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Abstract.- Industrial wastes contain a variety of toxic chemicals, including heavy metals, which are carcinogenic and mutagenic. Heavy metals when present beyond traces are toxic to humans. Initially these may combine with proteins and may not cause any poisoning but when their concentration exceed the threshold level, they become a real health concern. These toxic metals interact with essential cellular components through covalent and ionic bonding. At high levels, both essential and non-essential metals can damage cell membrane, alter enzyme specificity, disrupt cellular function and damage the structure of DNA. Chromium is one of the major components of tanneries waste. Several toxic effects are associated with exposure to chromium compounds, including increased incidence of certain cancers, toxic towards living cells, tissue and organisms serious damage to such major organs as lung, liver and kidneys, pulmonary fibrosis and chronic bronchitis, skin ulcers, allergic dermatitis, lung cancer and mutagenic effect on bacteria and impairment of primary immune responses. Chromium VI induces chromosomal aberrations, mutations and transformation in cultured mammalian cells, variety of DNA lesions such as DNA single-strand breaks, alkali-labile sites, and DNA-protein cross links in kidneys, liver and lung nuclei and Chinese hamster ovary, mouse embryo firoblast and osteosarcoma cell. Cr VI selectively inhibits the activity of enzymes such as glutathione reductase. Chromium is an industrial carcinogen. The potential cancer risk in human extends to all forms of chromium and to total chromium. Carcinogenic chromium VI is persistent environmental contaminant with potential threat for human exposure through drinking water. Exposure of human being to Cr caused chronic hepatitis, liver cirrhosis and there is a potential for increased respiratory cancer risk. Lung cancer rates and death rates increased by gradient level of exposure to trivalent (insoluble) and to hexavalent (soluble) chromium.

Key words: Chromium toxicity, hexavalent chromium, trivalent chromium, gentoxicity.

INTRODUCTION

One of the major threats facing humanity in the present times is environmental pollution. It is the result of industrialization, urbanization and phenomenal population growth (Fitzgerald, 1993). Cities are becoming more and more polluted every day due to increasing discharge of untreated industrial and municipal wastes into the rivers and

coastal waters.

The intensive development of industries and water disposal without efficient emission control, to protect the ambient environment, may cause the accumulation of high amount of heavy metals in soils, which cannot be degraded by any natural biological process. Consequently these harmful substances have entered the food chain of man. These heavy metals have long half-lives and accumulate in the tissues (Moore and Ramamoorthy, 1984) and have varying toxic potentials in the soil (Mukerjee, 1998). At low concentrations heavy metals are essential to living system but are toxic at sufficiently high supra optimal concentrations (Wood and Wang, 1985; Klerk and Weiss, 1987; Dean-Ross and Mills, 1989; Christensen, 1995; Bruins *et al.,* 2000). Their toxicity occurs through displacement of essential metals from their natural native binding sites or through ligand interaction. Toxicity results from alterations in conformational structure of nucleic acid and protein interference with oxidative phosphorylation and osmotic balance (Poole and Gadd, 1989).

Industrial wastes contain a variety of toxic chemicals (Gilberg, 1974), including heavy metals, which are carcinogenic and mutagenic (Moore and Ramamoorthy, 1984; Gilberg, 1974; Degraeve, 1981; Chang *et al.*, 1998; Hamilton *et al.*, 1998; Zhitkovich *et al.*, 1998). Heavy metals when present beyond traces are toxic to humans. Initially these may combine with proteins and may not cause any poisoning but when their concentration exceed the threshold level, they become a real health concern (Jaffar, 1988). These toxic metals interact with essential cellular components through covalent and ionic bonding. At high levels, both essential and non-essential metals can damage cell membrane, alter enzyme specificity, disrupt cellular function and damage the structure of DNA (Bruins *et al.*, 2000; Blasiak *et al.*, 1999).

Contamination of the ecosystem by heavy metals is extremely pernicious because these contaminants are environmentally persistent. Metallurgical industries, chemical plants, thermal power plants and mining activities are mostly responsible for discharging heavy metals into the environment. In addition to the domestic emission, there are heavy metals, which are transported over long distances within air masses and eventually contaminate ecosystems. These are deposited by dry and wet deposition into the terrestrial and aquatic ecosystems of Asia, Europe and other countries, as well as return fluxes from these ecosystems to the air (Ruhling *et al.*, 1992; Pacyna *et al.*, 1993; Ebinghaus *et al.*, 1995).

Industrial wastes laden with heavy metal are posing serious problems in Pakistan where the environmental awareness is abysmally low. Waste recycling treatments and disposal of effluents is not according to world standards. In the province of Punjab, there are about 46,000 industrial units of various categories, out of which 4,600 units are considered to be the major contributors of pollution (Khalil *et al.*, 1991).

In Kasur the effluents from tanneries are discharged in open fields, rendering the agricultural land into waste land and the atmosphere absolutely smelly and the air unbreathable. The water of Bangla Kamboan (Kasur) has been reported to be harmful even for irrigation (personal communication). Hasnain and Sabri (1992) have reported the presence of toxic metals in concentration much higher than permissible limits in the industrial wastewaters of Lahore.

Chromium

Chromium is the 21st most abundant element in the earth's crust (Krauskopf, 1979). It is a naturally occurring element found in soil, water, air, rocks, plant, volcanic dust, gases and other biological materials (Wood and Wang, 1985, Goyer, 1986). Another source of chromium is chemical emissions in the manufacturing industry. It is ash gray in colour and chromium compounds have no taste and odor. Cr is # 24 on the periodic table. The concentration ranges from 0.1 ng/m^3 in rural areas to 0.03 μg/m^3 in industrial cities. The ground state electron configuration for the chromium atom is $1s^2$ $2s^2p^6$ $3s^2p^6$$3d^5$ $4s^2$.

Seventy percent of the world's chromite ore ((Fe, Mg) O (Cr. Al, Fe)$_2$ O$_3$) occurs in South Africa, followed by Albania, Turkey, India, and Zimbabwe. The stratiform-type of chromium ore deposition also occurs in northern Finland. The size of the deposition may ensure production for the next

hundred years, whereas world production is about 11×10^6 t years^{-1} (International agency for Research on Cancer, 1990).

