7. ANALYTICAL METHODS

The purpose of this chapter is to describe the analytical methods that are available for detecting, measuring, and/or monitoring chromium compounds, their metabolites, and other biomarkers of exposure to chromium compounds. 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 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 lower detection limits and/or to improve accuracy and precision in detection.

7.1 BIOLOGICAL MATERIALS

Several methods are available for the analysis of chromium in different biological media, with some recent methods of chromium determination reported in Table 7-1. Multiple reviews on the subject provide more detailed descriptions of the available analytical methods (EPA 1984a; Fishbein 1984; IARC 1986a, 1990; Torgrimsen 1982; WHO 1988). Frequently used methods for determining low levels of chromium in biological samples include neutron activation analysis (NAA); mass spectrometry (MS); graphite spark atomic emission spectrometry (AES); and graphite furnace atomic absorption spectrometry (GFAAS) (Greenberg and Zeisler 1988; Plantz et al. 1989; Urasa and Nam 1989; Veillon 1989). A newly added technique includes the use of total reflection X-ray fluorescence (TXRF) for analysis of total chromium in the air (Adekola and Eletta 2007).

There are numerous issues and considerations in collecting and analyzing the chromium content in presented samples. Some of these issues include problems with collection, contamination, and determining accurate concentration levels of the chromium content in the samples. The determination of trace quantities of chromium in biological materials requires special precautionary measures, from the initial sample collection process to the final analytical manipulations of the samples.

Contaminates including dust contamination or losses of the samples during collection, transportation, and storage should be avoided (EPA 1984a). Chromium-containing grinding and homogenizing equipment should not be used for preparation of biological samples. Reagents of the highest purity should be used to

Table 7-1. Analytical Methods for Determining Chromium in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Plasma	Wet ashing with $HNO_3/HClO_4/H_2SO_4$; residue complexed with APDC and extracted with MIBK; evaporated residue dissolved deposited in HNO_3/HCE, and solution on a polycarbonate foil	PIXE	0.3 µg/L	87% at 4.5 µg/g	Simonoff et al. 1984
Blood, serum	Sample after wet digestion converted to a volatile chelate usually with fluorinated acetylacetone	GC/ECD	0.03 pg 0.5 pg 1.0 ng	No data	Fishbein 1984
Serum	$Mg(NO_3)_3$ added to serum, dried by Lyophilization, ashed, and dissolved in 0.1 N HCl	GFAAS	0.005 µg/L	103% at 0.30 µg/L	Randall and Gibson 1987
Blood	Diluted with 0.1% EDTA and 5% isopropanol	GFAAS-Zeeman-effect background correction	0.09 µg/L	No data	Dube 1988
Blood or tissue	Wet ashing with $HNO_3/HClO_4/H_2SO_4$	ICP-AES	1 µg/100 g blood 0.2 µg/g tissue	114% recovery at 10 µg/sample	NIOSH 1994a (Method No. 8005)
Erythrocytes	Dilution with Triton X100	GFAAS	No data	No data	Lewalter et al. 1985
Serum and urine	HNO_3 de-proteinization	GFAAS with pyrolytic graphite tube and Zeeman background correction	0.02 µg/L (serum) 0.1 µg/L (urine)	No data	Sunderman et al. 1989
Body fluids (milk, urine, etc.)	Dried sample ashed by oxygen plasma, H_2O_2 addition, drying, dilution in 1N HCl	GFAAS with tungsten iodide or deuterium arc or CEWM background correction	<0.25 µg/L	91% at 0.55 µg/L	Kumpulainen 1984
Urine	None	GFAAS	0.05 µg/L	91% at 0.22 µg/L	Randall and Gibson 1987

