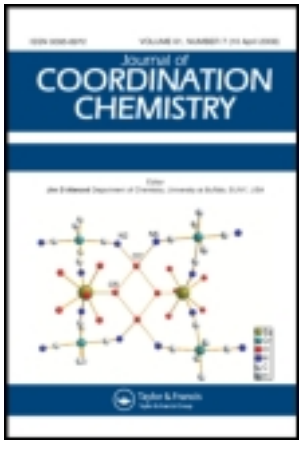

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REVIEW

Sources and toxicity of hexavalent chromium

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Chromium exists in oxidation states ranging from −IV to +VI, inclusively. The compounds exhibit a wide range of geometries including square planar, tetrahedral, octahedral, and various distorted geometries. Ore refining, chemical and refractory processing, cement-producing plants, automobile brake lining, catalytic converters for automobiles, leather tanneries, and chrome pigments contribute to the atmospheric burden of chromium. Hexavalent chromium is known to have 100-fold more toxicity than trivalent chromium, for both acute and chronic exposures because of its high water solubility and mobility, as well as easy reduction. The respiratory tract is the major target organ for hexavalent chromium following the inhalation exposure in humans. Chronic inhalation exposure to hexavalent chromium results in effects on the respiratory tract, with perforations and ulcerations of the septum, bronchitis, decreased pulmonary function, pneumonia, and nasal itching and soreness as reported. Chronic human exposure to high levels of hexavalent chromium by inhalation or oral exposure may produce effects on the liver, kidney, gastrointestinal, and immune systems, and possibly the blood. Dermal exposure to hexavalent chromium may cause contact dermatitis, sensitivity, and ulceration of the skin.

Keywords: Hexavalent chromium; Ulcerations of the septum; Bronchitis; Dermatitis; Ulceration of the skin

1. Introduction

Hexavalent chromium is a toxin typically originating from anthropogenic activity [1a]. However, chromium occurs naturally at high concentration in ultramafic rocks and is a common contaminant in surface and ground water [1b]. The ubiquitous occurrence of chromium in surface and ground water reflects its use as an important industrial metal in a variety of diverse processes (table 1). Characterized as a lustrous, silver–gray metal, chromium rarely occurs in the Earth's crust as the element (or metal) but almost only in compound form or ions in water. The chief commercial source of chromium is chromite ($FeCr_2O_4$) with chromium being mined as a primary product [2].

The main uses for chromium are metallurgical (67%), refractories (18%), and chemical (15%). Hexavalent chromium in ground water has generally been assumed to be anthropogenic (man-made) contamination, since it is used in a number of industrial applications, including electroplating, tanning, industrial water cooling, paper pulp production, and petroleum refining (table 2). Hexavalent chromium minerals have been

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Table 1. Uses of chromium.

Form	Uses
Chromium(0)	Stainless steel production, alloy production, metal and alloy manufacturing
Chromium(III)	Metal and alloy manufacturing, brick lining, chrome plating, leather tanning, textiles, copy machine toner
Chromium(VI)	Chrome plating, leather tanning, textiles, copy machine toner

Table 2. Sources of hexavalent chromium.

Uses	Hexavalent chromium chemicals
Pigments for paints, inks, and plastics	Lead chromate (yellow, chrome green, molybdenum orange), zinc chromate, barium chromate, calcium chromate, potassium dichromate, sodium chromate
Anti-corrosion coatings	Chromic trioxide (chromic acid), zinc chromate, barium chromate, calcium chromate, sodium chromate, strontium chromate
Stainless steel	Chromium (VI) is given off when stainless steel is cast, welded, or plasma torch cut
Textile dyes	Ammonium dichromate, potassium chromate, sodium chromate
Wood preservatives	Chromium trioxide
Leather tanning	Ammonium dichromate

found in nature and the ability of manganese(IV) oxide (MnO_2) to oxidize tetravalent chromium to hexavalent chromium is well-known [3–5]. The occurrences of hexavalent chromium are growing, particularly with state-wide hexavalent chromium sampling with lower detection limits mandated for water supplies by the California Department of Health Services (DHS) because of increasing health concerns [6].

Commonly associated with chrome plating, hexavalent chromium in water is converted through electroplating (decorative chrome plating) to the bright metallic chromium coating observed on plastic or metal products such as car bumpers or shower heads and a host of consumer and industrial products. It is also used to apply a hard smooth surface to machine parts, such as crankshafts and printer rollers. This process is known as ''hard'' chromium plating. The chromed layer is attractive and provides corrosion resistance, easy cleaning, and surface hardness. There are two types of chrome-plating baths: hexavalent and trivalent, although the latter are not common. Hexavalent chromium baths are widely used. A typical bath composition of a hexavalent chromium bath [7] is as follows: (i) electrolytic solution: chromic acid (H_2CrO_4); (ii) anode: lead with tin up to 7%; (iii) operating temperature: 45–60°C; (iv) plating current: 1.5–3.0 K amp m^{-2}. About 35% of used chromium is discharged in the effluent as trivalent and hexavalent chromium [7].

Tanning is a process of converting raw hides or skins into leather. The conversion of animal hides or skins into useful artifacts may be man's oldest technology [8]. A considerable quantity of basic chrome sulfate (known as tanning powder) which is manufactured from reduction of hexavalent chromium to trivalent chromium by sulfur dioxide is used in chrome tanning to convert polypeptide collagen strands in the hide to a cross-linked helix [8], which prevents penetration of water into leather pores [9] and supplies thermal stability. The options for cross-linking are threefold: they are intra

single helix, intra triple helix, and inter triple helix [8, 9]. The chromium fixation (cross-linking) can occur in two ways: (i) covalent reaction between one chromium ion and two carboxyl groups of collagen; (ii) hydrogen bonding between chromium species (monomer, dimer, etc.) and the protein, particularly along the polypeptide backbone. About 40% of used chromium is discharged in the effluent as hexavalent chromium and trivalent chromium. The trivalent chromium in soil may be oxidized to hexavalent chromium in the presence of manganese [10]. Hexavalent chromium is produced in leather during photoaging [11].

Chromium compounds are used in paint pigments [10]. Chromates of barium, lead, and zinc provide the pigments of lemon chromium, chromium yellow, chromium red, chromium orange, zinc yellow, and zinc green glass [7]. Chromium chemicals enhance the colors of fabrics and are used to achieve the brightly colored Cr-based paints for automobiles and buildings. The chromate of barium, lead, and zinc ($MCrO_4$) are toxic and these compounds are discharged in wastewater [7]. Potassium dichromate is used in the manufacture of waterproof glues and photography [10]. Almost all chemical laboratories (academic, research, and industry) discharge considerable amounts of chromium, both trivalent and hexavalent to the environment [10]. Hexavalent chromium is widely used in the laboratory as an oxidant. Dichromate (potassium dichromate is a primary standard, its solution is used to standardize secondary standards and quantitative estimation of metal ions) is a significant analytical tool. Chromium compounds have been used in the formulation of wood preservatives for about a century [12]. These are ''wolman compounds'' (based on sodium fluoride and nitrophenol with sodium dichromate or potassium dichromate), copper chromate (CC) (mixture of potassium dichromate and copper sulfate), copper–chromium–arsenic (CCA) (mixture of copper sulfate, sodium dichromate, and arsenic trioxide), copper–chromium–boron (CCB), copper–chromium–fluoride (CCF), and copper–chromium–phosphate (CCP), but the most extensive and commercially leading system has been the CCA [12]. Chromium compounds fulfill two principle functions in wood preservatives, these are (i) as a chemical fixative to prevent or reduce loss by leaching of other compounds of the preservative and (ii) as an anti-corrosion agent [12].

1.1. *The chromium cycle*

Naturally occurring and anthropogenic chromium can occur in various environmental media including surface and ground water, sea water, soil and sediments, rocks, and air. The chromium cycle consists of the chemical processes associated with chromium which occur in the environment.

