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EFFECTS OF CHROMIUM IN THE CANADIAN ENVIRONMENT

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ASSOCIATE COMMITTEE ON
SCIENTIFIC CRITERIA FOR
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NATIONAL RESEARCH COUNCIL OF CANADA
ASSOCIATE COMMITTEE ON SCIENTIFIC CRITERIA
FOR
ENVIRONMENTAL QUALITY

EFFECTS OF CHROMIUM IN THE CANADIAN ENVIRONMENT

SUBCOMMITTEE ON HEAVY METALS AND
CERTAIN OTHER COMPOUNDS

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This monograph contains the collective views of the panel members. The first draft of the monograph was revised, updated and edited by Dr. Jaworski prior to its final review.

The Associate Committee on Scientific Criteria for Environmental Quality was established by the National Research Council of Canada in response to a mandate provided by the Federal Government to develop scientific guidelines for defining the quality of the environment. The concern of the NRC Associate Committee is strictly with scientific criteria. Pollution standards and objectives are the responsibility of the regulatory authorities and are set for the purpose of pollution control. These may be based on scientific criteria as a starting point but they also take into account the optimal socio-economic impact of proposed measures as well as the state of existing technology.

The Associate Committee's program includes the evaluation of available information on the probability of effects of contaminants on receptors together with the related fundamental principles and scientific knowledge. In this work particular attention is directed to receptors and contaminants (and their interactions) important to Canada. This Canadian approach is necessary because evaluations made in other countries or regions will not always be applicable to the particular circumstances prevailing in Canada.

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Comments on Associate Committee documents are welcome and will be carefully reviewed by the Expert Panels. It is foreseen that these scientific criteria may be revised from time to time, as new knowledge becomes available.

UNITS

l = litre

ml = millilitre

m = metre

μm = micrometre = 10^{-6} metre

g = gram

μg = microgram = 10^{-6} gram

ng = nanogram = 10^{-9} gram

ppm = part per million = $\mu\text{g/g}$ = $\mu\text{g/ml}$ of dilute aqueous solution

ppb = part per billion = ng/g = ng/ml of dilute aqueous solution

DEFINITION

Criteria are cause/effect interrelations based strictly on the scientific evaluation of environmental harm.

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CHAPTER 1 INTRODUCTION

This document was written primarily to assess the environmental impact of chromium (Cr) and its compounds with a special regard to health effects on living organisms.

In many respects, Cr is an exceptional element. The predominant Cr-bearing mineral is chromite, a very inert mixed oxide of Fe, Cr and other elements. Nevertheless, Cr is found at low levels in almost all biological materials where it interacts strongly with biochemically important molecules to form very stable complexes. In spite of these low levels, Cr has been shown to be an essential element in mammalian metabolism.

Domestic and industrial uses of Cr are legion and involve forms of Cr which are more reactive than chromite. Hence it is necessary to examine whether man's activities can result in a significant change in the availability of Cr in the environment, and what are the possible consequences to living organisms.

Recently, the US National Academy of Sciences (NAS 1974) published an excellent volume on the health effects of Cr both in industry and in the environment as a whole. It is not the intent of this present document to duplicate this material. Rather, an attempt is made to translate the cause/effect data present therein into a Canadian context. Furthermore, work published in the interim (July 1972 to February 1975) has been critically evaluated in an attempt to present the current status of knowledge on the health effects of Cr and its compounds.

CHAPTER 2 SUMMARY

Chromium (Cr) was selected for study by the Subcommittee on Heavy Metals and Certain Other Elements of the NRC Associate Committee on Scientific Criteria for Environmental Quality because of its widespread use in industrial and domestic products and because of the toxicity of some of its chemical forms. Thousands of tons of Cr ore and concentrates are imported annually for the production of stainless steels, chrome plated metal, pigments for paints and inks, and a variety of chemicals. The two main chemical forms of Cr are Cr(III) (Cr^{+3}) and Cr(VI) (Cr^{+6}).

Industrial production and handling of certain Cr(VI) products have been associated with respiratory injury and cancer, as well as skin ulceration and the induction of allergic Cr dermatitis in occupationally exposed workers. Land dumping of wastes from industrial Cr(VI) production and electroplating operations has been responsible for groundwater contamination. Discharge of Cr(VI) wastes into streams and rivers has caused damage to aquatic ecosystems. Large amounts of Cr(III) and Cr(VI) are reintroduced into the environment as sewage and solid wastes by the disposal of consumer products containing Cr.

This report discusses the effects of emissions of Cr chemicals on the various living organisms in the environment. Naturally occurring levels and forms of Cr are compared with those resulting from industrial and domestic activities in order to assess their environmental impact. Background (natural) levels of Cr in air average 1 ng/m^3 , while urban air may contain $10\text{--}60 \text{ ng/m}^3$. Occupational exposure in some Cr-using industries may approach 10^7 ng/m^3 . Present control methods are aimed at reducing this level to below $5 \times 10^4 \text{ ng/m}^3$ Cr. Man-made aerosols characteristically have smaller-sized particles than naturally occurring aerosols; hence, they have a greater probability of penetrating into the lower respiratory tract.

Background levels of Cr in water average 1 ppb while municipal drinking water may contain 0.1-35 ppb Cr. Untreated industrial effluents may contain as much as 5×10^7 ppb Cr. Most of the Cr(III) present in water is adsorbed on particulate matter whereas Cr(VI) remains in solution, where it is a powerful oxidizing agent. The Cr(VI) is reduced to Cr(III) when it reacts with animal or vegetable material in the water.

Most foodstuffs contain 0.1-0.3 ppm Cr on a wet weight basis. Human soft tissues contain 0.03 ppm Cr and animal tissues contain 0.2 ppm Cr on a wet weight basis. Normal human dietary intake of Cr(III) averages 50 to 70 $\mu\text{g/day}$, with absorption ranging from 0.5 to 25%, depending on the type of food.

Cr(III) is the commonest naturally occurring form of Cr and is the form which functions biologically as an essential element in mammals where it maintains efficient glucose, lipid and protein metabolism. The toxicity of Cr(III) is low because of its poor membrane permeability and noncorrosive nature.

Cr(VI) is produced by the oxidation of Cr(III). Little Cr(VI) is found in nature, and the bulk of the Cr(VI) in the environment is a result of industrial and domestic emissions. Cr(VI) is a strong oxidizing agent and, since it easily crosses biological membranes, is highly toxic.

The scientific criteria (cause/effect relations) detailed in various chapters of the document are summarized below:

MICROSCOPIC ORGANISMS

Various microorganisms have been studied in solution culture: as little as 0.5 ppm Cr(III) interfered with normal metabolism in certain bacteria; T₁ phage (virus) and *E. coli* (bacterium) were irreversibly inactivated by 1-10 ppm Cr(III); Cr(VI) was toxic to certain yeasts and microbes even at the 0.1 ppb level. For various protozoans and rotifers, the aqueous Cr(VI) concentration at which 50% of the test organisms died in 96 hours (the 96-hour median lethal concentration, or LC50) was in the range of 0.5 to 20 ppm.

Biological oxidation by microorganisms in sewage sludges was adversely affected by as little as 50 ppm Cr(III) or 5 ppm Cr(VI) in the raw sewage.

All the above values are affected by the ionic strength (total content of dissolved salts) of the medium as well as by the nature and amount of suspended solids present in the medium and by the length of the testing period. Nevertheless, Cr(VI) was invariably the more toxic form.

AQUATIC ORGANISMS

Benthic organisms (bottom dwelling communities) exhibited varying tolerances to Cr pollution. *Daphnia* (a small crustacean) exhibited high sensitivity to both Cr(III) and Cr(VI) in solution culture. A 16% reproductive impairment was linked with 0.33 ppm Cr(III). This is significant because Cr(III) in waters contained in sediments can approach this level. Toxic thresholds ranging from 0.016 to 0.7 ppm Cr(VI) were reported for *Daphnia magna*.

In two years, oysters accumulated enough Cr from sea water containing 10 ppb Cr to suffer toxic and even lethal effects during the summer when their metabolic activity was at its highest. Other benthic organisms (crustaceans, worms, insect larvae, snails) exhibited 96-hour LC50 values ranging from 3 to 60 ppm Cr(III).

In fish, the 96-hour LC50 is a measure of acute rather than chronic exposure. A 12-week chronic toxicity study of salmon fingerlings showed that exposure to 0.2 ppm Cr(VI) in the stream water resulted in a 53% mortality while 0.2 ppm Cr(III) was below the mortality threshold. Rainbow trout survived 12 days exposure in laboratory tanks to 2.5 ppm Cr(VI) but accumulated significant quantities of Cr. In laboratory experiments with bluegills, fatheads and goldfish (species more resistant to Cr toxicity), the 96-hour LC50 ranged from 3 ppm Cr(III) (soft water) to 72 ppm Cr(III) (hard water), and from 18 ppm Cr(VI) (soft water) to 133 ppm Cr(VI) (hard water).

As for microorganisms, all the above values are affected by ionic strength, the amount of suspended solids, and the length of the testing period.

TERRESTRIAL VEGETATION

The main toxic action of Cr occurred in the roots. More than 0.1 ppm Cr(VI) in a solution culture interfered with the uptake and translocation of essential elements by the roots of soybeans. In solution culture, 1-5 ppm of either Cr(III) or Cr(VI) was the toxic threshold for a number of plant species. When compost was used for the growth medium, the toxic threshold was increased to 500 ppm for Cr(VI) and 5000 ppm for Cr(III). In contrast, the use of sand as the growth medium did not change the toxic threshold much from those for solution cultures. This increase in the toxic threshold was related to the greater ion exchange capacity of compost as compared to sand. Thus, the greatest risk of Cr toxicity to plants is in acidic sandy soil having low organic content.

The high Cr content of various fertilizer materials may lead to significant accumulation of Cr in agricultural soils.

Damage to foliage has been caused by application of droplets of Cr(VI) solutions as dilute as 50 ppm. Thus, industrial air emissions containing Cr(VI) aerosols would pose a significant risk to the surrounding vegetation.

ANIMALS

Pathological changes in the lungs of animals were induced when they were exposed to Cr(VI) aerosols at concentrations as low as 1 mg/m³ for more than 30 days. Chromium (III) oxide did not appear to be chemically active in the lung. However, cell culture studies showed that more than 52 ppm Cr(III) reduced the ciliary activity of the cells responsible for clearance of debris and particulate matter from the lungs of rabbits.

The dietary toxic threshold for Cr(VI) in experimental animals is higher than in man. In dogs fatal exposure was 0.1 g Cr(VI)/day in food for 2-3 months; 11.2 ppm in drinking water for four years caused significant tissue accumulation of Cr but no pathological effect; 6 ppm was the threshold for Cr accumulation in tissues. In rats, 134 ppm Cr(VI) in drinking water for 2-3 months caused kidney and liver lesions, while the toxic threshold for Cr(VI) in food of young rats was 0.1% (1000 ppm). Hence, Cr(VI), when blended in with food, is much less available than the Cr(VI) in drinking water.

Contact (dermal) allergic response to Cr was induced by repeated exposure to solutions containing 1000-3000 ppm of Cr(III) or Cr(VI) salts. Allergic or sensitized guinea pigs responded to as little as 10 µg Cr(III) or Cr(VI). Generally, Cr(VI) elicited the stronger response.

Highly concentrated solutions of Cr(VI) (30,000 ppm) caused skin lesions or ulcers only if the skin was abraded or had its natural oil removed beforehand. Cr(III) in concentrations as high as 100,000 ppm had no ulcerogenic effects.

For most mammalian experimental animals (mice, dogs, rabbits, cats, guinea pigs), the minimum injected fatal dose of Cr(VI) was 1-5 mg/kg body weight; lower doses, 0.2-0.5 mg/kg-body weight, produced marked kidney damage. The minimum lethal dose for Cr(III) was 0.26 g/kg in mice.

Repeated sublethal injections of Cr(VI) do not promote tolerance in mice but rather decrease the amount of the minimum lethal dose.

Intravenously injected Cr(III) passed the placental barrier in mice.

Inhalation of Cr-bearing dusts rarely produced respiratory cancer in experimental animals; however, implantation of Cr(VI) (in a carrier medium) in rat bronchial tubes did consistently produce cancers of the respiratory tract. Cr(VI) in

the form of a soluble pure salt is rapidly cleared from the lungs, while the slower release at a significant concentration from the implant produces cancer in surrounding tissues and perhaps mimics the carcinogenic effect of partially soluble Cr(VI) in a mixed dust. Cr(III) was inactive in the production of cancer.

HUMANS

The respiratory and renal systems were most susceptible to the action of Cr(VI).

Cr(VI) levels in air greater than 0.05 mg/m^3 were associated with a high risk of injury to nasal tissues. Levels as low as 0.01 mg/m^3 Cr(VI) produced strong irritation in the nose even after short exposures. Risk of injury to throat and lower respiratory tissues was low if Cr(VI) levels were kept below 0.05 mg/m^3 . In some individuals, where the lower respiratory tissues became Cr-sensitized, asthmatic attacks occurred at levels as low as 0.0025 mg/m^3 Cr(VI).

Respiratory tissues are considered to be susceptible to irritation by Cr(III) only if previously sensitized. High levels of Cr(III)-bearing dusts, $4\text{-}9 \text{ mg/m}^3$, have been associated with an increased incidence of bronchitis and pneumoconiosis in chrome-refractory workers and chromite miners.

The production of chromates from chromite and the production of chromate pigments have been associated with occupational respiratory cancer. In the chromate-producing industry, workers who developed respiratory cancer were exposed to estimated Cr levels ranging from 0.03 to 1.1 mg/m^3 for periods of 4-24 years. Workers producing chromate pigment who developed respiratory cancer had an estimated Cr exposure of $0.5\text{-}1.5 \text{ mg/m}^3$ for 6 to 9 years.

The lethal oral dose of Cr(VI) (single dose basis) for a 14-year-old boy was estimated to be 10 mg/kg -body weight. This concentration is much lower than that tolerated by test animals on a repeated basis over a period of several weeks or months. The most consistent manifestation of Cr(VI) poisoning was kidney damage. One family used drinking water estimated to contain 1-25 ppm Cr(VI) for 3 years without obvious deleterious effects. Drinking water containing 5 ppm or more Cr(VI) caused nausea when consumed on an empty stomach.

Repeated exposure to Cr compounds caused dermal sensitization in some workers. Such sensitized individuals reacted to solutions as dilute as 0.005% $\text{K}_2\text{Cr}_2\text{O}_7$. Cr(VI) is reduced to

Cr(III) in the skin; the Cr(III) is cleared from lower skin layers at a slow rate, and subsequent skin reactions have been observed years after the original exposure.

Concentrated Cr(VI) solutions (3-10% by weight) were corrosive to skin, causing slow-to-heal ulcers.

CHAPTER 3 BACKGROUND CHEMISTRY OF CHROMIUM

INTRODUCTION

Chromium is never found in nature as the pure metal, nor is it easily converted to that state as most of its naturally occurring forms are quite refractory. Table 3-1 gives a brief history of Cr and its uses. Udy (1956), Bacon (1968) and Rollinson (1973) have given comprehensive reviews of the properties and chemistry of Cr.

The main deposits of Cr consist of complex cubic isomorphous minerals called spinels whose general formula is $[\text{Fe}, \text{Mg}]\text{O} \cdot [\text{Cr}, \text{Al}, \text{Fe}]_2\text{O}_3$. Of these, chromite, $\text{FeO} \cdot \text{Cr}_2\text{O}_3$ is the highest in Cr content (theoretically 68% by weight Cr_2O_3). These minerals are the only commercially important Cr ores at present. While Canada does have Cr-bearing minerals, deposits are usually of low grade (see Appendix A). Hence, all Canadian requirements are filled by the importation of chromite ore and Cr concentrates such as ferrochromium and chromium trioxide. Cr is playing an increasingly important role in the metallurgical and chemical industries while Cr composite catalysts are proving their usefulness in many new applications.

Cr is also of great importance biologically as it has been shown to be an essential element in fungi and vertebrates (Altman and Dittmer 1974; Mertz and Roginski 1971). Cr in its common oxidation states (+3 and +6) is known to interact strongly with biologically important molecules. Depending on the form and mode of exposure, Cr compounds can exhibit beneficial to toxic and even lethal effects. This will be discussed in later chapters.

PROPERTIES OF Cr

The properties of Cr may be divided into three broad categories: physical, nuclear, and chemical. Listings of these properties may be found in Bacon (1968) and NAS (1974).

Table 3-2 lists important examples of Cr compounds and gives some of their properties and uses. The chemistry of Cr is dominated by the three commonest oxidation states: 0, +3 and +6, +3 being the most stable. The metal (0 oxidation state) is resistant to attack by oxidizing acids and a range of other chemicals, hence its use in corrosion-resistant alloys. Its reactivity increases with temperature, causing reactions considered insignificant at room temperature to become important in high temperature applications.

Table 3-1. History of Cr usage in industry.

Date	Event or process	Comment
1798	chromium discovery	charcoal reduction of natural $PbCrO_4$
1800	chromate manufacture	chromite roasted with lime and soda ash
1816	pigment manufacture	e.g. $PbCrO_4$
1820	mordant dyeing	
1858	chrome tanning	commercialized in 1884
1879	refractory brick	declining production
1910	metallurgy	corrosion resistant ferrous alloys
1926	chrome plating	increasing production

The chemistry of Cr relevant to health and environmental considerations is that of Cr(III) and Cr(VI) in the various environmental media.

NATURAL WATERS

Cr(III) is a positively charged ion, Cr^{+3} , which has a strong tendency to form very stable complexes with negatively charged organic or inorganic species. Hence, it is unlikely that appreciable quantities of uncomplexed Cr^{+3} will be found in aqueous solution as long as any anionic dissolved or particulate matter (e.g. decaying plant or animal tissue, silt or clay particles) is suspended therein. Even if there are no anionic species present, Cr can react with water itself in neutral solutions to form colloidal hydrous oxides. Figure 3-1 gives the solubility of Cr(III) as a function of pH (Nilsson 1971). Below pH 5, the Cr^{+3} hexaquo complex is stable. Above pH 9, soluble negatively charged hydroxides are formed. Cr(III) attains its minimum solubility in the pH range covered by natural waters. Dissolved Cr is therefore expected to be a poor indicator of the Cr(III) burden of natural waters. This is illustrated by Merritt (1974) who analyzed raw Ottawa River water at seven sites along the length of the river. Table 3-3 lists the results obtained for dissolved Cr and the distribution of Cr between aqueous and particulate phases. At Site 5, the dissolved Cr value gives a deceptive picture of the Cr burden of the stream. This will be discussed further in Appendix B.

Cr(VI) in aqueous solution exists almost exclusively in the form of oxo anions (CrO_4^{-2} , $\text{Cr}_2\text{O}_7^{-2}$). Hence, its chemistry is radically different from that of Cr^{+3} . In dilute solution (<1 ppm), the predominant form is CrO_4^{-2} which, being negatively charged, does not complex with anionic particulate matter. Hence, Cr(VI) is more mobile than Cr^{+3} which is largely associated with particulate matter and is subject to sedimentation or filtration. Cr(VI) compounds, however, are powerful oxidizing agents, especially in acidic solutions. The tendency is strong to react with oxidizable substances (usually organic molecules) to form Cr(III). Nevertheless, if the concentration of oxidizable substances is low in the water, then Cr(VI) may persist for considerable periods of time.

AIR

Natural geochemical processes cause Cr to be associated entirely with particulate matter in the air. Although gaseous forms of Cr have been produced experimentally

Table 3-2. Chemical compounds of chromium and their properties.

Oxidation state	Subclassification	Examples	Comments
0	metal	Cr	soluble in: halogens, halogen acids; insoluble in: bases and oxidizing acids
	intermetallic organometallic	CrFe, CrBe ₂ Cr(CO) ₆	volatile and colorless compound
1	-	Cr(dipyridyl) ⁺ ₃	very few such compounds
2	-	CrSO ₄ ·7H ₂ O	oxidizable in aqueous solution by O ₂ to Cr ₃
3 ^a	oxide	Cr ₂ O ₃	insoluble and very refractory
	basic salts	Cr ₂ O ₃ ·H ₂ O	soluble in acids and alkalis
	salts of organic acids	[Cr(OH)(H ₂ O)] ₂ SO ₄ Cr(acetylacetonate) ₃	used in tanning leather used as combustion modifier in some fuels
4	oxide	CrO ₂	ferromagnetic, used in computer and sound tapes
5	oxide	Cr ₂ O ₅	used as pigment in ceramics
6 ^a	oxide chromates	CrO ₃ Na ₂ CrO ₄	anhydride of chromic acid used to produce most Cr(VI) chemicals
	dichromates	K ₂ Cr ₂ O ₇	primary standard laboratory reagent

^a See Chapter 5 for a further discussion of these industrially important oxidation states of chromium.

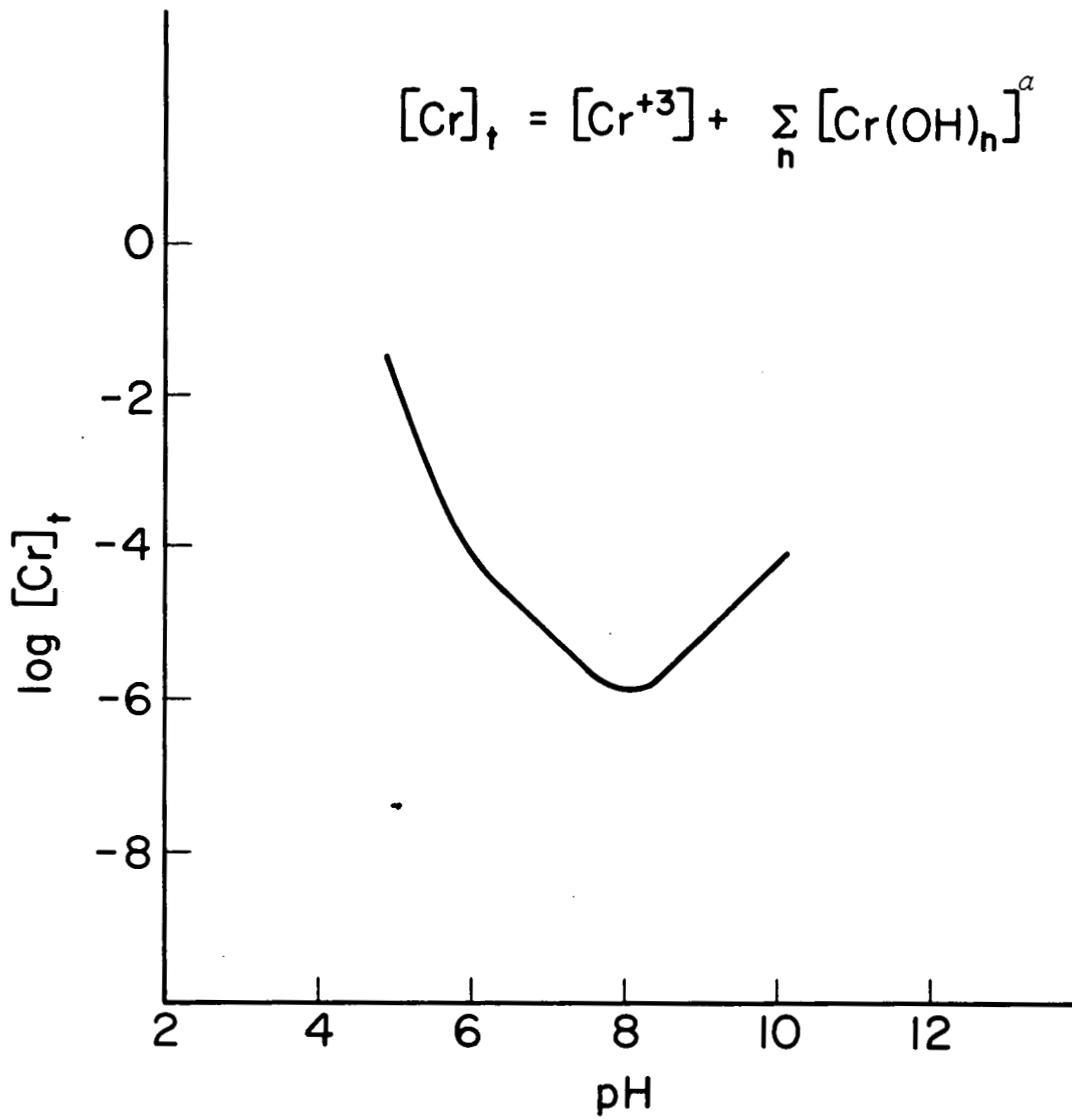


Fig. 3-1. pH dependence of Cr(III) solubility.

^a Total Cr concentration in solution is equal to the sum of the concentrations of Cr^{+3} and all Cr hydroxides.

Table 3-3. Analysis of Cr in Ottawa River water^a.

Site	Concentration of dissolved Cr (ppb)	Fraction of Cr in particulate matter
1	0.40	0.5
2	0.83	0.2
3	0.24	0
4	1.17	0.4
5	0.81	ca. 1.0
6	1.31	0.3
7	1.02	0.2

^a Data from Merritt (1974).

(CrO_2Cl_2 , $\text{Cr}(\text{CO})_6$), they are unlikely to be produced in nature and no attempt thus far has been made to detect them in industrial emissions. Bulk elemental analyses of soil and particulate matter filtered from air at remote sites (Rahn 1971; Duce *et al.* 1974, 1975) indicate that the most likely natural source of Cr in air is windblown soil. This observation may be misleading because many forms of industrial air pollution have bulk analyses nearly identical to those of soil particles (see Figure 3-2). Since the ratio of the Cr and Al or the Cr and Fe concentrations is often used to characterize aerosol particles, it can be seen that on the basis of bulk analyses, an oil soot particle may be classified as a soil particle. Hence, other properties, such as particle morphology, should also be considered when assessing the relative contributions of industrial and natural geochemical processes to Cr levels in the air.

Another indicator of the source of airborne particulate matter is the elemental composition expressed as a function of particle size. Cr associated primarily with relatively large particles ($>5 \mu\text{m}$ in diameter) originates most probably from windblown soil as soil-forming processes rarely produce particles smaller than $3 \mu\text{m}$ in diameter. Cr associated primarily with relatively small particles ($\leq 1 \mu\text{m}$ in diameter) originates most probably from smelting, incineration and other high temperature processes. Natusch and Wallace (1974) have pointed out that Cr is more concentrated on the surface of fly ash particles than in the interior. Thus, it stands to reason that as the particle size decreases and the surface area-to-weight ratio increases, the bulk concentration of Cr increases. Natusch and Wallace (1974) conclude that the surface of a fly ash particle is definitely not comparable to that of a soil particle. They consider that this surface concentration is an important factor from the standpoint of environmental health because it is the particle surface which is immediately in contact with extracting fluids and body tissues.

The level of Cr in sea salt is too low to make a significant contribution to the Cr levels in aerosols except at relatively high altitudes or in regions remote from any land masses. Although both Cr(III) and Cr(VI) have been found in airborne particulate matter, little information is available on the relative amounts of the two forms present in various aerosols.

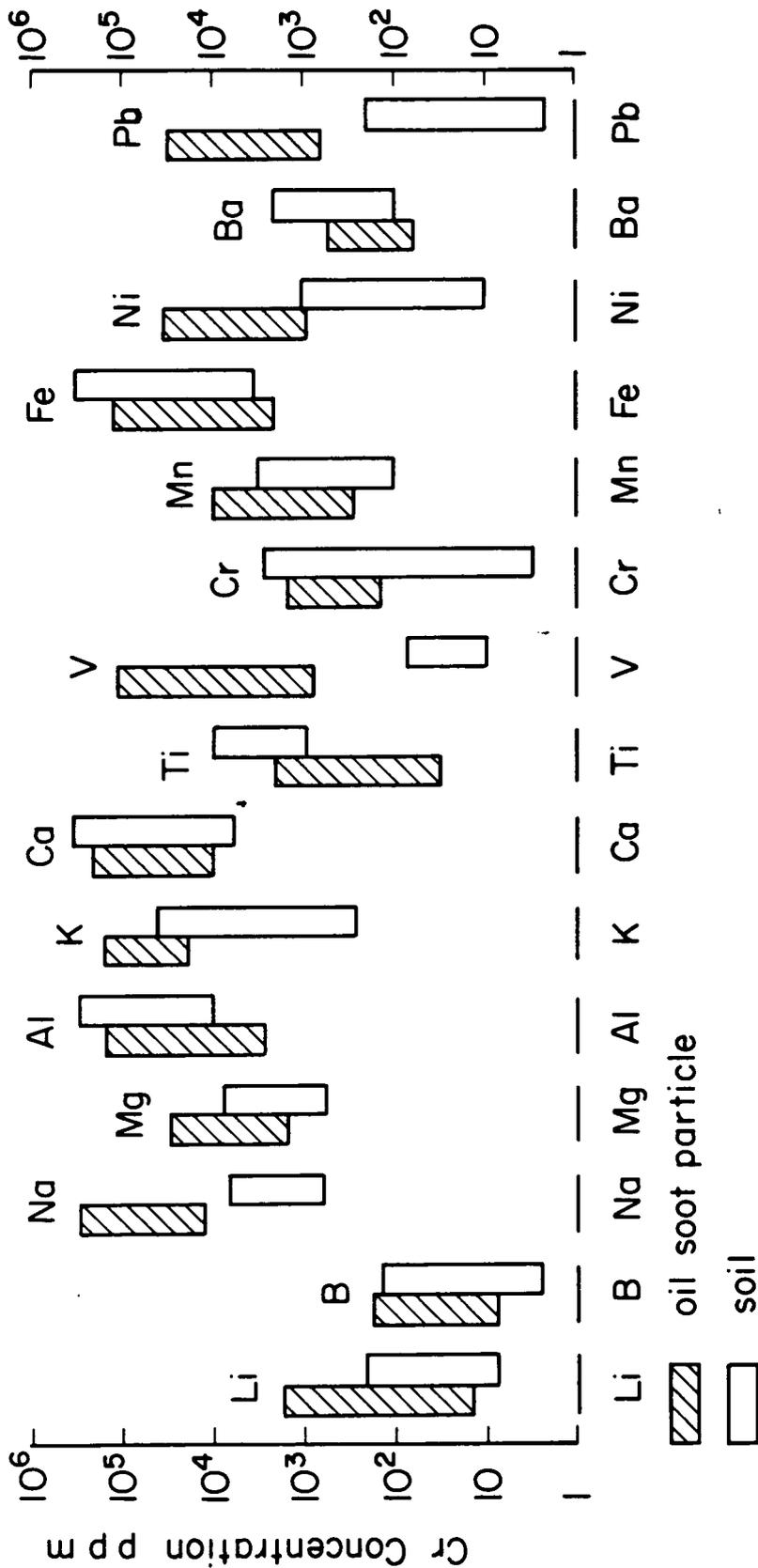


Fig. 3.2. Bulk elemental compositions* of soil and oil soot particles.
 *oil soot data from McHugh and Stevens 1972; soil data from Bowen 1966.