Table 7-1. Analytical Methods for Determining Chromium in Biological Materials

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	None	GFAAS with CEWM background correction and WM-AES	0.09 µg/L (CEWM-AAS) 0.02 µg/L (WM-AES)	No data	Harnly et al. 1983
Urine	No sample preparation other than addition of yttrium internal standard	ICP-AES	12 µg/L	77% at 13 µg/L	Kimberly and Paschal 1985
Urine	Sorption onto polydithiocarbonate resin, ash sorbate in low temperature oxygen plasma and dissolve in $HNO_3/HClO_4$	ICP-AES	0.1 µg/sample	100% recovery at 1 µg/50mL urine	NIOSH 1994b (Method 8310)
Urine	None	GFAAS	0.0052 µg/L	No data	Kiilunen et al. 1987
Urine	Sample spiked with standard chromium (standard addition)	GFAAS	0.03–0.04 µg/L	No data	Veillon et al. 1982
Urine	Diluted with water	GFAAS-Zeeman-effect-background correction	0.09 µg/kg	No data	Dube 1988
Milk powder	Mixed with water	GFAAS	5 µg/kg	134–141% at 17.7 µg/kg	Wagley et al. 1989
Tissue(Chromium(V))	Injection of sodium dichromate	EPR	0.1 mmol/kg	No data	Liu et al. 1994

AAS = atomic absorption spectrophotometry; APDC = ammonium pyrrolidine dithiocarbonate; CEWM = continuum source echelle monochromator wavelength-modulated; ECD = electron capture detector; EDTA = ethylenediaminetetraacetic acid; EPR = electron paramagnetic resonance spectroscopy; GC = gas chromatography; GFAS = graphite furnace AAS; H_2O_2 = hydrogen peroxide; H_2SO_4 = sulfuric acid; HCI = hydrochloric acid; $HClO_4$ = perchloric acid; HNO_3 = nitric acid; ICP-AES = inductively coupled plasma-atomic emission spectrometry; $Mg(NO_3)_3$ = magnesium nitrate; MIBK = methylisobutyl ketone; MS = mass spectrometry; PIXE = proton-induced X-ray emission spectrometry; XRF = X-ray fluorescence analysis; WM-AES = wavelength-modulated atomic emission spectrometry

avoid contamination, and the potential loss of chromium due to volatilization during wet and dry ashing should be minimized (EPA 1984a).

The determination of chromium in most biological samples is difficult because of the matrix interference and the very low concentrations present in these samples. Prior to 1978, numerous erroneous results were reported for the chromium level in urine using electrothermal atomic absorption spectrometry (EAAS) because of the inability of conventional atomic absorption spectrometry systems to correct for the high nonspecific background absorption. Similarly, the reported serum and plasma chromium concentrations of normal subjects have varied more than 5,000-fold since the early 1950s. The chromium levels in human serum or plasma as reported in the mid-1980s ranged from 0.01 to 0.3 μg/L, and the daily urinary excretion rate of chromium in healthy and nonoccupationally exposed humans is <1 μg/day (Anderson 1987; Harnly et al. 1983; Sunderman et al. 1989; Veillon 1989).

The problem of generating accurate data for chromium in biological samples is further complicated by the lack of Standard Reference Materials (SRM). Standards in chromium certified materials, such as brewer's yeast (DOC 1976c), bovine liver (DOC 1977b, 1982), human serum (DOC 1985, 1993b, 2003), urine (DOC 1993c), orchard leaves (DOC 1977a), spinach leaves (DOC 1976b, 1996), pine needles (DOC 1993a), oyster tissue (DOC 1983, 1989), and tomato leaves (DOC 1976a), have been issued by the National Institute of Standards and Technology (formerly the National Bureau of Standards). However, due to the previous lack of SRMs, older data should be interpreted with caution (EPA 1984a), unless the data are verified by interlaboratory studies (WHO 1988).

In addition to the consideration of contamination and potential loss of sample, it should be noted that chromium may exist as several different oxidation states in biological media. Two of the most common oxidation states are chromium (III) and chromium (VI). Each of these oxidation states displays very different physical and biological properties. In biological samples where chromium is generally present as chromium(III), the choice of a particular method is dictated by several factors, including the type of sample, its chromium level, and the scope of the analysis (Kumpulainen 1984).