In ground water and soil, chromium exists in two major oxidation states: the oxidized hexavalent chromium and the less-oxidized trivalent chromium. Trivalent chromium compounds are sparingly soluble in water, hexavalent chromium compounds are quite soluble. The resulting hexavalent chromium solutions are powerful oxidizing agents under acidic conditions, but less so under basic conditions. For example, chromic acid (H_2CrO_4) is often used in chemical labs to clean glassware by oxidizing organic residues. Thus hexavalent chromium is much more toxic and mobile in ground water than the relatively immobile trivalent chromium. Depending on the concentration and acidity, hexavalent chromium can exist either as chromate (CrO_4^{2-}) or dichromate ($Cr_2O_7^{2-}$). The common dissolved chromium species (all hexavalent chromium) are $HCrO_4^-$,

CrO_4^{2-}, and $Cr_2O_7^{2-}$ [13–15]. Which entity will dominate in a particular environment depends upon the specific conditions, for example, pH, Eh (redox potential), total concentration of chromium, and the overall aqueous chemistry.

Due to its anionic nature (as polyatomic ions) hexavalent chromium sorption in soils is limited to positively charged surface exchange sites, the number of which decreases with increasing pH. Fe and aluminum oxide (Al_2O_3) surfaces absorb CrO_4^{2-} at acidic and neutral pH [13]. The absorption of hexavalent chromium by ground water alluvium was owing to the Fe oxides and hydroxides. The adsorbed hexavalent chromium was, however, easily desorbed into uncontaminated ground water, indicating non-specific adsorption [2].

The presence of chromate (CrO_4^{2-}) and nitrate (NO_3^-) had little effect on chromium(VI) adsorption, whereas sulfate (SO_4^{2-}) and phosphate (PO_4^{3-}) inhibited adsorption. SO_4^{2-} and dissolved inorganic carbon (C) inhibited CrO_4^{2-} adsorption by amorphous iron oxyhydroxide [FeO(OH)] and soil. However, the presence of sulfate enhanced hexavalent chromium absorption to kaolinite. Barium chromate ($BaCrO_4$) (S) may form in soils at chromium-contaminated waste sites. No other hexavalent chromium precipitates have been observed at pH 1–9 [16].

1.2. *Electronic and molecular structure of hexavalent chromium compounds*

In aqueous solution hexavalent chromium exists as oxoforms in a variety of species depending on pH and the hexavalent chromium concentration [14]. For the oxo species of hexavalent chromium three main pH regions may be distinguished:

(1) H_2CrO_4 (pH < 0),
(2) $HCrO_4^-$ and $Cr_2O_7^{2-}$ (pH 2–6),
(3) CrO_4^{2-} (pH > 6).

The abundance of these forms is largely dependent upon concentration. In very acidic solutions two other forms have been detected, $Cr_3O_{10}^{2-}$ and $Cr_4O_{13}^{2-}$ [17]. The equilibrium between protons, water molecules, and the hexavalent chromium species are as follows:

$$H_2CrO_4 \rightleftharpoons HCrO_4^- + H^+ \quad K_1, \tag{1}$$

$$HCrO_4^- \rightleftharpoons CrO_4^{2-} + H^+ \quad K_2, \tag{2}$$

$$Cr_2O_7^{2-} + H_2O \rightleftharpoons 2HCrO_4^- \quad K_3, \tag{3}$$

$$HCr_2O_7^- \rightleftharpoons Cr_2O_7^{2-} + H^+ \quad K_4, \tag{4}$$

$$H_2CrO_4 \rightleftharpoons HCr_2O_7^- + H^+ \quad K_5. \tag{5}$$

The most important equilibria in hexavalent chromium aqueous solutions above pH 1.5 are deprotonation and dimerization reactions, which can be described by equations (2) and (3).

The electronic structure of the tetrahedral chromate ion, a predominant oxo species at neutral pH, is second only to the permanganate ion (MnO_4^-) in being thoroughly studied. The chromate ion forms a regular tetrahedron with the chromium–oxygen

distance being 1.66 Å [18]. The schematic energy level diagram for the higher filled (HOMO) and lower unfilled (LUMO) orbitals in the ground state established the $1t_1$ orbital to the highest occupied level and 2e to be the lowest unoccupied [17]. The highest occupied MO level $1t_1$ possesses pure oxygen character and the 2e orbital is mostly of chromium character [19].

1.3. *Reactivity of hexavalent chromium: mild, selective, and effective oxidants*

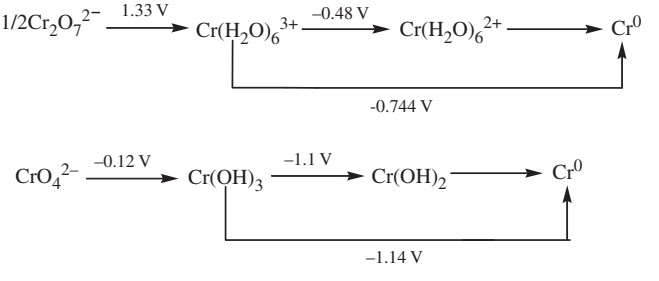
The reduction process of hexavalent chromium in acidic solution can be expressed by

$$Cr_2O_7^{2-} + 14H^+ + 6e^- = 2Cr^{3+} + 7H_2O \quad E_0 = 1.33\,V,$$

and in alkaline solution

$$CrO_4^{2-} + 4H_2O + 3e^- = Cr(OH)_3 + 5OH^- \quad E_0 = -0.12\,V.$$

The Latimer diagrams for acidic and basic solutions are:



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The reduction of hexavalent chromium involves the subsequent transformation from a labile tetrahedral and pseudo-tetrahedral oxyanion to an inert $Cr(H_2O)_6^{3+}$ complex. However, in a biological environment there are also potential ligands and/or reductants other than water. Thus the Latimer diagram is a general, simplified overview to both the intermediates and the final products, that is, the trivalent chromium complexes can also have other formulae, stability, and redox potentials [20–25].

Chromium can exist in 11 states ranging from −IV to +VI [26]. Trivalent chromium and hexavalent chromium have major environmental significance because of their stability in the natural environment. Hexavalent chromium has 100-fold more toxicity than trivalent chromium because of its high water solubility and mobility as well as easy reduction [27]. Thus the United States Environmental Protection Agency (USEPA) has laid down the maximum contaminant level (MCL) for hexavalent chromium in domestic water supplies to be $0.05\,mg\,L^{-1}$, while total chromium-containing trivalent chromium, hexavalent chromium and other species of chromium are regulated to be discharged below $2\,mg\,L^{-1}$ [28]. The toxicological effect of hexavalent chromium originates from the action of this form itself as an oxidizing agent, as well as formation of free radicals during the reduction of hexavalent chromium to trivalent chromium occurring inside the cell [29]. Research and carcinogenicity of hexavalent chromium have focused on the fact that chromate quickly pass through cellular and nuclear membranes, often *via* anion transporter routes, while the trivalent species are slower [30]. In fact trivalent chromium hydrolyzes smoothly at body pH to give an

insoluble hydroxide. Trivalent chromium is kinetically inert due to its d^3 electronic configuration, and the ligand-exchange process at trivalent chromium is very slow. After entry in the cytoplasm, chromate can either pass the nuclear membrane and be reduced to trivalent chromium or be reduced in the cytoplasm. Hexavalent chromium, not trivalent, reacts strongly with DNA; it is thought the reduction of hexavalent chromium to trivalent chromium, either in the cytoplasm, nucleus or the blood, produces free radicals which in turn can bind to DNA [31]. Breathing hexavalent chromium-containing material can cause perforation of the nasal septum, asthma, bronchitis, pneumonitis, inflammation of larynx, and liver and increased incidence of bronchiogenic carcinoma [32]. Skin contact of hexavalent chromium compounds can produce skin allergies, dermatitis, dermal necrosis, and dermal corrosion [33, 34].

Hexavalent chromium shows adverse effects on growth parameters and also causes accumulation of chromium in plants [35–38]; *via* plants it enters the food chain. Cr(VI) has been shown to induce a variety of DNA lesions such as strand breaks, DNA–protein cross-links, and DNA base modification [39–42]. Hexavalent chromium itself does not react readily with isolated DNA [43], but the reduction of hexavalent chromium by cellular reductants to its lower oxidation states, Cr(V), Cr(IV), and Cr(III), has been considered an important step [44]. While hexavalent chromium intermediates have been reported to induce DNA strand breaks *in vitro* and mutations in bacterial systems [45–47], free radicals generated by these chromium species at lower oxidation states may also be important in the mechanism of hexavalent chromium-induced carcinogenicity [48].