SOIL AND GROUNDWATER

Soil-groundwater parameters such as pH, rate of drainage, content of organic material, etc., play a major role in the chemistry of both Cr(III) and Cr(VI). In general, ionic equilibrium exists between soil and groundwater.

As mentioned before, Cr(III) tends strongly to be adsorbed onto clay particles and organic particulate matter, but can be mobilized if it is complexed with organic molecules, e.g. those produced by decomposing plant and animal matter in poorly drained soils (Mitchell 1964). Cr(III) present in minerals is mobilized to different extents, depending on the weatherability and solubility of the mineral in which it is contained (*ibid.*). It is interesting to note that the spinels, the minerals containing the highest concentrations of Cr(III), are also some of the most inert minerals known to man.

Cr(VI) is anionic in nature and is not strongly adsorbed by soil components. Hence, Cr(VI) is mobile in groundwater and has even been used in tracer form to follow groundwater flows (Todorovic and Filip 1967). Cr(VI) is quickly reduced to Cr(III) in poorly drained soils having a high organic matter content.

LIVING ORGANISMS

The chemistry of Cr(III) is characterized by limited mobility because, as a triply-charged cation, its movement across biological membranes is highly restricted unless its net charge is decreased by complexation. At physiological pH, Cr^{+3} is unstable and will either be complexed with some biological molecule or will form colloidal hydrous oxides. Cr(III) is known to bind strongly to proteins and, in sufficient concentration, forms cross-linkages between carboxyl groups of different protein molecules (Hörmann 1974). This is the basis for the process of chrome tanning of leather. Some low molecular weight complexes of Cr(III) with as yet unknown ligands increase the mobility of Cr(III) across membranes either by lowering the net positive charge of Cr (i.e. some of the ligands are anionic) or by taking advantage of some specific active transport mechanism functioning in cell membranes.

Cr(VI) is known to cross biological membranes with relative ease (Gray and Sterling 1950). Furthermore, it does not interact as strongly as Cr^{+3} with biological macromolecules because many of their functional groups are anionic. Hence,

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when Cr(III) and Cr(VI) are injected into the mammalian bloodstream, most of the Cr(III) either binds to the plasma proteins or forms a colloidal precipitate (Visek *et al.* 1953), while the Cr(VI) reacts only to a small extent with the plasma proteins and rapidly penetrates the membranes of red blood cells where it reacts with hemoglobin to yield a very stable complex (Gray and Sterling 1950). Further discussion of the effects of Cr(III) and Cr(VI) on various life forms will be discussed later.

ORGANOMETALLIC Cr COMPOUNDS

Little is known about the properties of organochromium compounds (i.e. compounds with direct Cr-C bonds). Even less is known about the interaction of such compounds with living organisms; nevertheless, their toxicity, from general considerations, is undoubted (Thayer 1974). It is unlikely that any process similar to the biological methylation of mercury is operative in the case of Cr, i.e. organochromium compounds are not expected to be produced in nature. However, increasing use of organochromium compounds in the chemical industry is predicted because of their usefulness in organic synthesis.

ANALYTICAL CHEMISTRY OF Cr

Analytic methodology is dealt with in Appendix B; however, a few comments should be made at this point. Improvements in analytical instrumentation and methodology have shown that, in many cases, previously accepted methods for Cr were in error due to contamination, interferences, and improper sample preparation. Nevertheless, it is impossible to judge the quality of analytical work by its date alone. The thoroughness of the investigator, purity of reagents, availability of standards, and the level of Cr to be determined must be considered. The following generalizations can be made:

1) Analyses of Cr in predominantly inorganic samples (e.g. metals, rocks, soils) have usually been quite reliable because of the relatively high Cr concentrations and straightforward sample preparation.

2) Analyses of Cr in predominantly organic samples, (e.g. animal and plant tissue) or in very dilute aqueous solutions (e.g. natural waters) have always been subject to some suspicion because of the low levels of Cr involved. Contamination is very difficult to eliminate. Cr levels in

air particulate and laboratory materials are usually greater than those in the samples. Lengthy procedures involving ashing to remove organic material increase the possibility of inadvertent gain or loss of Cr by the sample.

3) Wider acceptance of newer methods involving less sample manipulation before analysis is minimizing the sample contamination problem. Availability of standard reference materials for both plant and animal tissue (US National Bureau of Standards) has made it possible to check the accuracy and sensitivity of existing and proposed methods for analysis of environmental samples.

AVAILABILITY

The elemental concentration of Cr in a given environment is a poor measure of the effect which the Cr can have on an organism living in that environment. Depending on the physical and chemical state of the Cr, the same elemental concentration can have a wide variety of mobilities and reactivities, and thus have different effects.

The physical dimensions of airborne particles determine whether they can form stable aerosols. Particles larger than 10 μm in diameter tend to settle out quickly and, in general, do not penetrate deeply into man's respiratory tract (Natusch and Wallace 1974). On the other hand, relatively small particles-(less than 1 μm in diameter) tend to form stable aerosols and may be transported many miles before settling out. Furthermore, these particles show appreciable penetration deep into the lungs, i.e. small particles are more available than large particles.

The chemical effect of Cr compounds on living organisms is determined by the relative absorption upon exposure and the relative tendency to react with biologically important molecules in a harmful manner. Hence, a Cr compound which is extremely toxic when injected directly into the bloodstream may normally not be considered hazardous if it is poorly absorbed through the lungs, skin, or digestive tract. Therefore, Cr(VI) compounds are to be considered more available than Cr(III) compounds because of their higher rates of diffusion across the epidermis (Samitz *et al.* 1967).

The element Cr, unlike many other pollutants which are now being recognized (e.g. organic pesticides), is intrinsic to nature and its presence in the environment is not entirely a product of man's activities. In fact, this is probably why Cr at low levels now functions as an essential

element in some animal species. However, the redistribution and chemical transformation of Cr compounds, so as to change their availability to living organisms, is the main environmental risk associated with Cr. The availability of Cr from most minerals, especially chromite, is exceptionally low. Industrial and domestic activities result in much more available forms of Cr, e.g. Na_2CrO_4 .

CHAPTER 4 NATURAL LEVELS OF Cr IN THE ENVIRONMENT

INTRODUCTION

Natural or background concentrations of Cr in the environment are those which result from processes which do not involve human activity. For example, toxic substances leached by water from a derelict mine are considered to result from human activity even though the actual process of leaching is quite common in nature, i.e. in this case man has affected the extent or possibility of a geochemical process taking place.

The only way, at present, of arriving at estimates of Cr background concentrations is to measure the trace element concentrations in the environment at locations remote from industrial and domestic activity and assume these are natural. Strictly speaking, such values will only represent Cr levels at these remote locations but, to a reasonable approximation, they can also reflect background levels in populated industrial areas if natural regional variability is taken into account. It should not be assumed that all naturally occurring Cr levels are harmless and that all Cr levels in industrial and domestic wastes are harmful. The determining factor is the availability of Cr to be concentrated in living organisms beyond toxic levels.

NECESSITY OF DETERMINING NATURAL Cr LEVELS

Knowledge of natural Cr levels and the amounts of various chemical forms cycling naturally in the environment is required to assess

- (1) whether man's activities will have any impact on the availability of Cr in the environment;
- (2) what will be the magnitude and duration of effects resulting from various amounts and types of Cr emissions;
- (3) the source of dangerous levels of Cr emissions so that remedial action may be swiftly taken;
- (4) what forms of Cr at what permissible loading rates may be safely returned to the environment;
- (5) whether there are any significant trends with time in the levels of Cr in the environment.

Hence, the significance of any environmental cause/effect statement can only realistically be evaluated in the light of this prerequisite information.

BACKGROUND Cr LEVELS IN THE ENVIRONMENT

AIR

Remote-sampling data from a number of investigators point to a world background Cr concentration in lower atmosphere aerosols in the range of 0.3 to 2 ng/m³, with an average near 1 ng/m³ (see Table 4-1). It should be noted that natural levels of particulate matter (and hence Cr) in air are strongly influenced by meteorological conditions, e.g. strong winds tend to introduce more particulates into the air while rain and other forms of precipitation tend to reduce the amounts of particulates in air.

No reports exist which describe the chemical forms of Cr in aerosol particulate matter or rainwater. No studies have been performed to determine the presence or absence of gaseous Cr compounds in the air regardless of their origin.

WATER

The term "water" actually includes the following subclassifications: rainwater, river water, lake water, groundwater, sea water and drinking (processed) water. Bogen (1974) has measured the trace element content of rain and cloud water over Heidelberg and concluded that the bulk of the Cr had a natural origin. Table 4-2 contains values representative of natural Cr levels in rainwater.

Dissolved Cr is the Cr parameter most often determined in the trace elemental analysis of Canadian surface and groundwaters. The suspended solids are normally removed using a 0.45 μ m filter (CCIW 1974). Usually no attempt is made to determine Cr(III) and Cr(VI) separately. Hence, reported values represent the sum of the concentrations of both Cr oxidation states. Table 4-3 gives the distribution of dissolved Cr concentration values determined in Canadian streams and rivers. Over 99% of the samples analyzed contained less than 25 ppb dissolved Cr. However, the following should be emphasized:

- (1) Water sampling is not done on a continuous basis. Hence, batch discharges of even concentrated Cr wastes will usually be undetected (Oliver 1973).

Table 4-1. Natural Cr levels in air.

Location	Concentration (ng/m ³)	Reference
Twin Gorges, NWT	0.59-2.5	Rahn (1971)
Jasper Nat. Park, Alberta	0.32	Rahn (1971)
Riding Mountain Nat. Park, Manitoba	0.92	Rahn (1971)
Prince Albert Nat. Park, Saskatchewan	1.1	Rahn (1971)
Novaya Zemlya, USSR	0.34	Egorov <i>et al.</i> (1970)
Salehard, USSR	1.6	Egorov <i>et al.</i> (1970)
North Atlantic	0.07-1.1	Duce <i>et al.</i> (1975)
South Pole	2.5-10	Duce <i>et al.</i> (1974)
Algonquin Park	.88-1.9	Rahn (1971)

Table 4-2. Natural Cr levels in rainwater^a.

Location	Concentration (ng/cm ³)	Reference
Heidelberg, Germany	3.6	Bogen (1974)
Quillayute, Wash., USA	2.	Rancitelli and Perkins (1970)
Wraemore, England	2.9	Cawse and Peirson (1972)

^a Total dissolved and undissolved Cr.

Table 4-3. Dissolved Cr levels in Canadian streams and rivers^b.

Cr concentration (ppb)	Number of samples	Percent of samples
<10	4163	95.9
10-14	92	2.12
15-24	62	1.43
25-49	19	.44
50-99	4	.092
100-500	2	.046

^b Data courtesy of Environment Canada, Ottawa, Ontario.

- (2) As seen before, cationic adsorption of Cr(III) onto particulate matter results in a tendency to maintain fairly constant dissolved Cr values. This tends to mask Cr pollution if particulate matter is not analyzed as well (see Table 3-3).

Table 4-4 gives representative values for dissolved Cr in the Great Lakes. Generally, the values average 1 ppb.

Merritt (1971) has analyzed a sample of groundwater taken near the Chalk River National Laboratory and reports a Cr value of 0.02 ppb in sandy (low Cr) sediments. Brinkmann (1974) made an inventory of trace elements in groundwaters of the Netherlands and reported natural levels in the range < 0.5-2 ppb.

Drinking water generally contains the same Cr levels as the surface and groundwaters which serve as its source. Although some piping materials contain significant levels of Cr (corrosion resistant steel, 8-14%; cement, 5-120 ppm Cr) (Perone *et al.* 1974), little is leached into the flowing water. However, it should be noted that Cr(III) may be oxidized to Cr(VI) during the chlorination process (Bahensky and Kubanova 1974).

Generally speaking, the background or natural concentration of dissolved Cr in Canadian surface waters is approximately 1 ppb. Appreciably higher values than this level probably indicate the presence of Cr(VI) resulting from natural chromate mineral deposits or more probably from domestic and industrial sources. Low dissolved Cr values are not indicative of the absence of Cr pollution unless suspended particulate material and sediments are also examined.

AQUEOUS SUSPENDED PARTICULATE MATERIAL

The Cr suspended material in streams can either be intrinsic to the particles, e.g. Cr in a mineral, or it can be surface deposited from solution by cationic adsorption processes. The concentration of Cr expressed as a function of particle size (and hence surface area per unit weight of suspended material) permits the two types of Cr to be distinguished:

- (1) If the Cr concentration increases with decreasing particle size, then [Cr] adsorbed > [Cr] intrinsic;

Table 4-4. Dissolved Cr levels in Great Lakes waters (ppb).

Location	Median	Range	Reference
Lake Superior	1	d ^a - 18	Weiler and Chawla (1969)
Lake Huron	1.6	d - 19	Weiler and Chawla (1969)
Lake Erie	1.6	d - 14	Weiler and Chawla (1969)
Lake Ontario	0.7	d - 12	Weiler and Chawla (1969)
Great Lakes	5.8	-	Durum and Haffty (1963)
Great Lakes	-	0.1 - 10	Livingstone (1963)

^a d ≡ detection limit ≈ 0.2 ppb.

- (2) If the Cr concentration remains roughly constant, or decreases with decreasing particle size, $[Cr]_{\text{adsorbed}} < [Cr]_{\text{intrinsic}}$.

A recent study of the Ottawa and Rideau Rivers (Oliver 1973) has shown that there is a statistically significant positive correlation between particle surface area and Cr content. Hence, particle size must be taken into account when attempting to obtain a representative sample.

The natural or background concentration of Cr in suspended particulate material should be comparable in magnitude with the Cr concentration in the geological material forming the stream bed, i.e. in nature little contribution should be made from adsorbed Cr^{+3} (most Cr(III) minerals are poorly soluble in natural waters while Cr(VI) minerals are relatively rare). Existing data appear to support this (Turekian and Scott 1967): the average Cr content of suspended material in US streams was 199 ppm (range 37-460 ppm), while the average Cr content of crustal rocks is 125 ppm (range 30-400 ppm). Decaying plant and animal matter should not make a significant contribution due to the low levels of Cr normally found in these materials.

Fitchko and Hutchinson (1974a) have made a survey of the heavy metal concentrations in river mouth sediments around the Great Lakes in an attempt to determine the specific heavy metal inputs of Great Lakes tributaries. A condensation of their results is presented in Table 4-5. These investigators (Fitchko and Hutchinson 1974b) note that while metals adsorbed on the sediment are chemically bound, "various physical, chemical and biological (microbial) factors can bring about the redistribution and partial solution of heavy metals in sediments...back into the water column. If these processes are efficacious, then there is a potential danger of these heavy metals from sediments moving through biological food chains with subsequent biomagnification." Results of the Onondaga Lake Study (Onondaga County 1971) show that the Cr content of the interstitial waters of sediments (170 ppb maximum) was several times higher than the Cr in the water column, indicating that "mineral dissolution and/or desorption of ions takes place in the sediments". Resuspension of heavy-metal-bearing sediments during turbulent flow following heavy rainfalls has been held responsible for some fish kills in streams (Williams *et al.* 1973).

A study by Iskandar and Keeney (1974) has shown how core samples of lake sediments may be used, along with estimated rates of sediment accumulation, to estimate the heavy metal content of water systems as a function of time. In particular, an attempt was made to determine if the precultur-

Table 4-5. Chromium levels in river mouth sediments of Great Lakes^a.

Lake	Range of Cr levels (ppm dry weight)	Mean (ppm dry weight)
Superior	trace - 27.2	8.6
Michigan	trace - 2085	84.6
Georgian Bay	0.9 - 37.8	10.6
Huron	trace - 10.5	3.8
Detroit-St. Claire	5.0 - 89.4	20.5
Erie	3.4 - 156.8	29.5
Ontario	4.5 - 51.8	18.3

^a Note that many of these results almost certainly contain large Cr contributions from pollution sources.

al content of heavy metals was significantly less than the levels now being deposited in the sediments. In 4 out of 10 Wisconsin lakes, significant Cr accumulation was shown to have occurred in recent times.

SOILS AND BEDROCK

The composition of the soil and the availability of elements from it to plants is determined by the parent rock type as well as the actions of climate and living organisms in soil genesis. The older the soil the less may be the influence of the parent rock (Mitchell 1964). Water tends to leach elements from surface layers to lower soil layers while the action of plant roots and vascular systems is to transport minerals from lower soil layers and deposit them in the top layer via fallen branches, leaves, and fruit. As stated earlier, Cr, present usually as Cr(III) in the soil, is characterized by its lack of mobility except in cases where Cr(VI) is involved. Cr(VI) of natural origin is rarely found in soils. Fleischer (1972) has given bulk concentration ranges for Cr in various rock types (see Table 4-6). Lisk (1972) reported Cr levels of 1-1500 ppm in American soils; preliminary studies show a range of 20-125 ppm in Canadian soils (Morley 1975). Swaine and Mitchell (1960) have analyzed various types of soils for their bulk and extractable Cr as a function of sample depth and rate of drainage of the soil. The extractable Cr ideally is a measure of Cr availability to plants. It should be noted that the Cr available to plants is not directly related to the total Cr content in soils. Prince (1957) has shown this by comparing the Cr content of corn plants with the Cr content of the soil in which they were grown. The results, listed in Table 4-7, indicate that the highest and lowest accumulations of Cr in corn leaves were obtained in two different soils both having the same bulk content of Cr.

Many fertilizers contain appreciable levels of Cr. Table 4-8 lists levels of Cr in representative fertilizers from the extensive compilation of Swaine (1962). Repeated use of fertilizers with high levels of Cr can lead to high accumulations of Cr in soils. The use of wastes and sludges as fertilizers will be discussed later.

FOOD

The widespread use of fertilizers as well as the encroachment of industrial and domestic pollution on farm lands makes it questionable whether the levels of Cr reported in foods sampled nowadays are background (or natural) levels. However, the poor translocation of Cr(III) from plant roots (Huffman and Allaway 1973) and the high toxicity of Cr(VI) to plants (see later) make it unlikely that anything short

Table 4-6. Chromium levels in parent rocks (ppm dry weight).

Rock type	Normal Cr range	Average Cr level
Ultramafic igneous	1000-3400	1800
Basaltic igneous	40- 600	220
Granitic igneous	2- 90	20
Shale	30- 590	120
High carbon black shale	30-1000	100
Deep sea clay	-	90
Limestone	-	10
Phosphorite	300-3000	1000
Sedimentary iron ore	150- 800	-

Table 4-7. Noncorrespondence of bulk and available Cr in different soils.

Cr in soil (ppm dry weight)	Cr in corn leaves (ppm dry weight)
20	0.44
20	1.69
30	1.61
32	1.37
38	2.18
39	0.68
40	0.47
45	0.50
46	1.28
75	0.80

Table 4-8. Cr content of some typical fertilizer materials.

Type	Cr concentration (ppm)
Nitrogen fertilizers (urea, nitrates)	<5-3000
Phosphorus fertilizers	30-3000
Superphosphate	60-250
Bone meal	<20-500
Limestones	<1-200

of gross Cr pollution would change the Cr uptake into the food chain via plants. None of the plants normally used as food or animal feed are Cr accumulators (Allaway 1968).

The average Cr content of three species of Great Lakes fish (whole fish basis: alewife, spottail shiner, perch) was 1 ppm on a fresh weight basis (Lucas *et al.* 1970). Considering that the Cr concentration in Great Lakes waters averages 1-2 ppb (Weiler and Chawla 1969), this represents a substantial accumulation; however, the major portion of the Cr appears to be associated with the portions of the fish not consumed as food such as the skin, bones and organs. Knoll and Fromm (1960) showed that the edible portion of the rainbow trout, the muscle, accumulates little Cr even when the fish were exposed to Cr(VI). Schroeder *et al.* (1962) indicated that the levels of Cr found in the skin of the halibut (0.18 ppm fresh weight) were substantially higher than in the fillet (0.01 ppm fresh weight).

Table 4-9, which includes the results of three Canadian studies, contains a summary of representative bulk Cr concentrations in various food types. Table 4-10 lists the results of an attempt to give some indication of Cr availability from various foods in terms of the biological activity of their extracts. The values in Table 4-10 are taken from Toepfer *et al.* (1973) and do not take into account the effect of acid hydrolysis (which occurs in the digestion of food) nor the relative absorption of various forms of Cr across the gut. The entire order of biological activities in Table 4-10 would change drastically if the extractable Cr of the foods were considered on a dry weight basis or on the basis of the weight of that food yielding 100 calories of food value.

Processing of foodstuffs, especially refining, lowers the Cr content. Zook *et al.* (1970) have shown that the production of flour from wheat involves a Cr loss, probably due to removal of the bran and germ. Interestingly enough, these same investigators found that, in the production of bread from flour, a major portion of the Cr came from elsewhere than the flour. The stainless steel used in mixing containers in the baking industry may be the source of such extraneous Cr, e.g. Titus *et al.* (1930) found Cr contamination up to 0.12 ppm in food prepared and stored in vessels of chrome-nickel steel. Similarly, the relatively high levels of Cr found in some margarine products (Owlya 1974) may be due to the use of Cr-containing catalysts in the hydrogenation of polyunsaturated oils (Koritala *et al.* 1973). Wolf *et al.* (1974) found that successive steps in sugar refinement each reduced the Cr content of the sugar. These investigators also considered the relation of this finding to the statistical correlation found between excessive consumption of refined sugar and cardiovascular disease.

Table 4-9. Cr content of various foodstuffs ($\mu\text{g/g}$ fresh weight).

Foodstuffs	Ottawa/Hull ^a	Vancouver ^b	Halifax ^b	USA ^c
Milk and dairy products	0.11	.06	.05	.23
Meat, fish and poultry	.18	.07	.06	.23
Cereals	.15	.17	.06	.22
Potatoes	.26	.05	.04	.24
Leafy vegetables	.09	.11	.09	.10
Legumes	.10	.16	.06	.04
Root vegetables	.15	.06	.09	.09
Garden fruits	.23	.10	.05	-
Fruits	.07	.06	.05	.09
Oils and fats	.09	.09	.03	.18
Sugars and candy	.33	.17	.34	-
Drinks	.07	.03	.03	-
Condiments and spices	-	-	-	.33

^a Méranger and Smith (1972)

^b Kirkpatrick and Coffin (1974)

^c Toepfer *et al.* (1973)

Table 4-10. Relative biological values^a of Cr in selected foods.

Sample	Relative Cr biological value
Brewers' yeast	44.88
Black pepper	10.21
Calf's liver	4.52
American cheese	4.39
Wheat germ	4.05
Whole wheat bread	3.59
Cornflakes	3.01
White bread	2.99
Spaghetti	2.89
Beef	2.89
Wheat grain	2.96
Butter	2.81
Rye bread	2.67
Margarine	2.48
Oysters	2.43
Corn meal (yellow)	2.35
Chili peppers	2.27
Wheat bran	2.27
Chicken	2.16
Corn meal (white)	2.09
Shrimps	2.03
Lobster	1.95
Mushrooms	1.92
Haddock	1.86
Beer	1.77
Egg white	1.77
Skimmed milk	1.59

^a The relative amount of Cr extracted from 1 g (fresh weight) of sample by an ethanol-water mixture.

NATURAL GEOCHEMICAL-BIOLOGICAL CYCLING OF CHROMIUM

Figure 4-1 presents the available information on the steady-state amounts of Cr present in the various environmental media and the estimated annual turnover of Cr. Many of the quantities or turnover rates are undetermined while those that are present are only intended to be indicators of order of magnitude, i.e. these estimates may be high or low by a factor of up to 5-10.

The biologically important aspect of this Cr cycle is succinctly stated by Allaway (1968): "Cr is one of the few essential elements for which no accumulation against a concentration gradient is evident at any point in the biological cycle from soil to plant to animal". Huffman and Allaway (1973) and Huffman (1973) have given experimental evidence for the above statement by showing:

- 1) the bulk (>90%) of the Cr absorbed by most plants remains in the roots and is poorly translocated to the tops;
- 2) uptake and translocation of ^{51}Cr radioactive tracer differed only slightly between Cr(III) and Cr(VI);
- 3) rats fed ^{51}Cr -labelled bean leaves retained less than 0.5% of the dose after 48 hours;
- 4) the carrot, a root vegetable, when grown in $\text{Na}_2^{51}\text{CrO}_4$ solution absorbed some of the ^{51}Cr but more than 85% of this was located in the outer 1 mm of the root.

In a study of selected trophic levels of an East Tennessee deciduous forest ecosystem, Andren *et al.* (1973) found no tendency for significant bioaccumulation. Their results are shown in Figure 4-2.

The absorption of Cr(III) from water by mammals is usually less than 1% of the dose (Visek *et al.* 1953), while slightly more than 2% of the Cr(VI) dose in the water is absorbed by man (Donaldson and Barreras 1966). Hence, it appears unlikely that absorption from drinking water could be responsible for any significant Cr bioaccumulation, especially with the ppb levels of Cr present in potable waters.

There are several documented instances of Cr accumulation against a concentration gradient but various factors limit the importance of this bioaccumulation (see Table 4-11

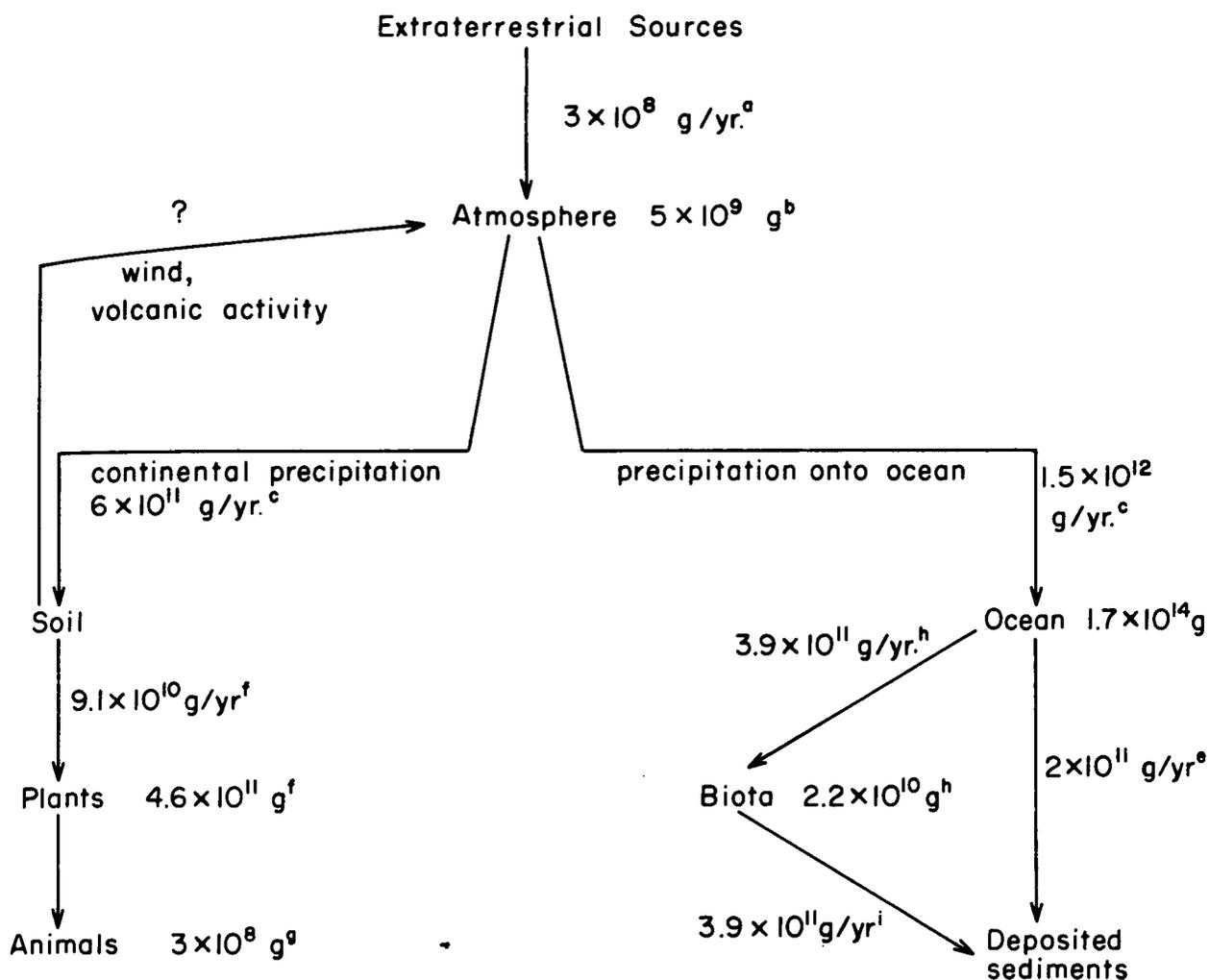


Fig. 4-1. Natural biogeochemical cycling of chromium.