The preceding factors, in combination with the desired precision and accuracy and the cost of analysis, should be considered in selecting a particular analytical method. Although the methods reported in Table 7-1 represent some of the more recent methods, they are not necessarily the ones most commonly used. A comparison of the various standard methods and approaches for maintaining sample purity and integrity during sampling, handling, and analysis are provided by Kumpulainen (1984).

7.2 ENVIRONMENTAL SAMPLES

Analytical methods for determining chromium in environmental samples are reported in Table 7-2. The three commonly used methods that have the greatest sensitivity for chromium detection in air are GFAAS, instrumental neutron activation analysis (INAA), and graphite spark atomic emission spectrometry (Schroeder et al. 1987). Measurements of low levels of chromium concentrations in water have been made by specialized methods, such as inductively coupled plasma mass spectrometry (ICP-MS), capillary column gas chromatography (HRGC) of chelated chromium with electron capture detection (ECD), and electrothermal vaporization inductively coupled plasma mass spectrometry (Gonzalez et al. 2005; Henshaw et al. 1989; Malinski et al. 1988; Parks et al. 2004; Schaller and Neeb 1987). A method using high performance liquid chromatography (HPLC) interfaced with direct current plasma emission spectrometer has been used for the determination of chromium(III) and chromium(VI) in water samples (Krull et al. 1983). An acid digestion procedure followed by AAS has been developed that can quantify chromium(VI) in soil, sediment, and sludge (Ayyamperumal 2006; Oygard et al. 2004). The preferred methods for digestion of environmental samples have been discussed by Griepink and Tolg (1989).

Many of the same issues with the biological samples are also present in environmental analysis, including issues of collection, contamination, and detection. Chromium may be present in both the trivalent and hexavalent oxidation states in most ambient environmental and occupational samples, and the distinction between soluble and insoluble forms of chromium(VI) is sometimes necessary. The quantification of soluble and insoluble chromium is done by determining chromium concentrations in aqueous filtered and unfiltered samples. However, soluble chromium(VI) may be reduced to chromium(III) on filtering media, particularly at low concentrations, and under acidic conditions. Teflon® filter and alkaline solution are most suitable to prevent this reduction (Sawatari 1986). Routine analytical methods are not available that can quantify the concentration of both chromium(VI) and chromium(III) in air samples when present at a total concentration of <1 $\mu g/m^3$ (EPA 1990a), although two methods described in Table 7-2 can determine chromium(VI) concentrations alone in air at a minimum detection limit of 0.1 ng/m^3 for a 20-m^3 sample (CARB 1990).

As in the case of biological samples, contamination and chromium loss in environmental samples during sample collection, storage, and pretreatment should be avoided. Chromium loss from aqueous samples

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Air (total chromium)	Air particulate matter collected on filter is cut out and irradiated with X-ray photons	XRF	0.017 µg/m^3	No data	Wiersema et al. 1984
Air (total chromium)	The collected particulates in filter dissolved in HNO_3, dried and redissolved in acidified water	ICP-AES	0.05–0.2 ng/m^3	No data	Barrie and Hoff 1985
Air (total chromium)	Particulate matter collected on cellulose ester filter, digested with aqua regia	ICP-AES	1 µg/m^3	87–102% at 0.5–100 µg	Lo and Arai 1988
Air (total chromium)	Air particulate collected on cellulose ester filter, wet wash with HCI/HNO_3	Flame atomic absorption	0.06 µg/sample	98% at 45–90 µg/sample	NIOSH 1994c (Method 7024)
Air (total chromium)	Sample collected on cellulose ester membrane filter dissolved in acid mixtures	ICP-AES	1 µg/sample	98% at 2.5 µg/filter	NIOSH 1994d (Method 7300)
Air (chromium(VI))	Sample collected on sodium carbonate-impregnated cellulose filter leached with sodium bicarbonate	Ion chromato-graphy/ coulometric	0.1 ng/m^3 for 20 m^3 sample	89–99% at 100 ng	CARB 1990
Air (chromium(VI))	Sample collected in filters containing sodium bicarbonate buffer at 15 L/minute	Ion chromato-graphy/ coulometric	0.01 ng/m^3 for 20 m^3 sample	94%	Sheehan et al. 1992
Occupational air (welding fumes)	The particular matter on filter wet ashed with H_2SO_4 and chromium(III) oxidized to chromium(VI) by addition of Na_2O_2; the centifuged solution was acidified with HCl and reduced to chromium(III) by SO_2; the solution was complexed with β-isoproyl tropolone in $CHCl_3$	HPLC-UV	10 pg	No data	Maiti and Desai 1986

