6. ANALYTICAL METHODS

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

Several methods are available for the analysis of chromium in different biological media. Some of the more recent methods for the determination of chromium are reported in Table 6-1. Several other reviews on the subject provide a more detailed description of the available analytical methods (EPA 1984a; Fishbein 1984; IARC 1986a, 1990; Torgrimsen 1982; WHO 1988).

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. Contaminations including dust contamination or losses of the samples during collection, transportation, and storage should be avoided. Biological samples collected with stainless steel scalpels, trays, and utensils are unacceptable for chromium analysis. Similarly, contamination or loss arising from sample containers should be avoided. Chromium-containing grinding and homogenizing equipment should not be used for preparation of biological samples. Reagents of the highest purity should be used to avoid contamination. The possible 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

Table 6-1. 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

Table 6-1. Analytical Methods for Determining Chromium in Biological Materials (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Urine	None	GFAAS	0.05 µg/L	91% at 0.22 µg/L	Randall and Gibson 1987
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_2SO4 =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

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 four most frequently used methods for determining low levels of chromium in biological samples are neutron activation analysis (NAA), mass spectrometry (MS), graphite spark atomic emission spectrometry (AES), and graphite furnace atomic absorption spectrometry (GFAAS). Of these four methods, only the GFAAS is readily available in conventional laboratories, and this method is capable of determining chromium levels in biological samples when an appropriate background correction method is used (Greenberg and Zeisler 1988; Plantz et al. 1989; Urasa and Nam 1989; Veillon 1989).

The problem of developing accurate data for chromium in biological samples is further complicated by the lack of Standard Reference Materials (SRM). Only recently have chromium certified materials, such as brewer's yeast (SRM-1569), bovine liver (SRM-1577), human serum (SRM-909), urine (SRM-2670), orchard leaves (SRM-1571), spinach leaves (SRM-1570), pine needles (SRM-1575), oyster tissue (SRM-1566), and tomato leaves (SRM-1573) been issued by the National Institute of Standards and Technology (formerly the National Bureau of Standards). Because of the lack of SRMs, the less recent data should be interpreted with caution (EPA 1984a), unless the data are verified by interlaboratory studies.

Another difficulty with the analytical methods used to detect chromium is the ability of the applied analytical method to distinguish between chromium(III) and chromium(VI). However, 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. These 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 6-1 are some of the more recent methods, they are not necessarily the ones most commonly used. A comparison of the various commonly used methods and the methods for the avoidance of contamination during sampling, sample handling, and analysis are provided by Kumpulainen (1984).

6.2 ENVIRONMENTAL SAMPLES

Analytical methods for determining chromium in environmental samples are reported in Table 6-2. Chromium may be present in both the trivalent and hexavalent oxidation states in most ambient environmental and occupational samples, and sometimes the distinction between soluble and insoluble forms of chromium(VI) is 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 μg/m^3 (EPA 1990a), although two methods described in Table 6-2 can determine chromium(VI) concentrations alone in air at a minimum detection limit of 0.1 ng/m^3 for a 20m^3 sample (CARB 1990). The three commonly used methods that have the best 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 (Henshaw et al. 1989; Malinski et al. 1988; Schaller and Neeb 1987). A method using high performance liquid chromatography 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 alkaline digestion procedure followed by UV-VIS spectroscopy has been developed which can quantify chromium(VI) in soil, sediment, and sludge (EPA 1997).

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 due to adsorption on storage containers should be avoided by using polyethylene or similar containers and acidifying the solution to the proper pH. The preferred methods for digestion of environmental samples have been discussed by Griepink and Toelg (1989).

Table 6-2. 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 chromatography/ 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 chromatography/ coulometric	0.01 ng/m^3 for 20 m^3 sample	94%	Sheehan et al. 1992

Table 6-2. Analytical Methods for Determining Chromium in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
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
Occupational air (chromium(VI))	Extract with 0.05M $(NH_4)_2SO_4$–0.5M $(NH_4)_2SO_4$.1M NH_3.	FIA-UV/VIS	0.11 ng	>90%	Wang 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	Spectrophotometry 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	Chromatography at 540 nm Spectrophotometry 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 simultaneous 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

Table 6-2. Analytical Methods for Determining Chromium in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Atomospheric 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 Nuclepore 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
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 1983 (Method 218.5)
Drinking water, groundwater and water effluents (chromium(VI))	Buffer solution introduced into ion chrom. Derivitized with dipenylcarbazide	Ion chromatography spectrophotometry 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

Table 6-2. Analytical Methods for Determining Chromium in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
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 EC for 10 minutes	Spectrophotometry at 583 nm	Lower than diphenylcarbazone 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 1983, 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 6010)
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)
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 50µg/L	EPA 1983, 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)

Table 6-2. Analytical Methods for Determining Chromium in Environmental Samples (*continued*)

Sample matrix	Preparation method	Analytical method	Sample detection limit	Percent recovery	Reference
Soil, sediment and sludges (chromium(VI))	Alkaline digestion extraction using Na_2CO_3 and NaOH	UV-VIS	No data	85–115%	EPA 1997 (Method 3060A and 7196A)

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; HCl=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

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 chromium is available. Where adequate information is not available, ATSDR, in conjunction with the National Toxicology Program (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.

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. There are studies correlating chromium in urine (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), 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); the usefulness of this method for detecting prior exposures is limited to a timespan of months (Simpson and Gibson 1992). Analytical methods to detect chromium concentrations in urine (Randall and Gibson 1987), whole blood (Dube 1988), serum/plasma (Simonoff et al. 1984), and tissue (Liu et al. 1994) have been reported. Generally, the detection limits are in the subppb to ppb range, and recoveries are good (>70%). These methods are sensitive enough to measure background levels in the general population.

Chromium induced DNA-protein complexes may be used as a biomarker of exposure as discussed in Section 2.12.2. These complexes can be detected by potassium chloride-sodium dodecyl sulfate mediated

precipitation. These methods have a number of inherent limitations including being tedious and 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. Chromium forms chromium-DNA complexes inside of cells and these complexes constitute a potential biomarker for the assessment of environmental or occupational exposure. Recently, 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 allows 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. Air contaminated with chromium(VI), particularly in occupational settings, are of great concern. Methods have been developed that can determine low levels of total chromium and chromium(VI) in the air (Barrie and Hoff 1985; CARB 1990; NIOSH 1994c, 1994d; Sheehan et al. 1992). These methods have detection limits in the ng/m^3 range with excellent recoveries (90% or better). 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 (EPA 1983, 1996a; Harzdorf and Janser 1984) with recoveries of 90% or greater being reported. Methods are available 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 (EPA 1997). Current analytical methods exist that are sufficient for measuring background levels of chromium in soil (EPA 1996b, 1997) and water (EPA 1983, 1986a, 1996a).

6.3.2 Ongoing Studies

No ongoing studies regarding the determination of different speciated forms of chromium (as opposed to total chromium content) in biological or some environmental media (e.g., soil, sediment) were found.