- a. Estimated from the data of Parkin and Tilles (1968) assuming the fallen material contains 3000 ppm Cr (NAS 1974).
- b. Estimated from the data of Rancitelli and Perkins (1970).
- c. Estimated from the data of Cawse and Peirson (1972) assuming deposition directly proportional to relative surface area (29% land, 71% ocean); may include some industrial pollution and thus tend to be high.
- d. Estimated from a mean Cr concentration of 0.1 ppb and an ocean mass of 1.7×10^{24} g.
- e. From the data of Bertine and Goldberg (1971).
- f. Estimated from the data of Bowen (1966) assuming the average Cr content of plants to be 0.4 ppm (dry weight); see also Allaway (1968).
- g. Estimated from the data of Bowen (1966) assuming the average Cr content of animals to be 0.15 ppm (dry weight).
- h. Estimated from the data of Bowen (1966) assuming an average Cr concentration of 3 ppm in ocean life.
- i. Assumes a steady state has been achieved.

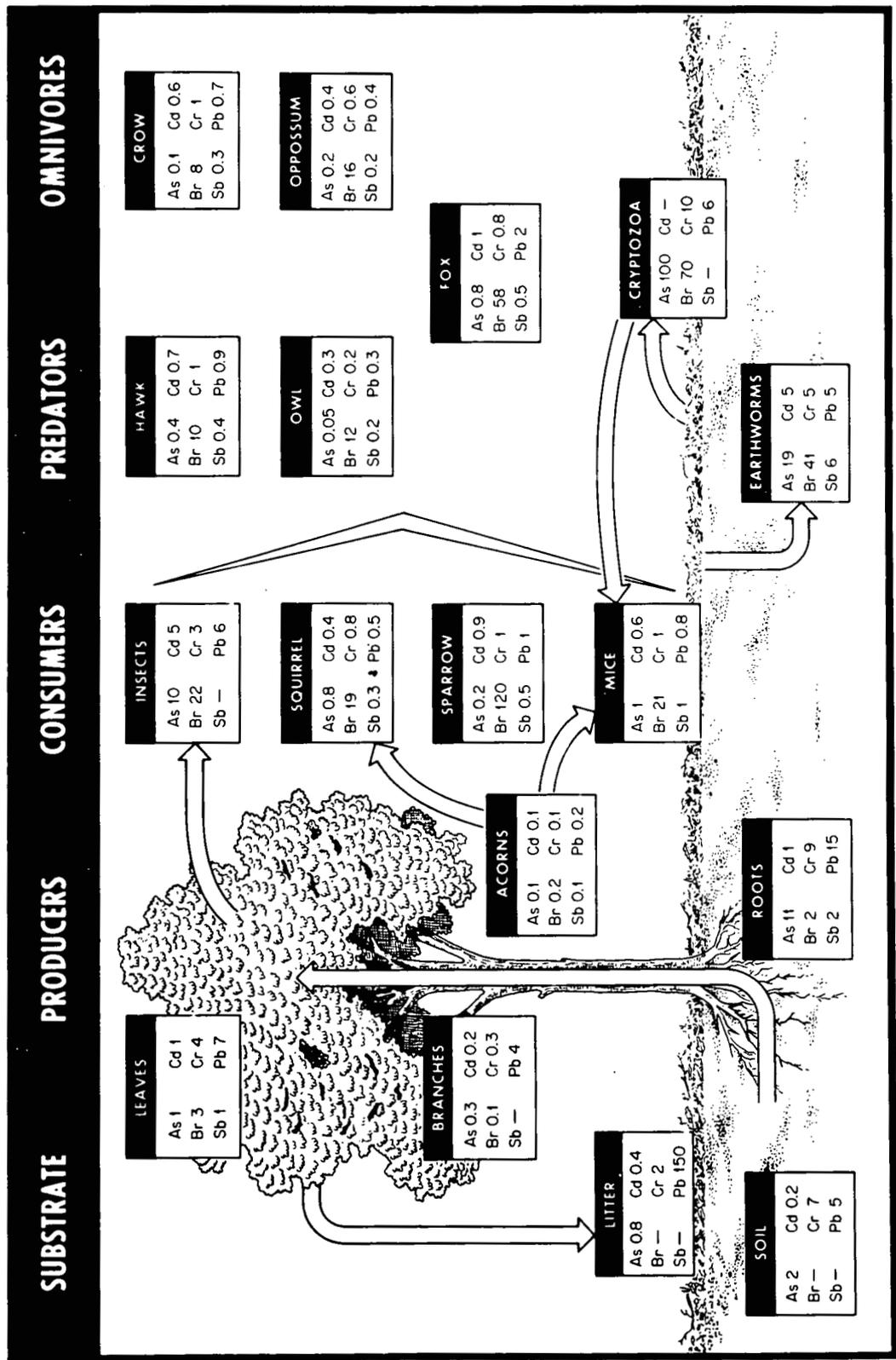


Fig. 4-2. Elemental concentrations (ppm, dry weight) for three nonmetals (As, Br, Sb) and three metals (Cd, Cr, Pb) in selected trophic levels of a deciduous forest ecosystem in East Tennessee.

for a summary of these cases). Generally, it can be stated that there is little tendency for Cr to accumulate along food chains in the trivalent inorganic form; however, organo-Cr compounds can have significantly different bioaccumulation tendencies. Very little is known about these latter compounds.

Table 4-11. Examples of bioaccumulation of Cr.

Organism	Accumulation factor	Comments	Reference
Human fetus heart, liver and spleen	10 (compared to adult tissues)	Levels decrease upon aging	Schroeder <i>et al.</i> (1962)
Snail	10 ⁶ (compared to ocean water)	not an important part of the food chain	Levine (1961)
<i>Leptospermum scoparium</i> (shrub)	10 ³ (compared to "normal" plants)	not an important part of the food chain	Lyon <i>et al.</i> (1969b)
Seaweed	10 ² (compared to ocean water)	little biomagnification afterwards	Boothe and Knauer (1972)

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CHAPTER 5 INDUSTRIAL AND DOMESTIC USES AND EMISSIONS OF Cr

INTRODUCTION

It is not the purpose of this document to indicate specific cases of environmental pollution involving Cr and its compounds. Rather, the intent here is to set forth in a general manner the concentrations and amounts of Cr which may be expected in various industrial and domestic situations. This is done in order to shed light on the significance of the cause/effect data which will follow in subsequent chapters.

Of all the metals used in industrial and domestic activities, Cr is the most ubiquitous (see Table 5-1). The widespread use of Cr makes it desirable to draw up an inventory of Cr sources and emissions. This is not possible since Canadian data are for the most part lacking or fragmentary. Hence, much of the following discussion is based on studies of Cr emissions in the United States (Gafafer 1953; GCA Corp. 1973).

INDUSTRIAL Cr EMISSIONS

AIR

It should be remembered that numerical values placed on emission rates and amounts are subject to considerable variability due to differing production processes and emission control practices. Hence, these numerical values should be considered only as order-of-magnitude indications.

Table 5-2 is a summary of the air emissions inventory for Cr in the USA as compiled by the GCA Corporation (1973). Major portions of overall Cr emissions to the air come from the ferrochromium and refractories industries, as well as from the combustion of coal.

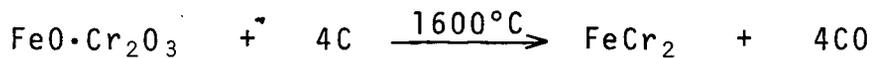
Gafafer (1953) has reported particle size distribution for the work areas of plants producing chromate chemicals and chromite refractories. Geometric mean particle sizes were 0.32-0.37 μm (chromate production) and 0.22-0.28 μm (refractory brick production). In addition, this same report gave the average Cr exposure of workers on an occupational basis and gave an analysis of the total Cr exposure in terms of (a) water-soluble hexavalent Cr, (b) chromite, (c) acid-soluble, water-insoluble Cr and (d) basic chromium sulfate. The greatest average exposure to Cr was 1.07 mg/m^3 , consisting of 83% chromite ore, 13% acid-soluble, water-insoluble Cr, and 4% water-soluble Cr(VI). The greatest average exposure to Cr(VI) was 0.17 $\text{mg Cr}/\text{m}^3$.

Table 5-1. Heavy metals found in major industries^a.

	Al	Ag	As	Cd	Cr	Cu	F	Fe	Hg	Mn	Pb	Ni	Sb	Sn	Zn
Pulp, paper mills, paperboard, building paper, board mills				X	X	X		X	X		X	X			X
Organic chemicals, petrochemicals	X		X	X	X		X	X	X		X		X	X	
Alkalis, chlorine, inorganic chemicals	X		X	X	X		X	X	X		X		X	X	
Fertilizers	X		X	X	X	X	X	X	X	X	X	X			X
Petroleum refining	X		X	X	X	X	X	X			X	X			X
Basic steel works, foundries			X	X	X	X	X	X	X	X	X	X	X	X	X
Basic non-ferrous metals-works, foundries	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Motor vehicles, aircraft-plating, finishing	X	X		X	X	X		X	X			X			
Flat glass, cement, asbestos products, etc.					X										
Textile mill products					X										
Leather tanning, finishing					X										
Steam generation power plants				X											X

^a After Dean *et al.* (1972).

The combustion of coal leads to the production of large quantities of fly ash. The 82% control quoted in Table 5-2 is deceptive because it was determined on the basis of weight. If the percentage control were reported in terms of the reduction in the number of particles per cubic metre of air emitted, the extent of control would definitely be less. The GCA Corporation report states that efficiency of particle removal tends to decrease as particle size decreases, i.e. small particles of high mobility and availability for deposition in the respiratory tract are not subject to the same control as are larger particles (Harrington 1974). It is desirable that emission control be reported on the basis of particle size. This is especially important because many elements including Cr have been shown to be concentrated in the smaller fly ash particles (Davison *et al.* 1974). Figure 5-1 illustrates this trend. Natusch and Wallace (1974) report that single particle fly ash analyses using a scanning electron microscope show that the Cr of the small particles is considerably more concentrated on the particle surface than in the interior bulk. This points strongly to the existence of gaseous Cr phases which subsequently condense on available surfaces. It is known that Cr metal has an appreciable vapor pressure at elevated temperatures (1 torr at 1616°C). Cr(CO)₆ is a volatile Cr compound which may be produced from Cr in the presence of carbon monoxide (Gerasimov and Sharifov 1958). No attempt has been made to measure Cr(CO)₆ production in the coking of chromite to produce ferrochrome (see chemical equation below).



At high temperatures, the carbonyl compound is unstable, but carbon monoxide concentrations remain high in the lower temperature flue gases.

SOIL

Large amounts of slag containing 2-6% Cr as Cr₂O₃ are by-products of ferrochrome and chromium steel production. The Cr is present in a sintered form which is relatively unavailable for incorporation into plants or microorganisms. Solid waste from chromate manufacture contains considerable Cr(VI) residue and has caused land reclamation problems when dumped on land without chemical treatment (Breeze 1973; Gemmel 1972, 1973). Runoff from such contaminated lands has caused serious pollution of nearby streams (Breeze 1973). Klein (1972) has demonstrated that the Cr content of a homogeneous tract of soil is dependent on the land use pattern: residential land had an average of 3.2 ppm Cr in the soil;

Table 5-2. Air emissions inventory of Cr (USA)^a.

Source	Uncontrolled emissions (g/kg of product)	Percent Cr in emissions	Percent emission control (% by weight)	Total emissions after control (10 ³ kg/y)
Ferrochrome produced by electric furnace	250	22	40	12,360
Ferrochrome handling	5	65	32	830
Refractory brick, noncast	75	17	64	1630
Refractory brick cast by electric furnace	112	17	77	173
Dichromate manufacture	15	30	90	92
Cr steel production	12	(18)	78	520
Cast iron production	38	(2)	99	2
Alloy and superalloy production	12	-	78	33
Coal combustion	-	-	82	1564

^a GCA Corporation (1973)

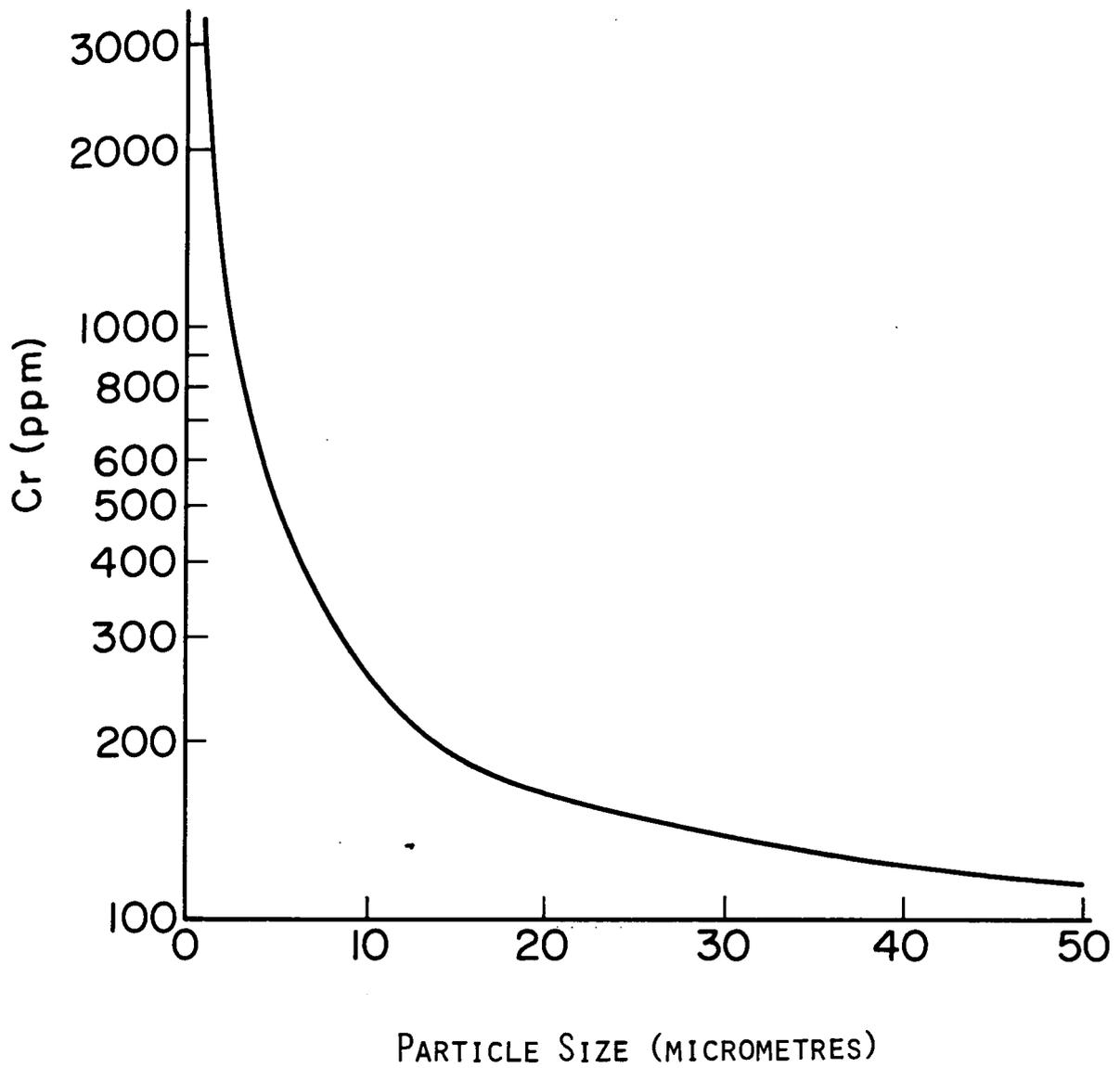


Fig. 5-1. Effect of particle size on Cr concentration in fly ash^a.

^a After Davidson *et al.* (1974).

agricultural land, 4.6 ppm; industrial land, 8.5 ppm; and airport land, 17.6. The last figure may result from the use of Cr compounds as antistatic agents in jet fuels (Lodwick 1964).

WATER

Surface and groundwater pollution by Cr results from emissions by industries using chromium chemicals. Table 5-3 lists uncontrolled waste water emissions of Cr for various processes. Technology exists which is able to reduce Cr in emissions to very low levels. To give some idea of the amounts of Cr chemicals used the following examples are put forth:

- 1) a moderately-sized cooling tower system which recirculates 10,000 gal/min, with a blowdown rate of 100 gal/min and operating at a residual chromate level of 30-35 ppm, discharges more than 7 tons of Cr(VI) annually (Shepherd and Jones 1971);
- 2) a tannery which processes 2500 hides a day puts out 820 kg of Cr(III) daily in its effluent. In addition, chrome-tanned leather itself contains 3-6% by weight chromium oxide (A.C. Lawrence Leather Co., quoted in WRSIC 1972);
- 3) oil well drilling mud is made to contain $2-5 \times 10^3$ ppm Cr(VI) to reduce corrosion fatigue in drilling pipe. This means 460 to 2700 kg of $\text{Na}_2\text{Cr}_2\text{O}_7$ are used per deep well drilled (Udy 1956, p. 406).

Newer electroplating technology (O'Sullivan 1975) permits the use of Cr(III) in the plating process, eliminating the need for the more toxic Cr(VI) and hence reducing waste disposal problems.

A proposed use of Cr-base catalysts is in the control of carbon monoxide emissions from internal combustion engines. Schlatter *et al.* (1973) have studied Cr catalysts for this application and have found them an attractive alternative to more expensive noble metal catalysts. However, Balgord (1973) has studied metallic emissions from such devices and concluded that under simulated driving conditions significant emissions of fine Cr-containing particles ($\sim 0.01 \mu\text{m}$ diameter) could occur under a broad range of controlled conditions at temperatures ranging from 185°C to 800°C.

Table 5-3. Aqueous chromium emissions.

Process	Uncontrolled emissions (ppm)	Comments	Reference
Electroplating	trace-600	as Cr(VI)	Cheremisinoff and Habib (1972)
Metal pickling	600	as Cr(VI)	Cheremisinoff and Habib (1972)
Metal bright dip	10,000-50,000	as Cr(VI)	Cheremisinoff and Habib (1972)
Leather tanning	40	as Cr(III)	Cheremisinoff and Habib (1972)
Cooling tower blowdown	10-60	as Cr(VI)	Cheremisinoff and Habib (1972)
Animal glue manufacture	475-600	acid waste Cr(VI)	Wang <i>et al.</i> (1973)
Textile dyeing	1	as Cr(III)	Klein <i>et al.</i> (1974)
Fur dressing and dyeing	20	as Cr(III)	Klein <i>et al.</i> (1974)
Laundry (commercial)	1.2	as Cr(VI) in Na ₂ SO ₄	Klein <i>et al.</i> (1974)

DOMESTIC Cr EMISSIONS

It is virtually impossible to follow Cr in the domestic sphere of activities. Table 5-4 gives a few examples of the occurrence of Cr in domestic activities. Examination of the list indicates how thoroughly Cr in its many forms has become of everyday use to man. Hence, it should not be unexpected that domestic activities contribute significantly to Cr pollution problems.

Recent investigations by the Department of Water Resources, City of New York (Klein *et al.* 1974) have shown that electroplating wastes accounted for only 43% of the total daily Cr burden of the city's sewer system and 24% of the total Cr emissions from New York City into the harbor (see Table 5-5). Analysis of residential waste waters and rainwater runoff indicated that these sources contributed 28% and 9%, respectively, of the daily sewer Cr burden, i.e. together almost as much as the electroplating industries. Other heavy metals, notably Zn, Cd and Cu, also exhibited over a 40% contribution from residential waste waters.

The above example is possibly an indication of what may be taking place in large Canadian cities. The significance of these findings is that rigid controls on industrial Cr emissions will not eliminate all or even the major portion of the Cr pollution from large population centres unless an effort is made to limit the unnecessary incorporation of heavy metals into consumer goods. It must nevertheless be remembered that industrial Cr emissions tend to be concentrated point sources while domestic Cr emissions are diffuse and dilute in nature. Hence, on a local basis, industrial Cr emissions are always the cause of greater concern than domestic emissions.

COMPARISON OF Cr EMISSIONS WITH NATURAL Cr LEVELS

The total estimated atmospheric emissions from US sources alone is 1.48×10^{10} g/year (GCA Corporation 1973), while the estimated background Cr content of the atmosphere is 5×10^9 g. Hence, it appears that Cr emissions to the atmosphere are significant on a global as well as a local scale, especially if particle size and chemical reactivity are taken into consideration. Background levels of Cr in the atmosphere have been estimated at 1 ng/m^3 . Cr levels in American urban centres have been reported to range from $10\text{-}60 \text{ ng/m}^3$, while workers engaged in the handling and use of Cr compounds are exposed to mean Cr levels of $0.1\text{-}1.7 \times 10^5 \text{ ng/m}^3$ (Gafafer 1953). The present internationally accepted threshold limit for exposure in sodium bichromate plants is $0.5 \times 10^5 \text{ ng Cr(VI)/m}^3$.

Table 5-4. Domestic sources of Cr emissions.

Source	Comment	Reference
Hot water heating system	300 ppm corrosion prevention	Udy (1956)
Fire sprinkler systems	300 ppm corrosion prevention	Udy (1956)
Tanned leather goods	3-6% Cr ₂ O ₃ by weight	Mertz (1969)
Textile dyeing and goods	both Cr(III) and Cr(VI) used	Udy (1956)
Pigments and paints	percent levels of Cr	Udy (1956)
Wood preservation	5000 ppm solution Cr(VI) used	Samitz <i>et al.</i> (1967)
Laundry detergents	can contain up to 10 ppm Cr	Czernielewski and Dudek (1972)
Chrome plated appliances		
Garbage incineration	170 ppm Cr in ash	GCA Corporation (1973)
Coal combustion	900-2600 ppm Cr in ash	GCA Corporation (1973)
Oil combustion	200-300 ppm Cr in ash	GCA Corporation (1973)

Table 5-5. Sources of heavy metals entering New York treatment plants^a.

Source	Percent of total weights received				
	Cr	Cu	Ni	Zn	Cd
Water supply	0	20	0	7	0
Electroplating	43	12	62	13	33
Other industry	9	7	3	7	6
Runoff	9	14	10	31	12
Residential	28	47	25	42	49
Unknown	11	0	0	0	0
Total kg/day	675	1160	510	1780	73

^a Klein *et al.* (1974)

The amounts of Cr added by industrial and domestic sources to streams, rivers and, eventually, the ocean again appear to be significant compared to the amount of Cr mobilized annually by weathering processes, e.g. New York City alone emits 4.4×10^8 g/year of Cr (Klein *et al.* 1974), while natural mobilization of Cr by weathering processes is estimated at 3.2×10^{10} g/year on a worldwide basis (Bertine and Goldberg 1971).

CHAPTER 6 INTERPRETATION OF CAUSE/EFFECT DATA

INTRODUCTION

Before discussing Cr cause/effect data for various life forms in detail, it is useful to examine some of the assumptions and hidden variables affecting the significance of the results. This chapter sets forth some of the questions to be asked when one is trying to relate experimentally derived cause/effect data to Cr levels in the environment.

ASSUMPTIONS AND HIDDEN PARAMETERS

Ideally, cause/effect data may be displayed graphically as in Figure 6-1, where the effect is the direct result of the applied Cr concentration. This picture may be modified by the following considerations:

- 1) Is the effect general or specific, i.e. is Cr the only element causing that effect and does it do so consistently?
- 2) What is the change in effect when the species, sex, age, nutritional state, previous history of the animal are changed?
- 3) What change in effect (or its functional dependence on concentration) is observed when the time between dosage and measurement of effect is changed, e.g. some lung cancers have a 10-20 year initiation period while most commonly used laboratory animals live only a fraction of that time.

The design of the experiment should provide controls for determining these factors.

The concentration values used are assumed to be the concentrations of the physiologically active species. This is often not the case:

- 1) The Cr concentration reported is usually the total Cr in the system. No correction for the availability of the compound is attempted. Hence, the results are strictly valid only for that chemical compound used and the specific route by which it is administered, e.g. equal amounts of Cr(III) given orally or intravenously will have different effects.

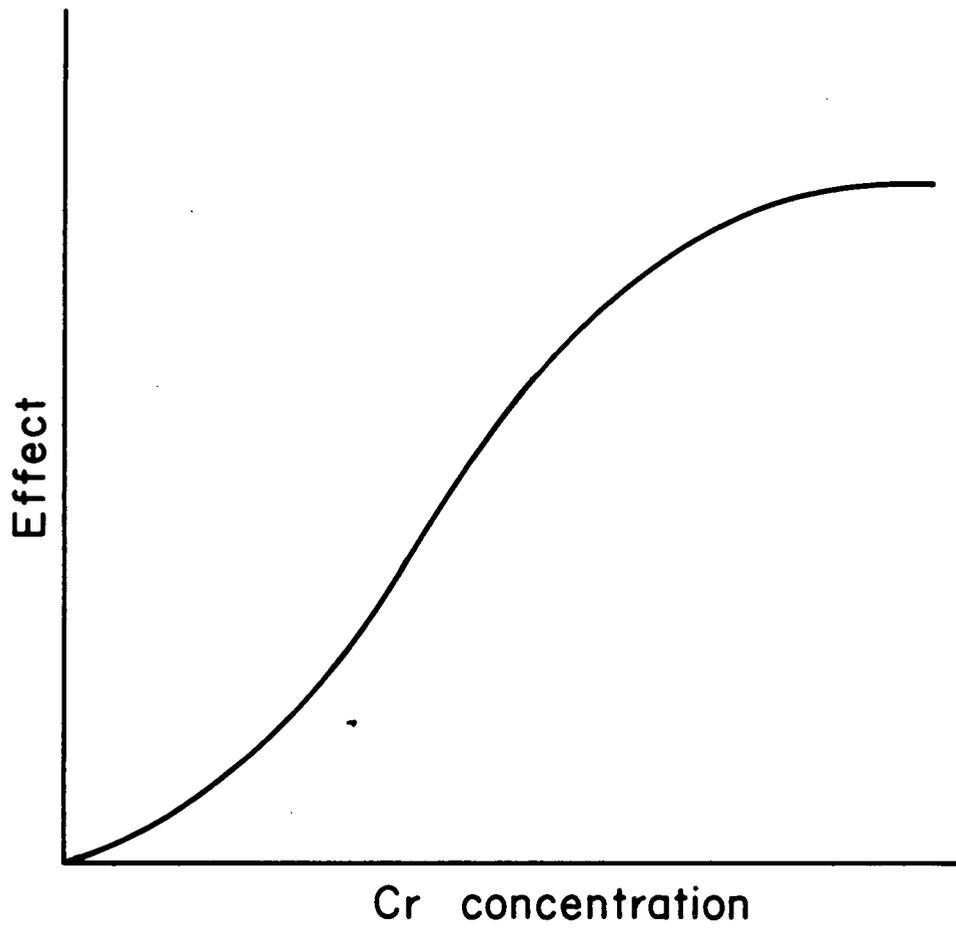


Fig. 6-1. Idealized graphical display of cause/effect data.

- 2) Pure compounds are used in laboratory simulations but in nature there are many other elements and compounds which may act in a synergistic or antagonistic manner, either by direct chemical interaction with the Cr (affecting its availability) or by interacting with the living organism to change its susceptibility to Cr. Purified artificial environments and diets may exclude essential nutrients which would normally be present.
- 3) The Cr dosage may be applied suddenly in laboratory simulations, while in the environment, concentration changes usually occur on a longer time scale. Shock loading may lead to unrealistically high (no time to adapt) or low (all absorption processes stop) observed toxicities.

The design of the experiment and the way in which results are reported^a can thus have a significant bearing on the apparent toxicity of a chemical compound. Hence, experimentally derived results can seldom be applied directly to determine the effects of Cr levels in the environment without a thorough knowledge of prevailing field conditions.

^a See D. Huff and I. Geis (1954) "How to Lie With Statistics" for an excellent discussion of personal bias introduced by incorrect use of statistics.

CHAPTER 7 EFFECTS OF CHROMIUM AT THE MICROSCOPIC LEVEL: BIOCHEMISTRY AND MICROSCOPIC ORGANISMS

INTRODUCTION

This chapter amplifies the section in Chapter 3 dealing with the chemistry of Cr in living organisms and gives examples of specific biochemical effects. Many of these effects, although important, are not easily related to symptoms observable on a macroscopic scale, i.e. the apparent state of health or disease of a living organism. These latter effects will be dealt with in the chapters on fish, plants, animals and man. Cr-deficiency symptoms will only be mentioned briefly, in as much as they elucidate some of the biochemistry of Cr.