The fate of chromium in soil is partly controlled by redox potential and soil pH. The behavior of four forms of chromium *i.e.* low trivalent forms (Cr^{3+} cation and CrO^-_2 anion) and two hexavalent forms ($Cr_2O_7^{2-}$ anion and CrO_4^{2-} anion) in soils are of most interest. Past studies indicate that chromium III is less toxic than chromium VI. It is relatively immobile and low in reactivity in the environment due to its strong adsorption capacity onto soils (Amacher and Baker, 1982; Bartlett and James, 1988). In contrast, chromium VI is highly unstable and mobile since it is poorly adsorbed onto soils under natural conditions (Bartlett and James, 1988; Weng *et al.*, 1994).

Adsorption and complication with MnO_2 inhibit the mobility of chromium III in the soil matrix; as a result, a large part of any chromium III will not be oxidized to chromium VI, even in the presence of MnO_2 and favorable pH condition of soils (Bartlett, 1991). Hexavalent chromium is an ionic form of the metallic element chromium (Cr) and is discharged by cement-producing plants; exhaust emission from catalytic converters in automobiles; waste from electroplating, leather tanning and textile industries; indiscriminate discharge into lakes and rivers; solid wastes from the manufacture of chromium compounds; consumer products such as inks, paints and papers; leather materials; stainless steel and a few other alloy metals; chrome-plated products; some toner powders used in copying machines. Higher exposure to chromium may occur to those who work in related chromium industries and those who smoke cigarettes (LCSS).

Chromium normally exists in oxidation states ranging from chromium II to chromium VI. The compounds of divalent chromium are usually blue and they demonstrate basic properties. Trivalent chromium compounds range from purple to green in color and are amphoteric. They are most abundant and most stable. The hexavalent chromium compounds are well known as laboratory reagent and manufacturing intermediates. They demonstrate acidic properties and their colours range from yellow to orange. The solubility equilibrium of hexavalent chromium compounds is complex and highly pH dependent (Weast, 1970; Dean, 1979, 1985; Linke, 1958). However, the three major forms of chromium that commonly exist in the environment are chromium (O), chromium (III) and chromium (VI.) Only the trivalent (III) and hexavalent (VI) forms are of biological significance. All forms of chromium can be toxic at high levels but hexavalent compounds are more toxic than its trivalent form. Metallic chromium O is relatively non-toxic. In the natural environment, Cr VI is less stable than Cr III; Cr VI will convert to Cr III in the presence of organic and inorganic reducing agent. It has been estimated that the average half-life for Cr VI in the ambient air is less than 24 hrs (an estimation of 13 hrs was given in 1988) (Research Triangle Institute, 1988).

The acid anhydride CrO_3 and acid chloride CrO_2Cl_2 and wide variety of metal chromates $MCrO_4$ and metal dichromate MCr_2O_7 are typical hexavalent chromium compounds. Cresser and Hargitt (1976) evaluated the chromate-dichromate equilibrium. Rieman *et al.* (1951) have evaluated the acid functions.

The solubility equilibria of hexavalent chromium and trivalent chromium compounds are complex (Table I) and are highly pH dependent (Weast, 1970; Dean, 1979, 1985; Linke, 1958).

On a global basis, chromium discharge to aquatic systems is mostly from the metal industries followed by domestic wastewater sources. The estimated maximum worldwide aquatic discharge is reported to be 239×10^3 t year^{-1} (Nriagu and Pacyna, 1988). Elevated levels of Cr in anthropogenically-polluted ecosystems are of serious human concern. Chromium is extensively discharged to the environment through a large number of industrial operations. Generally, the hexavalent form of chromate compounds is of greater industrial importance. Sodium chromate and dichromate are the principal substances engaged for the production of all chromium chemicals. Hexavalent chromium is used extensively in the chrome plating, manufacture of dyes and pigments, leather tanning and wood preservation industries. Chromium metal is used mainly in the making of steel and other alloys (Bell and Hipfner, 1997; Rodriguez-Pinero *et al.*, 1998),

Table I.- Solubilities of hexavalent and trivalent chromium compounds at 20-30°C.

Hexavelent compound	Solubility (mol kg^{-1} water)	K_{sp} [a]	Trivalent compound	Solubility (mol kg^{-1} water)	K_{sp} [a]
$(NH_4)_2CrO_4$	2.4		$Cr(CH_3OCOO)_3.H_2O$	Soluble	
$(NH_4)_2Cr_2O_7$	1.4		$(Cr(H_2O)_6)Cl_3$	2.2	
Li_2CrO_4	8.7		$(Cr(H_2O)Cl_2)Cl.2H_2O$	2.2	
$Li_2Cr_2O_7$	5.7		$Cr(OH)_3$		$1.2x10^{-28}$
Na_2CrO_4	5.2		$Cr(NO_3)_3.9H_2O$	5.2	
$Na_2Cr_2O_7.2H_2O$	9.7		$CrPO_4$		$2.4x10^{-23}$
K_2CrO_4	3.3		$CrK(SO_4)_2.12H_2O$	0.44	
$K_2Cr_2O_7$	0.44		$Cr_2(SO_4)_3.18H_2O$	3.1	
Rb_2CrO_7	2.6				
$Rb_2Cr_2O_7$	0.15				
Cs_2CrO_4	2.2				
$MgCrO_4.7H_2O$	5.2				
$CaCrO_4.2H_2O$	0.83				
$CaCrO_4$	0.00071				
$SrCrO_4$	0.0059	2.2×10^{-5} [a]			
$BaCrO_4$	0.000017	1.8×10^{-10}			
$PbCrO_4$	0.00000018	b			
Ag_2CrO_4	0.0000060	c			
$Hg2CrO_4$	Slightly soluble	2.0×10^{-9}			
$HgCrO_4$	Slightly soluble				
$HgCr_2O_7$	Insoluble [d]				
$CoCrO_4$	Insoluble				
$CuCrO_4$	3.6×10^{-6}				
$CuCr_2O_7.2H_2O$	Very soluble				
$ZnCrO_4$	Insoluble				
$ZnCr_2O_7.3H_2O$	Soluble				

[a] The K_{sp} for $SrCrO_4$ is the product of the equilibrium molar at a constant temperature, usually 298 K, *i.e.* $K_{sp} = (Sr^{2+})(CrO_4^{2-})$. The other K_{sp} values are similarly defined.