2. Sources of hexavalent chromium

2.1. *Natural sources*

In soil and rock

Chromium typically occurs in countries that have little use for it, whereas, most of the large industrial countries are deficient in it. Theoretically chromite is composed of 68% Cr_2O_3 (the oxide contains 68.4% chromium) and 32% FeO, however, Cr_2O_3 may be replaced by Al_2O_3, Fe_2O_3, MgO, CaO, and SiO_2, reducing the chromium concentration to as little as 40%. Commercial ores usually have a chromium concentration of at least 45%, with a Cr : Fe ratio greater than $2.5\,L^{-1}$ for metallurgical chromium. Most chromium ores are not adaptable to concentration processes, thus are marketed as lump $FeCr_2O_4$ following hand sorting or rough concentration. The ore is mainly marketed as ferrochromium that is produced *via* smelting in an electric furnace utilizing fluxes and carbon [2].

Primary chromium deposits are associated with magnesium (Mg) and nickel (Ni) in ultrabasic rocks or closely related anorthosite rocks; with rare exceptions, chromite is found in peridotites, anorthosites, and other similar ultramafic rocks. Almost all chromium deposits are formed *via* magmatic segregations in ultrabasic rocks, occurring as masses, lenses, and disseminations. During cooling of magma, chromite forms either by early crystal setting or later gravitational liquid accumulation. Chromite deposits occur in two basic forms: stratiform (or layered) and pod-shaped. About 98% of the deposits are stratiform in nature. The Stillwater complex in Montana and Bushveld

igneous complex in South Africa are examples of large-scale stratiform deposits. Smaller and high quality deposits also occur in Cuba, Pakistan, India, Yugoslavia, Greece, Brazil, and New Caledonia. Chromium-bearing rocks may have chromium concentration of 1000–3000 ppm, whereas gabbros and granite contain chromium at only about 200 and 5 ppm, respectively. Secondary deposits of chromium can develop as heavy mineral accumulations in placer and beach deposits (i.e., black sand). Under favorable tropical or subtropical conditions, lateritic soils may develop containing as much as 50% Fe and 2–4% chromium. Such reddish-colored soil results from the leaching of magnesium silicates from $FeCr_2O_4$ bearing ultramafic rocks [1b].

Chromite with a Mohs hardness index of 5.5 occurs in igneous rocks and to a lesser extent in sedimentary and metamorphic rocks. Host minerals including pyroxenes, amphiboles, micas, garnets, and spinel peridotites can become metamorphosed through hydrothermal alteration into serpentinite. As such, chromite commonly occurs within these rock bodies as euhedral crystals commonly form high concentrated zones of chromite within the serpentinites. Uvarovite is a green-colored chromium, garnet, and is frequently associated with chromite ore. Chromdravite is a chromium-bearing mineral of the tourmaline group. Chrome diopside is a bright-green variety of the mineral diopside (Cr_2O_3) which contains a small amount of elemental chromium. Picotite is a chromium-bearing spinel. A cherty-like rock called chrome chert is a chromite peridotite formed by the replacement of the silicate minerals by silica (SiO_2). Chromite has been found in placer deposits within certain sedimentary ore bodies. Chrome ochre is a bright-green chromiferrous clay containing 2–10.5% Cr_2O_3.

Chromite ore, one of the main sources of elemental chromium, can take on a high glass polish, is generally hard, lustrous, and has a steel gray color. Another economical chromium ore is crocoites, also called lead chromate ($PbCrO_4$). Crocoite is a bright, orange-red mineral with Mohs hardness index of only 2.5–3.0. The mineral has a specific gravity of 5.9–6.1. The fracture of crocoites is conchoidal. It forms as a secondary lead mineral in the zone of alteration in massive hydrothermal replacement deposits. Although crocoites are considered a rare mineral, the element chromium was first extracted from this chromium mineral [1b, 2, 3].

In crustal rocks, chromium concentration is estimated to be 140 ppm. However, carbonaceous meteorites have chromium concentrations estimated to be 3100 ppm. Chromium forms a variety of compounds such as fluorides ($CrF_2, CrF_3, CrF_4, CrF_5, CrF_6$), chlorides ($CrCl_2, CrCl_3, CrCl_4$), bromides ($CrBr_2, CrBr_3, CrBr_4$), iodides ($CrI_2, CrI_3, CrI_4$), oxides ($CrO_2, CrO_3, Cr_2O_3, Cr_3O_4$), sulfides ($Cr_2S_3$), selenides (CrSe), nitrides (CrN), and tellurides (Cr_2Te_3).

In surface and ground water

In natural water systems, chromium geochemically behaves in a unique manner [5]. Chromium(III) is the most common form of naturally occurring chromium, but is largely immobile with natural waters containing only trace amounts unless the pH is extremely low. Chromium can occur as hexavalent chromium and persist in polyatomic anionic form as CrO_4^{2-} under strong oxidizing conditions. Natural chromates are rare.

In atmosphere

Natural releases of chromium into the atmosphere arise from windblown sand, volcanic activity, forest fires, meteoric dust and sea salt spray or particles, although only windblown sand and volcanic activities are of importance. Worldwide fallout of

chromium to soil is estimated to be on the order of 4.6×10^4 metric ton Y^{-1} [49]. Bulk deposition of chromium is less than $0.2\,kg\,km^{-2}\,Y^{-1}$ in remote areas, $0.5–5\,kg\,km^{-2}\,Y^{-1}$ in rural areas, and generally more than $10\,kg\,km^{-2}\,Y^{-1}$ in urban areas.

2.2. *Anthropogenic sources*

The most common "metals" (usually not as the element) found at US superfund sites occur in the following order of frequency: lead, chromium, arsenic, zinc, cadmium, copper, and mercury (USEPA 1996 and 2000) [1a, 3]. In 1986, the USEPA established the national priority list (NPL) presenting approximately 1000 sites of which about 40% reported "metals" problems. Of these the most often cited "metals" in descending number of sites are lead, chromium, arsenic, zinc, cadmium, copper, and mercury. Of the superfund sites that contain elevated concentrations of these contaminants, 306 sites contain chromium as a major source of contamination. For chromium, sources of contamination include airborne sources (from industrial processes), contaminated soils, and direct chromium contamination in ground water.

The use of "heavy metals" in waterborne wood-preserving solutions has increased over time. In 1995, the use of heavy metals exceeded all other processes combined (AWPI 1996). The most widely used wood preserving formulation is copper–chromium–arsenate. Thus, nearly all wood preserving plants 20 years or older have reported soil and ground water contamination of varying degrees. Of these, about 71 are listed as Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA) NPL sites and at least 678 additional sites are known for wood-preserving operations currently or in the past (USEPA 1997). The most significant contaminant associated with Cu–Cr–AsO_4^{3-} (CCA) is hexavalent chromium [1a].

Anthropogenic chromium in soil

Soil, and under certain conditions, groundwater can become contaminated with chromium and other metals by direct infiltration of leachate from landfill disposal of solid wastes, sewage, or sewage sludge.

Anthropogenic chromium in agricultural materials

Chromium in food, mainly in the chromium(III) form, can be found in products, such as brewer's yeast, calf liver, cheese, and wheat germ. The total annual input of chromium into soils has been estimated to be between 4.35×10^5 and 1.18×10^6 metric tons [50]. From an agricultural perspective, chromium concentration in fertilizers, limestone, and animal manure is of interest. The amount of chromium is greater in fertilizers with phosphates [51]. The national research council of Canada (1976) reported chromium in phosphate fertilizers of approximately $30–3000\,mg\,kg^{-1}$. Although the amount of chromium entering the soil *via* phosphate fertilizers is uncertain, it is likely to exist as chromium(III). The amount of chromium utilized in phosphates and limestone (the latter being used to adjust soil acidity) is greater than what exist in native soils. Published values of chromium in limestone vary significantly ranging from $1–120\,mg\,kg^{-1}$, averaging $10\,mg\,kg^{-1}$. Animal manure contains little chromium.