BIOCHEMISTRY OF Cr(III)

As indicated in Chapter 3, the hexaquo Cr^{+3} complex is not stable at physiological pH and the tendency is strong to form colloidal hydrous oxides which are accumulated by the liver, spleen and bone marrow (Visek *et al.* 1953). In nature, Cr is a ubiquitous, and sometimes essential, component of living organisms. These organisms have within their metabolic systems many molecules which can bind Cr^{+3} , usually preventing its precipitation as the hydrous oxide.

LOW MOLECULAR*WEIGHT LIGANDS

Some naturally occurring low molecular weight ligands are the oxalate, phytate and citrate anions. Lyon *et al.* (1969a) isolated a $\text{Cr(III)}(\text{oxalate})_3$ complex from *Leptospermum scoparium*, a known Cr accumulator plant. Chen *et al.* (1973) concluded that the other complexes could be formed in the digestive tract of a herbivorous animal. In an *in vitro* experiment, these investigators showed that the oxalate complex increased Cr(III) transport across the rat intestine, phytate decreased the transport while citrate caused no significant change.

Rollinson *et al.* (1967) demonstrated that other biologically important ligands complex with Cr, maintaining it in a diffusible form at physiological pH. Specifically, the following order of decreasing complex stability was determined: pyrophosphate > methionine > serine > glycine > lucine > lysine > proline. As Mertz (1969) points out, the great affinity of pyrophosphate for Cr(III) is of special interest as ATP (adenosine triphosphate) and similar derivatives are important biological energy storage compounds. Janson and Cleland (1974b)

have shown that Cr-ATP (synthetically prepared) competes with the naturally occurring Mg-ATP as substrate for such enzymes as acetate kinase, pyruvate kinase and 3-phosphoglycerate kinase. Once Cr-ATP is bound to these enzymes it is biologically inert and inhibits the enzyme from participating in further biological catalysis. Janson and Cleland (1974a) have also investigated other Cr-nucleotide complexes and found that the enzyme specificities for the nucleotide bases were unchanged but the Cr derivatives bonded more strongly to the enzymes than did the corresponding natural Mg nucleotides. While it has not been conclusively shown that Cr-nucleotide complexes are formed *in vivo*, the high concentrations of Cr in RNA from diverse sources (Wacker and Vallee 1959) are a strong indication of their existence.

Cr interacts with the unsaturated fatty acids of certain foods and, in the presence of oxygen, accelerates the formation of rancid flavoring compounds by a free radical mechanism (Berger 1975). As little as 1.2 ppm Cr is required to halve the stability of fats present as soaps (Ohlson, quoted in Berger 1975). Research is needed to determine whether this free radical mechanism is operative *in vivo*, e.g. in the mammalian respiratory tract.

The most important natural Cr complex from the standpoint of mammalian nutrition is the glucose tolerance factor (GTF), a complex of Cr(III), several amino acids and nicotinic acid (Mertz *et al.* 1974). This complex, unlike other Cr(III) complexes, is readily absorbed across the gut (10-15% versus 0.5% for inorganic Cr(III)) and rapidly equilibrates with body stores of Cr(III) (Mertz 1969). Cr deficiency in mammals leads to a state resembling diabetes mellitus. No other Cr(III) complex, even when injected intravenously, counteracts Cr deficiency as effectively as GTF. Cr supplied by GTF, or some inferior alternate source, assists in binding insulin to the cell membrane of fat cells, possibly by initiating disulfide exchange between insulin and membrane receptors. The membrane-bound insulin of fat cells then stimulates these cells to absorb glucose from the bloodstream to form glycogen (Mertz and Roginski 1971). Cr and insulin appear to act in unison to influence many biological functions such as the incorporation of dietary amino acids into proteins (Roginski and Mertz 1967). GTF easily crosses the placental barrier while other Cr(III) complexes do not (Mertz 1969). In view of its high availability, GTF is a possible source of toxic amounts of Cr(III); however, insufficient quantities of the pure material have been isolated to perform feeding experiments (Mertz *et al.* 1974).

Cr(III) has been shown to interact strongly with the membranes of nerve axons (Goldman 1970). The ionic permeability of the membrane is changed to produce a marked reduc-

tion in axon excitability. The accumulation of injected Cr(III) complexes in skeletal tissue (Anghileri 1970) is again an indication of the affinity of Cr(III) for certain biological membranes.

Cr(III)-PROTEIN INTERACTIONS

The best known interaction of Cr with a biological molecule is the cross-linking of collagen molecules through their carboxyl side groups (Mertz 1969; Hörmann 1974) in the tanning of leather. Cr^{+3} is the only metal ion which forms stable enough complexes such that true cross-links are formed. Physiological Cr levels (0.1 ppm) are much lower than those required for tanning (10^4 ppm) but even at these low levels there is evidence that Cr(III) may stabilize proteins in their proper configurations (Mertz 1969). Higher than normal Cr concentrations may alter protein configurations by nonspecific binding. These altered proteins are postulated to cause Cr dermatitis by an immune response of the body against altered forms of its own proteins (Shmunis *et al.* 1973).

In mammals Cr is present in the blood complexed to transferrin (siderophilin) which is postulated to be the normal carrier protein for up to 80% of serum Cr (Hopkins and Schwarz 1964). Jett *et al.* (1968) have shown that Cr(III) injected intravenously in increasing quantities binds to siderophilin, serum albumin, two alpha proteins and gamma globulin. These diverse proteins together probably serve the double purpose of Cr transport and limiting the availability of Cr for nonspecific binding. The overflow of their collective binding capacity leads to nonspecific binding to proteins and membranes (see above) or to the formation of colloidal hydrous oxides which are filtered out by the liver (Visek *et al.* 1953).

Cr has been shown to be capable of increasing the activity of certain enzymes, e.g. succinic-cytochrome C dehydrogenase, or satisfying the requirement of a metal cofactor in other enzymes, e.g. phosphoglucomutase (Mertz 1969). Cr *in vitro* has been shown to be active as the metal cofactor in cholesterol synthesis and in maintaining the activity of trypsin and rennin, two protein-splitting enzymes (Mertz 1969).

Excessive levels of Cr predictably inhibit the action of enzymes and hormones almost certainly through nonspecific binding which changes the three-dimensional configuration of the active site, e.g. Cr-trypsin and Cr-insulin ratios of 1:1 are optimal while Cr-protein ratios of 10:1 *in vitro* are sufficient to initiate the formation of cross-links.

Cr(III)-NUCLEIC ACID INTERACTIONS

The highest natural concentration of Cr ever reported in animal tissue was 1080 ppm in a beef liver fraction consisting of 70% RNA and 30% protein (Wacker and Vallee 1959). Since then, Cr has been shown to be regularly present in nucleic acid fractions from diverse sources regardless of the exclusion of exogenous Cr during purification. Eisinger *et al.* (1962) studied the interaction of Cr(III) with DNA using a pulsed NMR technique and concluded that the Cr^{+3} was bound to the DNA at exterior sites, probably the phosphate groups. In this case, the Cr^{+3} was added to a solution containing the DNA. Cr^{+3} bound naturally to DNA could conceivably be bound at other sites. As with the proteins, Cr appears to help maintain the ordered three-dimensional structure of the nucleic acids (Mertz 1969); however, it is not yet possible to postulate a special role for Cr in the functioning of DNA and RNA.

Cr *in vivo* interacts with other essential trace metals. O'Flaherty *et al.* (1974) studied the effect of Cr on serum ceruloplasmin activity, copper, and zinc in male rats and reported the following:

- 1) Cr-supplemented drinking water (10 ppm Cr) significantly reduced serum copper levels and ceruloplasmin activity; zinc supplementation reversed this trend.
- 2) Manganese (50 ppm) in the presence of Cr (10 ppm) in the water significantly raised serum copper and ceruloplasmin activity even though there was no copper supplementation, i.e. manganese reversed the effect caused by Cr.

In contrast, Popov (1969) reported that some disorders caused by lack of manganese can be treated with Cr. Cr(III) probably binds to the same site on the transferrin (siderophilin) molecule as does iron (Hopkins and Schwarz 1964), but interferes with iron binding only as the protein becomes saturated with iron and Cr.

Factors other than trace metals can affect Cr metabolism, e.g. Pekarek *et al.* (1974) observed a decrease in the baseline fasting serum Cr level from 1.49 ppb in controls to 0.45 ppb in subjects infected with sand fly fever. The rates of glucose clearance from the blood paralleled the decrease in blood Cr level.

BIOCHEMISTRY OF Cr(VI)

Some plants grown in solutions containing Na_2CrO_4 were shown to contain Na_2CrO_4 in their xylem sap; however, all the Cr present in the root, stem and leaf tissues was in the form of Cr(III) complexes (Lyon *et al.* 1969a). Chromate injected into the mammalian blood stream rapidly diffuses into the red blood cells where it reacts to form Cr(III) which then binds to the globin fraction of hemoglobin (Gray and Sterling 1950). Cr(VI) has no proven biological function and Cr(III) is assumed to be the biologically active form of Cr in nature.

Cr(VI), nevertheless, does interact with biological material, but never with beneficial effects, e.g. bacterial urease is inhibited by 1-10 ppm Cr(VI) (Mertz 1969). Cr(VI) does not interact as strongly with proteins as does Cr(III). This is because CrO_4^{2-} , being an anion, is not attracted to the negatively charged surfaces of protein molecules. This accounts for the greater mobility of Cr(VI) over Cr(III) in biological systems, i.e. the protective binding of Cr by metal-binding proteins and other molecules is not effective for Cr(VI). Hence, Cr(VI) can diffuse into tissues not normally accessible to Cr(III), perhaps as the monovalent anion HCrO_4^- (Trama and Benoit 1960). Once Cr(VI) has diffused into the tissues it may react to form Cr(III) and participate in potentially harmful nonspecific binding to important biological molecules. It is the greater mobility of Cr(VI), coupled with its strong oxidizing ability, that makes it generally more toxic than Cr(III).

In vitro studies using isolated rat liver or ox heart mitochondria have shown that even low levels of Cr(VI) can powerfully inhibit respiratory chain activity (Broughall and Reid 1974):

- 1) 1.56 ppm Cr(VI) completely inhibited within 2-3 minutes the respiration of glutamate and alpha-oxoglutarate; inhibition of the enzymes alpha-oxoglutarate dehydrogenase and glutamate dehydrogenase is responsible for this observation.
- 2) 21 ppm Cr(VI) completely inhibited respiration of beta-hydroxybutyrate, malate and pyruvate, possibly through inhibition of NADH dehydrogenase.
- 3) 156 ppm Cr(VI) completely inhibited succinate dehydrogenase.

All the above enzymes play a vital role in the extraction of energy from the chemical breakdown of foods.

Another *in vitro* study (Chiraseveenuprapund and Rosenberg 1974) indicated that 52 ppm Cr(VI) was sufficient to inhibit glutathione reductase in bovine thyroid slices. This caused decreased tissue glutathione levels and upset normal metabolism by stimulating organic binding of iodine, i.e. acting in the manner of a thyrotropic agent.

Venitt and Levy (1974) studied the effect of Cr(VI) on bacterial DNA and concluded that chromates acted in a mutagenic manner by directly modifying DNA bases in such a way that base pair errors arise at subsequent cell divisions. These investigators postulated that attack occurred at G-C base pairs, causing G-C to A-T transitions at a subsequent round of DNA replication. The Cr(VI) levels for such effects to be readily observable were rather high (10^3 ppm).

Cr AND MICROSCOPIC ORGANISMS

In nature, microscopic organisms (bacteria, protozoans, etc.) form important parts of aquatic and soil food chains. These organisms are now being used to biologically degrade industrial and domestic wastes. At ppb levels Cr can fill the metal ion requirement of some microorganisms for continued growth (Mertz 1969). Higher levels of Cr in the aqueous growth medium may be toxic to these microorganisms if there are not sufficient natural ligands present to reduce the Cr availability, i.e. an increase in the dissolved and/or suspended solids in the aqueous growth medium usually decreases Cr toxicity for both Cr(VI) and Cr(III) (WPR 1970).

The results of laboratory tests on various microorganisms are shown in Table 7-1. Effects range from depressed growth and inability to utilize various nutrients to irreversible inactivation and inability to reproduce.

Table 7-2 gives the results of laboratory and field studies aimed at determining the effects of Cr on biological oxidation in sewage sludge. Single exposures of up to 500 ppm Cr(VI) have been tolerated for short periods of time, i.e. upon clearance of Cr(VI) the microorganisms were still able to reproduce. Maximum permissible continuous levels of Cr(VI) and Cr(III) are much lower (1 and 15 ppm respectively) in order that digestion processes not be adversely affected. The paper by Bailey *et al.* (1970) reviews some of the previous work in the field and the reader is referred to this work for further information.

By inhibiting oxygen absorption by sludge microorganisms, the presence of Cr(III) tends to produce artificially low values for BOD, e.g. 3 ppm and 20 ppm Cr(III) reduced BOD

Table 7-1. Effects of Cr on microorganisms.

Microorganism	Form of Cr	Concentration (ppm)	Temperature (°C)	Effects and Comments	Reference
Cornebacterium ^a	Cr ⁺²	0.52	-	decreased growth	Clarke (1958)
T ₁ phage ^b	Cr(III)	1-10	-	irreversible inactivation	Puck <i>et al.</i> (1951)
<i>E. coli</i> ^a	Cr(III)	1-10	-	irreversible inactivation	Puck <i>et al.</i> (1951)
Salmonella (mutant) ^a	Cr(III)	0.5	-	inability to utilize sugars	Corwin <i>et al.</i> (1966)
Salmonella (wild)	Cr(III)	26	-	inability to utilize sugars	Corwin <i>et al.</i> (1966)
Staphylococcus ^a	Cr(VI)	1	-	observed toxicity	Henry and Smith (1946)
Yeast and microbes	Cr(VI)	0.0001	-	toxic in some cases	Henry and Smith (1946)
Bacteria	Cr(VI)	1	-	inability to reproduce	Ingols <i>et al.</i> (1964)
<i>Bacillus subtilis</i> ^a	Cr(VI)	2	37	increased mutation rate	Nishioka (1974)
<i>V. microstomata</i> ^c	Cr(VI)	0.53	20	96-hour median inhibitory limit	Sudo and Shuicki (1973)
<i>C. campyllum</i> ^c	Cr(VI)	12.9	20	96-hour median inhibitory limit	Sudo and Shuicki (1973)
<i>Opercularia</i> sp. ^c	Cr(VI)	20.9	20	96-hour median inhibitory limit	Sudo and Shuicki (1973)
Rotifer	Cr(VI)	3.1	20	96-hour median lethal dose	Buikema <i>et al.</i> (1974)
Rotifer	Cr(VI)	12.0	5	96-hour median lethal dose	Schaefer and Pipes (1973)
Rotifer	Cr(VI)	8.9	15	96-hour median lethal dose	Schaefer and Pipes (1973)
Rotifer	Cr(VI)	7.4	20	96-hour median lethal dose	Schaefer and Pipes (1973)
Rotifer	Cr(VI)	5.5	25	96-hour median lethal dose	Schaefer and Pipes (1973)
Rotifer	Cr(VI)	4.4	30	96-hour median lethal dose	Schaefer and Pipes (1973)

^a Bacterium^b Virus^c Protozoan

Table 7-2. Effects of Cr on biological oxidation in sewage sludges.

Form of Cr	Concentration (ppm)	Suspended solids	Effect or comment	Reference
Cr(III)	50	?	nontoxic limit for 14-day anaerobic digester	Bailey <i>et al.</i> (1970)
Cr(III)	90	?	maximum Cr level before complete inhibition of 14-day anaerobic digester	Bailey <i>et al.</i> (1970)
Cr(III)	15	3514 mg/l	severe inhibition of nitrification in activated sludge processing	Bailey <i>et al.</i> (1970)
Cr(VI)	1	?	maximum continuous Cr influx for acceptable Cr loss ^a in effluent	Lawson and Fearn (1970)
Cr(VI)	5	4000 mg/l	increase in COD of effluent	WPR (1970)
Cr(VI)	5	2000 mg/l	nitrification almost completely inhibited	WPR (1970)
Cr(VI)	50	?	maximum tolerated on a continuous basis	Moore <i>et al.</i> (1961)
Cr(VI)	100	?	maximum tolerated slug dose	Barth <i>et al.</i> (1963)
Cr(VI)	500	?	survival of shock load for 1 hour	English <i>et al.</i> (1964)

^a i.e. for Cr in effluent to be less than 0.2 ppm.

by 50% and over 90% respectively, but normal BOD values were obtained after addition of EDTA to complex with the Cr (Morgan and Lackey 1958).

Cr AND FERMENTATION TECHNOLOGY

Yeasts, certain bacteria, and some fungi have the capability of fermenting waste organic material into useful by-products (protein, alcohols, etc.). Crossland (1974) and Gorman (1974) have pointed to the possibility of producing large quantities of edible proteins from solid wastes. However, the presence of significant levels of Cr and other heavy metals in almost all domestic and industrial wastes could pose serious problems. The fermentation process could be adversely affected, e.g. as little as 0.1 ppb Cr(VI) (Henry and Smith 1946) and 200 ppm Cr(III) (Mertz 1969) can be toxic to some yeasts. Furthermore, toxic compounds could be synthesized by the fermenting organisms while detoxifying or reacting to the Cr, e.g. brewers' yeast metabolizes Cr(III) into the GTF which is 20 times more available to mammals than inorganic Cr(III) (Mertz and Roginski 1971); as little as 1 ppm Cr caused a doubling of the amounts of aflatoxin produced by *Aspergillus flavus* growth on defatted corn germ (Lillehoj *et al.* 1974).

CHAPTER 8 EFFECTS OF Cr ON AQUATIC ORGANISMS

INTRODUCTION

Three excellent reviews dealing with the measurement of pollutant toxicity to fish have been published by Sprague (1969, 1970, 1971). Skidmore (1974) recently discussed some of the factors affecting the toxicity of pollutants to fish. The following is a partial summary of the general ideas presented in these articles.

Although the incipient LC50^a is the effect usually measured in laboratory trials, other effects may be better indicators of toxicity: growth rate, respiration rate, swimming speed, behavior, reproductive ability. Short-term (24- to 72-hour) studies yield misleading information on the relative toxicities of various chemicals. Mixtures of toxic substances can exhibit less-than-additive, additive, or more-than-additive effects. Some progress has been made in calculating the joint toxicity of a mixture of pollutants. Increasing the water hardness usually decreases heavy metal toxicity. The effect of temperature is unpredictable because both absorption and excretion or detoxification are temperature dependent processes. Figure 8-1 illustrates how the time at which the effect is measured can yield either increasing or decreasing toxicity with increasing temperature. On the other hand, Schaefer and Pipes (1973), using the rotifer as a test animal, showed that the effect of temperature on the LC50 for Cr(VI) decreased as exposure time was lengthened (see Fig. 8-2). Water temperature can also affect the apparent toxicity of a given Cr level because the solubility of oxygen, essential for fish life, is temperature dependent. The pH of the water has a marked effect on the relative amounts of $\text{Cr}(\text{H}_2\text{O})_6^{+3}$ and $\text{Cr}(\text{OH})_3$ present. A change of one pH unit changes the $\text{Cr}(\text{H}_2\text{O})_6^{+3}$ concentration by a factor of 1000.

Environmental concentrations of toxic substances vary with time; in the laboratory, concentrations are usually kept constant throughout the test period. Some success has been achieved at estimating the effective toxicity of water

^a Median lethal threshold concentration, the level of the toxic substance which is lethal for 50% of the individuals exposed for periods sufficiently long that acute lethal action has ceased.

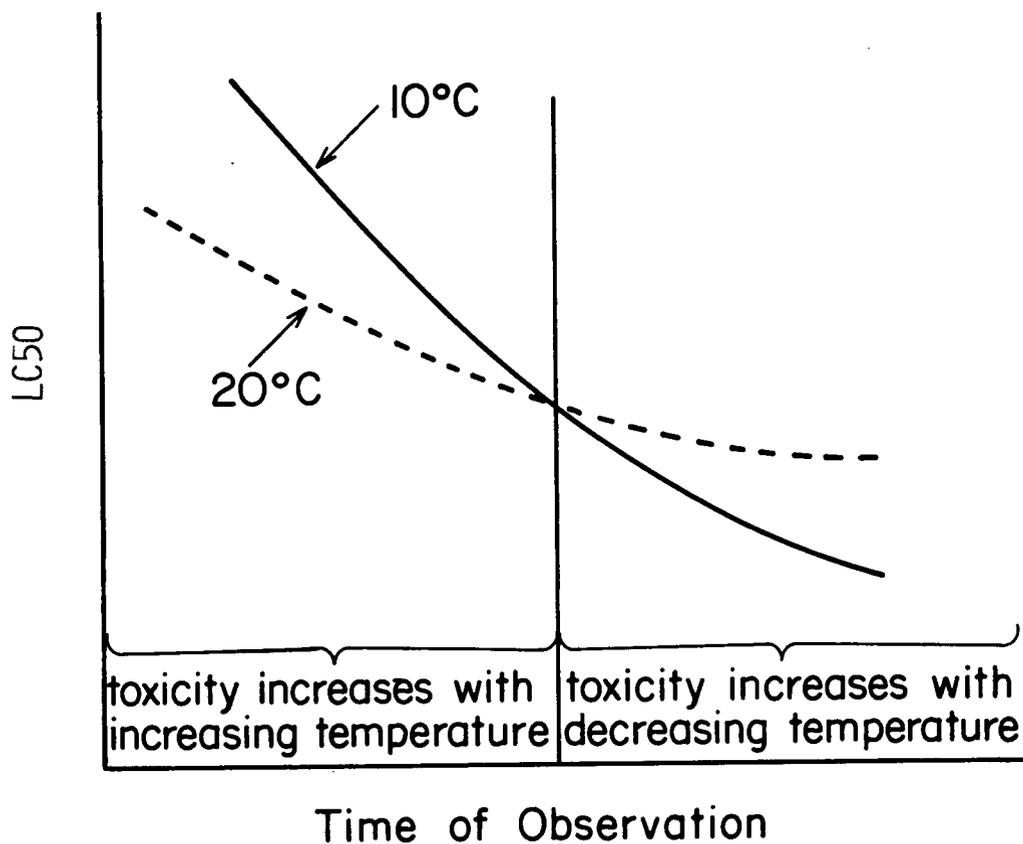


Fig. 8-1. Time-temperature dependence of toxicity.

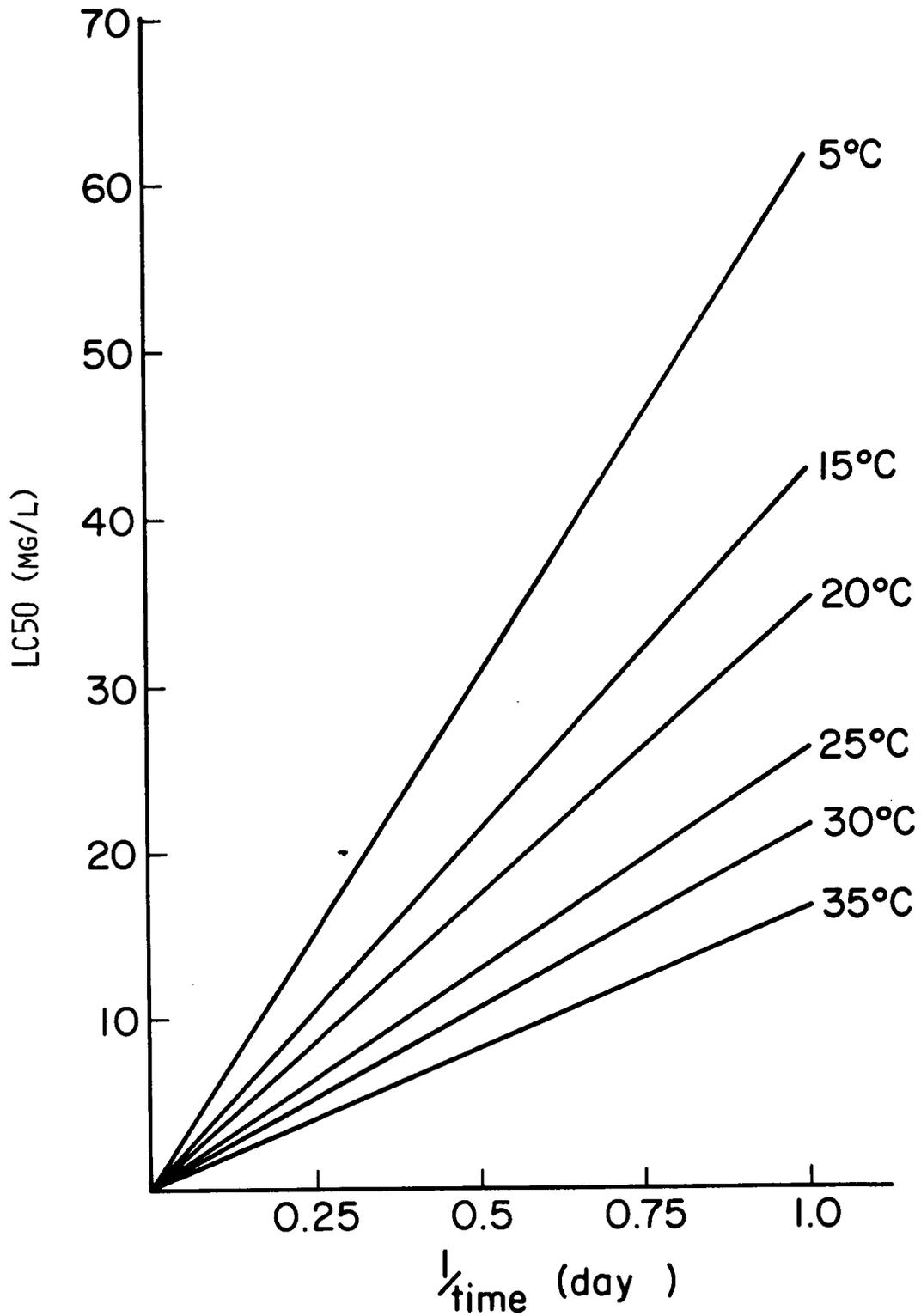


Fig. 8-2. Diminished effect of temperature with increasing time before measurement.^a

^a After Schaefer and Pipes (1973).

containing fluctuating levels of chemicals by integrating the effect as a function of concentration over the specified period of time. Acclimation of the test organism to sublethal levels of Cr may reduce the apparent toxicity of Cr but may also increase the susceptibility to other toxic chemicals.

The problems with experimental conditions and design such as described above have doubtless contributed to the conflicting cause/effect data on Cr contained in the earlier literature. Improvements may be expected as increasing attention is paid to the standardization of the test organism (species, sex, age), the composition of the basic growth medium, the test conditions (temperature, nutrition, etc.), and the actual effect monitored. The data reproduced in Table 8-1 were chosen to indicate:

- 1) the relative toxicity of Cr compounds;
- 2) the relative susceptibility of aquatic organisms to Cr toxicity;
- 3) Cr levels for comparison with those found in uncontaminated and polluted waters.

Often the "safe" concentration of a toxic substance is taken as 0.1 to 0.03 times the 96-hour LC50.

Several general points can be drawn from Table 8-1:

- 1) Smaller organisms, with the exception of most of the insects, are more susceptible to Cr toxicity than larger organisms.
- 2) Increasing water hardness drastically reduces Cr(III) toxicity but does not have as large an effect on Cr(VI) toxicity. (Cr(III) forms stable complexes with many anions. Pickering and Henderson (1966) report the presence of a visible precipitate after the addition of Cr(III) to hard (360 mg/l) water.)

MICROSCOPIC ORGANISMS

These organisms often form important links in the food chain, e.g. the rotifer is excellent fish food (Schaefer and Pipes 1973). Buikema *et al.* (1974) also point out that rotifers and similar organisms consume large amounts of bacteria and algae; hence, their disappearance could lead to a blooming of fouling microbial organisms.

Table 8-1. Effects of Cr on aquatic organisms.

Organism	Form of Cr	Water hardness (mg/l)	pH	Temperature (°C)	Dissolved O ₂ (mg/l)	96-hour LC50 (ppm)	Reference
Rotifer ^a	Cr(VI)	25	7.4-7.9	20	-	3.1	Buikema <i>et al.</i> (1974)
Rotifer	Cr(VI)	81	7.4-7.9	20	-	15.0	Buikema <i>et al.</i> (1974)
Bristle worm	Cr(III)	50	7.6	17	6.2	9.3	Rehwooldt <i>et al.</i> (1973)
Crustacean (Amphipoda)	Cr(III)	50	7.6	17	6.2	3.2	Rehwooldt <i>et al.</i> (1973)
Mayfly	Cr(III)	50	6.8	18.5	8.0	2.0	Warnick and Bell (1969)
Midge larva	Cr(III)	50	7.6	17	6.2	11.0	Rehwooldt <i>et al.</i> (1973)
Dansel fly nymph	Cr(III)	50	7.6	17	6.2	43.0	Rehwooldt <i>et al.</i> (1973)
Caddis fly larva	Cr(III)	50	7.6	17	6.2	50.0	Rehwooldt <i>et al.</i> (1973)
Caddis fly larva	Cr(III)	50	6.8	18.5	8.0	64.0	Warnick and Bell (1969)
Snail eggs	Cr(III)	50	7.6	17	6.2	12.4	Rehwooldt <i>et al.</i> (1973)
Snail (adult)	Cr(III)	50	7.6	17	6.2	8.4	Rehwooldt <i>et al.</i> (1973)
Fatheads	Cr(VI)	20	7.5	25	7.8	17.6	Pickering and Henderson (1966)
Fatheads	Cr(VI)	360	7.5	25	7.8	27.3	Pickering and Henderson (1966)
Bluegills	Cr(VI)	20	7.5	25	7.8	118.	Pickering and Henderson (1966)
Bluegills	Cr(VI)	360	7.5	25	7.8	133.	Pickering and Henderson (1966)

Table 8-1. (cont'd).

