Environmental Health Perspectives
Vol. 92, pp. 13–16, 1991

The Analytical Biochemistry of Chromium

by Sidney A. Katz*

The essentiality and carcinogenicity of chromium depend on its chemical form. Oxidation state and solubility are particularly important in determining the biological effects of chromium compounds. For this reason, total chromium measurements are of little value in assessing its nutritional benefits or its toxicological hazards. Aqueous sodium carbonate-sodium hydroxide solutions have been successfully used for extracting hexavalent chromium from a variety of environmental and biological matrices while preserving its oxidation state. Typical recoveries are 90 to 105% in samples spiked with both trivalent and hexavalent chromium. Determination of hexavalent chromium after extraction with sodium carbonate-sodium hydroxide solution, coupled with the determination of total chromium after nitric acid-hydrogen peroxide digestion, has been applied to the evaluation of chromium speciation in airborne particulates, sludges, and biological tissues.

In general, the bioavailability of an element is determined by its chemical form. Oxidation state and solubility are particularly important factors for bioavailability. For this reason, it is frequently necessary to establish the trace element composition of agricultural, biological, clinical, and environmental materials in both qualitative and quantitative terms. In this reference frame, chromium is of special interest because it is both an essential nutrient and a carcinogen. Dietary deficiency of trivalent chromium has been associated with faulty sugar metabolism in the human, and the inhalation of some moderately insoluble hexavalent chromium compounds has been correlated with increased incidences of lung cancer.

Chromium is an essential trace element in the human diet. It is poorly absorbed, and the concentrations of chromium in various tissues and fluids are normally quite low. A quarter of a century ago, Mertz (*1*) proposed a biochemical role for trivalent chromium (Cr^{III}) in the metabolism of glucose. Subsequently, a glucose tolerance factor was identified as a complex of trivalent chromium with niacin and three amino acids (*2*).

The available data show trivalent chromium compounds to be less toxic than those of hexavalent chromium (*3–5*). The carcinogenicity of various hexavalent chromium (Cr^{VI}) compounds in human and animal models is well documented. Acute and chronic toxicity problems associated with exposure to hexavalent chromium compounds include ulceration of the skin, perforation of the nasal septum, inflammation of the larynx, as well as damage to the kidneys and lungs. These effects are not observed with exposure to trivalent chromium compounds.

When chromium forms chemical compounds, it demonstrates several oxidation numbers. The most abundant and most stable compounds are those of trivalent chromium. Those of hexavalent chromium are well known and well characterized. Chromium compounds demonstrating other oxidation numbers are unstable. In acid media, trivalent chromium exists as the hydrated cation or as simple inorganic complexes. Many trivalent chromium compounds are water insoluble in the pH range of 4 to 11, but trivalent chromium forms stable complexes with many "bioligands" (*6*).

Trivalent chromium is readily oxidized to the hexavalent form in alkaline media. Many of the resulting chromates are insoluble in water. The chromates condense to dichromates and to higher polymers in acid media. The dichromates are usually more soluble than the corresponding chromates. The dichromates are strong oxidizing agents in acid media. Their tendency to be reduced to the trivalent state increases with decreasing pH.

The toxic manifestations of chromium exposure appear to be determined by the bioavailability and biochemical interactions of specific chromium compounds rather than by chromium *per se*. For this reason, it is frequently necessary to selectively determine the amounts of these compounds, rather than the total chromium content, in a wide variety of agricultural, biological, clinical, and environmental matrices.

*Department of Chemistry, Rutgers University, Camden, NJ 08102.

The analytical chemistry of chromium makes use of molecular, atomic, and nuclear spectroscopic techniques as well as chemical and electrochemical methodologies. Atomic absorption spectroscopy has found frequent application for the determination of chromium (*7–9*), but this technique lacks specificity with respect to the oxidation state of the chromium. Atomic absorption spectroscopy coupled with appropriate chemical pretreatments, however, allows some degree of specificity in the selective determination of tri- and hexavalent chromium.

Selective determinations of tri- and hexavalent chromium are necessary for compliance monitoring in the workplace. The current National Institute for Occupational Safety and Health (NIOSH) recommendation (*10*) for airborne chromium limits is: *a*) Carcinogenic Cr^{VI} shall be controlled in the workplace so that the airborne workplace concentration of Cr^{VI}, sampled and analyzed according to recommended procedures, is not greater than 1 μg Cr^{VI}/m^3 of breathing zone air. *b*) Noncarcinogenic Cr^{VI} shall be controlled in the workplace so that the airborne workplace concentration is not greater than 25 μg Cr^{VI}/m^3 of breathing zone air determined as a time-weighted average (TWA) exposure for up to a 10-hr workday, 40-hr work week and is not greater than 50 μg Cr^{VI}/m^3 of breathing zone air as determined in any 15-min sample.