Anthropogenic chromium in sewage sludge

Chromium derived in influent from natural, domestic, or industrial sources can concentrate in the organic resowing at sewage treatment facilities. Nearly 100% of chromium(III) can be removed from waste water sludge depending on the process and age of the sludge. However, only 26–48% of hexavalent chromium has been removed [52]. Non-industrial sources contributed about 48% of chromium in the influent to 12 sewage works in New York [53].

Chromium-containing effluents can be released *via* metal plating, anodizing, ink manufacture, dyes, pigments, glass, ceramics, glues, tanning, wood preservatives, textiles, and corrosion inhibitors in cooling water [51]; both chromium(III) and chromium(VI) may occur in waste water derived from these sources. Past practice, notably with plating operations, was to dispose of chromium waste water into dry wells. Also, it is not surprising that hexavalent chromium is predominant in plating wastes. Currently, such waste water is either treated on-site to reduce the chromium concentrations or at sewage treatment works. The hexavalent chromium is thus reduced by organic matter and the resultant sludge exhibits hexavalent chromium type compounds [54].

Anthropogenic chromium in coal and fly ash disposal

The largest amount of chromium applied directly to the land surface is by disposal of trapped and bottom fly ash (table 3) [50]. The disposal of large quantities of this ash on soil can result in elevated chromium concentrations relative to natural background levels, notably around coal-fired power plants. However, little chromium appears to be taken up by vegetation.

Anthropogenic chromium in mining and smelter wastes:

Although mining operations occur throughout the US, most of the mining for metals is situated in the western region of the country. In the US, the metals which constitute a significant domestic presence are iron, copper, lead, zinc, gold, and silver. No significant chromium mining operations are performed in the US. Despite this, chromium is noted as one of the contaminants of concern at some mine sites, such as Clear Creek/central city site in Colorado, and the Eagle Mine, located in Gilman, Colorado (USEPA, 1995b). However, internationally, chromium production has increased 10-fold during the period from about 1930 to 1980 [55]. In fact since 1900, chromium production has doubled each decade. By 1984 production reached 10.16 million metric tons.

Table 3. Chromium concentration in coal and fly ash.

Material concentration ($mg\,kg^{-1}$)	Chromium
Coal	15
Fly ash	152
Bituminous	172
Sub-bituminous	50
Lignite	43

Source: S.M. Testa [2] and references therein.

Emissions of chromium from chromate smelters can result in large quantities of CrO_4^{2-} waste; the coal valley in northern England was reported to be intensely phytotoxic [56a, 56b, 56c].

Anthropogenic chromium in roadside soils

Sources of chromium that affect roadside soils include chromium derived from the wear of chromium-containing asbestos brake linings in vehicles and aerosols produced from chromium catalysts used in emission reduction systems for treating exhaust fumes.

Anthropogenic chromium in water

In seawater, chromium is estimated to be at a concentration of 0.6 ppb. Surface water chromium concentrations are estimated as 1 ppb. Often, immobilization occurs by precipitation into solid phases as a result of changes in the pH or oxidation state (Eh). Chromium normally exists as the relatively immobile chromium(III) under reducing conditions and the highly mobile and toxic chromium(VI) under oxidizing conditions. Therefore, redox manipulation is the preferred method of immobilizing chromium [5]. Chromium(VI) can be reduced to chromium(III) by several means and chromium(III) will not easily re-oxidize to hexavalent chromium. The degree of chromium immobilization and the success of the proposed in-place treatments depend upon the general chemical processes including complex formation, surface sorption, and precipitation.

Anthropogenic chromium in the atmosphere

The largest total chromium released to the atmosphere is as suspended particles derived from metallurgical and chemical manufacturing industries and combustion of natural gas, oil, and coal. Other sources of chromium emissions in the environment can be derived from various consumer products, industrial wastes, cement producing plants, and exhaust emissions from catalytic converters. In a 1973 national survey, ferrochromium production was the most important with an estimated 11,213 metric tons Y^{-1}, even after control of air pollution. The production of refractory brick products was also noted as an important source releasing 1478.7 metric tons Y^{-1} to the atmosphere, followed closely by combustion of coal which released 1418.8 metric tons Y^{-1} with steel production releasing 471.7 metric tons Y^{-1}. However, a more recent study conducted over a decade later concluded that the iron and steel industry is the largest anthropogenic source of chromium emissions worldwide [50].

Chromium also occurs naturally as a trace component in most crude oils and can be found in residual and distillate oils. Chromium(III) is emitted from oil combustion, sewer sludge incineration, cement production, municipal waste incinerations, and refractories. In California, hexavalent chromium emissions are primarily from chromium plating. Chromium electroplating operations are conventionally conducted in baths containing chromic acid. During the plating process, bubbles of gas are emitted through the surface of the bath. These bubbles are entrained into the air. Firebrick lining of glass furnaces is another source of hexavalent chromium emissions. Lead chromate, a pigment used in yellow paint (i.e., for traffic lane demarcation), makes up a minor source of hexavalent chromium emissions.

2.3. *Uptake and transformation of chromium by biota*

Chromium can be taken up by biota from the air, water, and soil.

Bacteria: Microorganisms have the potential to accumulate chromium and reduce hexavalent chromium to chromium(III). High levels of hexavalent chromium are toxic to microorganisms. Chromium can be accumulated by bacteria periphytic to a crab (*Helice crassa*) carapace and by sewage fungus and may be contributing to the presence of chromium in the food chain. The active uptake of chromate by the sulfate transport system has been shown in *Neurospora crassa*.

The reduction of hexavalent chromium under anaerobic conditions by an *Enterobacteria cloacae* strain isolated from activated sludge was reported by Wang *et al*. [56d]. The rate of chromate reduction was found to depend on cell density and chromate concentration.

Plants: Chromate is reduced to chromium(III) at the surface of root cells. Dissolved hexavalent chromium may be taken up by plants without immediate reduction. For instance, chromate was found in the xylem sap of *Leptospermum scoparium* but not in the soluble plant fraction. Also, it has been shown that chromium(VI) enters the plant through root by active transport in barley (*Hordeum vulgare L.*). Chromium uptake is a metabolically mediated process *via* the sulfate pathway and thus chromium(VI) is readily transported through the plant.

Higher concentrations of chromium have been reported in plants growing in high chromium-containing soils compared with plant growing in normal soils. However, most of the increased uptake of chromium results in accumulation in the roots, and only a small fraction of total chromium is translocated to the above-ground part of edible plants. Leaves usually contain higher chromium concentrations than grains.

Plant tissues that accumulate iron also accumulate chromium. Most chromium accumulated by roots was present in a soluble, non-particulate form in the plant vacuoles [56b, 56c]. Although chromium is largely retained in the roots of plants, the oxidation state of chromium, the pH, and the presence of humic substances and plant species affect plant uptake and transport. Chromium is more available to plants growing in a neutral to basic soil than in an acidic soil, probably due to the stability of hexavalent chromium under these conditions.

Aquatic animals: A large number of bioaccumulation studies of chromium have been with finned fish. The assertion that chromium is ingested by fish (and perhaps bivalves) *via* food uptake was proposed by Kimbrough *et al*. [56e]. However, several researchers found that dissolved chromium passes readily through the gill membrane of fish and rapidly accumulate in other organs. Other researchers have shown that fish skin mucus contains molecules (such as protein-bound sulfhydril groups) capable of reducing hexavalent chromium non enzymatically.

3. Toxicity of chromium

The toxicity and fate of chromium in the body varies with its oxidation state. Chromium(III) and elemental chromium seem relatively innocuous.

Hexavalent chromium is a potent sensitizer of the skin. It also induces sensitization of the respiratory tract (although the phenomenon is not very frequent), induces mutations *in vitro* and *in vivo* and causes cancer in experimental animals and in humans [57–59].