Organism	Form of Cr	Water hardness (mg/l)	pH	Temperature (°C)	Dissolved O ₂ (mg/l)	96-hour LC50 (ppm)	Reference
Goldfish	Cr(VI)	20	7.5	25	7.8	37.5	Pickering and Henderson (1966)
Guppies	Cr(VI)	20	7.5	25	7.8	30.0	Pickering and Henderson (1966)
Fatheads	Cr(III)	20	7.5	25	7.8	5.07	Pickering and Henderson (1966)
Fatheads	Cr(III)	360	7.5	25	7.8	67.4	Pickering and Henderson (1966)
Bluegills	Cr(III)	20	7.5	25	7.8	7.46	Pickering and Henderson (1966)
Bluegills	Cr(III)	360	7.5	25	7.8	71.9	Pickering and Henderson (1966)
Goldfish	Cr(III)	20	7.5	25	7.8	4.10	Pickering and Henderson (1966)
Guppies	Cr(III)	20	7.5	25	7.8	3.33	Pickering and Henderson (1966)

^a See also Table 7-1

Note: The first two listings are microorganisms; the next two are benthic organisms; the next five are aquatic insects or insect larvae; the remaining twelve are fish.

The data contained in Tables 7-1 and 8-1 indicate that certain species of these microscopic organisms are quite sensitive to Cr, with Cr(VI) exhibiting greater toxicity than Cr(III).

OLIGOCHAETES (WORMS), CRUSTACEANS, AND MOLLUSCS

These organisms usually live in bottom sediments. Since Cr(III) concentrates in sediments and interstitial water (Onondaga County 1971), these benthic organisms are subjected to higher Cr(III) concentrations than others found only in the water column or in flowing streams.

Certain species, e.g. *Daphnia* (crustacean), exhibit high sensitivity to Cr. Biesinger and Christensen (1972) reported a 16% reproductive impairment in *Daphnia* due to 0.33 ppm Cr(III).^a Toxic thresholds ranging from 0.016 to 0.7 ppm Cr(VI) have been reported for *Daphnia magna* (McKee and Wolf 1963). More resistant species have toxic thresholds approaching 1 ppm Cr(VI). Cr(VI) threshold toxicities for marine benthic organisms are of the same order of magnitude as those for freshwater organisms: e.g. marine polychaete worm - 1 ppm, prawn - 5 ppm, crab - 10 ppm (Raymont and Shields 1963).

Molluscs are known to accumulate heavy metals, including Cr, and some species have been used as biological pre-concentrators in pollution studies to follow the movement of heavy metals (Navrot *et al.* 1974). Over a period of 2 years, oysters accumulated large enough amounts of Cr from water containing 10 ppb Cr to exhibit toxic and even lethal effects in the summer when their physiological activity was at its highest (Haydu 1972).

INSECTS AND LARVAE

The values quoted in Table 8-1 indicate that insects are less susceptible to Cr toxicity than most other aquatic organisms. Ingols *et al.* (1964) studied the division of insect egg cells in solutions containing Cr(VI) at ppm levels and found that while many of the cells began to divide, they could not complete the division and had chromosome damage. Interestingly enough, the cells continued to respire at the normal rate for several hours, which was 3 to 5 times the normal period between cell divisions. Thus, an examination of the respiration rate alone could lead to the erroneous conclusion that Cr(VI) was without effect.

^a A Cr(III) concentration of 0.17 ppm in interstitial waters has been reported (Onondaga County 1971).

FISH

The complex series of events involved in the toxic actions of Cr on fish and the lack of critical studies make it impossible to be certain of the relative toxicities of Cr(III) and Cr(VI) as well as what would constitute safe levels of these chemicals (Trama and Benoit 1960). The 96-hour LC50 values listed in Table 8-1 indicate that Cr(III) is more toxic than Cr(VI). Olson (1958) exposed a large number of chinook salmon fingerlings to low levels of Cr(III) and Cr(VI) for 12 weeks and obtained strikingly different results. Under the test conditions (water hardness 70 mg/l, pH 7.7-8.0, temperature 8-16°C), 0.2 ppm Cr(III) did not increase the mortality over controls (0.8% mortality), whereas 0.2 ppm Cr(VI) caused a 53% mortality. The full toxic effect of Cr(VI) became evident only after 5 weeks, or nearly 9 times longer than the common 96-hour LC50 test. Thus, it would appear that the 96-hour test is still a measure of acute and not chronic toxic effects. While indications are that salmon may be more sensitive to Cr toxicity than most fish, this appears to be generally true of the more highly prized game and food fish.

Olson also found that a retarded growth rate could serve as an early indicator of chronic toxic effects in fish. A sharp retardation in growth was observed after only 2 weeks in fish exposed to 0.2 ppm Cr(VI). Significant growth retardation was observed at levels as low as 0.016 ppm Cr(VI). Reducing the temperature had the effect of increasing the apparent toxicity of Cr(VI).

Trama and Benoit (1960) developed equations describing the equilibrium between $\text{Cr}_2\text{O}_7^{2-}$, CrO_4^{2-} and their various protonated forms as a function of pH of the water. There was a strong correlation between the calculated HCrO_4^- concentration and the fish mortality. This appears reasonable since the monovalent anion HCrO_4^- would be expected to cross biological membranes more easily than the doubly charged CrO_4^{2-} anion. In dilute solution $\text{Cr}_2\text{O}_7^{2-}$ reacts with water to form CrO_4^{2-} and protons (H^+). Although the calculations of Trama and Benoit (1960) are not exact because they do not take into account the effects of ionic strength on ionic equilibria, their use in extracting correlations is entirely valid because only relative concentration values are considered.

Knoll and Fromm (1960) studied the accumulation and elimination of Cr(VI) by the rainbow trout. The fish were exposed for 12 days to 2.5 ppm Cr(VI) (containing some ^{51}Cr tracer) in tap water held at 14°C. At the end of this period

the fish were returned to fresh tap water until analysis. The principal findings may be summarized as follows:

- 1) The main path of absorption of Cr(VI) was through the gills, with little Cr being taken up through the gut or the skin.
- 2) The Cr levels in the blood were almost always lower than in the surrounding water, indicating that Cr(VI) probably entered through the gills by passive diffusion. However, some tissues such as the kidney accumulated 15 ppm Cr or more.
- 3) After Cr(VI) exposure stopped, blood, liver, stomach, pyloric caeca and posterior gut lost Cr rapidly.
- 4) Kidney and spleen were slower to lose Cr; spleen exhibited no significant decrease even after the fish had been in fresh water for 25 days.

While the uptake and elimination of Cr(III) by fish has not been studied extensively, it is known that fish, when irritated by toxic levels of Cr^{+3} , secrete large amounts of mucus which complexes with the metal ion and thus reduces the ionic diffusion through the skin (Carpenter 1927). The main toxic action of Cr^{+3} is presumed to arise from the coagulation or cross-linking of the mucus secreted by the gills, or from damage to the gill tissue which in turn interferes with respiratory function, resulting in death by suffocation (Doudoroff and Katz 1953). Increased mucus secretion tends to protect the gills.

Other toxic effects of Cr have been recorded:

- 1) Necrosis of gut tissue has been observed in bass exposed to 65 ppm Cr(VI) for 6 days (Fromm and Schiffmann 1958).
- 2) As little as 2 ppm Cr(VI) produced significant changes in the hematocrit^a of the rainbow trout (Schiffmann and Fromm 1959) and in the blood specific gravity of the bluegill (Abegg 1950).

^a The fraction of whole blood volume occupied by blood cells.

SUMMARY

Water hardness, temperature, dissolved oxygen, species, as well as age of the test organism, all play a role in modifying Cr effects in fish and other aquatic life. Trivalent Cr appears to be more acutely toxic to fish than hexavalent Cr; the reverse is true in long-term chronic studies.

CHAPTER 9 CHROMIUM AND VEGETATION

INTRODUCTION

Plants take part in the transmission of Cr from the soil to man by acting as food for man and feed for animals consumed by man. Low level Cr supplementation has been reported to stimulate plant growth in some cases (Pratt 1966), but this is not sufficient to prove the essentiality of Cr to plants. (See Bowen 1966, page 102, for a discussion of essentiality.) Higher levels of Cr applied to soil both as Cr(III) and Cr(VI) caused toxic symptoms but the soil type had a marked effect on the intensity of these toxic effects. Several excellent reviews have been written on the chemistry of trace elements in the soil (Mitchell 1964; Swaine and Mitchell 1960; Lisk 1972).

AVAILABILITY OF Cr TO PLANTS

Recent laboratory studies (Breeze 1973; Huffman and Allaway 1973) have shown that Cr(III) and Cr(VI) are equally available to plants grown in nutrient solutions (i.e. no soil was present). When, however, inorganic and organic soil components are added to the system, Cr(VI) remains mobile and available while Cr(III) is less available because it becomes adsorbed or complexed. Cr(VI) is so mobile in soils that it has been used as a tracer in the study of groundwater movement (Todorovic and Filip 1967). Most forms of Cr(III) are so readily immobilized by soils having moderate to high ion exchange capacity that Wentink and Etzel (1972) have suggested that Cr(III) be removed from electroplating effluents by percolation through beds of soil. The mobility of Cr(III) in soils can be greatly increased by forming soluble anionic complexes such as Cr-EDTA (Todorovic and Filip 1967).

Field studies by Prince (1957) showed that the total Cr content of the soil is a poor measure of the Cr availability to corn. In fact, Table 4-7 shows that one of the highest Cr contents in corn leaves was obtained from the soil containing the least total Cr. Various ion extracting solutions, e.g. 2.5% acetic acid, have been used in attempts to simulate the action of plants in absorbing trace elements from soils (Mitchell 1964), but seldom with complete success. The following generalizations can be made:

- 1) Poorly-drained soils containing decaying organic matter usually have more extractable Cr than well-drained soils (Kee and Bloomfield 1962). Cr(III) present in natural oxide form is quite inert and is not mobilized under these condi-

tions. However, Cr present in lattice imperfections in more easily weathered minerals and in hydrous oxide form (e.g. land-dumped industrial waste) is subject to limited mobilization under these conditions.

- 2) The clay content of the soil contributes significantly to the extractable Cr. Generally, 40-50% of the extractable Cr is contained in the clay fraction even though this fraction contains only 10-20% of the total amount of Cr in the soil (Mitchell 1964).
- 3) Cr absorbed by plants tends to remain primarily (>90%) in the roots and is poorly translocated to the leaves (Huffman and Allaway 1973). Hence, correlations between Cr levels in plant tops and Cr levels in the soil are poor because the wrong tissues are being monitored.
- 4) Sandy soils with little organic content have very low ion exchange capacity and hence the availability of Cr(III) from such soils tends to be very high.

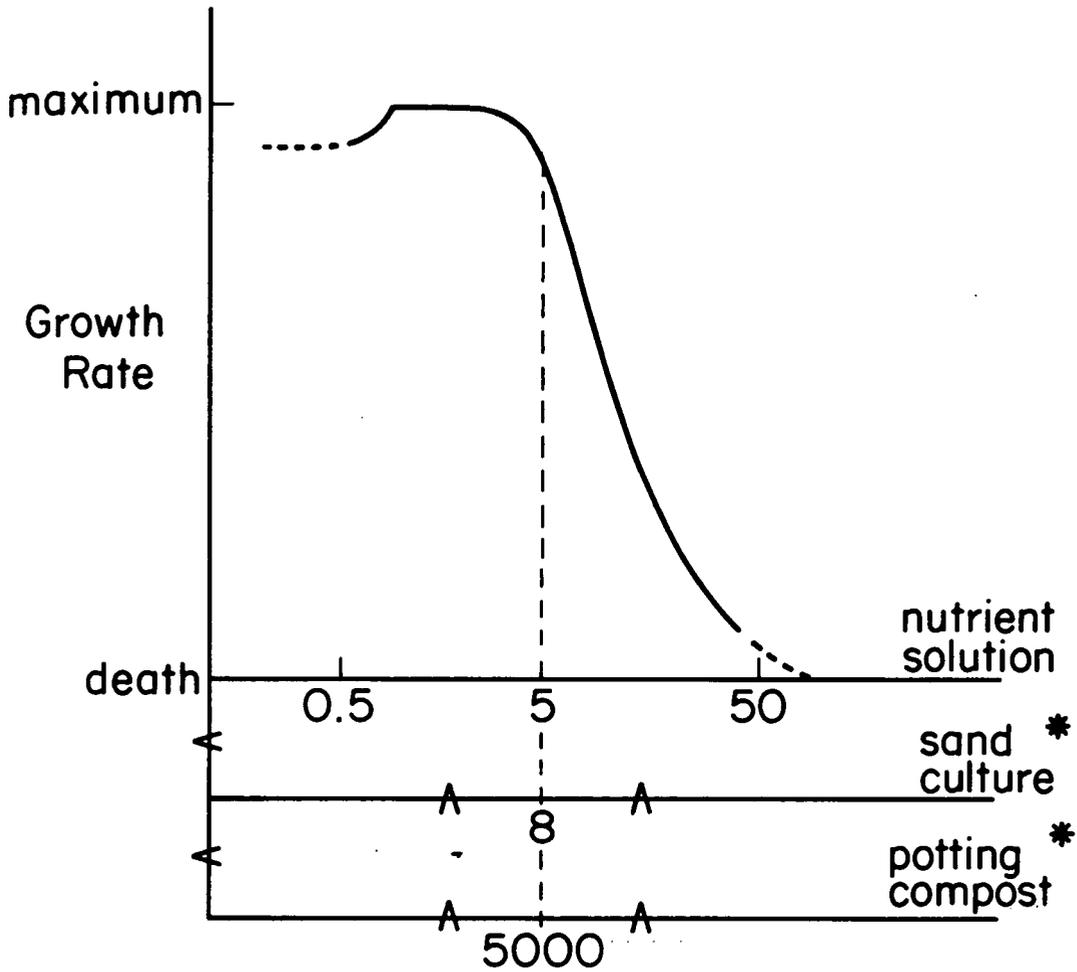
EFFECTS OF Cr ON PLANTS

Pratt (1966) reviewed the literature on the interaction of Cr with plants. Information from this review, along with more recent data, is given in Table 9-1. The data indicate that 1-5 ppm present in available form in the soil solution either as Cr(III) or Cr(VI) is the toxic threshold for a number of plant species.

When Cr was added to various soils the toxic limit for Cr(VI) varied from 5 ppm ($\mu\text{g/g}$ air-dried soil) for tobacco grown in sandy soil (Soane and Saunder 1959) to 500 ppm for *L. perenne* grown in a potting compost (Breeze 1973). Toxic limits for Cr(III) varied from 8 ppm for sugar beets grown in sand (Hewitt 1953) to 5000 ppm for *L. perenne* grown in potting compost (Breeze 1973). The sand is relatively inert and does not change the Cr(III) or Cr(VI) toxic thresholds much from the values observed in solution culture. The potting compost contains organic material which binds Cr(III) to reduce its availability, and which also reduces some of the Cr(VI) to Cr(III) which is subsequently bound and detoxified. Figure 9-1 gives a schematic view of the effect that addition of Cr(III)-binding material has on the concentration axis of a cause/effect diagram for Cr and plants.

Table 9-1. Effects of Cr on plants.

Plant species	Cr form	Cr concentration (ppm)	Growing medium	Observed effects	Reference
Corn	Cr(III)	0.5	solution culture	stimulation of growth	von Scharrer and Schropp (1935)
Corn	Cr(III)	5	solution culture	moderate toxicity	von Scharrer and Schropp (1935)
Corn	Cr(III)	50	solution culture	severely stunted growth	von Scharrer and Schropp (1935)
<i>L. perenne</i>	Cr(III)	10	solution culture	increased plant mortality	Breeze (1973)
<i>L. perenne</i>	Cr(VI)	10	solution culture	increased plant mortality	Breeze (1973)
Barley	Cr(VI)	50	soil	severely stunted growth	Voelcker (1921)
Barley	Cr(VI)	500	soil	death	Voelcker (1921)
Oats	Cr(VI)	5	sand	iron chlorosis	Hunter and Vergnano (1953)
Sugar beet	Cr(VI)	8	sand	iron chlorosis	Hewitt (1953)
Tobacco	Cr(VI)	5	sand	retarded stem development	Soane and Saunder (1959)
Corn	Cr(VI)	10	sand	stunted growth	Soane and Saunder (1959)
Sweet orange seedling	Cr(VI)	75	soil	no toxicity	Vanselow (1951)
Sweet orange seedling	Cr(VI)	150	soil	observed toxicity	Vanselow (1951)
Soybean	Cr(VI)	5	loam soil	inhibition of uptake of Ca, K, Mg, P, B, Cu	Turner and Rust (1971)



Cr III concentration (ppm) in various media

Fig. 9-1. Schematic growth-response curve for plants exposed to Cr(III) in different growth media.

*Note: Due to lack of data, scales may differ from that for the nutrient solution.

The main toxic action of Cr is considered to occur in the roots where the Cr concentration is the highest (Pratt 1966). Turner and Rust (1971) found that Cr(VI) interfered with the uptake and translocation of essential elements in plants:

- 1) More than 0.1 ppm Cr(VI) in nutrient solution decreased concentrations and total contents of calcium, potassium, phosphorus, iron, and manganese in the tips, and of magnesium, phosphorus, iron, and manganese in the roots.
- 2) Cr interfered with the accumulation of calcium, potassium, magnesium, phosphorus, boron, and copper by plant tops.

Hewitt (1953) showed that Cr(III) aggravated iron deficiency chlorosis, i.e. interfered with iron metabolism. Painting the plant leaves with an FeSO_4 solution permitted recovery from chlorosis in 5 days. The visual symptoms of Cr toxicity are consistent with inhibited root function: stunted growth, curled and discolored leaves, and poorly developed root systems (Pratt 1966).

Hunter and Vergnano (1953) observed that subtoxic levels of Cr (2 ppm Cr(VI) in sand culture) increased the degree of specific nickel symptoms in oats as well as the degree of nickel uptake by the plants; however, the Cr levels in the plant tops were not raised above those observed in healthy plants growing on soil of "normal" Cr content. Hence, for low levels of Cr in the growth medium (0-15 ppm), little Cr was transported to the tops even though moderate toxicity was observed. At higher levels of Cr in the growth medium (25 ppm), the concentration of Cr in the plant tops was increased by almost two orders of magnitude, possibly indicating that some homeostatic mechanism had been overcome.

In most of the environment there is no problem with Cr toxicity in land plants. In fact, it is quite possible for plants to thrive on soils so low in Cr that a negative Cr balance results in animals which consume those plants. As Allaway (1968) states: "Cr is one of the few essential elements for which no accumulation against a concentration gradient is evident at any point in the biological cycle from soil to plant to animal... A controlled increase in the amount of Cr moving from soils to plants to man would be desirable and might result in a decreased incidence of diabetes... None of the food or feed crops at the present time has been identified as an effective Cr accumulator". Hence, it would appear that while there is a tendency for insufficient Cr to be passed

from plants to animals in the food chain, this cannot be rectified by adding Cr to the soil because toxic levels of Cr will be reached before significantly larger quantities of Cr are translocated from the roots to the edible portions.

Aquatic plants are strongly affected by Cr(VI). Significant growth inhibition of diatoms and algae starts in the concentration range of 0.03 to 6.4 ppm (Hervey 1949; Bui *et al.* 1971). Diatoms exhibited the lowest toxicity threshold (0.03-0.3 ppm) while euglenoids (0.32-1.6 ppm) and chlorococcales (3.2-6.4 ppm) were less sensitive. Giant kelp exhibited a 20-30% reduction in photosynthesis after 7-9 days exposure to 1 ppm Cr(VI); 5 ppm produced 50% inactivation of photosynthesis in 4 days (Clendenning and North 1960).

FIELD STUDIES OF Cr TOXICITY

There are no recorded examples of direct deleterious effects on plants which can be attributed primarily to Cr toxicity (Pratt 1966; Anderson *et al.* 1973). Ultrabasic soils contain high concentrations of Cr and nickel. In most cases, the nickel is responsible for the bulk of the toxic effects although Cr has been shown to be a potentiator of nickel toxicity (Hunter and Vergnano 1953). Studies on Rhodesian chromite-bearing soils and plants (Wild 1974) indicated that certain accumulator plants contained up to 48,000 ppm Cr and 5200 ppm nickel in the ash of their leaves. However, these accumulator plants do not form an important part of the food chain. Ultrabasic soils are found in highly localized areas because they are formed from narrow dikes of intrusive ultrabasic rock. Hemphill (1972) stated that applications of limestone increased the soil pH sufficiently that Cr(III) availability was lowered to subtoxic levels.

ADDITION OF Cr TO SOIL: FERTILIZERS, SEWAGE SLUDGES, INDUSTRIAL WASTES

FERTILIZERS

Swaine (1962) reported the Cr content of various fertilizer materials; Table 9-2 lists some of these which contained high levels of Cr, most probably in the form of Cr(III). Included in the list are some industrial by-products and wastes which have some value as phosphate fertilizers. In some of these materials Cr is present in the relatively unavailable form of Cr(III) mixed oxides; however, this is not considered to be the case with the phosphate materials. While soils of high organic content and relatively high pH (most soils are neutral to slightly acidic) can contain up to

Table 9-2. Fertilizers high in Cr content^a.

Fertilizer	Concentration (ppm)
Phosphoric acid	600
Waste phosphoric acid from metal electroplating	7000
Phosphate rock	1000-100,000
Serpentine superphosphate	100-1000
Basic furnace slags	1000-5000
Aluminum industry waste	500-1000

^a Data from Swaine (1962).

Table 9-3. Cr content of typical municipal sewage sludges^b.

Sludge type	Cr (ppm dry weight)
Milorganite	1400-3900
Raw sewage sludge	31-10,000

^b Data from Swaine (1962) and Nadkarni and Morrison (1974)

5000 ppm Cr(III) and still support healthy plant life because of the low Cr availability (Breeze 1973), significant Cr toxicity can be expected if there is a change in conditions. Since most food crops are harvested by collecting the plant tops, the bulk of the Cr (>90% of the Cr in the plant) remains in the soil with the roots, i.e. successive cropping is an ineffective way to lower Cr levels in soil. Furthermore, repeated cropping may eventually increase the availability of Cr by increasing the fraction of the Cr present as low molecular weight organic complexes in decaying root tissues.

SEWAGE SLUDGES (WASTES HAVING SOME VALUE AS FERTILIZER)

Sewage sludges have been used as fertilizers on a commercial basis for over 30 years (Swaine 1962). Table 9-3 lists the Cr content of various waste sludges. These wastes are desirable as fertilizers because of their nitrogen and phosphorus content (Webber 1972b); however, the presence of heavy metals in these sludges can more than counteract the advantages of this convenient means of waste disposal (Purves 1972; Horvath 1972; Kick *et al.* 1971). Le Riche (1968) showed that the treatment of garden soil with municipal sewage sludge raised the acid-extractable soil Cr from 0.2-0.7 ppm to 2.0-3.5 ppm.

Fujihara *et al.* (1973) report that as little as 10-100 ppm Cr(III) in loam soil significantly affected the populations of soil microorganisms and decreased the evolution of carbon dioxide (i.e. interfered with microbe respiration). Morrissey *et al.* (1974) showed that 1000 ppm Cr(III) introduced into the soil by sludge significantly reduced ammonium ion utilization and nitrate production in the soil. After a period of 6 weeks, the inhibitory effects of Cr were no longer evident, i.e. the soil microorganisms had either adapted to the higher Cr level, or the availability of Cr had decreased; however, 10,000 ppm Cr completely blocked the above nitrogen transformations. On the other hand, Webber (1972a) reported the absence of toxicity to plants from sludges containing 4400-8800 ppm Cr(III) when applied either as a single heavy dressing of 125 metric tons per hectare or as annual dressings of 31 metric tons per hectare. Similarly, Andrezewski (1970) used ground Cr-tanned leather (containing 3-5% Cr₂O₃) as a nitrogen supplement for soils without toxic effects. Because characteristics of soils vary widely, it is advisable that each case should be carefully investigated before a decision is taken on the possible application of a particular sludge.

LANDFILL OF INDUSTRIAL WASTES (HAVING NO TRUE FERTILIZER VALUE)

Landfill appears to be a feasible means for disposing of waste containing large amounts of Cr(III). The low mobility and availability of Cr(III), especially in oxide form, e.g. slags and incinerated sludges, make it relatively harmless to plants and underground water supplies. The situation is different with Cr(VI); if Cr(VI) is not reduced to Cr(III) before land dumping, uncontrollable situations may arise. Perlmutter *et al.* (1963) and Pinder (1973) described the extent and movement of Cr(VI)-contaminated groundwater resulting from recharge basin seepage at a Long Island plating plant. The slug of Cr(VI)-contaminated groundwater, first discovered in 1942, contained up to 14 ppm Cr(VI), extended 1400 metres from the recharge basin and reached depths of 20 metres or more below the land surface. Pinder (1973) estimated that a nearby stream would continue to be contaminated with Cr(VI) from groundwater seepage for a period of 7 years after further Cr(VI) discharge into the ground was halted.

Breeze (1973) and Gemmel (1972, 1973) described land and river pollution caused by the dumping on land of waste material from the processing of chromite ore into chromate. The highly alkaline waste which contained large amounts of Cr(VI), mostly CaCrO_4 , was extremely toxic to plants; 200 ppm of this mixed waste in sand inhibited plant growth by 50%. Runoff from the waste heaps carried 3-5 tons of Cr(VI) per year into a nearby river, strongly affecting the downstream algal and macrophyte flora. Even 1.5 km downstream the flora showed little sign of recovery and the concentration of Cr(VI) in the river water (0.2-0.5 ppm) had undergone negligible dilution. Dilution of the land-dumped solid waste with soil or application of a thick topsoil covering were ineffective in alleviating the pollution problem. Gemmel (1973) concluded that the key to reducing the pollution problem was complete chemical reduction of the Cr(VI).

Wilms *et al.* (1973) proposed to treat metal pickling waste liquor by adsorbing the Cr(VI) onto ferric hydroxide flocks and reducing the remaining Cr(VI) to Cr(III) which would be precipitated as Cr(OH)_3 . While this permits liquid effluents to pass standards and produces a minimum of sludge per unit of Cr(VI) originally present, a problem remains with the Cr(VI)-containing solid wastes which will eventually be dumped on land.

ABSORPTION OF Cr BY FOLIAGE

While the predominant route of Cr absorption is *via* the roots, studies by Levi *et al.* (1973) showed that Cr(III) and Cr(VI) applied to leaves remained at the treated areas without any significant translocations. A solution containing as little as 50 ppm Cr(VI) produced visible leaf necrosis 24-48 hours after application. In most localities, it is unlikely that the absorption of Cr from aerosols by leaves will add significantly to Cr levels in plants. Nevertheless, Cr in dustfall and acid rainfall in areas where industries are involved with Cr chemicals could damage foliage or significantly raise Cr levels in plants.

CHAPTER 10 Cr AND ANIMALS

INTRODUCTION

Cr is an essential element in mammals. Schroeder (1968) and Mertz (1969) indicated that adequate Cr nutrition improved growth and longevity and, along with insulin, helped to maintain correct glucose, lipid, and protein metabolism. Two reports presented evidence that Cr supplementation of animal diets reduced the incidence of atherosclerosis (Schroeder *et al.* 1970; Novakova *et al.* 1974). Schroeder *et al.* (1962) showed that wild and domestic animals had higher tissue levels of Cr (0.15-0.20 ppm) than man (0.02-0.03 ppm); but, in view of the very small amounts of Cr required to fill metabolic needs, the significance of these differing levels is uncertain.

To date, toxic effects of Cr in animals have almost entirely been observed in controlled experiments in order to evaluate the effects of such variables as concentration, chemical form, duration, and route of administration. Extrapolation of such data to man may be unjustified unless adjustments are made for differences in physical characteristics (size, shape, etc.), biochemical metabolism and metabolic rate, and differences in living habits (see Chapter 6).

There are three main routes by which Cr can enter the body of an animal: (1) the respiratory system, (2) the gastrointestinal tract, and (3) the skin.