[b] Reported values range from 2.8×10^{-11} to 1.8×10^{-14}

[c] Reported values range form 9.0×10^{-12} to 1.1×10^{-12}.

[d] Soluble, molarity of saturated solution $>1 \times 10^{-1}$; insoluble, molarity of saturated solution $< 1 \times 10^{-3}$

in electroplating; in the manufacturing of dyes and pigments and in leather tanning. It is well known fact that chromium is essential for leather quality, such as strength, elasticity and thickness and for making bricks in furnaces (Grozza, 1984). In addition to the above-mentioned sectors, chromium is also used for water treatment, as a rust and corrosion inhibitor, in toners for copying machines, magnetic tapes and also in catalysts (International agency for Research on Cancer, 1990; USEPA, 1984).

Chromium was first determined to be essential for animals by Schwartz and Mertz (1963). Chromium is an essential co-factor for optimum function of insulin in mammalian tissue, required for normal metabolism of carbohydrates, proteins, and lipids, and as an active component of glucose tolerance factor (GTF) (Mertz, 1969, 1992), although GTF activity has been extracted form several biological sources, but is primarily from brewer's yeast.

Chromium is thought to potentiate the action of insulin by facilitating insulin-receptor binding at cell surfaces, thus enhancing insulin sensitivity and responsiveness in peripheral tissues and reversing the effects of clinical hyperglycemia (Mooradin and Morley, 1987; McCarty, 1993; Morris *et al.*, 1993). Chromium increases the absorption of insulin, and helps to reduce body fat and build lean muscle. Biologically active *in vivo* forms of Cr known as low-molecular-weight chromium-binding substance (LMWCr), is a naturally occurring Cr-containing polypeptide (Davis and Vincent, 1997).

Insufficient dietary intake of chromium leads

to increase in risk factors associated with diabetes and cardiovascular diseases including elevated circulating insulin, glucose, triglycerides, total cholesterol, reduced HDL-cholesterol and impaired immune function. Chromium has also been shown to alleviate the diabetic symptoms and neuropathy of a patient on total parenteral nutrition (Jeejeebhoy *et al.*, 1977). Chromium was shown to be essential nutrient more than three decades ago when it was shown that rats fed on a Torula yeast-based diet developed impaired glucose tolerance that was reversed by an insulin-potentiating factor whose active component was shown to be trivalent Cr (Mertz and Schwarz, 1959; Mertz *et al.*, 1994).

The essentiality of dietary Cr has been demonstrated in numerous species, such as the mouse (Schroeder *et al.*, 1963), rat (Schwarz and Mertz, 1963; Schroeder *et al.*, 1965; Mertz *et al.*, 1965), guinea pig (Seaborn *et al.*, 1994), turkey (Rosebrough and Steele, 1981), fish (Shiau and Lin, 1993), pig (Lindemann *et al.*, 1995) as well as children (Gurson and Sauer, 1973). For optimal health, as a supplement, women should take between 200-400 µg daily and men should take between 400-600 µg daily. On the other hand, elevated amounts of chromium may be hazardous to fauna and flora (Nriagu *et al.*, 1988; Bartlett and Kimble, 1976; Losi *et al.*, 1994) (Table II).

In human, Cr deficiencies result from metabolic and physical stresses, such as pregnancy, carbohydrate loading, extreme physical exertion, trauma, and disease (Borel *et al.*, 1984; Anderson, 1988, 1994, 1998), which increase glucose metabolism, accelerating Cr mobilization and urinary loss, and deplete body stores of Cr.

Table II.- Signs and symptoms of chromium deficiency.

Function	Species
Impaired glucose tolerance	Human, rat, mouse, squirrel Monkey, guinea pig, cattle
Elevated circulating insulin	Human, rat, pig, cattle
Glycosuria	Human, rat
Fasting hyperglycemia	Human, rat, mouse
Impaired growth	Human, rat, mouse, turkey
Hypoglycemia	Human
Elevated serum cholesterol and triglycerides	Human, rat, mouse, cattle, pig
Increased incidence of aortic plaques	Rabbit, rat, mouse
Increased aortic intimal plaque area	Rabbit
Nerve disorders	Human
Brain disorders	Human
Corneal lesions	Rat, squirrel monkey
Ocular eye pressure	Human
Decreased fertility and sperm count	Rat
Decreased longevity	Rat, mouse
Decreased insulin binding	Human
Decreased insulin receptor number	Human
Decreased lean body mass	Human, pig, rat
Elevated percentage of body fat	Human, pig
Impaired humoral immune response	Cattle
Increased morbidity	Cattle

Chromium requirements and intake

The Estimated Safe and Adequate Daily Dietary Intake (ESADDI) for chromium for children 7 years to adult is 50 to 200 µg per day (National Research Council, 1989). Anderson *et al.* (1991) demonstrated that consumption of normal diets in the lowest quartile of intake (less than 20 µg per day) led to detrimental effects on the glucose and insulin values in subjects with marginally impaired glucose tolerance (90 min glucose between 100 to 200 mg/dl following an oral glucose load of 1g/kg). Consumption of these same diets by control subjects (people with good glucose tolerance) did not lead to changes in glucose and insulin variables. This is consistent with previous studies demonstrating that the requirement for Cr is related to the degree of glucose intolerance. Not only is the dietary intake of chromium important, but also the total diet consumed. For example, increased intake of simple sugars lead to increased losses of supplemental chromium (Kozlovsky *et al.*, 1986). This becomes a double-edged sword since high sugar foods are often also low in chromium. Diets high in simple sugars lead to elevated levels of circulating insulin and once insulin increases, chromium is mobilized. Chromium does not appear to be reabsorbed by the kidney and is lost in the urine.