The carcinogenicity of different hexavalent chromium compounds differs markedly. Strontium chromate ($SrCrO_4$) is apparently more carcinogenic by far than any other chromium compound, and calcium chromate ($CaCrO_4$) and zinc chromate ($ZnCrO_4$) are also relatively potent. These chromates are relatively insoluble in water. Different lead chromates are apparently carcinogenic but with a low potency.

In epidemiological studies on cancer in human, elevated lung cancer risks have been clearly and consistently observed in CrO_4^{2-} production (exposure mainly to chromium(III) and water-soluble hexavalent chromium compounds), in CrO_4^{2-} pigment production (exposure mainly to water-soluble and water-insoluble chromates), and also in chromium plating using chromic acid.

3.1. *Health effects among chromium-plating workers*

3.1.1. Effects on nasal mucosa and skin. Exposure to chromic acid (like other hexavalent chromium compounds) may induce nasal irritation, which in its extreme form may lead to nasal perforation [60–63]. Information on exposure concentrations and durations that cause the different nasal problems is very scanty. Nasal irritation in chromium-plating workers exposed to chromic acid mist at concentrations $>1\,\mu g\,m^{-3}$ had a high frequency of nasal perforations among workers exposed to peak chromic acid concentrations $>20\,\mu g\,m^{-3}$ [64a].

Scar formation was estimated to appear in nasal septum at cumulative exposure of 0.4–1 $\mu g\,m^{-3}$ for months (i.e., exposure to, e.g., 40–100 $\mu g\,m^{-3}$ for 10 months or 4–10 $\mu g\,m^{-3}$ for 100 months), while nasal septum perforation started to appear after an exposure of 1–3 $\mu g\,m^{-3}$ for months. Although chromic acid is acutely irritating, it seems that the risk of nasal ulceration increases with the time of exposure [63].

Because of the known carcinogenicity of hexavalent chromium compounds in general, possible precancerous lesions in the nasal mucosa have been investigated among chromium-plating workers. No increase in the frequency of micronuclei as an indication of genotoxic action was observed in exfoliated cells of the nose.

Chromic acid is irritating to the skin and induces skin ulceration which may lead to "chromium holes" and scar formation. Chromic acid exposure leads to skin ulcers only when there is a pre-existing cut, abrasion, or other defect in the protective epidermis [65]. Both sensitization and irritation may be behind a contact dermatitis on chromium-plating workers.

3.1.2. Lung cancer. Suspicion of an increased risk of cancer among chromium-plating workers was raised in a small study performed on decorative chromium-plating workers in the UK [60]; 49 lung cancer deaths were observed, while only 35 were expected. Based on studies published in the 1980s and earlier, the International Agency for Research on cancer concluded in 1990 that there is sufficient evidence of carcinogenicity of hexavalent chromium compounds as encountered in CrO_4^{2-} pigment production and chromium-plating industries.

3.1.3. Other respiratory diseases. Some studies have reported elevated respiratory symptoms and decreased pulmonary function among chrome-plating workers. No clear-cut picture emerges from the mortality from non-malignant respiratory diseases in the cohort studies (on lung cancer). Bronchial asthma is a rare disease after exposure to hexavalent chromium compounds, and cases have been reported also among chromium-plating workers [59–61]. Ingesting large amounts of hexavalent chromium can cause stomach upsets and ulcers, convulsions, kidney and liver damage, and even death. Hexavalent chromium is so toxic because reduction produces chromium(V), a known carcinogen that will lodge in any tissue to form cancerous growths. There are reports that chromium(V) is also a factor leading to premature senility in parts of Russia.

The acidity and action of enzymes on hexavalent chromium promotes the formation of small quantities of chromium(V). However, as the size of this is normally too large to be adopted by a tissue, the chromium(V) is likely to get lodged in some of the fine capillaries in the kidneys, intestines, or lungs [59–62]. During the passage, hexavalent chromium will continue to oxidize, leaving deposits of relatively safe chromium(III) and completely unsafe chromium(V) behind.

3.2. *Hexavalent chromium transport through anionic channels*

Transport of chromate through the cellular membrane (figure 1) with preservation of the hexavalent chromium oxidation state can be regarded as a part of pre-redox equilibrium involving hexavalent chromium species [65f]. The structural similarity of chromate, sulfate, and phosphate is an important factor in biological transport of hexavalent chromium. Assimilation of free anions takes place through the ionic channels. Arginine plays a significant role in CrO_4^{2-} binding by anion transporters through the formation of a network of hydrogen bonds. The function of Arg can also

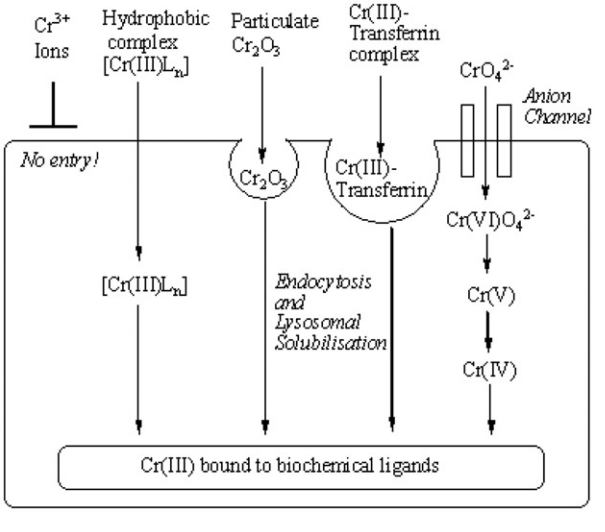


Figure 1. Cellular uptake and reduction of chromium compounds.

be considered in changes of coordination number of chromium on its redox pathway as the stabilizing factor of hexavalent chromium complexes by hydrogen bonding. Five-coordinate hexavalent chromium species can form and interact with the channel components in a way depicted in figure 2 [65a]. Studies on anion channel proteins have shown that they function as H^+/sulfate or Na^+/sulfate co-transporters and act in a narrow pH range of 5.0–5.5. Therefore, the chromium(VI)–Arg interactions are likely to involve comparable amounts of CrO_4^{2-} and $HCrO_4^-$ (the pK_a value for $HCrO_4^-$ at 25°C and $I = 0.5\,mol\,L^{-1}$ is 5.80). Thus, the anion–cation interaction and coordination abilities of tetrahedral anions may play a key role in transport in biological systems and may shed light on pre-redox equilibria of hexavalent chromium species.

3.3. *Molecular mechanism of hexavalent-chromium-induced carcinogenesis*

Waste water from different industries involving steel manufacture, chrome plating, and leather tanning is rich in chromium content. During tanning of leather, hexavalent chromium is reduced to chromium(III) that remains as polynuclear complexes bridging the protein chains by using carboxylate groups of the protein chains. This complexation prevents putrefaction. Chromium(III) present in tannery waste is gradually oxidized to hexavalent chromium by dissolved oxygen. Chromium as chromate also enters into the water bodies from cooling towers.

Chromium(III) can exist as $Cr(OH)^{2+}$, $Cr(OH)_2^+$, $Cr(OH)_3$, $Cr(OH)_4^-$, depending on pH. Chromium(III) being kinetically inert forms complexes with the surrounding ligands very slowly. Under oxidizing conditions chromium(III) is oxidized to chromium(VI) which can enter into the cell as chromate through SO_4^{2-} uptake pathway. Chromium(VI) undergoes stepwise reduction in interaction with biological thiol compounds. Intermediate states like chromium(V) and chromium(IV) are formed during the reduction of chromium(VI) to chromium(III) [65f]. These intermediate states are labile forming complexes with available biomolecules (including genetic molecules) acting as ligands. The intermediates are finally reduced to chromium(III) complexes. The bound ligands at chromium(IV) and chromium(V) are ultimately retained with Cr(III). It is believed that during the reduction of chromium(VI) to chromium(III) *via* chromium(V) and chromium(IV) interaction with DNA is responsible for carcinogenesis. It is suggested that glutathione reacts with chromium(VI) leading to the stepwise reduction of chromium(VI) through one-electron transfers. In these one-electron