RESPIRATORY SYSTEM

Breathing of aerosols leads to deposition of a portion of the aerosol particles in the respiratory tract. Akatsuka and Fairhall (1934) estimated that 17-20% of the 200 mg of chromic carbonate dust inhaled by domestic cats over a period of 4 months was retained in the respiratory tract. Natusch and Wallace (1974) calculated the probable relative deposition of aerosol particles in various regions of the respiratory tract of man (and like species) as a function of particle size. Their results, shown in Fig. 10-1, indicated that particles with a diameter of 2 micrometres (μm) or greater were deposited in the upper respiratory tract (nose and pharynx), while smaller particles penetrated more deeply into the system and hence had a greater probability of deposition in the trachea, bronchial tubes, and alveoli. Ciliary action tended to remove particles from all pulmonary regions except the alveoli. Because of this difference in deposition efficiencies, bulk analysis of particulate matter filtered from air without determining the particle size distribution is insufficient to describe the availability of Cr, e.g. 10 ng of ZnCrO_4 , equivalent to 10^6 particles 0.1 μm in diameter, would have a high

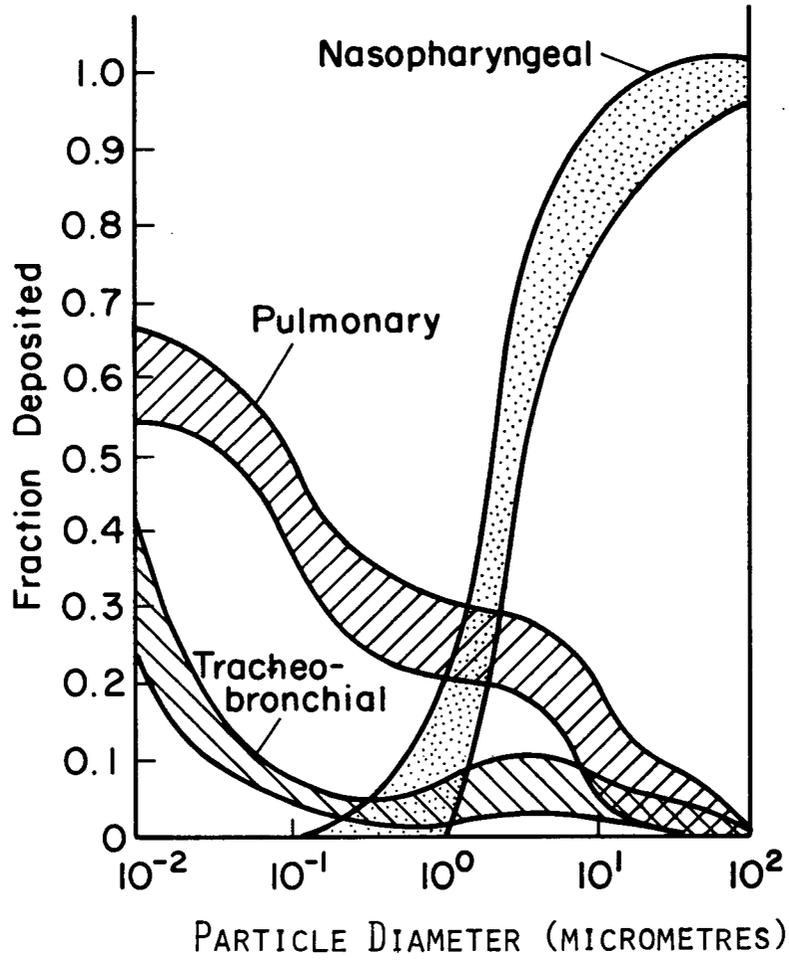


Fig. 10-1. Respiratory deposition efficiencies for inhaled particles (Natusch and Wallace 1974).

probability of penetrating deeply into the respiratory tract. The same 10 ng $ZnCrO_4$, present as a single particle 10 μm in diameter, would probably not even reach the lower respiratory tract.

Once deposited in the respiratory tract, Cr(III) and Cr(VI) behave differently. Baetjer *et al.* (1959) injected Na_2CrO_4 and $CrCl_3$ in powder form intratracheally into guinea pigs and found that after 30 days, 30% of the Cr(III), but only 2.4% of the Cr(VI) were still present in the lung tissue. More Cr appeared in the red blood cells, liver, kidney, and spleen following Cr(VI) rather than Cr(III) injection into the trachea. Nevertheless, Baetjer *et al.* (1959) showed that an individual who had worked in a chromate-producing plant still had high concentrations of soluble Cr in his lung tissues 23 years after exposure had ceased. This was partially explained by the fact that 11-19% of the Cr(VI) injected intratracheally into guinea pigs was reduced *in situ* to Cr(III). The scarring effect accompanying the reduction of Cr(VI) to Cr(III) was described by Nettesheim and Szakal (1972). The poorer clearance of Cr from scar tissue as compared with normal tissue may be related to the long retention of high levels of Cr. Nettesheim and Szakal (1972) postulated that the scarring of alveolar tissue may be a preliminary step in carcinogenesis.

Table 10-1 contains an overview of cause/effect data related to the inhalation of Cr compounds. The mechanisms and time scales for manifestation of these effects are but poorly understood. Generally, acute inhalation of chromates elicits an inflammatory reaction leading to bronchopneumonia, alveolar epithelial changes and atrophy, as well as benign tumor formation. The combined effects of these pathological changes lead to rapid death in cases of high exposure (see Table 10-1). Tissues other than the lungs may be affected, e.g. hyperplasia of lymph nodes and atrophic changes in spleen, liver and intestinal mucosa. Existing studies indicate that simple inhalation of chromate dusts has not generated a significantly higher incidence of malignant tumors in test animals even when cofactors such as influenza virus or artificial smog were also introduced. Nevertheless, epidemiological studies of various occupations do implicate Cr(VI) as contributing to the increased hazard of lung cancer.

Chromium metal has been shown to affect alveolar macrophage activity. The macrophages are present in lungs to ingest lung debris and then remove it by migrating to the bronchial tubes where they are swept out of the respiratory tract by ciliary action. Cr metal coating on teflon particles 5 μm in diameter was shown to stimulate the phagocytic activity of

Table 10-1. Effects of respiratory exposure to Cr compounds.

Material	Species	Cr dosage mg/m ³	Duration	Effects and comments	Reference
Dichromate	cat	4-8.5	2-3 h for 5 days	bronchial pneumonia and death	Lehmann 1914
Dichromate	rabbit	4-8.5	2-3 h for 5 days	no visible lung damage	Lehmann 1914
Chromate chemicals	rabbit, cat	1-50	14 h for 1-8 months	pathological changes in lungs	Lukanin 1930
Chromic acid mist	guinea pig	190	0.5-3 h/day, 45 days	pathological changes in lung mucosa, submucosa and in spleen and kidney	Galloro 1938
Mixed dust containing chromates	mice	0.75	4 h/day, 5 days/week, 1 year	no obvious harmful effects	Baetjer 1956
	mice	8-14	1/2 h/day, intermittently	fatal to some strains	Baetjer 1956
	mice	3.6	37 h over 10 days	fatal	Baetjer 1956
	rats	3.6	37 h over 10 days	barely tolerated	Baetjer 1956
Chromate roast	rabbits	3-4	44-53 h	lung inflammation, benign tumor production	Steffe and Baetjer 1965
	guinea pigs	"	"	"	"
	rats	"	"	"	"
Cr ₂ O ₃	rats	25	5-1/2 h/day, 5 days/week 10-160 weeks	no increased lung tumor incidence	Nettesheim et al. 1970
CaCrO ₄	mice	30	-	rapid weight loss, fatty atrophy of liver and intestines death	Nettesheim et al. 1970
CaCrO ₄	mice	13	6 months	growth stunted, increased incidence of pulmonary adenomas, epithelial necrosis of bronchial mucosa, massive increase in pulmonary subepithelial connective tissue; lymph node hyperplasia	"

macrophages *in vitro* (Camner *et al.* 1974). It was postulated that molecules such as serum proteins were modified by adsorption on the Cr metal surface and this stimulated macrophage activity. Casarett *et al.* (1971) studied *in vitro* the phagocytic response of pulmonary cells to the presence of Cr₂O₃ particles. They regarded that "early changes in alveolar cells may be considered as initial events related to the etiology of ultimate pulmonary effects (pathology)". Neither Cr metal nor Cr₂O₃ produced obvious cytotoxic effects immediately after accumulation in alveolar phagocytes (Camner *et al.* 1974; Casarett *et al.* 1971). Waters *et al.* (1975) found that the viability of rabbit alveolar macrophages *in vitro* was reduced at Cr(III) levels above 52 ppm, e.g. 40% reduction in viability at 350 ppm Cr(III). These Cr levels can be readily achieved at the surface of a dissolving particle of a Cr salt. Decreased macrophage viability is also accompanied by increased lysis of the cells. Study is required to determine whether Cr chemicals can cause *in vivo* phagocytolysis (premature rupture of phagocytic cells) or inhibit their mobility which would inhibit the clearance of Cr from the lungs and place a stress on the pulmonary immune response system. Polak and Frey (1973) showed that the mobility of macrophages isolated from the peritoneal exudate of guinea pigs made hypersensitive to Cr was inhibited by Cr salts regardless of their valency. Hence, significant inhibition of the clearance of Cr particulates from the lungs may be expected in Cr-sensitized individuals. High levels of chromite ore dusts are known to irritate and damage the lungs, probably *via* a medium involving the interaction of particle surfaces with macrophages: chromite ore miners develop a benign pneumoconiosis (Sluis-Cremer and Du Toit 1968); bronchitis and emphysema may occur in chromite workers (Worth and Schiller 1954). Some fractions of the chromite dust may cause Cr sensitization in the lungs:

- 1) Hueper (1952) reported that rats exhibited elevated Cr levels in blood after 18 months of inhaling finely powdered chromite dust, i.e. significant quantities of Cr may be mobilized from the chromite over a period of time.
- 2) Grogan (1957) obtained evidence that chromite ore undergoes oxidative dissolution in aerated physiological media to form Cr(VI). This Cr(VI) may then cause Cr hypersensitivity and inhibit macrophage mobility for clearance of Cr from the lungs.

Further information and discussion of Cr inhalation studies using test animals are contained in NAS (1974), on pages 74 to 79. Long-term (> 5 years) low-level chronic inhalation studies on Cr or any of its compounds have not yet been reported. The only long-term data are those concerned with the epidemiology of Cr injury in workers handling and processing various Cr materials (see Chapter 11).

DIGESTIVE TRACT

In general, inorganic Cr(III) and Cr(VI) are poorly absorbed by the gastrointestinal tract (Mertz 1969). Approximately 0.5% of an oral dose of CrCl_3 was absorbed by man and 2-3% was absorbed by rats. Chromate was absorbed to a slightly higher extent: 2.1% of an oral dose by man and 3-6% by rats. Since these values were obtained by analysis of urine samples assuming that negligible excretion of Cr occurred in the gut, true absorption values may be slightly higher. Nevertheless, Mackenzie *et al.* (1958) showed that rats fed CrCl_3 or K_2CrO_4 absorbed 9 times more Cr(VI) than Cr(III). Huffman and Allaway (1973) grew bean plants in $^{51}\text{Cr(VI)}$ solution and then fed the leaves to rats. Less than 0.5% of the ^{51}Cr fed to the rats in the leaves was retained after 48 hours. Mertz (1969) examined the daily total intake of Cr by man (80 μg) and the daily total of Cr excreted in the urine (8.4 μg) and concluded that the overall percent absorption for Cr must have been higher than 0.5%. Complexation of the Cr, especially the Cr(III), can change its percent absorption. Chen *et al.* (1973) showed that complexation with oxalate caused a significant increase in Cr(III) absorption while phytate significantly decreased Cr(III) absorption. Citrate and EDTA had no effect. It is interesting to note that Lyon *et al.* (1969b) has proved the existence of a Cr(oxalate)_3 complex in one plant species. The most readily absorbed Cr(III) complex and the one which equilibrates the most quickly with body stores of Cr is the glucose tolerance factor, GTF (estimated percent absorption varies from 10-20%). Other details of absorption, tissue distribution, and excretion of Cr have recently been reviewed (NAS 1974).

Table 10-2 presents representative cause/effect data involving the ingestion of Cr by animals. Cr(III) is noncorrosive and tends either to adsorb on food fibres or to precipitate in an insoluble form in the digestive tract. Hence, little toxicity to mammals is expected from ingestion of Cr(III) even in comparatively large amounts. Cr(VI), in contrast, being a strong oxidizing agent, is quite corrosive. The high concentration of HCl present in the stomach, as well as organic material from food, are probably responsible for converting much of the ingested Cr(VI) to Cr(III). This would account for the absence of accumulation of Cr except when fed in drinking water at concentrations exceeding 5-6 ppm Cr(VI) (see Table 10-2).

Gross and Heller (1946) reported that young rats were more susceptible than mature animals to Cr(VI) toxicity. High levels of Cr(VI) (above 0.125% in feed) were needed to impair reproductive function and cause sterility. Stunted growth and roughness of fur were also noted. Kucher and Shabanov (1967) found that in rabbits poisoned by addition of $\text{K}_2\text{Cr}_2\text{O}_7$ to feed, hyaluronates, chondroitin sulfates and neutral mucopolysaccha-

rides accumulated in soft tissues causing pericapillary sclerosis. Blood-tissue barriers, permeable under normal conditions, were blocked by this accumulation, preventing normal transport of metabolites. One manifestation of this condition was the inhibition of insulin production in the pancreatic islets because of damage to the β -cells contained therein.

The longest exposure of experimental animals to Cr was that in which dogs were given up to 11.2 ppm Cr(VI) in their drinking water for a period of 4 years (Anwar *et al.* 1961). While there were significant increases in tissue levels of Cr, there was no histological evidence of toxicity. However, reproductive function was not assessed.

SKIN CONTACT

Chromium compounds, even in small amounts, have produced contact dermatitis in some sensitized animals. The severity of the reaction has been found to depend on two factors: (1) the relative diffusibility of the compounds across the dermis (Samitz *et al.* 1967), and (2) the degree of dissociation or the solubility of the compound under physiological conditions (Gross *et al.* 1968). Sensitization is achieved by repeated exposure to relatively high levels (1000-3000 ppm) of Cr(III) or Cr(VI) salts. Abrasion of the skin before or during exposure increases the severity of the reaction. Once sensitized by either Cr(III) or Cr(VI), the subject reacts to both forms of Cr but Cr(III) generally elicits the greater response. As little as 10 μ g or less Cr(VI) (as $K_2Cr_2O_7$) or Cr(III) (as $Cr_2(SO_4)_3$) elicited allergic responses in sensitized guinea pigs (Jansen and Berrens 1968). Several studies have indicated that complexes of Cr(III) with proteins (Katz *et al.* 1974) and with amino acids (Shmunis *et al.* 1973) and not the Cr moiety itself cause the allergic reaction. While in extreme cases of allergic response some tissue necrosis was observed (Shmunis *et al.* 1973), these did not result in malignant growths. Tissues slowly returned to normal after the Cr was removed. Nevertheless, in some cases enough Cr was left after the original response had subsided to produce "flare-up" reactions at test sites even 3 to 4 years after exposure ceased (Fregert and Rorsman 1964). This is especially likely with Cr(III) which is cleared from the skin more slowly than Cr(VI) (Pedersen and Naversten 1973).

Chromic acid and its anhydride are highly corrosive, producing skin ulcers and necrosis by a mechanism independent of any allergic response. Samitz and Epstein (1962) studied the process of Cr(VI) ulceration and found that a 30,000 ppm Cr(VI) solution would consistently cause skin lesions only if the skin was broken or had its natural oil reduced beforehand. Repeated applications of Cr(VI) produced more consistently

severe results than single doses. Cr(III) in concentrations as high as 10^5 ppm had no ulcerogenic effects. Any ulcerogenic activity of Cr(III) solutions was attributed to the acidity of the solution and not to the action of Cr(III) itself.

Lewin (1907) found that dermally applied Cr(VI) could be taken up by rabbits at a sufficient rate to cause systemic poisoning. Analogous examples for man are given in Chapter 11, but skin ulceration is far more common than systemic poisoning.

INJECTION AND INSERTION

The introduction of Cr compounds either by injection of solutions or by insertion of solids into various tissues of a test animal yield equivocal toxicity data because (1) the trauma or systemic shock accompanying the injection or insertion may be the main cause of death, masking any specific effects of the Cr dose, (2) this procedure bypasses many of the body's natural defence mechanisms. Nevertheless, some valuable information can be derived on the action of precisely known amounts of various Cr compounds. Table 10-3 indicated that the minimum fatal dose of Cr(VI) given by direct injection lies in the range 1-5 mg/kg-body weight for most mammals. Because of the ease with which Cr(VI) passes membranes, injection of Cr(VI) is a more realistic dosing procedure than injection of Cr(III), i.e. the body's natural defence mechanisms are not as effective in stopping the influx of Cr(VI) as they are for Cr(III). Lower doses of Cr(VI) (0.2-0.5 mg/kg-body weight) produce marked necrosis of the kidneys. Cr(VI) poisoning of the kidneys leads to a significant decrease in the ascorbic acid content of the kidneys (Simavoryan 1971). The ascorbic acid probably protects the tissues from the oxidative action of Cr(VI), and depletion of tissue levels of ascorbate would increase susceptibility to Cr(VI) toxicity at a later date. Evan and Dail (1974) studied the acute effects of injected doses of Cr(VI) in rats. They found the following sequence of progressive changes leading to nephron damage (dosage level 10-20 mg/kg-body weight):

- 1) swelling and loss of microvilli
- 2) formation of intracellular vacuoles of varied sizes
- 3) mitochondrial swelling
- 4) cytoplasmic liquefaction and loss of cells lining the nephron surface.

Berndt (1975) studied the mechanism of potassium dichromate nephrotoxicity by using both *in vitro* and *in vivo* methods. Quantitatively, the alterations in tissue electrolytes and water were always larger after *in vivo* rather than *in vitro* administration, even when the tissue concentrations of dichromate were approximately the same. It was concluded that dichromate

Table 10-2. Effects of ingestion of Cr compounds.

Material	Species	Cr dosage ^a	Duration	Effects and comments	Reference
K ₂ CrO ₄	dogs, cats, and rabbits	1.9-5.5 mg/kg of body weight	1-23 months	no pathological changes observed	Lehmann 1914
K ₂ CrO ₄	dogs	0.1-0.2 g/day	3 months	fatal	Brard 1935
K ₂ CrO ₄	rats	134 ppm in water	2-3 months	kidney and liver lesions	Gross and Heller 1946
ZnCrO ₄	mature rats	1% in feed	2-3 months	toxic threshold	" "
"	young rats	0.1% in feed	2-3 months	toxic threshold	" "
K ₂ CrO ₄	young rats	0.1% in feed	2-3 months	toxic threshold	" "
K ₂ CrO ₄	rats	0-5 ppm in water	1 year	no tissue accumulation of Cr	Mackenzie <i>et al.</i> 1958
"	rats	10-25 ppm in water	1 year	significant accumulation, no pathological changes	" "
CrCl ₃	rats	25 ppm in water	1 year	significant accumulation, no pathological changes	" "
K ₂ CrO ₄	dogs	0-6 ppm in water	daily	no liver or kidney accumulation	Anwar <i>et al.</i> 1961
"	dogs	11.2 ppm in water	4 years	accumulation but no pathological changes	" "
CrCO ₃	cats	1 g/day	17 weeks	no deleterious effects	Akatsuka and Fairhall 1934
K ₂ CrO ₄	dogs	5 mg/kg body weight, daily	1-12 months	changes in lipid metabolism in aorta; increase in acidic and decrease in neutral mucopolysaccharides	Morozova and Veklenko 1967

^a Assumed weights of test animals: mouse - 0.3 kg; rat - 0.6 kg; guinea pig - 1 kg; rabbit - 2-3 kg; cat - 3-5 kg; dog - 20-30 kg.

Table 10-3. Effects of insertion and injection of Cr compounds.

Material	Species	Cr exposure ^a	Duration	Effects	Reference
K ₂ Cr ₂ O ₇	monkey	6-230 mg ^b	single dose	fatal	Hunter and Roberts 1933
K ₂ Cr ₂ O ₇	dog	210 mg ^b	single dose	rapidly fatal	Brard 1935
K ₂ Cr ₂ O ₇	guinea pig	10 mg ^b	single dose	lethal	Ophiils 1912
K ₂ Cr ₂ O ₇	rabbits	10-45 mg ^b	single dose	80% fatality	Hasegawa 1938
K ₂ Cr ₂ O ₇	rabbits	20 mg ^b	single dose	lethal	Ophiils 1912
K ₂ Cr ₂ O ₇	rabbits	0.8-2 mg ^b	single dose	nephritis	Ohta 1940
Na ₂ CrO ₄	rabbits and guinea pigs	50-150 mg ^c	single dose	rapid death	Priestly 1877
ZnCrO ₄	mice	.022 mg/month ^c	10 months	tolerated	Baetjer 1956
ZnCrO ₄	mice	0.75 mg ^c	single dose	fatal	Baetjer 1956
BaCrO ₄	mice	0.52 mg/dose ^c	9 doses, 6 weeks apart	tolerated	Baetjer 1956
K ₂ CrO ₄	dogs	5000 mg ^c	single dose	fatal	Gmelin 1926
K ₂ CrO ₄	dogs	500 mg ^c	single dose	survived	Gmelin 1926
K ₂ Cr ₂ O ₇	dogs	210 mg ^c	single dose	rapidly fatal	Brard 1935
K ₂ Cr ₂ O ₇	dogs	17 mg/dose ^c	2 doses	marked kidney damage	Hepler and Simmonds 1945a
CrCl ₃	dogs	2 mg ^c	single dose	renal function not impaired	Collins <i>et al.</i> 1961
CrCl ₃	dogs	.17 mg/min ^c	500 minutes, continuous	renal function not impaired	Collins <i>et al.</i> 1961
CrCO ₃	hamster	2.5 mg/kg ^c	single dose	6% malformation rate	Gale 1974
CrCl ₃	mice	0.26 g/kg	single dose	minimum lethal dose	Merck Index 1960

^a i.e. in terms of weights of Cr only; assumed weights of test animals: mouse - 0.3 kg; guinea pig - 1 kg; rabbit - 2-3 kg; dog - 20-30 kg; monkey - 10-15 kg.

^b Subcutaneous

^c Intravenous

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affected the kidney by having an indirect effect on renal transport, e.g. the nephrotoxin affected blood flow in the kidney in such a way as to compromise renal transport mechanisms. Significant changes were observed in kidney function *in vitro* at two Cr(VI) levels: at 0.1 ppm there was a significant stimulation of tetraethylammonium chloride uptake; at 10 ppm accumulation of paraaminohippurate and tetraethylammonium chloride was significantly inhibited and the normal distribution (intracellular/extracellular) of water and electrolytes was disrupted.

Yoshikawa (1970) showed that it was not possible to increase the tolerance of mice to injected Cr(III) by pretreatment with sublethal doses. Instead, a marked sensitization was observed: pretreatment of mice with 12 mg/kg caused a 250% increase in mortality when pretreated and non-pretreated animals were given a challenge dose of 120 mg/kg Cr(III).

By successively larger intravenous injections of $^{51}\text{CrCl}_3$, Jett *et al.* (1968) showed that Cr(III) binds successively to siderophilin, serum albumin, two alpha proteins, and gamma globulin in the blood of the rat. Intracutaneously injected CrCl_3 exhibited a much lower clearance rate than Na_2CrO_4 ; half of the Cr(VI) was cleared in 10-15 minutes with only 15% left after 2 days. After 2 days 60% of the Cr(III) remained and 5-12 days were required for disappearance of 50% of the dose (Pedersen and Naversten 1973). Fregert and Rorsman (1964) reported that Cr-sensitive individuals exhibited "flare-up" reactions at test sites as long as 3-4 years after a subcutaneous injection of CrCl_3 .

Visek *et al.* (1953) studied the distribution of Cr(III) and Cr(VI) in rats after intravenous injection. In certain cases the liver, kidney, and spleen were observed to accumulate significant fractions of the initial dose; however, no attempt was made to study the effects on the accumulating organs. Diab and Sörenmark (1972) studied the distribution in mice of intravenously injected CrCl_3 and concluded that there was a significant transfer of Cr across the placental barrier to the bone and skin of the fetus. The radioautographic method used by Diab and Sörenmark (1972) is more sensitive than the analytical methods used by Visek *et al.* (1953) thus explaining the negative results reported by the latter. Anghileri (1970) showed that intravenous injection of a Cr(III)-alloxanthin complex resulted in a 20% retention of the dose after 24 hours. Over 50% of this retained amount was associated with the skeletal tissue and it was postulated that the Cr(III) formed a complex with the crystal lattice of the bone. Grogan (1958) found that Cr(VI) injected into 1-year-old hens did penetrate into the nuclei of the red blood cells. Repeated small doses were more effective at increasing Cr levels in the nuclei. Cr(VI) also penetrated the leukocytes and platelets. The bulk

of the injected Cr was excreted via the kidneys (Mertz 1969), although there was evidence that the small intestine is a minor excretory pathway as well (Diab and Sörenmark 1972).

CANCERS PRODUCED EXPERIMENTALLY IN ANIMALS BY Cr COMPOUNDS

The complex series of processes involved in carcinogenesis is poorly understood and this has made it difficult to design definitive experiments. Attempts at producing significant incidence of cancer in test animals often employed acute doses of Cr. The damage done by such high doses may have inhibited the growth or even the production of cancers (Hueper 1958), i.e. low-level chronic exposure for longer periods of time may be a more efficient procedure for the production of cancers. The following is an overview of the attempts at production of cancers in experimental animals.

Vollmann (1940) and Schinz and Vehlinger (1942) reported that intraosseous sarcomas can be elicited by implantation of metallic Cr. Bischoff and Bryson (1964) pointed out in their review that smooth metallic surfaces have the ability to induce tumors. Hueper (1955) failed to obtain conclusive results after injecting powdered Cr metal into various tissues of test animals.

Hueper and Payne (1959) reported that various chromate chemicals (CaCrO_4 , CrO_3 , ZnCrO_4 , SrCrO_4) exhibited significant carcinogenic activity when mixed with sheep fat and implanted in either the pleural cavity or the thigh muscle. The overall carcinogenic activity appeared to parallel the increasing solubility of the Cr(VI) compounds in water.

As stated earlier, simple inhalation of various Cr-bearing dusts has not been linked with a significant incidence of malignant tumors, although benign growths were usually increased. Laskin *et al.* (1970) succeeded in producing malignant lung tumors in the rat by implanting a pellet of stainless steel mesh containing the Cr compound mixed with a cholesterol carrier in the bronchial tube. The Cr compounds which produced carcinomas were: process residue (from chromate production), CaCrO_4 , and CrO_3 ; however, Cr_2O_3 was inactive.

The main conclusions drawn from animal studies by NAS (1974) were:

- 1) Cr(VI) chemicals are carcinogenic under certain conditions;
- 2) Cr(III) chemicals by themselves do not appear to be carcinogenic;
- 3) Current data are insufficient to permit direct dose-response correlations.

CHAPTER 11 EFFECTS OF Cr COMPOUNDS ON HUMAN HEALTH

INTRODUCTION

To date there is no published report on the possible adverse effects due to normal background levels of Cr in air, water, soil, and food. Absorption, metabolism, and excretion of Cr(III) and Cr(VI) have been discussed in detail (Schroeder *et al.* 1962; Schroeder 1968; Mertz 1969; Underwood 1971; NAS 1974).

The main health risk involving Cr is in industry where respiratory and epidermal injuries have been caused by Cr(VI) compounds. Cr(III) is considered to be less harmful, its main effect being a form of contact dermatitis in Cr-sensitive individuals.

EFFECTS OF Cr ON THE RESPIRATORY SYSTEM

RESPIRATORY INJURY

Cr(VI), either as finely powdered chromate or as chromic acid mist, can lead to ulceration of the nasal mucosa and perforation of the nasal septum. Perforation of the nasal septum results when the overlying mucous membrane of the septum is destroyed, cutting off the blood supply to the cartilage and resulting in necrosis of that cartilage. An overview of the dose-response data in the literature for this type of injury is contained in Table 11-1. Cr(VI) levels in air greater than 0.05 mg/m³ indicated a high probability of injury to nasal tissues, while levels as low as 0.01 mg/m³ produced strong irritation of the nose even if the exposure was of short duration (see Table 11-1). The main industries involved were those producing chromate chemicals from chromite ore, and those using Cr(VI) solutions in the electroplating of Cr metal. At the high temperatures used to produce Al₂O₃ (alumina) by sintering bauxite mixed with limestone, Cr(III) may undergo conversion to Cr(VI) if sufficient oxygen is present. Thus, Budanova *et al.* (1974) observed that workers involved with such processes were indeed exposed to Cr(VI) in dust emissions and exhibited the following symptoms: atrophic rhinitis, allergic rhinitis, and bronchial asthma. It is not known whether similar Cr(VI) exposures exist in Canadian production facilities. Bidstrup (NAS 1974) reported that, in addition to injury of the nasal mucosa, irritation and redness of the throat and generalized bronchospasm resulted from the inhalation of Cr(VI) mists or dust. It is difficult to set a lower limit below which such effects are not observed but a reasonable estimate is in the range of 0.05 to 0.10 mg Cr(VI)/m³. Generalized irritation of the lower respiratory tract occurred at these lower levels in sensitized individuals while higher

Table 11-1. Ulceration and perforation of the nasal mucosa by Cr(VI).