Chromium absorption via the gastrointestinal tract

Chromium absorption is inversely related to dietary intake (Anderson *et al.*, 1996). At daily

dietary intakes of 10 μg, chromium absorption is approximately 2% and at intakes of 40 μg is 0.5%. This leads to absorption of approximately 0.2 μg per day, which appears to be a minimal basal level. At dietary intakes above 50 μg Cr per day, chromium absorption is approximately 0.4%. The form of chromium also influences the absorption, *i.e.* absorption of chromium from Cr chloride is usually in the region of 0.4 % (Anderson *et al.*, 1983) and Cr from Cr picolinate approximately 1.2% at intakes of approximately 1000 μg per day (Campbell *et al.*, 1999). Chromium incorporation into rat tissues was shown to vary widely depending upon form (Anderson *et al.*, 1996). The highest concentrations of chromium were found in the kidney followed by liver, spleen, heart, lungs and gastrocnemius muscles. In 1996 Anderson *et al.* fed a low Cr diet containing 30±5 ng Cr/g of diet in control rat and rats given 5000 ng of Cr/g of diet in the form of Cr chloride, Cr histidine and Cr nicotinate for three weeks, all displayed similar tissue levels of Cr. Rats given chromium in the form of Cr alum (Cr potassium sulfate), Cr nicotinic acid histidine, Cr picolinate, Cr acetate, Cr glycine and a Cr nicotinc acid glycine, cystine glutamic acid complex all displayed significantly greater levels of tissue Cr than the control animals.

In addition to form, oxidation state and route of administration, ascorbic acid, carbohydrates, phytate, oxalate, aspirin, antacids and indomethacin also alter Cr absorption (Stoecker, 1999). Ascorbic acid was shown to significantly increase Cr absorption in humans (Offenbacher, 1994), with similar results in rats (Seaborn and Stoecker, 1992). Using radioactively labeled Cr chloride, animals fed starch were shown to have higher Cr absorption than those fed sucrose, fructose or glucose (Seaborn and Stoecker, 1992). Phytate has been reported to have either no effect on Cr absorption (Keim *et al.*, 1987) or an inhibitory effect (Chen *et al.*, 1973). Oxalate also inhibits Cr absorption. Prostaglandin inhibitors such as aspirin and indomethacin enhance Cr absorption (Kamath *et al.*, 1997) and antacids, such as Maalox and Tums, inhibit Cr absorption (Davis *et al.*, 1995).

Chromium excretion

Chromium levels in the blood and urine are biomarkers of exposure, reflecting the amount of chromium, which is internalized in the body. However, whereas chromium in erythrocytes is diagnostic for internal exposure to chromium VI, it is not possible to distinguish, on the basis of urinary chromium, whether exposure to chromium VI or chromium III has occurred (Kortenkamp, 1997). In fact, as the result of chromium VI reduction in the blood, all chromium detectable in the urine of exposed individuals is chromium III (International agency for Research on Cancer, 1990 Kerger *et al.*, 1997; Minoia and Cavalleri, 1988).

Absorbed chromium is excreted primarily in the urine and only small amounts are lost in the hair, perspiration or bile. Therefore, urinary Cr excretion can be used as an indicator of Cr absorption. Cr excretion alters with various stresses including high sugar intake, exercise, infection, pregnancy, lactation and physical trauma (Anderson, 1994). The more serious the stress, the greater the losses of chromium. Increase in exercise intensity leading to greater degrees of stress assessed by the stress hormone, cortisol, correlated with increase in urinary Cr losses (Anderson *et al.*, 1991). While urinary Cr losses are an indicator of stress and recent occupational exposure, they cannot be used to assess Cr status.

Urinary Cr losses for subjects consuming normal diets are approximately 0.1 to 0.3 μg per day with urinary chromium: creatinine ratio in the region of 0.1 to 0.2 ng Cr per mg of creatinine (Anderson *et al.*, 1982, 1983, 1991; Randall and Gibson, 1987). Supplementation of control subjects with 200 μg of Cr as Cr chloride for three months increased the chromium: creatinine ratio to 1.43 mmol/mmol of creatinine (Anderson *et al.*, 1982). Levels from nutritional studies that lead to improvements in glucose, insulin and blood lipids are in the same range as occupational exposed subjects such as tannery workers.

Beneficial effects of supplemental chromium

The beneficial effects of supplemental Cr on human subjects have been reviewed by Anderson (1998). There are more than 30 studies reporting beneficial effects on subjects with varying degrees of glucose intolerance ranging from marginally elevated to those classified with overt diabetes.

Subjects with varying levels of blood lipids have also been shown to improve following Cr supplementation with the greatest improvements in total cholesterol, HDL-cholesterol and triglycerides in subjects with the highest initial levels. In the past ten years, Cr has been shown to improve the signs and/or symptoms of diabetes in people with glucose intolerance (Cefalu *et al.*, 1999) and type 1 (Ravina *et al.*, 1995), type 2 (Anderson *et al.*, 1997) and gestational (Jovanovic *et al.*, 1999). The amount of supplemental Cr shown to have beneficial effects in these studies ranged from 200 to 1000 μg per day (Anderson *et al.*, 1991; Ravina *et al.*, 1999). Involving 180 subjects with type 2 diabetes mellitus (type 2 DM), Cr effects were greater at 1000 μg per day than at 200 μg per day. The most dramatic improvements were shown in hemoglobin A1C, which is a reliable indicator of long-term glucose control. Hemoglobin A1C in the placebo group was 8.5±0.2%, 7.5±0.2% in the 200 μg group and 6.6±0.1% in the group of subjects receiving 1000 μg of Cr as Cr picolinate per day for 4 months. Laboratory values for control subjects normally range from 5.2 to 6.2%. Improvement in women with gestational diabetes were also greater in a group receiving 8 μg per kg body weight per day compared with those receiving 4 μg per kg body weight (Jovanovic *et al.*, 1999). Steroid-induced diabetes that could not be controlled by oral hypoglycemic medications and or insulin was also improved to acceptable levels in 47 of 50 people given 600 μg of Cr as Cr picolinate per day for 2 weeks followed by a daily Cr maintenance dose of 200 μg (Anderson *et al.*, 1997). Insulin sensitivity of obese subjects with a family history of diabetes also improved following 1000 μg daily of supplemental Cr as Cr picolinate (Cefalu *et al.*, 1999).

Safety of supplemental chromium

Trivalent Cr, the form of Cr found in foods and nutrient supplements, is considered one of the least toxic nutrients. The reference dose established by the U.S. Environmental Protection Agency for Cr is 350 times the upper limit of the Estimated Safe and Adequate Daily Dietary Intake (ESADDI). The reference dose (RfD) is defined as "an estimate (with uncertainty spanning perhaps an order of magnitude) of daily exposure to the human population, including sensitive subgroups, that is likely to be without any appreciable risk of deleterious effects over a lifetime" (Mertz *et al.*, 1994).