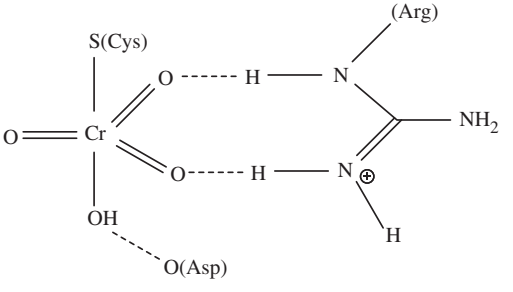
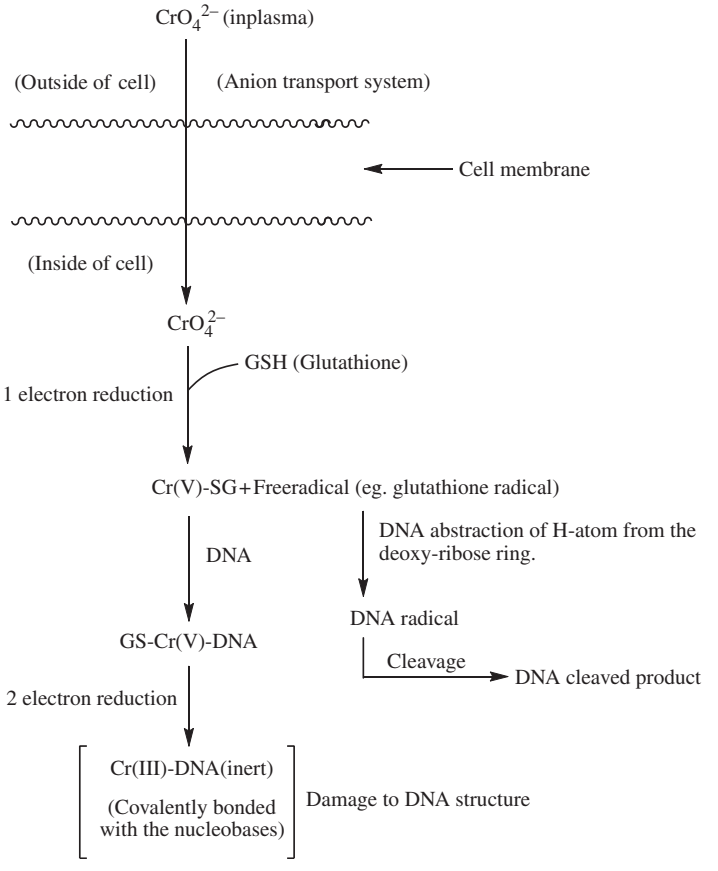


Figure 2. One possible mode of interaction of chromium(VI) transient complex with protein amino acids.

transfer steps, different free radicals are generated and these show the cytotoxic action (scheme 1).

3.3.1. Reduction of chromium by low molecular weight molecules. Chromate salts produce the greatest genotoxic response in the shortest period of time [65c, 65d]. Chromium salts also exhibit the ability to form mutation-inducing cross-links between DNA and protein [65e].

The complex intracellular redox cycling of chromium is thought to produce a range of reactive species as well as producing DNA–protein cross-links. Generally, Cr(VI) on entry into cells is rapidly reduced by interaction with any of a number of low molecular weight thiols, from glutathione (GSH) to cysteine, as well as a range of other reductants, such as ascorbic acid, hydrogen peroxide, or cytochrome P450 reductase. Of these, GSH [66, 67], cysteine [68], lipoic acid [69], and diol containing molecules such as NAD(P)H [65c], ribose, fructose, and arabinose [70, 71] have been shown to reduce chromium(VI) *in vitro* at physiological pH. The reduction process itself occurs either by sequential single-electron transfers, progressively reducing Cr(VI) to Cr(V) and then



Scheme 1. Schematic representation of uptake-reduction model for chromate-induced carcinogenic action. Chromium(VI), chromium(V), chromium(IV) are labile while chromium(III) is inert.

Cr(III), or by a two-electron transfer to Cr(IV), then by single-electron transfer to Cr(III) [65c, 66b]. These reactions can produce a variety of other reactive intermediates and provide the mechanism for cross-linking of DNA to proteins by a bifunctional Cr(III) intermediate. Both the oxidative DNA damage caused by redox reactive intermediates and, more importantly, the Cr(III)-mediated DNA–protein cross-links [65e] can cause mutations, initiating the process of carcinogenesis. Similarly, the interaction of reactive species may also alter cell-signaling pathways causing alterations in gene regulation [65c].

Ascorbate and GSH may be the most likely candidates as non-enzymatic chromium(VI) reductants, especially because of their ubiquitous occurrence in mammalian cells. Glutathionyl radical ($GS^{\cdot}$) and chromium(V) and chromium(IV) complexes were generated in the reduction of chromium(VI) by GSH. These complexes can be isolated as solids [66, 71]. Stable chromium(V) and chromium(IV) solids can be used as model compounds to study the role of intracellular chromium(IV) and chromium(V) in the mechanism of chromium(VI)-induced carcinogenesis.

Reduction of chromium(VI) by ascorbate is kinetically favored over GSH and intratracheal injection of chromium(VI) has been reported to generate ascorbate but not GSH in rat lung [72]. The chromium(V) and chromium(IV) generated by ascorbate reduction have been shown to react with hydrogen peroxide to produce hydroxyl radicals ($OH^{\bullet}$), which caused DNA strand breaks [73].

Reduction of hexavalent chromium by cellular reductant enzymes, organelles, and intact cells

A variety of enzymatic and non-enzymatic factors function as chromium(VI) reductants. These factors include microsomes, mitochondria, and several flavoenzymes, such as glutathione-reductase (GSSG-R), lipoyl dehydrogenase, and ferredoxin–$NaDP^{+}$ oxidoreductase [74]. Among these, glutathione reductase is discussed here as an example. In the presence of NADPH, glutathione reductase reduces chromium(VI) to generate chromium(V), which was identified as a chromium–NADPH complex. During the reduction process, molecular oxygen is reduced to O_2^-, which generates hydrogen peroxide *via* dismutation.

3.3.2. Free radical generation

Thiyl radical generation

Using ESR spin trapping, the formation of chromium(V) and glutathione-derived thiyl radicals ($GS^{\cdot}$) were detected in the reaction of chromium(VI) with GSH. The thiyl radicals generated by this reaction may cause direct cellular damage. These radicals may also react with other thiol molecules to generate O_2^- radicals (equations (6) and (7)) [75, 76].

$$GS^{\bullet} + RSH \rightarrow RSSR^{-} + H^{+}, \tag{6}$$

$$RSSR^{-} + O_2 \rightarrow RSSR + O_2^{-}. \tag{7}$$

The generation of O_2^- radicals leads to the formation of hydrogen peroxide and O_2^- is able to cause additional oxygen radical generation, for example, by reducing chromium(VI) to chromium(V) and a subsequent reaction with hydrogen peroxide as discussed in the following sections.

Hydroxyl radical generation by chromium(V) reaction

The $OH^{\bullet}$ radical is generated in the reaction of chromium(V) with hydrogen peroxide *via* a Fenton-like reaction (equation (8)).

$$Cr(V) + H_2O_2 \rightarrow Cr(VI) + OH^{\bullet} + OH^{-}. \quad (8)$$

Chromium(V)–NADPH generated by the reaction of chromium(VI) with NADPH was used to verify the above reaction [70, 71, 74, 77]. A mixture of chromium(VI), NADPH, and hydrogen peroxide generated both chromium(V) and $OH^{\bullet}$ radical. The reactivities of chromium(V) species depend on their structure [78]. For example, tetraperoxochromate $\{[Cr(O_2^{2-})_4]^{3-}\}$ has a tetrahedral structure with all covalent bonds fully occupied by O_2^{-}. The $\{[Cr(O_2^{2-})_4]^{3-}\}/H_2O_2$ complex does not easily split H_2O_2 to generate $OH^{\bullet}$ radical. On the other hand, a chromium(V) complex, such as chromium(V)–NADPH, is octahedral, with one vacant site. Hydrogen peroxide can attach to the vacant coordination site and form a long-lived complex to generate $OH^{\bullet}$ radicals.