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Occupational air (chromium (VI))	Extract with 0.05 M $(NH_4)_2SO_4$–0.5 M $(NH_4)_2SO_4$.1 M NH_3.	FIA-UV/VIS	0.11 ng	>90%	Wang et al. 1997a
Welding fumes (total chromium(VI))	Air particulate collected on PVC filter is extracted with hot 3% Na_2CO_3 and 2% NaOH, acidified with H_2SO_4 and complexed with diphenyl carbazide	Spectro-photometry at 540 nm	0.05 µg/sample	No data	NIOSH 1994e (Method 7600); Zatka 1985
Welding fumes (total chromium(VI))	Air particulate collected on PVC filter, extracted with H_2SO_4 and complexed with diphenylcarbazide	Chromato-graphy at 540 nm Spectro-photometry at 540 nm	3.5 µg/sample	No data	NIOSH 1994f (Method 7604)
Simultaneous determination of chromium(III) and chromium(VI) in water extract from metal fumes	Sample solution at pH 5 reacted with disodium ethylenediamine tetraacetic acid at 50 °C for 1 hour	HPLC on anion exchange column with Na_2CO_3 eluting solution and simultan-eous UV and AAS detection	0.2 ng by UV for chromium(VI) 2.0 ng by UV 5.0 ng by AAS for chromium (IV) 5 ng by AAS for chromium (III)	95–105% at 0.002–2.0 µg	Suzuki and Serita 1985
Atmospheric deposition (snow); determination in soluble (chromium(VI)) and particulate (chromium(III)) part	The melted snow filtered through Nucleopore filter; the filtrate acidified with HNO_3; and dried by freeze-drier; residue dissolved in HNO_3; this preconcentrated solution placed in plastic tubes; both plastic tube and Nucleopore filter irradiated with protons	PIXE	2 µg/L (soluble portion) 26 µg/L (snow particle)	No data	Jervis et al. 1983; Landsberger et al. 1983
	Either the above Nucleopore filter or the preconcentrated liquid placed in plastic vial is irradiated by thermal neutron	INAA	5 µg/L (soluble portion) 115 µg/g (snow particle)	No data	Jervis et al. 1983; Landsberger et al. 1983