This conservative estimate of safe intake has a much larger safety factor for trivalent Cr than almost any other nutrient. The ratio of the RfD to the ESADDI or RDA is 350 for Cr, compared to less than 2 for zinc, roughly 2 for manganese, and 5 to 7 for selenium (Mertz *et al.*, 1994). Anderson *et al.* (1999) demonstrated a lack of toxicity of Cr chloride and Cr picolinate in rats at levels several thousand times the upper limit of the estimated safe and adequate daily dietary intake for humans (based on body weight). There was no evidence of toxicity in their study and there have not been any reported toxic effects in any of the human studies involving supplemental Cr.

Toxicity of chromium

Several toxic effects are associated with exposure to chromium compounds, including increased incidence of certain cancers, toxic towards living cells, tissue and organisms (Becker *et al.*, 1991; Deschamps *et al.*, 1995; Langard and Norseth, 1986; Langard, 1990; Leonard and Lauwerys, 1980) serious damage to such major organs as lung, liver and kidneys (Langard and Norseth, 1986; Kim and Na, 1991; Tandon, 1982), pulmonary fibrosis and chronic bronchitis, skin ulcers, allergic dermatitis, lung cancer and mutagenic effect on bacteria (Baruthio, 1992; Nishioka, 1975; Nestmann *et al.*, 1979; Green *et al.*, 1976; Petrilli and DeFlora, 1977) and impairment of primary immune responses (Graham *et al.*, 1978). The inhalation of Cr containing fumes, dusts and particles is primarily associated with respiratory tract damage (Deschamps *et al.*, 1995; Stern, 1983; Ishikawa *et al.*, 1994a,b), whereas exposure to forms that are internalized is associated with toxicity to other organs including liver and kidneys (Kirschbaum *et al.*, 1981; Tandon *et al.*, 1978). Epidemiological studies on occupational exposure to Cr compounds provide the primary source of information on Cr toxicity and carcinogenicity in humans (Deschamps *et al.*, 1995; Ishikawa *et al.*, 1994a,b; Costa *et al.*, 1996; Tailoli *et al.*, 1995), and the route of exposure

can influence which organs are most affected (Deschamps *et al.*, 1995; Ishikawa *et al.*, 1994a; Kirschbaum *et al.*, 1981; Tandon *et al.*, 1978). During the First World War, it was established that chromium compounds cause lung cancer (Shupack, 1991) and the carcinogenicity of chromium VI has been well established (Baruthio, 1992; IPCS, 1988).

Chromium had binding affinities to biomolecules, living systems and cause toxicity to biological life. Chromium trioxide and other chromium VI salts are moderately toxic substances by ingestion; 1 to 15 gs may be a fatal dose in humans. Ingestion of non-lethal doses of these compounds can cause stomach, liver and kidney damage. In spite of that 100 ppm (Cr VI) for 6 weeks in drinking water followed by a 140 days clearance period results in a significant accumulation of Cr in body tissues but does not affect water consumption or growth of the animals over the 6-weeks exposure period (Coogan *et al.*, 1991).

Acute oral toxicity

Oral chromium VI is not genotoxic at doses which greatly exceed the drinking water standards. Thus, ingestion of a bolus dose of 5 mg chromium VI by human volunteers did not result in any increase of DNA-protein crosslinks in peripheral blood lymphocytes (Kuykendall *et al.*, 1996). Administration up to 20 mg chromium VI, either in drinking water or by lavages, failed to produce any effect in the mouse bone marrow micronucleus assay or in the rat hepatocyte DNA repair assay (Mirsalis *et al.*, 1996). No adverse effects resulting from long-term exposures to chromium VI with drinking water. No adverse effect was observed in dogs fed 11.2 mg/l chromium VI for 4 years (Anwar *et al.*, 1961) or rats fed 25 mg/1 year (MacKenzie *et al.*, 1958) or 134 mg/6 months (Borneff *et al.*, 1968). Moreover, no health effect was reported in a family accidentally exposed to chromium VI at 1 mg/l in well water for 3 years (USEPA, 1996).

The NIOSH Registry data on the acute oral toxicity of trivalent chromium compounds range from 1900 to 3300 mg kg^{-1} body weight. By comparison, the LD_{50} for sodium chromate, a hexavalent compound, is reported to be 50-150 mg kg^{-1} body weight. Hexavalent chromium appears to be 10-100 times more toxic than the trivalent chromium compounds by the oral route of acute exposure (Table III).

Table III.- Oral acute toxicity of some chromium compounds in the rat.

Compound	LD_{50}	
	mg Cr kg^{-1}	mmol Cr kg^{-1}
$CrCl_6.H_2O$, chromium (III) chloride	1870	7.0
$Cr(CH_3CO_2)_3.H_2O$, chromium (III) acetate[a]	11260	46
$Cr(NO_3)_3 9H_2O$, chromium (III) nitrate	3250	8.1
CrO_3, chromium (VI) oxide	80-114	0.80-1.1
Na2CrO4, sodium chromate (VI)	52	0.32
$Na_2Cr_2O_7$, sodium dichromate (VI)	51	0.39
$K_2Cr_2O_7$, potassium dichromate (VI)	57	0.39
$(NH_4)_2Cr_2O_7$, ammonium dichromate (VI)	54	0.43

[a] Minimal bioavailability because of stability of this complex.

Chronic oral toxicity

Chromium VI can be ingested with drinking water, other beverages and food. Certainly, many food components have reducing properties. It has been shown that chromium VI is efficiently reduced when drinking water is used to prepare common beverages (Kerger *et al.*, 1996). Another way of exposure of the digestive tract is swallowing of chromium VI refluxed from airways via the muco-ciliatory escalator in individuals exposed by inhalation.

