Analytical Methods for Quantifying Cyanide and Cyanide Compounds[a]

Investigator	Affiliation	Research description	Sponsor
Fennell, TR	Research Triangle Institute	Development of a liquid-chromatography/ mass spectrometry analytical method for the detection and quantification of adducts formed between hemoglobin and reactive chemicals such as cyanide.	NCI
Goodridge, CF	CC Technology, Inc.	Development of a handheld rapid device for the detection of cyanide exposure. The device takes advantage of the strong complexing that occurs between cyanide and gold nanoparticles for extracting cyanide from blood or saliva. Detection of cyanide is accomplished by releasing cyanide from the nanoparticles through a reaction with a releasing agent such as sulfuric acid and then quantifying the released cyanide using an inexpensive Raman spectrometer.	NIH

[a]FEDRIP 2004

NCI = National Cancer Institute; NIH = National Institutes of Health