Hydroxyl radical generation by hexavalent chromium reaction

Tetravalent chromium is the other reactive chromium intermediate generated in the reduction of hexavalent chromium by cellular reductants, such as ascorbate and GSH. Tetravalent chromium is able to generate $OH^{\bullet}$ from hydrogen peroxide *via* a Fenton-like reaction (equation (9)) [78a].

$$Cr(IV) + H_2O_2 \rightarrow Cr(V) + OH^{\bullet} + OH^{-}. \quad (9)$$

The reaction of hexavalent chromium with ascorbate was used as a source of tetravalent chromium. Hydrogen peroxide reacts with both pentavalent chromium and tetravalent chromium competitively to generate $OH^{\bullet}$ radicals. By adding equations (8) and (9) the net reaction becomes that described in equation (10).

$$Cr(IV) + 2H_2O_2 \rightarrow Cr(VI) + 2OH^{\bullet} + 2OH^{-}. \quad (10)$$

Hydroxyl radical generation by a hexavalent chromium and trivalent chromium mediated the Haber–Weiss reaction:

Xanthine and xanthine oxidase were used as a source of O_2^{-} radicals; O_2^{-} was able to reduce hexavalent chromium to pentavalent chromium (equation (11)), which in turn reacted with hydrogen peroxide to generate $OH^{\bullet}$ and hexavalent chromium (equation (8)).

$$O_2^{-} + Cr(VI) \rightarrow Cr(V) + O_2. \quad (11)$$

By combining equations (8) and (11), we obtain equation (12).

$$O_2^{-} + H_2O_2 \rightarrow O_2 + OH^{\bullet} + OH^{-}. \quad (12)$$

Similar to hexavalent chromium, trivalent chromium is also able to generate $OH^{\bullet}$ radicals *via* the Haber–Weiss cycle [79a, 79b, 79c].

The metal chelators, deferoxamine, 1,10-phenanthroline, and EDTA, decreased the $OH^{\bullet}$ generation, showing that proper coordination of trivalent chromium is required for trivalent chromium-mediated $OH^{\bullet}$ generation. The Haber–Weiss mechanism of $OH^{\bullet}$ generation could become particularly significant during phagocytosis when macrophages and their cellular constituents generate a large quantity of O_2^{-} radicals

during respiratory burst. It has been reported that a significant portion of oxygen consumed by phagocytes is converted to O_2^- [79d, 79e]. The finding that hexavalent chromium or trivalent chromium can act as a Haber–Weiss catalyst may provide a basis for the critical role of molecular oxygen in the genotoxic and carcinogenic reactions of chromium(VI)-containing particles.

3.3.3. Cr–DNA adducts. Small Cr–DNA adducts are the most abundant form of Cr(VI)-induced genetic lesions in mammalian cells [65e] and were found responsible for all mutagenic damage generated during Cr(VI) reduction with cysteine [78c] and ascorbate [78d]. The majority (50–75%) of adducts generated during *in vitro* Cr(VI) reductions are binary Cr–DNA complexes [78e, 78f]. Binary adducts (figure 3) are only weakly mutagenic [78d, 78g], and their existence in cells is uncertain because of the presence of numerous Cr(III)-binding small molecules. Ternary Cr–DNA adducts can be disrupted during DNA isolation [78e], which produces binary adducts and further complicates the assessment of the real levels of these small adducts. The predominant form of Cr–DNA complexes in cells are ternary adducts (cross-links), which include Cr(III) bridging DNA and small cellular molecules (L–Cr–DNA). Four major forms of ternary adducts are glutathione–Cr–DNA, cysteine–Cr–DNA, histidine–Cr–DNA, and ascorbate–Cr–DNA complexes [78e]. All ternary adducts were much more mutagenic than binary adducts, and ascorbate–Cr–DNA cross-links were the most potent premutagenic Cr–DNA modifications [78d, 78g]. Ternary adducts are formed through attack of DNA by preformed ligand–Cr(III) complexes. Binary adducts can also be generated in the direct reaction of newly formed Cr(III) with DNA [78f], but the possibility that a fraction of binary adducts results from the reaction of intermediate Cr forms, particularly Cr(IV), cannot be excluded. Cr(V) complexes exhibit little or no direct binding to DNA [78h], and the presence of Cr(V) in Cr(VI) reduction reactions is not required for the formation of Cr–DNA adducts *in vitro* [78d, 78e] or in cells.

DNA–protein and DNA interstrand cross-links

The formation of DNA–protein cross-links (DPC) by Cr(VI) is well-established in various biological systems [64b] and in the *in vitro* reactions. The overall yield of DPC in cells was estimated to be less than 1% of all Cr–DNA adducts, but it could be significantly higher *in vitro*. The availability of sensitive methodologies led to the frequent use of DPC measurements as a biomarker of Cr(VI) exposure in humans and aquatic species [64c]. The biological significance and repair of Cr-induced DPC remain

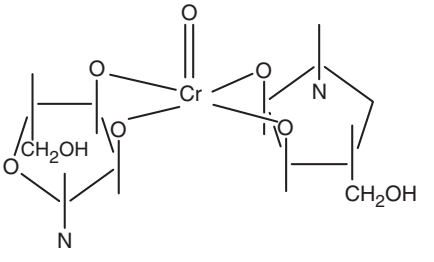


Figure 3. Proposed structure for the chromium(V)–nucleoside complex.

largely unknown; however, a very large size of these lesions would likely represent a major obstacle for the replication and transcription processes. Interstrand DNA cross-links have been detected only under certain *in vitro* conditions [64d, 64e], and on the basis of the severe steric restrictions for the intercalation of octahedral Cr(III) complexes, it was argued that interstrand cross-links were probably produced by Cr(III) oligomers. The presence of oligomeric Cr(III) forms is very unlikely inside the nucleus and the lack of Cr(VI) hypersensitivity in cross-link repair-deficient ERCC4(XPF)-null CHO cells [64f] provided a strong argument that DNA cross-linking is probably an *in vitro* phenomenon arising under conditions of high Cr(III) and low ligand concentrations. If formed, the interstrand cross-link would represent a potent block for cellular DNA replication [64g].

3.4. *Roles of free radical reactions in hexavalent chromium-induced carcinogenesis*

Using λ Hind III DNA digest, DNA damage induced by a mixture of hexavalent chromium and ascorbate with and without hydrogen peroxide has been assessed. DNA strand breaks were detected by agarose gel electrophoresis. A significant amount of DNA strand breaks occurred when DNA was incubated with hexavalent chromium and ascorbate. The amount of DNA strand breaks depended on relative concentrations of hexavalent chromium and ascorbate. Addition of hydrogen peroxide drastically enhanced the DNA damage. $OH^{\bullet}$ radical can interact with guanine residues at several positions to generate a range of products, of which 8-hydroxy-deoxyguanosine (8-OHdG) is important. Using single-cell gel electrophoresis, hexavalent chromium is able to cause DNA damage in the human prostate cell line, LNCap. Hexavalent chromium-induced DNA damage was stronger in Ras (+) cells, which over-express Ras protein than a wild type. NF-κB is considered a primary oxidative stress response transcription factor that functions to enhance the transcription of a variety of genes. The reduction of hexavalent chromium to low oxidation states is required for hexavalent chromium-induced NF-κB activation. Hydroxyl radicals generated by pentavalent chromium and hexavalent chromium-mediated Fenton-like reactions play a prominent role in the mechanism of hexavalent chromium-induced NF-κB activation [73].