Ex vivo studies of chromium VI-reducing capacity of human digestive tract (DeFlora *et al.*, 1997), indicated that saliva reduces 0.7 to 2.1 mg chromium VI/individual/day and gastric juice reduces at least 80.3 to 84.5 mg chromium VI/individual (DeFlora *et al.*, 1987). Reduction of chromium VI by gastric juice is due to thermostable components of gastric secretions and is favoured by low pH. The reaction is complete within 10-20 min, and at least half of it is accomplished in 1 min (DeFlora *et al.*, 1997). It is also estimated that the daily removal of chromium VI with fecal bacteria is 15.4 to 33.4 mg/individual (DeFlora *et al.*, 1997). Intestinal bacteria contain high amounts of GSH (Owens *et al.*, 1986), which is an efficient reductant for chromium VI (DeFlora and Wetterhahn, 1989). Since bacterial GSH is released extracellularly

(Owens *et al.*, 1986), it is likely that chromium VI is additionally reduced in the intestinal lumen.

There is very poor intestinal absorption of orally introduced chromium VI (Kerger *et al.*, 1996, 1997; MacKenzie *et al.*, 1959; Donaldson and Barreras, 1966). In case chromium VI escapes reduction by saliva and gastric juice and sequestration by intestinal bacteria, it will be absorbed by the intestine, released into the blood of the portal system, and carried to the liver. The blood and in particular RBC has a considerable capacity of sequestering and reducing chromium VI. The human liver can reduce as much as 3.3 g chromium VI/individual (DeFlora *et al.*, 1997).

In most studies of the mechanisms of Cr VI toxicity, rodents or purified reducing agents have been used to elucidate potential reductive processes and their implications. Several cellular components in rodents can reduce Cr VI at physiological pH (Connett and Wetterhahn, 1983; Mikalsen *et al.*, 1991; Standeven and Wetterhahn, 1991, 1992). A significant physiological role for rodent microsomal enzymes is questionable, because of high k_m for chromate and extreme O_2 sensitivity. It is assumed that ascorbate is among the prominent nonenzymatic reductants of Cr VI in rodents (Standeven and Wetterhahn, 1991, 1992). In contrast, previous studies with liver and lung tissues from several humans demonstrated significant differences between rodents and humans with respect to microsomal Cr VI reduction. Specifically, the human system was much less oxygen-sensitive, had a much lower apparent K_m for chromate, and was not mediated by cytochromes P450 but rather by unidentified flavoproteins (*e.g.*, P450 reductase) and cytochrome b_5. There was little interindividual variability with respect to human hepatic microsomal Cr VI reduction (Myers and Myers, 1998; Pratt and Myers, 1993), suggesting that the reducing enzymes are present at similar levels in different individuals. Since the levels and nature of reductants can significantly influence the levels and types of reactive intermediates generated, it is questionable if the rodent studies should be extrapolated to humans as a means to thoroughly understand Cr toxicity.

Some hexavalent chromium compounds also induce tumors in rodents at the site of injection. Of the chromium carcinogens, the insoluble hexavalent chromium compounds appear to be the most potent in experimental animals. In contrast soluble and insoluble trivalent chromium compounds are usually not active as carcinogens.

MacKenzie *et al.* (1958) have evaluated the chronic toxicity of chromium compounds. They reported that rats that received either trivalent $CrCl_3$ or hexavalent $K_2Cr_2O_7$ at concentration up to 0.025 mg ml^{-1} in drinking water for a 1-year period showed no significant adverse effects, other than 20 % reduction in water consumption. Schroeder *et al.* (1965) reported no adverse effects in male and female rats during lifetime exposure to 5-ppm (0.005 mg ml^{-1}) chromium acetate in drinking water. Bread baked with 1-5 % (10-50 mg ml^{-1}) chromium III oxide was fed to rats for 5 days a week for 2 years. This chronic feeding exposure was correlated with dose-dependent decrease in the weights of livers and spleens and with a possible increase in the frequency of mammary fibrosarcomas (Ivankovic and Preussmann, 1975). The incidence of mammary fibrosarcomas was 7/180 (3.9%) in the exposed animals versus 3/120 (2.5%) in the control group. The low chronic oral toxicity of trivalent chromium compounds may be related to the low bioavailability of trivalent chromium from the compounds investigated.

Dermal toxicity

Dermal contact is the only possible route of exposure in humans to chromium VI. It is well known that direct skin contact with chromium VI may produce irritating and ulcerating effects and elicit an allergic response, characterized by eczema and dermatitis, in sensitized individuals (US Department of Health and Human Services, 1993). No significant increase in mortality for skin cancer (Becker *et al.*, 1991) or specifically for malignant melanoma (Langard *et al.*, 1990) could be detected in chromium VI-exposed workers.

High incidence of skin ulceration and nasal septum perforation was frequent consequences of occupational exposure to hexavalent chromium compounds in some industrial facilities for tanning, electroplating and chromate production. Tissue damage, irritation lesions of the skin and respiratory tract and cell mediated allergic reactions are also

caused by the exposure of hexavalent chromium (Samitz, 1955; Samitz *et al.*, 1962). The chrome ulcer generally occurred on exposed areas of the workers' bodies in contact with vapours, fumes and dusts containing hexavalent chromium. The nasal septum was frequently involved. The lesions were multiple or single and typically crusted and painless. Chrome ulcer sometimes extended to the underlying tissues and became very painful. The chrome ulcers were slow to heal and left atrophic scars on the skin and perforations of the nasal septum.

Samitz (1961) and Mali *et al.* (1963) reported that the trivalent form was not active in the elicitation of the allergic response. Fregert and Rorsman (1964, 1965, 1966), however, have observed positive epidermal tests with high concentrations of trivalent salts (0.5 M $CrCl_3$.$6H_2O$ and 0.1 M Cr_2 $(SO_4)_3$). Cohen (1966) found that all of his 44 chromate-sensitive subjects responded positively to intradermal testing with a 0.25% (9.4×10^{-3}) solution of $CrCl_3$, a trivalent compound. Samitz and Shrager (1966) reported that 5 % (0.125 M) Cr2 (SO_4) $_3$, 5% (0.187 M) $CrCl_3$ and 5% (0.0735 M) Cr_2 $(SO_4)_3$ produced positive reactions on intact skin only occasionally, and that positive reactions to these trivalent chromium compounds were more frequent when the epidermis was stripped away with tape prior to testing. These divergent results make difficult the identification of the species of chromium responsible for the allergic contact dermatitis.