Source of Cr(VI)	Dose (mg/m ³ Cr(VI))	Duration	Effects and comments	Reference
Chromium plating (USA)	0-2.8 mg/m ³	0-7 h/day; 25-36 y	>0.05 mg/m ³ Cr(VI) daily, resulted in definite injury to nasal tissues	Bloomfield and Blum 1928
Chromate manufacture (USA)	0.005-0.17	8 h/day, 0.5-3 y	39.3% had perforated nasal septum	Gafafer 1953
"	0.005-0.17	8 h/day, 3-10 y	55.4% had perforated nasal septum	" "
"	0.005-0.17	8 h/day, over 10 y	69.6% had perforated nasal septum	" "
Chromate chemical plant	<0.25 ^a :26-51 ^a >.52 ^a	8 h day 8 h day 8 h day	52% had perforated nasal septum 52% had perforated nasal septum 69% had perforated nasal septum	Mancuso 1951 " " " "
Chromium plating	0.0015	8 h day	4 out of 33 men had perforated nasal septum	Lumio 1953
5% Chromic acid mist	0.045-0.6	8 h day	ulceration and atrophic rhinitis of nasal passage	Gresh 1944; Zvaifler 1944
Chromic acid test aerosol	0.01-0.024 0.0025-0.004	short period short period	strong irritation of nose response elicited from sensitive persons	Kuperman 1964
Handling of chromates	0.06-0.08	work day	some nasal septum perforation	Vigliani and Zurlo 1955
Chromium plating	0.18-1.4	0.5-12 months	7 out of 9 men had some degree of mucosal ulceration; 4 out of the 7 had a perforated nasal septum	Kleinfield and Rosso 1965
Chromium plating	0.4	6.6 y (ave.)	14 out of 77 men had papillomas of the oral cavity and larynx	Hanslian <i>et al.</i> 1967

^a Contained 50-80% Cr(III); value reported is total Cr.

concentrations were required to affect unsensitized subjects (Bidstrup, private communication). When the lower respiratory tissues became sensitized, asthmatic attacks followed and these recurred on subsequent exposure even to very low Cr levels, e.g. Kuperman (1964) noted a response in sensitive individuals to as little as 0.0025 mg/m^3 . Meyers (1950) reported that acute exposure to high levels of chromic acid mist ($20\text{-}30 \text{ mg/m}^3$) resulted in cough, chest pain, dyspnea, pleural effusion and loss of weight. Repeated episodes of chemical irritation may be expected to lead to chronic bronchitis. Prolonged inhalation of chromate dust may result in chronic irritation of the respiratory tract producing the following symptoms: hyperemia, chronic catarrh, congestion of the larynx, polyps of the upper respiratory tract, chronic inflammation of the lungs, emphysema, tracheitis, chronic bronchitis, chronic pharyngitis and bronchopneumonia (NAS 1974).

Cr(III) in combination with other compounds may contribute towards mixed-dust pneumoconiosis. Although this theory has not been firmly substantiated, a number of studies support its hypothesis. Sluis-Cremer and Du Toit (1968) reported that chromite miners developed a benign pneumoconiosis from the inhalation of chromite dust but there was no evidence of pulmonary fibrosis. Worth and Schiller (1954) reported bronchitis and emphysema in workers involved in the production of chromite refractories. Mikov (1967) reported that of 85 chrome-refractory workers exposed to $4.5\text{-}9.2 \text{ mg/m}^3$ of Cr(III) ($3.8\text{-}5.6 \times 10^3$ particles/cm³), 18.8% developed chronic bronchitis after 5 years of exposure and 5.9% developed mild pneumoconiosis after 9 years of exposure. Pierce and Scheel (1965) reported that some workers engaged in the production of ferrochrome exhibited the following symptoms: chronic pulmonary disease, chills, fever, and elevated erythrocytic sedimentation rate. These investigators noted the presence of silica but stated that working conditions were not conducive to the production of acute silicosis. Graham-Jones and Warner (1972) reported that Cr₂O₃ may be a contributing factor to the mixed-dust pneumoconiosis observed for metal dressers in steelworks.

RESPIRATORY CANCER

Epidemiological studies point to an increased risk of lung cancer among workers engaged in the production of chromates from chromite ore. These studies were discussed in two excellent reviews (Enterline 1974; NAS 1974). An overview of the data is presented in Table 11-2. All these studies were hindered by the fact that the long latent period for chromate cancerigenesis made it difficult to retrieve accurate records on the fate of individuals exposed to Cr(VI), e.g. typical estimates of the latent period are 10.6 years (Mancuso 1951), 14.5 years (Machle and Gregorius 1948) and 21 years (Bidstrup

Table 11-2. Comparison of cancer death rates in individuals exposed and not exposed to chromate chemicals.

Cause of death	Ratio ^a	Comments	Reference
Lung cancer	123-308 ^b	chromate workers, Germany 1929-1938	Alwens 1939
Cancer of bronchi and lungs	29.2	US chromate workers (1930-1947) vs	Machle and Gregorious 1948
Cancer of oral region	5.4	US oil refinery workers (1933-1938)	" " "
Cancer of digestive tract	2.0	" "	" " "
All cancers	5.3	" "	" " "
Lung cancer	15.2	Ohio chromate workers (1931-1949) vs	Mancuso 1951
		Ohio males dying between 1937 and 1947	
Lung cancer	3.6	British chromate workers (1951-1956)	Bidstrup and Case 1956
Nonlung cancer	1.3	" "	
Respiratory cancer	29	all ages	Gafafer 1953
"	40	age 15-44	" "
"	30	age 45-55	" "
"	20	age 55-74	" "
Respiratory cancer	9.4	US chromate workers (1937-1960)	Taylor 1966

^a Ratio = $\frac{\text{Death rate of exposed individuals}}{\text{Death rate of unexposed individuals}}$

^b Estimated

and Case 1956). It is interesting to note that Baetjer *et al.* (1959) reported that the lung tissues of a chromate worker still contained 64.8 ppm Cr (dry weight) 23 years after exposure to Cr(VI) had ceased. These levels were 300 times higher than the values reported for normal individuals not exposed to Cr(VI) (Schroeder *et al.* 1962). Case studies of 10 confirmed cases of pulmonary cancer (Gafafer 1953) indicated that these workers were exposed to estimated Cr levels ranging from 0.03 to 1.1 mg/m³ for periods of 4 to 24 years.

While the main process implicated in chromium pulmonary carcinogenesis has been the production of chromate from chromite ore, periodic reports of lung cancer in the chromium plating industry (Favre *et al.* 1972; Royle 1975) and in the chrome pigment industry (Baetjer 1956) indicate that chromium carcinogenesis may not be limited to the chromate manufacturing industry.

In a recent study of 24 workers producing chromate pigments, Langard and Norseth (1975) found three cases of bronchial carcinoma. They concluded that such workers had 38 times higher risk of respiratory cancer than the general population. The workers who developed carcinomas had an estimated exposure of 0.5 to 1.5 mg Cr/m³ for 6 to 9 years. In two of the three cancer cases tobacco smoking may have been a contributing factor. Nevertheless, Maltoni (1973) showed that chromate pigments were carcinogenic by subcutaneous injection into rats.

TOXICITY OF CHROMIC ACID IN THE CHROMIUM PLATING INDUSTRY

Royle (1975) studied in great detail the epidemiology of the effects of chromic acid (Cr(VI)) in the chromium plating industry. His survey of 54 plating plants in England showed that in all but the two largest plants the chromic acid levels in air were less than 0.03 mg CrO₃/m³; air in the two largest plants often contained over 0.1 mg CrO₃/m³. Automated plating plants had the lowest work area exposure levels to chromic acid, 0.0-0.009 mg/m³. Dust samples from automated plating plants had 0.0-3.9 mg of CrO₃ per gram of dust, while most of the other plants had 0.3-97.0 mg of CrO₃ per gram of dust.

A retrospective mortality-morbidity study among workers from the above plants was carried out for the period February 1969 to May 1972. In all, 1238 chromium platers and 1248 controls, matched for age, sex, and smoking habits, were available for study. The main results are shown in Table 11-3. This is the first study which suggests that exposure to chromic acid may cause cancer at sites other than the respiratory tract. Other retrospective studies are needed to confirm the observed

Table 11-3. Retrospective mortality-morbidity study of workers involved in chromium plating (Royle 1975).

Disease or condition	Incidence among chromium platers (%)	Incidence among controls (%)	Statistical significance (%)
Lung cancer	1.37	0.78	not significant
Gastrointestinal cancer	0.73	0.31	not significant ^a
Other cancers	1.05	0.54	not significant ^a
Total cancers	3.15	1.63	5.0%
Blood in phlegm	7.9	5.1	5.0%
Perennial nasal catarrh	20.6	15.7	0.5%
Cough with phlegm from lower respiratory tract	16.6	12.2	1.0%
Bronchial asthma	13.1	9.8	2.5%
Nasal ulcers	12.8		0.1% ^b
Nasal perforation	4.7		0.1% ^b

^a The number of cases in each category was too small to allow a reliable statistical comparison.

^b The value here describes the significance of the trend toward greater incidence of injury with increasing exposure time.

disease patterns. It should be noted that a larger number of controls (93, or 8.3%) than platers (36, or 3.6%) had been engaged in asbestos processing. This may account for the lack of significant difference in incidence of respiratory cancer.

ACUTE SYSTEMIC EFFECTS

Cr(VI) absorbed through the skin or the digestive tract in large amounts can be lethal. Cr(VI) is a nephrotoxin, but at high levels it apparently has an effect on the central nervous system as well. Brieger (1920) reported the death of 12 persons who used anti-scabietic ointment in which sulfur had accidentally been replaced by Cr(VI). The effects included necrosis of the skin at sites of application, nausea, vomiting, shock, and coma. Albumen and blood were present in the urine. The main finding at post-mortem was tubular necrosis of the kidneys. Bidstrup (unpublished data) reported the death of an individual from tubular necrosis of the kidney after being splashed with large amounts of chromic acid. Major (1922) describes a fatal nephritis following the treatment of a carcinoma of the face with crystals of CrO_3 . Anuria developed about 48 hours later and the patient subsequently died from tubular necrosis.

Sander and Camp (1939) described the case of an infant who had consumed some paint containing a pigment made from a relatively insoluble Cr compound. The symptoms, namely convulsions, stupor and dilated pupils, suggested an encephalitic condition. The urine and feces contained Cr but there was no evidence of nephritis. Goldman and Karotkin (1935) described the main symptom of acute $\text{K}_2\text{Cr}_2\text{O}_7$ ingestion as an enlarged tender liver.

Kaufman *et al.* (1970) reported a detailed clinical study of a 14-year-old boy who had consumed approximately 1.5 g $\text{K}_2\text{Cr}_2\text{O}_7$ (10 mg Cr(VI)/kg). The following clinical parameters were found to deviate significantly from normal values:

white blood cell count	(increased)
plasma fibrinogen concentration	(decreased)
blood calcium concentration	(decreased)
blood phosphorus concentration	(increased)
blood urea nitrogen concentration	(increased)
blood creatinine concentration	(increased)
blood CO_2 concentration	(decreased)
blood bilirubin concentration	(increased)

Despite blood transfusions, peritoneal dialysis and treatment with chelating agents, the patient became comatose 5 days after

ingesting the Cr(VI) and died on the eleventh day. Comparison of the ingested 10 mg/kg Cr(VI) dose with oral doses tolerated by animals (see Table 10-2) indicates that man is by far more sensitive to Cr(VI) poisoning than test animals.

EFFECTS ON DIGESTIVE TRACT

Cr(III) is expected to have little effect on man's digestive system even at relatively high levels (see Chapter 10), although its presence at high levels in the form of glucose tolerance factor may cause significant tissue accumulation of Cr (Mertz *et al.* 1974).

Oral ingestion of Cr(VI) compounds may lead to intense irritation of the gastrointestinal tract resulting in violent epigastric pain, nausea, vomiting, severe diarrhea, and hemorrhage (Kaufman *et al.* 1970; Partington 1950; Goldman and Karotkin 1935). With large doses, circulatory collapse, unconsciousness, and death may follow rapidly.

Stocks and Davies (1960) determined the levels of trace elements in soil samples taken from gardens of houses where people had died of cancers in North Wales, Cheshire and Devonshire, England. They found high levels of Cr to be associated with excess stomach and intestinal cancer. Berg and Burbank (1972), on the other hand, could find no correlation between the levels of Cr in US water supplies and cancer mortality. However, it is generally accepted (Schroeder *et al.* 1962) that the bulk of the Cr normally ingested is present in food and not in water. Davids and Lieber (1951) reported that a family used Cr(VI)-contaminated well water (1-25 ppm) as drinking water for 3 years without deleterious effects. McKee and Wolf (1963) reported the following experiment:

A volunteer used a 10 ppm solution of Cr(VI) as his only fluid for drinking for 15 days. In all, 235 mg of Cr(VI) were ingested. Three periods of nausea were noted. The experiment was continued for 14 more days at levels of 2.5-5 ppm Cr(VI). At 5 ppm, mild nausea resulted from drinking freely on an empty stomach. Under similar conditions, 2.5-3.5 ppm Cr(VI) did not produce nausea.

EFFECTS ON SKIN

Injury to skin by Cr compounds can be classified into two main categories: corrosive ulceration and contact dermatitis. There is no doubt that handling of certain Cr compounds causes appreciable absorption via the pores of the skin, e.g. Baetjer (1959) found 11.9 ppm Cr (dry weight basis)

in the skin of a chromate worker who was last exposed to Cr(VI) 3 years prior to the tissue analysis. A comprehensive list of industries with potential occupational exposures to Cr has recently been compiled (NAS 1974).

Cr DERMATITIS

This dermatitis may be due to a direct irritant effect or to an allergic reaction; the latter also occurs in persons who do not work with hexavalent chromium. Direct skin irritation occurs at points of contact with clothing, e.g. neck or wrists where Cr(VI) dust or mist particles may gather. Proper industrial hygiene can eliminate such exposure.

Occupational dermatitis in chromate-using industries is attributed to sensitization to Cr(VI) which is present in at least trace amounts in many industrial processes. Newhouse (1963) reported that workers in an automobile plant exhibited a higher sensitivity to Cr(VI) than control subjects (36% response vs. 7.6%). The cause of this hypersensitivity in assembly line workers was traced to a chromate dip used on nuts, bolts, washers, and screws. Engel and Colman (1963) found that 65 of 250 workers engaged in wet sanding of a $ZnCrO_4$ primer paint on car bodies had developed dermatitis. Winston and Walsh (1951) reported Cr dermatitis in men engaged in the servicing of diesel locomotives. The recommended Cr(VI) level in the diesel engine coolant is 500-1000 ppm (Shepherd and Jones 1971). Anderson (1960) reported cases of Cr dermatitis involving cement and oil as the Cr carrying medium. In a recent study, Perone *et al.* (1974) found a low incidence of Cr hypersensitivity in American cement workers.

The Cr hypersensitivity of individuals may be established by patch tests (Denton *et al.* 1954). Generally, the concentration of $K_2Cr_2O_7$ in the test solution is 0.1-0.5% (350-1750 ppm Cr(VI)). One very sensitive patient reacted to both a 0.005% $K_2Cr_2O_7$ solution and a more dilute solution containing 0.0001-0.0004% $K_2Cr_2O_7$ extracted from cement (Sullivan 1969).

While Cr(III) elicits allergic reactions in Cr-sensitive individuals, the reaction is much less pronounced than for Cr(VI) because of the slower rate of diffusion of Cr(III) across the skin (Samitz *et al.* 1967). Fregert and Rorsman (1964) reported positive patch test reactions in 11 of 17 chromate-sensitive individuals tested with a 0.5 M solution (26,000 ppm) Cr(III). With a 0.07 M solution of $CrCl_3$, only 4 of 22 chromate-sensitive individuals reacted positively. Samitz *et al.* (1967) found that $CrCl_3$ solutions in the concentration range 0.01-0.2 M rarely produced positive patch tests.

The type of skin reaction in Cr dermatitis varies from superficial reddening of the skin to more serious eczematous eruptions. The lesion may mimic conventional types of dermatitis and the connection with occupational exposure to chromates may not be discovered in such cases. It has been shown that Cr(III) and Cr(VI) penetrate the skin by passive diffusion (Samitz *et al.* 1967); the proposed pathway is via the sweat glands to the deeper layers of skin; Cr(VI) is then reduced to Cr(III) which reacts with the skin protein to form an antigen-antibody complex. This would explain the tendency for these skin lesions to be localized around sweat glands. The chronic character of this dermatitis is explained by the fact that the primary inflammatory lesion occurs in the deeper layers of the skin, the antigen-antibody complex being removed more slowly than if the reaction had occurred in the superficial layers.

CORROSIVE ULCERATION

Ulceration is the commonest effect of occupational exposure to Cr(VI). It is generally accepted (Gafafer 1953) that cuts or skin abrasions, however minor, are necessary before the Cr(VI) can cause the lesion (Samitz and Epstein 1962). The causative agent is either a particle of Cr(VI) material or the evaporated residue of a concentrated solution of Cr(VI) (e.g. electroplating solutions contain ~ 10% $K_2Cr_2O_7$ or approximately 1 mg Cr(VI) per drop.

The lesion begins as a painless papule which, if left untreated, forms an ulcer with a raised hard edge. The base is covered with exudate or finely-adhering crusting. Untreated, the ulcers may penetrate deeply into soft tissue, or become the site of secondary infections which are slow to heal and leave an atrophic scar. Malignant (cancerous) change never occurs as a result of chrome ulceration. Chrome ulceration can be prevented by wearing protective clothing such as gloves or rubber coats. Several preparations containing EDTA, ascorbic acid or thiosulfate are used in the immediate treatment of tissues exposed to concentrated Cr(VI) solutions, e.g. Canada Chrome and Chemicals Ltd. recommends sodium calcium edetate as the most effective treatment for chrome ulceration. On the basis of patch tests, Edmundson (1951) concluded that chrome ulcers of the skin do not cause sensitization to Cr(VI).

APPENDIX A CHROMIUM-BEARING MINERALS IN CANADA

Thayer (1956) presented an excellent review of the mineralogy and geology of Cr. Through an ability to substitute for Al^{+3} and Fe^{+3} , Cr occurs as a minor element in such a wide variety of silicates that a complete list of Cr-bearing minerals would be very extensive. The Geological Survey of Canada (Traill 1970) listed only Cr(III)-bearing minerals in its catalogue of Canadian minerals. Generally, these minerals are associated with ultrabasic rocks, the main deposits of which are located in British Columbia, Manitoba, Ontario, and Quebec, usually away from urban centres and farm lands. Table A-1 lists the main Cr(III)-bearing minerals in Canada. Chromite is the only member of the group which has possible economic value. Most Canadian deposits of chromite are too low in Cr_2O_3 content (12-16%) and too high in iron-to-Cr content to be useful without significant area beneficiation. The chrome deposit in Canada most likely to become commercially useful is the Bird River Deposit in Manitoba. Downes and Morgan (1951) discussed the possibility of utilizing low-grade Canadian chromite deposits.

Table A-1. Main Cr-bearing minerals in Canada.

Mineral	Composition	Cr content (%) ^a	Main Canadian deposits ^b
Chromite	$(\text{Mg,Fe})(\text{Cr,Al,Fe})_2\text{O}_4$	10-45	British Columbia, Ontario, Quebec, Newfoundland, Manitoba
Copiapite	complex hydrous Fe sulfate	5	British Columbia, Alberta
Halotrichite	$(\text{Fe,Mg,Ni})(\text{Al,Cr})_2(\text{SO}_4)_4 \cdot 22\text{H}_2\text{O}$	5	Nova Scotia
Kammerite	$\text{H}_4\text{Mg}_2(\text{Cr,Al})_2\text{SiO}_4$	trace-6	Quebec
Magnesiochromite	$(\text{Mg,Fe})(\text{Cr,Al})_2\text{O}_4$	~ 38	British Columbia, Quebec
Muscovite	$(\text{OH})_2\text{KA1}_2(\text{AlSi}_3\text{O}_{10})$	trace-5	British Columbia, Manitoba, Ontario, Quebec
Serpentine	$\text{H}_4\text{Mg}_3\text{Si}_2\text{O}_4$	trace-3	British Columbia, Ontario, Quebec, Newfoundland
Stichtite	$2\text{MgCO}_3 \cdot 5\text{Mg}(\text{OH})_2 \cdot 2\text{Cr}(\text{OH})_3$	15	Ontario, Quebec
Uvarovite	$\text{Ca}_3(\text{Cr,Al})_2(\text{SiO}_4)_3$	18	Quebec

^a Thayer (1956)

^b Traill (1970)

APPENDIX B ANALYTICAL METHODOLOGY

INTRODUCTION

This section outlines some of the advantages and drawbacks of techniques which may be applied to the analysis of Cr. Serfass and Muraca (1956) have reviewed methods of wet chemical analysis while Willard, Merritt and Dean (1974), in an excellent treatise, have reviewed general aspects of instrumental analysis. Filby (1973) has recently published a thorough discussion of the problems involved in trace metals analysis. Details of the procedures may be found in the references quoted.

ERRORS IN Cr ANALYSIS

Errors may be introduced at any of the three major steps of an analysis.

SAMPLING AND SAMPLE STORAGE

It is very difficult to obtain meaningful and representative environmental samples, e.g.:

- (i) Bulk analysis of particulate matter filtered from aerosols does not allow adequate characterization of particles of respirable size. It is well known that Cr is most highly concentrated in the smallest particles collected from ambient air (Natusch *et al.* 1974).
- (ii) The tendency of Cr^{+3} to adsorb on particulate matter in natural waters makes the measurement of dissolved Cr a poor indicator of the Cr burden of these waters.
- (iii) The assumption that Cr is present in cationic form is not justified as Cr(III) forms cationic, neutral and anionic complexes with various naturally occurring ligands. Hence, direct preconcentration of Cr in natural waters using a cationic ion exchange resin will likely occlude much of the Cr, especially if a significant fraction of the Cr is in the form of Cr(VI).

The sample may be contaminated by sampling utensils or by storage containers, e.g.:

- (i) Versieck and Speecke (1972) found Cr contamination from stainless steel scalpels and hypodermic needles used in biopsy work. Liver tissue

was contaminated with as much as 0.02 ppm and 11 ppm Cr, respectively, by a stainless steel scalpel and a stainless steel needle. (The normal Cr level in human liver was quoted at 0.008 ppm.) The Cr contamination of a venous blood sample withdrawn with a disposable stainless steel needle was as high as 85 ppb. (The quoted range of Cr levels in blood was 2-15 ppb.)

- (ii) Glass containers cleaned with chromic acid solution absorb Cr(VI) and detectable amounts of Cr are still leached from such glassware even after 10 rinsings with distilled water (Laug 1934; Henry and Smith 1946).
- (iii) Many filtering materials contain appreciable amounts of Cr which may be transferred chemically or physically to the sample giving a high blank (Filby 1973):

Delbag	0.19 ppm Cr
Millipore	17.6 ppm Cr
Nucleopore	0.57 ppm Cr

- (iv) Urban air may contain 0.03 $\mu\text{g}/\text{m}^3$ of Cr in the form of particles containing 100-1000 ppm Cr, depending on particle size (Davison *et al.* 1974). Hence, contamination by atmospheric dusts may be significant.

Chromium may be lost by adsorption on the walls of containers. This tendency is especially strong for Cr(III) (see Chapter 3). Benes and Steinnes (1975) showed that in some natural waters as much as 50% of the Cr(III) present is lost in 100 hours by adsorption on the walls of polyethylene containers. Cr(VI) can react with the surface of some plastic containers (it is used as a mordant in the dyeing of some plastics).

Contamination by leaching and loss by adsorption on container walls are eliminated if the sample is kept in solid form. Some laboratories freeze aqueous samples for storage purposes. Emission spectroscopy, neutron activation, spark-source mass spectrometry and X-ray fluorescence are capable of analyzing solid samples directly, thus eliminating dissolution and storage problems. However, there may be difficulties involved in homogenizing certain solid samples.

Improper sampling and storage practices may change the forms of Cr present in the sample, e.g.:

- (i) Sampling of Cr(VI) by bubbling air through water or various absorbing solutions is subject to

error caused by the presence of SO_2 in the air. Cr(VI) is reduced by dissolved SO_2 and this would yield low Cr(VI) results.

- (ii) Halogen anions in appreciable concentrations (> 100 ppm) reduce Cr(VI). Hence, natural water samples should be stabilized with HNO_3 and not HCl .
- (iii) Benes and Steinnes (1975) found that the ratio of Cr(III) to Cr(VI) in natural waters may be changed substantially during storage for one week or more.

SAMPLE TREATMENT PRIOR TO ANALYSIS

Most methods for elemental analysis (except neutron activation and X-ray fluorescence) require that organic material be absent from the sample. Gorsuch (1970) gave detailed procedures for the destruction of organic matter in samples; however, significant amounts of Cr may be gained or lost in the process, e.g.:

Cr losses:

- (i) Dry ashing at 450°C and higher has been shown to lead to significant volatilization of organically bound Cr from plant material (Bagliano *et al.* 1972) and from various sugar products (Wolf *et al.* 1974). Wolf (1975) suggested that the organically bound Cr was the biologically important species and recommended that low-temperature (oxygen plasma) ashing be used to minimize Cr losses by volatilization.
- (ii) Wet ashing techniques involving evaporation to dryness may result in the formation of chromyl chloride (CrO_2Cl_2 , boiling point 117°C). Chromyl chloride may be formed by the action of HCl on CrO_3 or by heating dichromate in sulfuric acid solutions containing chlorides. Losses of Cr may be minimized by keeping the chloride concentration low (< 100 ppm) or by maintaining Cr in the Cr(III) state.
- (iii) Cr losses may also be incurred during preconcentration steps which do not recover Cr quantitatively. The use of isotope dilution methods (either radioactive tracer or a stable isotopic spike) permits such losses to be quantified and corrected for.

Cr contamination:

- (i) Most ashing aids, e.g. nitric, sulfuric and perchloric acids, sodium and potassium nitrate, contain ppb level Cr impurities which may significantly contribute to the reagent blank and cause the theoretical detection limit to be unattainable. Wolf *et al.* (1972) reported that in the analysis of blood for Cr the blank from such sources as distilled water and glass surfaces amounted to almost 50% of the analytical signal.

ERRORS IN ANALYSIS

Even if the sensitivity and precision of the chosen technique are satisfactory for a given analysis, errors may still result if sample and standard are sufficiently different in composition (matrix effect). Peterson and Manning (1971) showed that in atomic absorption spectroscopy the sensitivity of Cr in river water is suppressed compared to Cr standards prepared in distilled water (see Fig. B-1). While standard addition methods may minimize matrix effects involving inter-element effects, errors associated with interference will persist. Some environmental standard reference materials are listed in Table B-1.

METHODS FOR THE ANALYSIS OF CHROMIUM

The choice of analytical method in any determination is governed by the type and quality of the information required, the rate at which the analyses must be performed, and the cost limitations involved. Table B-2 compares various aspects of the major techniques for the analysis of Cr. These techniques are classifiable by the type of information or data resulting from their application. Bulk analysis techniques only yield elemental concentrations averaged over the whole of the sample. This information is often not enough to characterize the sample, e.g. in Fig. 3-2 it is shown that oil soot and soil particles in atmospheric aerosols have almost identical elemental analyses and could be confused with one another if the morphology of the particles is not taken into account as well. Some techniques can yield more specific information useful in characterizing a sample, e.g. the bulk concentration of various compounds or oxidation states of the element in the sample, or the physical location and concentration of various elements in localized regions of the three-dimensional structure of the sample.

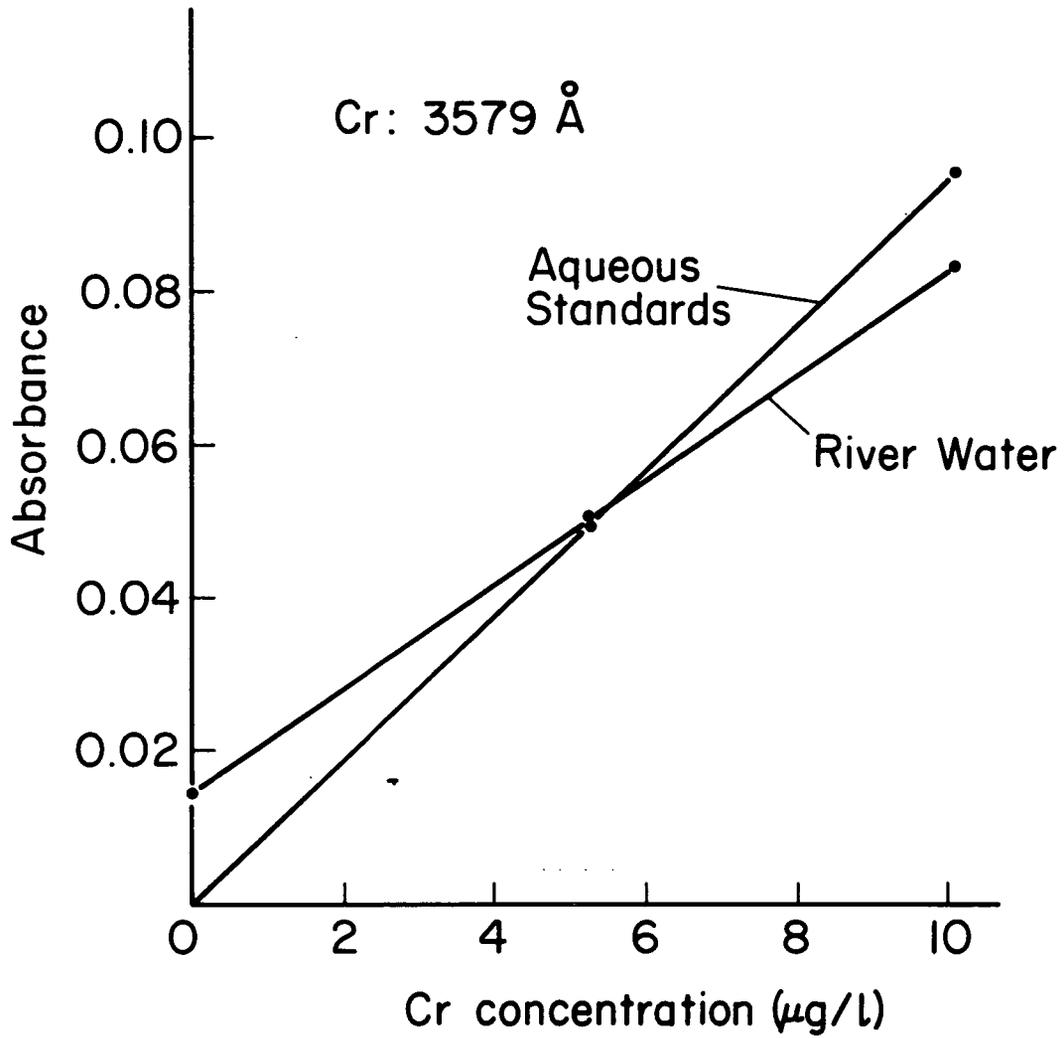


Fig. B-1. Calibration curves for Cr, indicating a matrix effect.