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Drinking water, surface water, and certain domestic and industrial effluents (dissolved chromium(VI))	Complex chromium(VI) in water with APDC at pH 2.4 and extracted with MIBK	Furnace AAS	2.3 µg/L	No data	EPA 1983c (Method 218.5)
Drinking water, groundwater and water effluents (chromium(VI))	Buffer solution introduced into ion chromatograph. Derivitized with dipenylcarbazide	Ion chromato-graphy spectro-photometry at 530 mm	0.3 µg/L	100% at 100 µg/L	EPA 1996a (Method 7199)
Waste water and industrial effluent for chromium(VI) only	Buffered sample mixed with $AlCl_3$ and the precipitate separated by centrifugation or filtration	DPPA at pH 10–12	30 µg/L	90% at 0.2 mg/L	Harzdorf and Janser 1984
Waste water 1986 (chromium(VI))	Sample mixed with a masking agent and cetyltrimethyl-ammonium bromide solution at pH 4.7–6.6, heated in water bath at 50 °C for 10 minutes	Spectro-photometry at 583 nm	Lower than diphenyl-carbazone method	No data	Qi and Zhu 1986
Water (total chromium)	Calcium nitrate added to water and chromium is converted to chromium(III) by acidified H_2O_2	GFAAS or ICP/AES	1.0 µg/L (GFAAS) 7.0 µg/L	97–101% at 19–77 µg/L	EPA 1983a, 1986a (Method 218.2 and 7191)
Industrial wastes, soils, sludges, sediments, and other solid wastes (total chromium)	Digest with nitric acid/hydrogen peroxide	ICP-AES	4.7 µg/L	101% at 3.75 mg/L	EPA 1996b (Method 6010b)
Oil wastes, oils, greases, waxes, crude oil (soluble chromium)	Dissolve in xylene or methyl isobutyl ketone	AAS or GFAAS	0.05 mg/L	107% at 15 µg/L	EPA 1986b (Method 7190)

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

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Groundwater, domestic and industrial waste (chromium[VI])	Chromium(VI) is coprecipitated with lead sulfate, reduced, and resolubilized in nitric acid	AAS or GFAAS	0.05 mg/L (AAS) 2.3 µg/L (GFAAS)	93–96% at 40 µg/L	EPA 1986c (Method 7195)
Groundwater-EP extract, domestic, and industrial waste (chromium[VI])	Chelation with ammonium pyrrolidine dithiocarbonate and extraction with methyl isobutyl ketone	AAS	No data	96% at 50 µg/L	EPA 1983b, 1986d (Method 218.4 and 7197)
Water, waste water, and EP extracts (chromium(VI))	Direct	DPPA	10 µg/L	93% at 5 mg/L	EPA 1986e (Method 7198)
Soil, sediment and sludges (chromium(VI))	Acid digestion extraction using HNO_3	AAS	No data	85–115%	Ayyamperumal 2006; Oygard et al. 2004

AAS=atomic absorption spectrophotometry; $AlCl_3$=aluminum chloride; APDC=ammonium pyrrolidine dithiocarbonate; $CHCl_3$=chloroform; DPPA=differential pulse polarographic analysis; EAAS=electrothermal atomic absorption spectrometry; EP=extraction procedure (for toxicity testing); FIA/uv/vis=flow injection analysis-ultraviolet/visible spectroscopy; GFAAS=graphite furnace atomic absorption spectrometry; H_2SO_4=sulfuric acid; HCI=hydrochloric acid; HNO_3=nitric acid; HPLC=high pressure liquid chromatography; ICP-AES=inductively coupled plasma-atomic emission spectrometry; INAA=instrumental neutron activation analysis; MIBK=methylisobutyl ketone; Na_2O_2=sodium peroxide; NaOH=sodium hydroxide; Na_2CO_3=sodium carbonate; $(NH_4)_2SO_4$=ammonium sulfate; NH_3=ammonia; PIXE=proton-induced X-ray emission spectrometry; SO_2=sulfur dioxide; UV=ultraviolet; XRF=X-ray fluorescence analysis

due to adsorption on storage containers should be avoided by using polyethylene or similar containers and acidifying the solution to the proper pH.

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 chromium compounds 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 chromium compounds.