Systemic toxicity

The initial toxic signs of ingesting hexavalent chromium compounds by humans are abdominal pain, vomiting, diarrhea and intestinal bleeding (Sanz *et al.*, 1990). These are followed by renal failure resulting from tubular necrosis (Michie *et al.*, 1991). Hepatic failure secondary to primary hepatocellular damage, encephalopathy, methaemoglobinaemia and hemolysis are frequent complications (Pedersen and Morch, 1978). Aggressive dialysis appears to be the best therapy for chromate ingestion (Saryn and Reedy, 1988), and the administration of ascorbic acid has been recommended to reduce the highly toxic hexavalent chromium to the less toxic trivalent form (Korallus *et al.*, 1984; Ellis, 1982).

Gregus and Klaasen (1986) have shown that the highest concentrations of radiotracer chromium were in the kidneys when the rats were administered intravenous injection of chromium III chloride. The relative distributions of chromium from hexavalent sodium dichromate between kidney and liver, however, was shown to depend upon the route of administration, *i.e.* intraperitoneal injection favoured accumulation in the liver while subcutaneous injection favoured accumulation in the kidney (Kim and Na, 1991).

The chromium from chromates intra-tracheally administered to rats is also capable of damaging kidneys and livers. Bragt and van Dura (1983) found that the concentrations of chromium in the kidneys and livers reflected the solubilities of the compounds after single doses to rats of Na_2CrO_4, $ZnCrO_4$ and $PbCrO_4$, having equivalent hexavalent chromium contents. The more soluble the compounds, the greater the accumulation in these organs. Similarly, Salem and Katz (1989) reported increased chromium concentrations in the kidneys and livers of rats that received whetlerite, a material containing both soluble and insoluble hexavalent chromium, and insoluble trivalent chromium, by intratracheal administration.

Toxicokinetics

Hopkins (1965) reported a biphasic urinary elimination pattern for ^{51}Cr administration to rats as single intravenous doses of $CrCl_3$ containing either 0.1 or 0.01μg of chromium. Mertz *et al.* (1965) reported a triphasic elimination pattern based on the whole body counting of ^{51}Cr in rats that received single doses of chromium III chloride by intravenous injection. The half times for the three components of the elimination pattern were 0.5, 5.9 and 83.4 days, respectively. Sayato *et al.* (1980) compared the urinary elimination of chromium from rats injected with ^{51}Cr-labeled sodium chromate and rats injected with ^{51}Cr-labeled chromium III chloride. They found that the ^{51}Cr from the hexavalent compound was excreted more rapidly than the ^{51}Cr from the trivalent compound. The respective half times that they reported were 22.24 and 91.79 days. Yamaguchi *et al.* (1983) also found that the urinary excretion of chromium from rats injected with hexavalent chromium from potassium

dichromate was more rapid than that from rats injected with trivalent chromium from chromium III nitrate.

Bragt and van Dura (1983) reported biphasic elimination patterns based on whole body counting of ^{51}Cr in rats that received intratracheal doses of ^{51}Cr-labeled chromates. The half times for the fast phase and slow phase elimination of chromium from sodium chromate (a soluble hexavalent chromium salt) were 5.0 and 71.1 days, respectively. For lead chromate (an insoluble hexavalent chromium compound) the corresponding half times were 1.9 and 94.8 days. Bragt and van Dura (1983) also reported that the clearance of ^{51}Cr from the blood was biphasic. The half times for the fast phases of blood clearance were 4.6 and 2.4 days for the rats that received $Na_2{}^{51}CrO_4$ and $Pb^{51}CrO_4$, respectively.

The biphasic blood clearance patterns and multiphasic urinary excretion patterns for chromium suggest the existence of several slow-releasing storage compartments. The distribution of chromium to/from these compartments is complicated by the possibility of different transports mechanisms of hexavalent and trivalent chromium and the potential for reduction of the former to the latter. Nearly 50 years ago, Gray and Sterling (1950) described the selective affinities of hexavalent and trivalent chromium for red cells and plasma proteins, respectively, and others (Zacharais, 1959; Borguet *et al.*, 1990) have subsequently investigated the nature of the chromium-protein interactions. Chromium appears to be mainly bound to transferring and to a lesser extent, to albumin. It is quite clear that some of the compartments to/from which chromium is distributed include kidney, liver, spleen and bone.

Cytotoxicity

Biedermann and Landolph (1990) evaluated the cytotoxicity of soluble and insoluble hexavalent and trivalent chromium compounds using diploid human fibroblastic cells derived from foreskins. The hexavalent chromium compounds were found to be 1000 times more toxic to these cells than were the trivalent chromium compounds. Cytotoxic and genotoxic events within the cell appear to involve the reduction of hexavalent chromium to the trivalent chromium state after penetration of the cell. Popper and Woldrich (1991) proposed that the cytotoxic of chromates to pneumocytes was caused by oxygen radicals possibly formed during an oxidative process and Susa *et al.* (1992) found that the cytotoxicity of chromates to HeLa cells (originating from human cervical carcinoma) was decreased when hexavalent chromium was reduced to the trivalent state with biologically occurring thiols. All six of the hexavalent chromium compounds studied $CaCrO_4$, Na_2CrO_4, CrO_3, $CaCr_2O_7$, $K_2Cr_2O_7$ and Na_2CrO_4, caused cytotoxicity in the 0.1-1.0 µM concentration range. The average concentrations of these hexavalent chromium compounds that allowed 50 % cell survival were 0.5 µM. The four trivalent chromium compounds studied $CrCl_3 .6H_2O$, Cr_2O_3, Cr_2Cl_3 and Cr_2S_3 induced dose-dependent cytotoxicity over a wider concentration range of 10-500 µM. The average 50% survival concentration was 50 µM (Biedermann and Landolph, 1990). Some typical results are summarized in Table IV.

Table IV.- Cytotoxicity of Cr (VI) and Cr (III) compounds.