According to the NIOSH criteria documents (*11*), noncarcinogenic Cr^{VI} is the chromium in the mono- and dichromates of hydrogen, lithium, sodium, potassium, rubidium, cesium, and ammonium, and in chromium trioxide. The chromium in all other hexavalent chromium compounds is identified as carcinogenic Cr^{VI}. NIOSH has apparently identified the water-soluble hexavalent chromium compounds as noncarcinogenic Cr^{VI} and many of the water-insoluble hexavalent chromium compounds as carcinogenic Cr^{VI}.

The American Conference of Governmental Industrial Hygienists (ACGIH) threshold limit values (*12*) for the various chemical forms of chromium are 0.5 mg/m^3 for chromium metal, 0.5 mg/m^3 for divalent chromium compounds, 0.5 mg/m^3 for trivalent chromium compounds, and 0.05 mg/m^3 for hexavalent chromium compounds. Both the qualitative and the quantitative determinations of the various chemical forms of airborne chromium in the workplace environment are necessary for an accurate assessment of the potential for an inhalation hazard. The concentration of insoluble hexavalent chromium is of major significance.

It is not possible to separate the various chemical forms of chromium from each other in a single step with a single reagent. It is, however, possible to selectively extract chromium compounds from some airborne dusts and to obtain information on the specific valence states that relate to the NIOSH and ACGIH exposure limits.

Extraction of the sample with nitric acid and hydrogen peroxide dissolves the chromium as the trivalent cation. Quantification with atomic absorption spectroscopic techniques gives a measure of the total chromium in the dust. Extraction of a second specimen with 0.1 M, pH 7.0, acetate buffer selectively isolates the soluble hexavalent chromium compounds. Most trivalent chromium compounds and many metal chromates are insoluble under these conditions. The soluble chromates are quantified by either atomic absorption spectroscopy or spectrophotometry of the diphenylcarbazide chromophore.

Extraction of a third specimen with 3% sodium carbonate-1% sodium hydroxide solution solubilizes both the soluble and the insoluble hexavalent chromium compounds in the dust. Trivalent chromium compounds are insoluble under these conditions, and oxidation of Cr^{III} to Cr^{VI} does not take place. The insoluble hexavalent chromium compounds are transposed to soluble sodium chromate, and the soluble hexavalent chromium compounds dissolve under these conditions. Quantification by either atomic absorption spectroscopy of spectrophotometry of the diphenylcarbazide chromophore gives a measure of the total hexavalent chromium in the dust.

Application of these selective extractions to the determination of chromium in airborne dust allows identification of its speciation as follows: *a*) insoluble trivalent chromium = difference between total chromium and total hexavalent chromium; *b*) soluble hexavalent chromium = chromium extracted by pH 7 buffer; *c*) insoluble hexavalent chromium = difference between total hexavalent chromium and soluble hexavalent chromium.

Nitric acid-hydrogen peroxide extracts and sodium carbonate-sodium hydroxide extracts of dust were quantified by atomic absorption spectroscopy, and the results were compared to those obtained from dust samples spiked with Cr^{III} and Cr^{VI}. Precision of the results and recovery of the spikes were acceptable. These data are summarized in Table 1.

Naranjit et al. (*13*) and Ehman et al. (*14*) have used ion exchange techniques to isolate hexavalent chromium from aqueous extracts of welding fume residues and airborne particulates, respectively. Naranjit et al. obtained 99% recovery of Cr^{III} from an anion column and 99% recovery of Cr^{VI} from a cation column with welding fume extracts buffered in the 3 to 5 pH range (*13*). Ehman et al. obtained 95% recoveries using an anion resin to isolate "bioavailable" hexavalent chromium from acidified EDTA extracts of airborne particulates (*14*).

Although the proposed drinking water standard for chromium is 100 ppb regardless of chemical form, information on the chemical speciation of chromium in wastewater, surface water, and drinking water is often needed for environmental quality and public health risk assessments. Subramanian (*15*) has developed an APDC-MIBK (ammonium pyrrolidinecarbodithioate-methyl isobutylketone) extraction scheme that allows the determination of total chromium and of hexavalent chromium in natural waters by electrothermal atomization atomic absorption spectroscopy. The conditions for

Table 1. Quality assurance/quality control exercise.