Table B-1. Recommended environmental standard reference materials.

Matrix	Recommended standard	
Rock, soil	USGS geological standards ^a	BCR-1
Plant	NBS orchard leaves	(SRM 1571)
Animal tissue	NBS bovine liver ^b	(SRM 1577)
Coal	NBS coal	(SRM 1630)
Fly ash	NBS Fly ash	(SRM 1631)

^a U.S. Geological Survey.

^b Cr value is provisional and not yet certified.

Table B-2. Techniques available for analysis of Cr

Method	Initial cost (x \$100)	Information		Quality of data (%) precision & accuracy	Simultaneous multielement capability	Required sample preparation	Matrix effect	Optimum detection limit	
		Bulk elemental analysis	Bulk compound analysis					absolute	relative
Gravimetry	5	x		0.1%	No	chemical isolation of Cr	large	10 ⁻³	5 ppm
Titrimetry	2	x		0.1	No	"	large	10 ⁻⁴	5 ppm
Catalytic	2	x	x	1-5	No	"	large	10 ⁻⁹	10 ppb
Colorimetric	5	x		3-5	No	"	large	10 ⁻⁸	50 ppb
Gas chromatography	100	x	x	10-20	No ^a	"	large	10 ⁻¹¹	10 ppb
Electrochemistry	1-100	x	x	0.1-5	No ^a	"	large	10 ⁻¹²	.005 ppb
Emission spectroscopy	100-1000	x		15-30	Yes	removal of organic material	moderate	10 ⁻⁹	5 ppm
Atomic absorption	40-200	x		5-10	No	"	moderate	10 ⁻¹²	0.01 ppb
Neutron activation	500-1000	x		5-10	Yes	none	none	10 ⁻¹²	0.1 ppb
Spark source mass spectroscopy	2000-3500	x		5-30	Yes	removal of organic material	negligible	5x10 ⁻⁹	50 ppb
X-ray fluorescence	500-1000	x		1-5	Yes	none; preconcentration if necessary	can be corrected for	10 ⁻⁹	100 ppm
ESCA	400-1500	x	x	1-5	Yes	none; preconcentration if necessary	can be corrected for	10 ⁻⁹	1000 ppm
Electron microprobe	500-1000		x	3-10	Yes	"	"	10 ⁻¹⁵	100 ppm
Ion microprobe	3500		x	10-15	Yes	polishing of surface of sample	large	10 ⁻¹⁷	1 ppm
Laser microprobe	20-100		x	30-50	Yes	removal of organic material	moderate	10 ⁻¹⁶	1 ppm

^a Limited numbers of elements in special cases.

The quality of the data, i.e. the degree of precision and accuracy, may vary from order-of-magnitude data used in screening procedures to high precision and accuracy data required in research and clinical applications. The detection limits listed in Table B-2 are defined as follows:

- (1) The absolute detection limit is the smallest amount of Cr which yields an analytical signal equivalent to twice the background or noise.
- (ii) The relative detection limit is the smallest concentration of Cr which supplies enough Cr to equal or exceed the absolute detection limit when the largest practical sample size is used.

The detection limits in Table B-2 are applicable where significant interferences are not present and where state-of-the-art equipment is used. Depending on the composition of the sample and the equipment used, these nominal detection limits may vary by several orders of magnitude.

The sample throughput (i.e. the rate of data acquisition) of these methods may range from several hundred to several hundred thousand samples per year depending on the technique itself and the degree of automation attained. The cost of an analytical system will increase as: 1) the required information or data becomes more specific, 2) the required quality of the data increases, and 3) the versatility and data acquisition rate increase.

METHODOLOGY

BULK ANALYSIS METHODS

Wet Chemical Procedures

Although wet chemical procedures have fallen out of favor because of the amount of time and technical expertise required, they are the only absolute methods (i.e. not requiring a comparative standard) available for use as referee techniques. Furthermore, sample pretreatment and preconcentration are based on wet chemical procedures.

Gravimetric determination of Cr involves the dissolution of the sample, isolation of Cr by a standard separations scheme, precipitation of hydrous Cr oxide and controlled ignition to Cr_2O_3 .

Volumetric procedures involve removal of interfering elements, such as cerium, followed by oxidation of Cr with sodium peroxide to form Cr(VI) ($\text{Cr}_2\text{O}_7^{2-}$). The dichromate is reduced with excess iron(II) which is then backtitrated with permanganate. Tungsten, vanadium, molybdenum, arsenic, uranium, nickel, cobalt, or iron in small amounts do not interfere as they are all oxidized to their highest valence before the iron(II) is added and are reoxidized by titration with permanganate. Large amounts of these elements (relative to the amount of Cr) must be removed by application of a suitable chemical separations scheme.

Colorimetry and Spectrophotometry

Colorimeters and spectrophotometers are amongst the most reliable and best calibrated analytical instruments. Vanderlinde *et al.* (1975) surveyed 63 different instruments and found that 80% of these instruments gave readings which differed by 4% or less.

Cr may be determined directly by oxidizing Cr to Cr(VI) and measuring the absorbance of the alkaline Cr(VI) solution near 366 nanometres (nm). The concentration detection limits listed in Table B-2 can be improved by using larger path length cells. The cerium(VI) and uranyl ions interfere because their spectral absorption bands overlap that of Cr(VI).

A method which improves sensitivity by nearly an order of magnitude involves the formation of a magenta-colored Cr(VI)-diphenylcarbazide complex. The following elements may interfere with the determination, depending on their concentrations relative to Cr: vanadium, iron, molybdenum, copper, and mercury; they may be removed beforehand by extraction as cupferrates (Sandell 1965).

The colorimetric analysis of aqueous samples requiring little pretreatment may be automated such that several samples can be processed per minute. The Technicon Autoanalyzer (Willard, Merritt and Dean 1974) is a commercially available unit which may be modified to perform all the chemical manipulations and colorimetric measurements involved in the diphenylcarbazide analysis of Cr. As many as seven other analyses may be performed simultaneously on the same sample using the autoanalyzer. A typical application of the diphenylcarbazide method is the determination of Cr(VI) in aerosols (Abell and Carlberg 1974). Other colorimetric Cr methods are described by Serfass and Muraca (1956) and Sandell (1965).

Kneebone and Freiser (1975) described a catalytic method for the determination of Cr(VI) in air. The method involves the catalytic effect of Cr(VI) in the sample on the oxidation rate of 3,3-dimethoxybenzidine by hydrogen peroxide. The rate of the reaction, measured by following the absorbance of the reacting mixture at 450 nm, is directly proportional to the Cr(VI) present in the sample. However, lead(II), Cr(III), copper(II), iron(III) and vanadium(V) are major interferences and hydrochloric acid has a marked effect on the oxidation rate. In the absence of interferences, the detection limit is 1 ng Cr(VI).

Gas Chromatography

Solvent extraction of the Cr(III)-trifluoroacetyl-acetone complex followed by gas chromatography can be used for analyzing biological fluids such as blood and urine containing subnanogram amounts of Cr. The electron capture detector is sensitive down to 10^{-11} g of Cr in complexed form (Savory *et al.* 1972); however, this detector is not very selective and responds to extraneous organic species in addition to the Cr complex. More reliable analyses of picogram quantities of Cr using a gas chromatograph-mass spectrometer system were reported by Wolf *et al.* (1972). Ross and Shafik (1973) described a similar system with a flame photometric detector for the determination of nanogram quantities of Cr in urine.

The main drawback in the above procedures is the difficulty of achieving quantitative extraction of Cr from the biological sample. This can result in a 25% error and a relatively poor analytical precision of $\pm 14\%$ (Savory *et al.* 1972).

Electrochemical Methods

Electrochemical methods respond to uncomplexed Cr, i.e. the activity of Cr in the solution, and hence metal-binding molecules and ions, must be avoided when total Cr is desired. On the other hand, electrochemical procedures provide a very elegant and precise means of measuring uncomplexed or available Cr in aqueous media. Gilbert (1972) published an excellent review which emphasized the great improvements in detection limits resulting from recent advances in the field of electronics. Table B-3 indicates the approximate concentration ranges over which various electroanalytical methods are applicable in the analysis of Cr.

Table B-3. Analytical ranges for electrochemical methods^a.

Method	Concentration range (molarity) ^b
Titrimetry: zero-current potentiometry	$10^{-1}-10^{-3}$
constant " "	$10^{-1}-10^{-3}$
null-point "	$10^{-2}-10^{-6}$
Amperometry	$10^{-3}-10^{-6}$
Coulometry	$10^{-2}-10^{-6}$
Electrogravimetry	$10^{-1}-10^{-2}$
Direct coulometry	$10^{-2}-10^{-5}$
Direct potentiometry	$10^{-1}-10^{-7}$
Polarography: direct current	$10^{-3}-10^{-6}$
pulse	$10^{-4}-10^{-7}$
single sweep	$10^{-4}-10^{-6}$
Anodic stripping voltametry	$10^{-4}-10^{-9}$
Conductance	$10^{-1}-10^{-5}$

^a After Gilbert (1972)

^b 10^{-3} M = 52 ppm Cr.

There is no specific ion electrode for Cr(III) (Buck 1974), but potentiometric measurements can be made indirectly, e.g. with a Ni^{2+} -sensitive electrode after removal of Ni^{2+} from the solution by using dimethylglyoxime.

The main drawback of the electroanalytical methods is the amount of experience and sample processing required to achieve meaningful results. Nevertheless, these methods yield results that surpass the precision, accuracy and detection limits of the most sophisticated spectroscopic techniques at a fraction of the cost, e.g. Rossley and Higgins (1974) describe the construction of a research quality polarograph using only \$33 worth of components.

Walker (1971) described the construction of specific ion microelectrodes capable of ionic measurements inside a single living cell (electrode tip diameter was 1-5 microns). Purdy (1965) discussed and reviewed the application of polarography and amperometric titrations to the study of Cr in biological systems. Crosmun and Mueller (1975) presented a simple method for the determination of Cr(VI) in natural waters by differential pulse polarography. The method permits determination of 35-2000 ppb Cr(VI) in the presence of up to 0.62 ppm Cu(II) and 0.55 ppm Fe(III).

Emission Spectroscopy

Bedrosian *et al.* (1968) reported a method for the direct analysis of unashed, dried biological samples using a DC arc excitation source with photoplate detection. The presence of molecular interferences required that the less sensitive Cr emission line at 283.5 nm be used. The absolute and relative detection limits were 10^{-8} g and 0.5 ppm respectively, while the precision was approximately $\pm 15\%$. The samples analyzed were 25 mg each of dried liver, blood, bone, and plant leaves.

Morrison *et al.* (1969) described the use of ashing techniques to achieve sample preconcentration. Using the same analytical system as above, they improved the relative detection limit by two orders of magnitude (1-5 ppb). The reduction of molecular interferences by ashing permitted use of the principal emission line at 425.4 nm.

Sugimal (1975) used DC arc (15 amp) emission spectroscopy with photoplate detection to determine 19 elements including Cr in particulate matter filtered onto glass fibre mats from the air. At a Cr concentration of $0.031 \mu\text{g}/\text{m}^3$ in air the relative standard deviation of the method was 7%. The useful range of the method was $0.005\text{-}0.15 \mu\text{g}/\text{m}^3$ Cr in air.

The use of a photoplate or a bank of photomultipliers for detecting spectral line intensities permits simultaneous analysis of 30 or more elements. It is now possible to perform several multielement analyses per minute (the equivalent of 10 to 50 individual determinations per minute) using photomultiplier detection and computerized data acquisition and calculation.

While the precision of analysis using the DC arc is characteristically lower than optimum ($\pm 15-30\%$), the technique retains its popularity because the sensitivity is high, solid samples can be analyzed directly, and there is relative freedom from matrix effects. Dickinson and Fassel (1969) described an induction-coupled plasma excitation source for use in emission spectroscopy of aspirated solutions. The reported detection limit was 1 ppb. The high temperature of the plasma minimizes matrix effects, e.g. the emission signal observed for excited Cr atoms at 425.4 nm changed by less than 10% when the Na concentration of the solution was varied from 0 to 7000 ppm (Larson *et al.* 1975).

Morrison and Talmi (1970) described a radio-frequency induction furnace source for emission spectroscopy which utilizes thermal volatilization and helium plasma excitation to produce an emission spectrum. An absolute Cr detection limit of 10^{-9} g was reported. A precision of 1-4% for evaporated liquid samples and 4-10% for powdered samples was routinely obtained. Hambidge (1969) used a static argon arc to determine Cr in ashed blood serum. The absolute detection limit was 10^{-9} g Cr. Further developments in plasma excitation sources may be expected.

Atomic Absorption Spectroscopy

This technique is one of the most rapid, sensitive, and inexpensive single-element techniques available. It is the method of choice when only a few elements need be determined in each sample.

In the analysis of Cr by flame atomic absorption a fuel-rich air-acetylene flame is usually used to limit the formation of Cr oxides. Absorbance is measured at 357.9 nm. Large amounts of Fe in the sample interfere with Cr atomization in the flame and suppress the Cr signal. This effect may be reduced by adding ammonium chloride (1000 ppm) to the aqueous samples and standards. In all cases the oxidation state of Cr should be the same in sample and standard. Depression of the Cr signal by phosphate is overcome by the addition of Ca (1000 ppm). The use of a nitrous oxide-acetylene flame reduces matrix or inter-element effects because of the higher

flame temperature, but does not improve the Cr detection limit significantly over the air-acetylene flame. The detection limit for direct aspiration into the air-acetylene flame is approximately 10 ppb (CCIW 1974). If the Cr is first oxidized to Cr(VI) and then extracted into methylisobutyl ketone before aspiration, the reported detection limit is improved fiftyfold (0.2 ppb) (CCIW 1974).

Sources permitting greater sensitivity than the flame are based on the atomization of the entire sample in a single pulse. This produces a high concentration of Cr atoms which, with modern electronic techniques, can be measured quite accurately. Davidson and Secrest (1972) described the use of a graphite furnace atomizer for the analysis of Cr in biological fluids. The absolute and relative detection limits were 2×10^{-12} g and 0.01 ppb respectively. The precision of analysis in the range 0.3-0.7 ng Cr was 5%. Kahn (1972) and Pekarek *et al.* (1974b) achieved similar results. Wolf *et al.* (1974) stated that as much as 80% of the Cr in a sample may be lost during the ashing step in the graphite furnace. They minimized this by preashing the sample before introducing it into the furnace.

To avoid matrix and background effects with flameless atomic absorption spectroscopy, the sample and standard must be closely matched (see Fig. B-1). Tessari and Torsi (1972) reported significant inter-element effects in the determination of Cr using a carbon rod analyzer. High levels of phosphate, iron and cobalt caused a significant decrease in Cr signal strength.

Monkman *et al.* (1972) described the procedures used by the Canadian Air Pollution Control Directorate for the determination of Cr in airborne particulate matter by flame and graphite furnace atomic absorption spectrometry.

Atomic Fluorescence

In fluorescence spectroscopy an external light source is used to excite the atoms of the sample which then emit their characteristic emission spectra. This emission spectrum or fluorescence is measured by placing the monochromator axis at right angles to the incident or exciting radiation. The lower background signal strength achieved by using this configuration leads to an improved detection limit.

In principle, any atomic absorption instrumentation may be converted to perform atomic fluorescence simply by the addition of an external light source at right angles to the axis of the monochromator. Thus, Johnson *et al.* (1975) used a xenon arc

excitation source with flame atomic absorption instrumentation to analyze simultaneously for 18 elements, including Cr, in aqueous solutions. The reported Cr detection limit (using the 357.9 nm fluorescence line) was 0.0015 ppm compared to 0.005 ppm for conventional flame atomic absorption spectroscopy.

Neutron Activation

Neutron activation with gamma ray spectrometry has often been used as a referee technique because there is no matrix effect and practically any sample can be analyzed without pretreatment. This means that the possibility of sample loss or contamination is greatly reduced. Upwards of 40 elements may be determined simultaneously. Once the sample is irradiated it may be counted without further chemical treatment; however, it is often necessary to allow short-lived high-activity nuclides to decay to negligible levels (1-3 weeks) in order to reduce spectral background. Since the radioisotope measured, ^{51}Cr , has a 27.7-day half-life, the decay period results in an acceptable loss of analytical sensitivity. Detection limits for Cr after irradiation (4 hours at 10^{12} neutrons/cm² flux) and a 1- to 3-week decay period are in the range 0.04-0.65 ppm and 10-500 ng for various environmental samples (Eckhoff *et al.* 1971). Improved detection limits can be achieved by chemically separating the Cr from the other elements, hence reducing the spectral background and vastly improving the signal-to-noise ratio (Becker and La Fleur 1971). Because only ^{51}Cr (gamma ray energy 0.320 MeV) is measured, subsequent contamination of the sample with Cr in the chemical separation step does not affect the analytical results, i.e. use of expensive high purity chemicals is not required provided all chemical manipulations are performed after irradiation of the sample. High flux irradiation coupled with radiochemical separation of Cr and sophisticated counting apparatus can lower the absolute detection limit to the picogram range (10^{-12} g). Several symposia have featured the use of nuclear activation techniques in environmental and clinical applications (IAEA 1967, 1972; Vogt *et al.* 1971).

Spark-Source Mass Spectrometry

This method accepts inorganic material for direct analysis while organic matrices must be ashed prior to analysis. During analysis, the sample is volatilized and ionized by a high voltage discharge between two electrodes composed of a compressed mixture of sample and graphite. More than 50 elements can be determined simultaneously in a given sample. The relative and absolute detection limits for direct analysis are 50 ppb and 5 ng respectively. The precision of the method is approximately 10-15%.

Isotope dilution spark-source mass spectrometry involves the addition of a known amount of a stable isotope (e.g. ^{53}Cr) to the sample. Measurement of the altered isotopic ratios yields the original amount of Cr present. Detection limits remain roughly the same as above but the precision is better ($\pm 3-5\%$).

The main advantage of the spark-source method is that the high energy spark reduces matrix effects to insignificant levels, i.e. the relative sensitivities of the elements remain constant despite major changes in the composition of the sample. Hence, there is no need to carefully match the sample and standard as in atomic absorption. This factor, along with the simultaneous multielement capability, make the method ideal for trace element survey work (Brown and Vossen 1971).

X-Ray Fluorescence

The sample is bombarded with high energy X-rays and the elements in the sample are induced to emit their characteristic X-rays. The method essentially determines the elemental composition of a dense sample (metal, rock) to a depth of several micrometres without chemically or physically altering the sample. The absolute Cr detection limit is several ng, but the relative detection limit is usually 100 ppm because of the small volume of the sample actually being analyzed. Materials which are either very thin or not dense, e.g. freeze-dried plant or animal tissue, are more efficiently sampled than dense materials because the exciting X-rays penetrate the entire thickness of the sample. Hence, Reuter (1975) reported a concentration detection limit of 10 ppm Cr in freeze-dried plant material. Analyses of dense samples containing less than 100 ppm Cr may be performed by chemically separating the Cr from the sample matrix and depositing it in a thin layer on a suitable substrate. This preconcentration places enough Cr in the volume sampled to give a measurable signal. Thus, the method may also be used to preconcentrate Cr from aqueous solutions. Campbell *et al.* (1966) reported an absolute detection limit of 0.22 μg for Cr collected from aqueous solution in an ion-exchange-resin-loaded filter paper. Beyermann *et al.* (1969) reported an improved absolute detection limit of 5 ng for Cr extracted from urine as the oxinate complex and subsequently evaporated onto a brass substrate. Wobrauschek and Aiginger (1975) used a special instrument geometry to perform total-reflection X-ray fluorescence on evaporated solutions. The reduction in background signal achieved by using this technique permitted the analysis of as little as 5 ng of Cr in 5 μl of solution (i.e. a 1 ppm solution).

Birks and Gilfrich (1972) reviewed the application of X-ray fluorescence to the analysis of particulate matter filtered from air and water as well as the preparation of suitable calibration standards.

PROBE ANALYSIS METHODS

Probe techniques permit multielement analysis to be performed on micron-size particles or localized regions of larger samples. The diameter of the spot analyzed varies with the technique used: electron microprobe - 0.2-300 μm , laser microprobe - 5-35 μm , ion microprobe - 1-10 μm . Microscope attachments permit selection of sampling site to a high degree of precision ($\pm 1-2 \mu\text{m}$). Andersen (1973) edited an excellent review of microprobe analyses.

Electron Microprobe

In this method the sample is bombarded with an intense beam of high energy electrons which causes each element in the sample to emit its characteristic X-rays. Correction must be made for the absorption of X-rays before they emerge from the surface of the sample. The volume sampled is usually 1-10 cubic micrometres (1-10 μm^2 area, 1 μm depth), which translates into 10^{-11} - 10^{-10} g of sample. Since the relative detection limit is usually 100-2000 ppm, or 0.01-0.2% (Heinrich 1966), the absolute detection limit for most elements, including Cr, is 10^{-15} - 10^{-14} g. This amount can be observed *in situ* and nondestructively because the method, being a close analog of X-ray fluorescence, does not alter the chemical or physical form of the sample. In many cases the Cr in a sample is localized in the form of a precipitate or an inclusion (e.g. particulate matter in the lung). On a bulk scale the Cr content may be at the ppm or ppb level but on a microscopic level Cr may comprise several percent of the included particle, readily permitting elemental characterization by electron microprobe, e.g. Natusch and Wallace (1974) reported that Cr levels on the surface of fly ash particles, compared to those in the bulk of the particles, were high enough to make electron probe analysis possible.

Proton Microprobe

Horowitz and Grodzins (1975) described the use of a collimated beam of high energy protons to excite X-rays from a sampling spot as small as 1 μm in diameter. High energy protons, when compared with high energy electrons, have a much greater probability of interacting with matter to produce X-rays. Hence, it was possible to determine as little as 0.1 ppm Cr in a

sample area 1 μm in diameter (absolute detection limit about 10^{-19} g). Using this method the above investigators were able to follow Cr concentration changes along the length of single human hairs.

Laser Microprobe

A high energy pulse of laser (coherent) light is used to vaporize 10^{-12} - 10^{-6} g of sample. The intense heat from the energy of the laser pulse causes a multielement emission spectrum to be produced by the vaporized elements. Secondary excitation sources may be used to increase the strength of the signal observed. Using an ultrasensitive detection system the absolute detection limit for Cr falls in the range 10^{-16} - 10^{-8} g. This translates into a relative detection limit of 0.1-10 ppm. The amount of sample vaporized by each laser pulse varies widely and results in poor precision (± 30 -60%). Nevertheless, the laser microprobe may be used as a relatively inexpensive attachment (\$5000-10,000) to existing emission spectroscopic equipment permitting simultaneous multielement analysis of interesting inclusions in a sample.

Ion Microprobe

The ion microprobe is capable of analyzing successive atomic layers of a sample surface. Inert gas or oxygen ions are accelerated to high energies and are used to bombard the sample surface producing secondary ions from the sample material. These secondary ions are then analyzed according to their mass to give the elemental composition of the sample. Organic material produces major interferences, but these may be minimized by improvements in the resolution of the instrument. Most work to date has been done on geological and metallurgical matrices. McHugh and Stevens (1972) used this method in the analysis of single oil soot particles.

The various elements exhibit widely differing sensitivities in a given matrix. Furthermore, these sensitivities change drastically when the matrix is changed. Hence, well-characterized standards are absolutely necessary for each matrix type analyzed.

Some ion microprobes act as ion microscopes, i.e. they use the secondary ions to produce an image of the sample surface which indicates the spatial location of the desired element on the surface of the sample. Correlation of the spatial locations of several elements implies compound formation.

Depending on the matrix and instrumentation used, the absolute detection limit for Cr ranges from 10^{-18} to 10^{-12} g. This translates into a relative detection limit of 1-100 ppm because of the small size of sample (10^{-12} - 10^{-8} g). Currently, the method is still being developed and is best used to obtain qualitative or semiquantitative data on minute samples or sample surfaces.

ELECTRON SPECTROSCOPY FOR CHEMICAL ANALYSIS (ESCA)

In this surface analysis technique, high-energy photons, usually X-rays, bombard the sample and cause the various elements present to eject electrons. The energy of the incident radiation is precisely known and hence measurement of the residual energy of the ejected electrons gives, by difference, their binding energies. In this way it is possible not only to perform elemental analysis but also to determine the oxidation states of the elements present (Baitinger and Amy 1974). The method is nondestructive and samples to a depth of approximately 30 angstroms. The absolute and relative detection limits are comparable to X-ray fluorescence, 10^{-9} - 10^{-6} g and 1% (by weight) respectively.

INTERLABORATORY COMPARISONS

Systematic errors present in a method may lead to very precise but inaccurate results. Because the development of certified standards for many classes of materials is scientifically exacting and quite expensive, very few are available. This has made it difficult to uncover and correct systematic errors. Hence, attempts have been made to check analytical methods through interlaboratory comparisons of analyses performed on carefully homogenized samples representing various environmental matrices. Table B-4 lists some typical results of interlaboratory comparisons. The following points can be made:

- 1) Agreement among the various laboratories decreases as the level of Cr decreases in the sample.
- 2) Fly ash, coal and other solids are more difficult to homogenize than liquids such as fuel oil.
- 3) The presence of complex organic material drastically interferes with Cr analysis making it impossible to achieve the optimum detection limits. This is exemplified by the analysis of Cr in blood samples.

Table B-4. Interlaboratory comparisons of Cr analyses.

Matrix	Mean Cr concentration	Relative standard deviation (%)	Number of values	Range	References
Lunar soil	2670 ppm	10	13	2080-3030 (7000) ^a	Morrison 1971
Fly ash	161 ppm	54	10	(80) ^a , 100-330, (500) ^a	von Lehmden <i>et al.</i> 1974
Coal	6.44 ppm	52	5	3.4-12	von Lehmden <i>et al.</i> 1974
Fuel oil	0.90 ppm	14	7	0.7-1.0, (4.0) ^a	von Lehmden <i>et al.</i> 1974
Animal bone	1.97 ppm	35	3	1.2-2.4, (19.1) ^a	Heinonen and Suschny 1972
Animal blood	0.22 ppm	118	5	0.02-0.54	Heinonen and Suschny 1972
Synthetic aqueous standard	0.075 ppm ^b	21	8	0.05-0.10	Wales and McGirr 1973
	0.050 ppm ^b	27	7	0.04-0.08	Wales and McGirr 1973
	0.020 ppm ^b	63	6	0.01-0.05	Wales and McGirr 1973
	0.010 ppm ^b	68	4	0.0-0.01	Wales and McGirr 1973

^a Value rejected by application of statistical test.

^b True value of Cr concentration, not an average.

Table B-5 lists concentrations of Cr in different samples of blood determined by various techniques. Even allowing for a considerable range of Cr values in the blood of normal individuals, it is still obvious that certain values are in error. The values reported before 1967 are indicative of problems involving contamination, standardization, and spectral interference. On the other hand, the value reported by Pekarek *et al.* (1974b) may represent the loss by volatilization of a significant portion of organically complexed Cr as discussed by Wolf *et al.* (1974).

Table B-5. Reported Cr concentrations in blood.^a

Author	Year	Concentration (µg/l)	Method
Urone <i>et al.</i> (Anal. Chem. <u>22</u> ; 1317)	1950	50	Colorimetric; diphenylcarbazine
Monacelli <i>et al.</i> (Clin. Chim. Acta. <u>1</u> ; 577)	1956	180	Emission spectrometry; photographic detection
Volod'ko, L.V. <i>et al.</i> (Vestn. Akad. Nauk. Belarusk <u>1</u> ; 107)	1962	200	" "
Schroeder, H.A. <i>et al.</i> (J. Chron. Dis. <u>15</u> ; 941)	1962	520;170	Colorimetric; diphenylcarbazine
Wolstenholme, N.A. (Nature <u>203</u> ; 1284)	1964	1000	Dry ashing; SSMS
Feldman, F.J. <i>et al.</i> (Anal. Chim. Acta. <u>38</u> ; 489)	1967	29	Solvent extraction; atomic absorption with air-hydrogen flame
Hambidge, K.M. (In "Newer Trace Elements in Nutrition", Dekker, N.Y.)	1971	7	Low temperature ashing; static DC argon arc emission spectroscopy
Cary, E.E. <i>et al.</i> (J. Agr. Food Chem. <u>19</u> ; 398)	1971	7	Solvent extraction; atomic absorption
Pekarek, R.S. <i>et al.</i> (Anal. Biochem. <u>59</u> ; 283)	1974	0.72	Direct analysis; graphite furnace atomic absorption

^a Table from Mertz (1975).

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