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. There are studies correlating chromium in urine (Cocker et al. 2007; Gylseth et al. 1977; Kilburn et al. 1990; Lindberg and Vesterberg 1983a; McAughey et al. 1988; Minoia and Cavalleri 1988; Mutti et al. 1985b; Sjogren et al. 1983; Tola et al. 1977), blood (Kilburn et al. 1990; Lewalter et al. 1985; McAughey et al. 1988; Wiegand et al. 1988), hair (Randall and Gibson 1987, 1989; Takagi et al. 1986), hair in children (Chiba et al. 2004), nails (Takagi et al. 1988), and erythrocytes (Lukanova et al. 1996) to occupational exposure levels. Since chromium is an essential element, levels of chromium compounds have to be relatively high in humans before they signify an increase due to exposure. Hair has been useful in determining chronic occupational exposure to chromium in high concentrations (Randall and Gibson 1989), although the utility of this method for detecting prior exposures has a limited timespan of months (Simpson and Gibson 1992). Analytical methods to detect chromium concentrations in urine (Randall and Gibson 1987), whole blood (Case et al. 2001; Dube 1988; Fahrni 2007), serum/plasma (Simonoff et al. 1984), and tissue (Fahrni 2007; Liu et al. 1994) have been reported. Generally, the detection limits are in the sub ppb to ppb range, and recoveries are good (>70%).

Chromium induced DNA-protein complexes were prepared as a biomarker of exposure, as discussed in Section 3.12.2. These complexes can be detected by potassium chloride-sodium dodecyl sulfate mediated precipitation. These methods have a number of inherent limitations including tedious methodology and being subject to considerable interindividual and interlaboratory variations (Singh et al. 1998b). Only one study has attempted to utilize this biomarker, and it was found that volunteers exposed to chromium in drinking water showed no increase in protein-DNA crosslinking in blood cells (Kuykendall et al. 1996). This suggests that this procedure may not be sensitive enough for use in environmental monitoring unless an individual has received a potentially toxic level of exposure. In addition, chromium forms chromium-DNA complexes inside of cells, and these complexes constitute a potential biomarker for the assessment of environmental or occupational exposure. A novel method has been described for the sensitive detection of chromium-DNA adducts using inductively coupled plasma mass spectrometry (Singh et al. 1998b). The detection limits of this method are in the parts per trillion range and allow for the detection of as few as 2 chromium adducts per 10,000 bases, which coupled with the low DNA sample requirements, make this method sensitive enough to measure background levels in the population. There are no data to determine whether there are age-specific biomarkers of exposure or effects or any interactions with other chemicals that would be specific for children.

Methods for Determining Parent Compounds and Degradation Products in Environmental Media. Methods are available and in use for detecting chromium in air, water, and soil environments. Air contaminated with chromium(VI), particularly in occupational settings, is of great concern. Methods have been developed that can determine low levels of total chromium and chromium(VI) in the air, with detection limits in the ng/m^3 range and excellent recoveries (90% or better) (Ashley et al. 2003; Barrie and Hoff 1985; CARB 1990; NIOSH 1994c, 1994d; Sheehan et al. 1992). These methods are sufficient to determine background chromium levels in the environment and levels at which health effects may occur. Chromium can be detected in water at concentrations in the ppb range (Abu-Saba and Flegal 1997; EPA 1983a, 1996a; Harzdorf and Janser 1984 Parks et al. 2004) and household and bottled drinking water (Al-Saleh and Al-Doush 1998), with recoveries of ≥90% being reported in some studies. In addition, there are also methods that can differentiate chromium(VI) from chromium(III) in water samples (EPA 1986c). A reliable analytical method for extracting and quantifying chromium, including chromium(VI), from soil surfaces has also been reported (Ayyamperumal et al. 2006; Oygard et al. 2004). Analytical methods exist that are sufficient for measuring background levels of chromium in soil (Ayyamperumal et al. 2006; EPA 1996b; Finley and Paustenbach 1997; Oygard et al. 2004) and water (EPA 1983a, 1983b, 1983c, 1986a, 1996a; Finley and Paustenbach 1997) and also water samples collected from various geological sites of interest (Gonzalez et al. 2005; Parks et al. 2004).

7.3.2 Ongoing Studies

Analytical methods for the detection of chromium compounds at increasingly lower concentrations are currently under development. Targeted areas of interest include air, water, and soil monitoring, with special emphasis being placed on populations considered vulnerable or potentially at risk, such as children and occupational workers. Additionally, more reliable methods to separate chromium(III) analysis from chromium(VI) analysis in collected samples is a source of interest and active research.