Specimen, extraction	Percent (SD)		
	Cr^{VI}	Cr^{III}	Total Cr
Five 250-mg specimens, alkaline extraction	2.05 (0.02)		
Five 125-mg specimens, alkaline extraction	2.05 (0.05)		
Five 100-mg specimens, acid extraction			2.65 (0.14)
Five 250-mg specimens, double alkaline extraction then acid extraction of the residue	2.20 (0.03)	0.61 (0.005)	
Five 250-mg spiked[a] specimens, alkaline extraction then acid extraction of the residue	2.44 (0.03)	1.00 (0.04)	

[a]Five 250-mg specimens were spiked with 1.00 mg of hexavalent chromium and 1.00 mg of trivalent chromium.

the selective extraction of Cr^{VI} from natural or drinking water samples are different from those under which Cr^{VI} plus Cr^{III} are extracted. Duplicate samples were extracted under each set of conditions, and the Cr^{III} was determined by difference. Spike recoveries of Cr^{VI} and Cr^{III} ranged from 95% to 105%, and the results obtained for total chromium in National Bureau of Standards standard reference material and National Research Council of Canada Certified Reference Materials were in good agreement with the reference values. Some of these results are presented in Table 2.

Recognizing the lack of evidence for the toxicity of trivalent chromium compounds, the U.S. Environmental Protection Agency (*16*) has published a proposal that applies the Resource Conservation and Recovery Act EP Toxicity test to hexavalent rather than total chromium for the assessment of hazardous wastes. The procedure for distinguishing between tri- and hexavalent chromium is based on the isolation of Cr^{VI} from the EP extract by coprecipitation of lead chromate with lead sulfate and subsequent quantification by electrothermal atomization atomic absorption spectroscopy.

Table 2. Chromium contents of standard reference materials/certified reference materials (*15*).

Reference sample[a]	ng Cr/mL, mean (SD)		
	Cr^{VI}	Cr^{III}	Total Cr
NBS SRM 1643			
Certified value[b]			17.3 (2.0)
Observed value	2.1 (0.3)	16.0	18.1 (0.6)
NBS SRM 1643			
Certified value			18.9 (0.4)
Observed value	1.4 (0.3)	16.4	17.8 (0.3)
NRCC SLRS-1			
Certified value			0.36 (0.04)
Observed value	0.11 (0.04)	0.11	0.22 (0.05)

[a]NBS SRM, National Bureau of Standards standard reference material; NRCC, National Research Council of Canada.
[b]Only total chromium values are certified.

The current methodologies for the determination of trivalent chromium and hexavalent chromium appear to be adequate for most air and water samples. The highly variable composition of sludges, sediments, and soils, however, presents some formidable problems in preserving its compounds from such matrices. When acid digestion or acid extraction is used for the quantitative recovery of chromium, hexavalent species are usually fully or partially reduced to trivalent forms. Most compounds of trivalent chromium and many metal chromates are of limited water solubility within three pH units of neutrality. The compounds of hexavalent chromium are efficiently solubilized by alkaline extractants, but the potential for the oxidation of Cr^{III} to Cr^{VI} is significantly increased. Good recovery of hexavalent chromium spikes from a humus-rich soil amended with a digested municipal sewage sludge has been obtained by extraction with a 3% sodium carbonate-1% sodium hydroxide solution (*17*), but the solubilization and recovery of hexavalent chromium from such matrices has not been adequately addressed.

The biological tissues are perhaps the least-adequately addressed of the matrices with respect to the chemical speciation of their chromium contents. Some unsophisticated polarographic studies on the amounts of hexavalent and trivalent chromium extracted from chrome-tanned leathers with alkali under nitrogen atmospheres were reported over a quarter century ago (*18*). Clearly, more work on tissues and fluids is needed. Aqueous solutions of tetramethylammonium hydroxide have been successfully used to prepare liver, kidney, and hair samples for the determination of cadmium, copper, and zinc by atomic absorption spectrometry (*19*). Similarly, Lumatom tissue solubilizer, a toluene solution of tetraalkyl ammonium hydroxide, has been reported to be superior to acid digestion in preparing muscle biopsies for cadmium, copper, and manganese determinations by atomic absorption spectroscopy (*2*). These quaternary ammonium hydroxides should be investigated with respect to their suitabilities as reagents in preparing fluids and tissues for chromium speciation determinations.

Carpenter (*21*) has used subtilisin, a potent proteolytic enzyme, to prepare samples of kidney and liver tissues for the determination of their cadmium, copper, lead, and thallium contents by atomic absorption spectroscopy. Recoveries of spikes for all four metals ranged from 95 to 102%, and the results obtained from samples prepared by plasma ashing agreed well with the results obtained from samples prepared by enzymatic digestion of the tissue matrix. The application of enzymatic digestion to the preparation of tissues for chromium speciation determinations should be further investigated.

At present, reliable methodologies for the determination of chromium speciation are lacking for some matrices. These deficiencies must be corrected, and standard methods for the determination of trivalent and hexavalent chromium in tissues and in sludges, sediments, and soils must be developed.