AP-1 activation

Hexavalent chromium can stimulate the activity of another important transcription factor AP-1. This is a dimeric, sequence-specific DNA-binding protein composed of jun and fos products. A number of mitogen-activated protein kinase (MAPK) members participate in the activation of AP-1 hierarchically through divergent kinase cascades. Hexavalent chromium is capable of inducing AP-1 activation. The induction of AP-1 by hexavalent chromium is associated with phosphorylation of MAP kinase p38 and JNK, but not extracellular-signal-regulated kinase (ERK). Aspirin, an antioxidant inhibits the activation of AP-1 and NF-κB induced by hexavalent chromium.

p53 activation

More than 50% of human cancers contain mutations in the tumor suppressor protein p53 gene. This transcription factor is considered as one of the oxidative stress response transcription factors and can be activated in response to a variety of stimuli, such as UV, γ radiation, and nucleotide deprivation. Hexavalent chromium is able to activate

p53 in human lung epithelial cells (A549) by increasing the protein level and enhancing both DNA-binding activity and transactivation ability of the protein [80]. SOD, by enhancing the production of hydrogen peroxide from O_2^-, increased p53 activity. Deferoxamine, a metal chelator, inhibited p53 activation by chelating hexavalent chromium to make it capable of generating radicals from hydrogen peroxide. NADPH, which accelerated the one-electron reduction of hexavalent chromium to pentavalent chromium and increased $^{\bullet}OH$ radical generation, enhanced p53 activation. Thus $^{\bullet}OH$ radicals generated from hexavalent chromium reduction in A549 cells are responsible for hexavalent chromium-induced p53 activation (figure 4).

Tyrosine phosphorylation

Tyrosine phosphorylation is an important step in the regulation of many key cellular functions. It is involved in the control of cell proliferation, differentiation, cell-cycle regulation, cell-signal transduction, metabolism, transcription, morphology, adhesion, ion channels, and cancer development. Hexavalent chromium can increase tyrosine phosphorylation in human epithelial A549 cells in a time-dependent manner. N-acetylcysteine (NAC), a general antioxidant, inhibited hexavalent chromium-induced tyrosine phosphorylation. Catalase (a scavenger of hydrogen peroxide), sodium formate, and aspirin (scavengers of $^{\bullet}OH$ radical) also inhibited the increased tyrosine phosphorylation induced by hexavalent chromium. Hydrogen peroxide and $^{\bullet}OH$ radicals generated by cellular reduction of hexavalent chromium are responsible for the increased tyrosine phosphorylation induced by hexavalent chromium.

Apoptosis

Apoptosis is a process in which cell death is initiated and completed in an orderly manner through activation and/or synthesis of gene products necessary for cell destruction. Chromium(VI) is able to cause apoptosis. In the apoptotic signaling

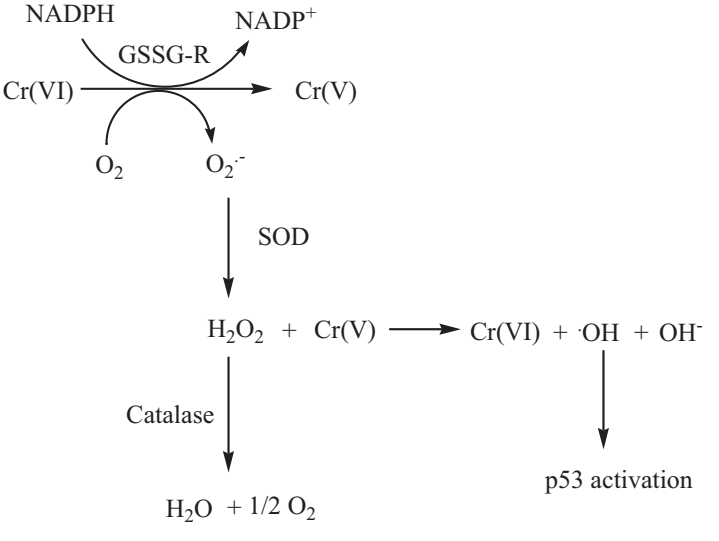


Figure 4. Schematic representation of possible mechanism of $^{\bullet}OH$ radical generation in Cr(VI)-stimulated A549 cells.

pathway, ROS generated from both chromium(VI) reduction and p53 activation play an important role. The chromium(VI)-derived ROS initiate apoptosis before activation of p53 protein (figure 5). ROS generated by hexavalent chromium may play a dual role in the mechanism of chromium(VI)-induced carcinogenesis: genetic damage and apoptosis. The chromium(VI)-induced carcinogenesis may depend on the balance of these two opposite processes [80b].

4. Removal technologies of chromium

Conventional methods for removing dissolved heavy metal ions include chemical precipitation [81], chemical oxidation and reduction [82], ion exchange [83], solvent extraction, membrane separation, cementation, evaporation, and foam separation. The chemical precipitation method involves a two-step process. The first step is the reduction of chromium(VI) under acidic conditions, followed by the precipitation of chromium(III) hydroxide. Commonly used reducing agents are sulfur dioxide, sodium sulfite, sodium bisulfate, and ferrous sulfate. An ion exchanger is a solid capable of exchanging either cations or anions from the surrounding materials. Commonly used matrices for ion exchange are synthetic organic ion-exchange resins. Chromium-removal efficiencies by electrochemical precipitation are greater than 99% and the residual chromium concentration is $<0.5\,\mathrm{mg\,dm^{-3}}$. In solvent extraction of chromium, several ion-association systems have been used such as triphenylsulfonium, triphenylphosphonium, tetraphenylstibonium, and triphenylselenium cations. The solvents used for the extraction of chromium(VI) are diethyl ether, isobutyl ketone, ethyl acetate, hexane, tri-n-butyl phosphate, and chloroform. Different strippants are used for the stripping of extracted chromium(VI) such as sodium hydroxide, sodium chloride, sodium nitrate, and sodium sulfite. However, these high-end processes have significant disadvantages, including incomplete metal removal, requirement of expensive equipment and monitoring systems, high energy requirements and/or generation of toxic sludge or other waste products that require disposal [84]. This has led to the

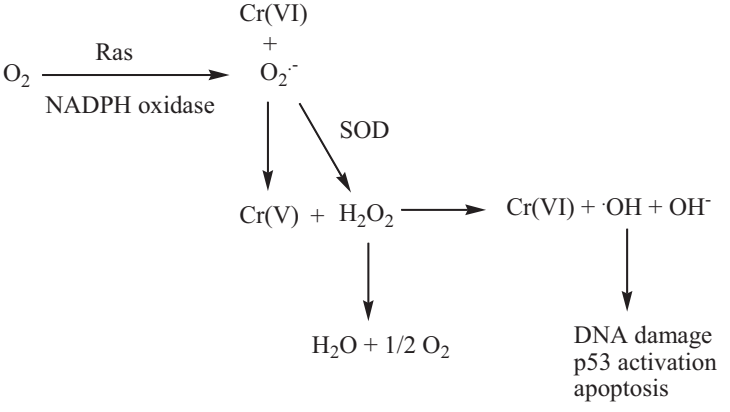


Figure 5. Schematic representation of possible mechanism of ROS generation in Cr(VI)-stimulated Ras(+) cells.

development of alternative low-cost technologies for the removal of Cr(VI) and other heavy metals from industrial effluents. In recent years, biosorption research has focused on using readily available biomass that can accumulate heavy metals. This process involves the use of biological materials that form complexes with metal ions using their functional groups. This process can be applied as a cost-effective way of purifying industrial wastewater whereby drinking water quality can be attained. A large number of materials have been investigated as biosorbents for hexavalent chromium removal. The tested biosorbents can be classified into the following categories, bacteria, fungi, algae, yeast, and agricultural products. Various functional groups present in the biosorbent material are responsible for biosorption. Several recent publications reported the utilization of locally available biological materials that can remove heavy metals from aqueous environments [85–88].

5. Conclusion

In this comprehensive review, the emphasis is on outlining the occurrences and carcinogenicity of hexavalent chromium. Hexavalent chromium contamination in ground water has generally been assumed to be anthropogenic contamination, since it is used in a number of industrial applications, including electroplating, tanning, industrial water cooling, paper pulp production, petroleum refining, paint and pigments, laboratory oxidant, and wood preservatives. Breathing of Cr(VI) containing material can cause perforation of the nasal septum, asthma, bronchitis, pneumonitis, inflammation of larynx and liver and increased incidence of bronchogenic carcinoma. Skin contact with Cr(VI) compounds can produce skin allergies, dermatitis, dermal necrosis, and dermal corrosion. A number of treatment methods for the removal of metal ions from aqueous solutions have been reported mainly reduction, ion exchange, solvent extraction, reverse osmosis, chemical precipitation, adsorption, and biosorption. Biosorption emerges as a potential technique for hexavalent chromium removal.

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