The development of analytical methodologies and the subsequent validation of analytical data for chromium speciation requires appropriate standard or certified reference materials. Such reference materials are available for total chromium in a range of biological and environmental matrices. In the absence of reference materials for trivalent and hexavalent chromium, method development and data validation must be assured by other means of analytical quality control including interlaboratory comparisons, standard addition experiments, radiotracer methods, and comparisons of the results obtained by different analytical procedures. To this end, continued cooperation among scientists in the academic, commercial, governmental, and industrial sectors is needed. It is hoped that this will be one of the outcomes of this conference, and it will lead to resolving some of the current deficiencies in the analytical biochemistry of chromium.

REFERENCES

1. Schwarz, K. and Mertz, W. Chromium(III) and the glucose tolerance factor. Arch. Biochem. Biophys. 85: 292–295 (1959).
2. Mertz, W. Effects and metabolism of glucose tolerance factor. Nutr. Rev. 33: 129–135 (1975).
3. Rinehart, W. E., and Gad, S. C. Current concepts in occupational health: metals–chromium. Am. Ind. Hyg. Assoc. J. 47: 696–699 (1986).
4. Norseth, T. The carcinogenicity of chromium and its salts. Br. J. Ind. Med. 43: 649–651 (1986).
5. Basu, D., Blackburn, K., Harris, B., Neal, M. W., and Stoss, F. W. Health Assessment Document for Chromium, EPA-600/8-83-014F. U.S. Environmental Protection Agency, Research Triangle Park, NC, 1984.
6. Samitz, M. H., and Katz, S. A. A study of the chemical reactions between chromium and skin. J. Invest. Dermatol. 43: 35–43 (1964).
7. Christian, G. D., and Feldman, F. J. Atomic Absorption Spectroscopy: Applications in Agriculture, Biology, and Medicine. Wiley-Interscience, New York, 1970.
8. Katz, S. A., and Jenniss, S. W. Regulatory Compliance Monitoring by Atomic Absorption Spectroscopy. Verlag Chemie International, Deerfield Beach, FL, 1983.
9. Van Loon, J. C. Selected Methods of Trace Metal Analysis: Biological and Environmental Samples. John Wiley and Sons, New York, 1985.
10. United States Department of Health, Education and Welfare. Occupational Exposure to Chromium(VI), HEW Publication NIOSH 76–129. U.S. Government Printing Office, Washington, DC, 1976.
11. National Institute for Occupational Safety and Health. Criteria for a Recommended Standard: Occupational Exposure to Chromium (VI), United States Department of Health, Education and Welfare, Washington, DC, 1975.
12. ACGIH Documentation of Threshold Limit Values, 4th ed. American Conference of Government Industrial Hygienists, Inc., Cincinnati, OH, 1980.
13. Naranjit, D., Thomassen, Y., and Van Loon J. C. Development of a procedure for studies of the chromium (III) and chromium (VI) contents of welding fumes. Anal. Chim. Acta 110: 307–312 (1979).
14. Ehman, D. L., Anselmo, V. C., and Jenks, J. M. Determination of low levels of airborne chromium (VI) by anion exchange treatment and inductively coupled plasma spectroscopy. Spectroscopy 3: 33–35 (1988).
15. Subramanian, K. S. Determination of chromium (III) and chromium (VI) by ammonium pyrrolidinecarbodithioate-methyl isobutyl ketone furnace atomic absorption spectrometry. Anal. Chem. 60: 11–15 (1988).
16. Environmental Protection Agency. Hazardous Waste Management System: identification and listing of hazardous wastes. Fed. Reg. 45: 72029–72034 (1980).
17. Kazi, T., and Katz, S. A. The biocycling of some micronutrients and some toxic elements from sewage sludges used as agricultural fertilizers. Presented at the Second International Conference on Health and Disease, Karachi, February 1987.
18. Samitz, M. H., and Gross, S. Extraction by sweat of chromium from chrome tanned leather. J. Occup. Med. 2: 12–14 (1960).
19. Murthy, L. Menden, E. E., Eller, P. M., and Petering, H. G. Atomic absorption determination of zinc, copper, cadium, and lead in tissues solubilized by aqueous tetramethylammonium hydroxide. Anal. Biochem. 53: 365–372 (1973).
20. Julshamn, K., and Andersen, K. J. A study on the digestion of human muscle biopsies for trace metal analysis using an organic tissue solubilizer. Anal. Biochem. 98: 315–318 (1979).
21. Carpenter, R. C. The determination of cadmium, copper, lead, and thallium in human liver and kidney tissue by flame atomic absorption spectrometry after enzymatic digestion. Anal. Chim. Acta 125: 209–213 (1981).