Cr (Vi) as $K_2Cr_2O_7$		Cr (III) as $CrCl_3 .H_2O$	
µM	% lethality	µM	% lethality
0.05	16	2.5	25
0.1	50	5.0	13
0.2	92	10.0	25

Chromium toxicity and metabolism under low-level chronic-exposure conditions renal and hepatic alterations have been reported in humans and experimental animals exposed to high doses of chromium (Tandon *et al.*, 1978; Kaufman *et al.*, 1970; Major, 1922; Evans and Dail, 1974; Franchini *et al.*, 1978) but organ damage is not always apparent in experiments using low-level exposures (MacKenzie *et al.*, 1958; Glaser *et al.*, 1985, 1986, 1988; Diaz-Mayans *et al.*, 1986). Symptoms may include clammy, cyanotic skin, sore throat, gastric burning, vomiting, and diarrhoea. Chromic acid is irritating to the skin, and prolonged contact can cause ulceration. Inhalation of chromate dust or chromic acid mist can result in severe irritation of the nose, throat, bronchial tubes, and lungs and may cause coughing, labored breathing, and swelling of

the larynx. Respiratory function has been one of the numerous studied effects on the health of stainless steel welders (American Thoracic Society, 1979, 1987). Welding activity may involve atmospheric exposure to a number of fume components (British Occupational Hyiene Society Committee on Hygiene Standars, 1980; Cotes *et al.*, 1989). Fogh *et al.* (Fogh *et al.*, 1969) reported eye contact with chromium trioxide and its solutions can cause severe burns and possible loss of vision.

Occupational exposure to chromium VI compounds has been related to an increased risk of lung cancer and according to Houtman (1996) chromium also seemed to counteract the development of cardiovascular disease and hypertension. Many other studies address occupational exposure to chromium (Koponen *et al.*, 1981; Tsuchiyama *et al.*, 1997; Green *et al.*, 1977; Kiilunen, 1994). The respiratory tract is the only target for chromium VI (Houtman, 1996). The amount of chromium tended to be higher in upper lobes of the lungs than lower lobes. Further the concentration of the chromium is significantly higher in man than in woman and that smoking also elevated the chromium amount significantly and increase in chromium concentration depends upon occupational exposure to metal containing dust. The interlobular distribution in lungs is however, not influenced by sex, age and smoking habitat (Huvinen *et al.*,1996). Carcinogenesis in individuals performing certain occupational activities, which involve exposures to levels in air that were several orders of magnitude higher than those found in the natural environment (International Agency for Research on Cancer, 1990; US Department of Health and Human Services, 1993). It is well known that the respiratory tract has important non-specific defense mechanisms (Kiilunen, 1994). In addition, the chromium VI reducing mechanisms occur in the lower respiratory tract. In particular, the epithelial lining fluid (ELF) including the surfactant and bronchial-bronchiolar secretions that can be recovered by bronchioalveolar lavage has an overall reducing capacity of 0.9-1.8mg chromium (VI) (DeFlora *et al.*, 1997; Petrilli *et al.*, 1986). The ELF of both humans (Cantin *et al.*, 1987) and rats (Suzuki and Fukuda, 1990) is known to be particularly rich in antioxidants.

Within the respiratory tract, chromium VI is reduced in the epithelial lining fluid, pulmonary alveolar macrophages, bronchial tree and peripheral lung parenchyma cells. Hence, lung cancer can only be induced when chromium VI doses overwhelm this defense mechanism. The efficient uptake and reduction of chromium VI in red blood cells explains its lack of carcinogenicity at a distance from the point of entry into the body. All experimental and epidemiological data and the underlying mechanisms, point to the occurrence of thresholds in chromium carcinogenesis (Lou *et al.*, 1996; Godet *et al.*, 1996).

Pulmonary alveolar macrophages (PAM) are sweeping cells, which can reduce ~ 2 μg chromium VI/10^6 cells in rats (DeFlora *et al.*, 1986) and 4.4 μg chromium VI/10^6 cells in humans (Petrilli *et al.*, 1986). Based on the knowledge that 23×10^9 PAM populate terminal airways in humans, De Flora *et al.* (1997) estimated an overall chromium VI reducing capacity of 136 mg per individual. Since $1\text{-}5\times10^6$ PAM are removed every hour from human terminal airways via the muco-ciliatory escalator (Kiilunen, 1994), it can be calculated that the PAM population, which is renewed every day, will reduce 0.1-0.5 μg chromium VI. Thus, even in cases of long-term exposure, which require a continuous detoxification of inhaled chromium VI, the respiratory tract appears to have efficient reducing mechanisms, which, however, are not as formidable as those of the digestive tract.

The interaction between airborne exposure to chromium VI and cigarette smoke is an intriguing problem, which was not clarified by epidemiological studies (Langard, 1993). No synergistic effect between cigarette smoking and stainless welding was observed in the induction of chromosome damage in lymphocytes (Jelmet *et al.*, 1994). Smokers usually show higher urinary chromium levels than non-smokers (Kortenkamp, 1997), which might be ascribed to enhanced retention of particulates in the bronchial tree. Alternatively this finding may be interpreted as a stimulation of chromium VI reduction in smoke, leading to an increased urinary excretion of chromium III. In fact, a cigarette smoke condensate (CSC) decreased the mutagenicity of chromium VI in bacteria, presumably due to the presence of reducing agents

in CSC. At the same time, chromium VI inhibited the metabolic activation of either CSC or benzo(a)pyrene to mutagenic derivatives (Petrilli and DeFlora, 1982). The antagonistic effect between chromium VI and benzo (α)pyrene diol epoxide was recently shown by evalutating the frequency of HPRT mutants in cultured human fibroblasts (Tesfai *et al.*, 1998). Moreover, the PAM from current smokers exhibited a chromium VI reducing capacity, which was significantly higher than that of either ex-smokers or never smokers (Petrilli *et al.*, 1986). Similarly, preparation of peripheral lung parenchyma from smokers was significantly more efficient than those from non-smokers in decreasing chromium VI mutagenicity (Owens and Hartman, 1986). Therefore, there are some lines of evidence suggesting that, at least in certain steps of the carcinogenesis process, there is a less than additive effect between chromium VI and cigarette smoke. Using an acellular system by reacting a CSC, chromium VI and plasmid DNA, it has also been shown that this mixture results in an enhanced generation of OH radicals and induction of DNA single-strand breaks (Liu *et al.*, 1999).

Several hexavalent compounds of chromium, including chromium trioxide, are listed in IARC Group 1 (carcinogenic to humans) and are classified as "select carcinogens" under the criteria of the OSHA Laboratory Standard. Long-term exposure to chromium trioxide or chromium VI salts may cause ulceration of the respiratory system and skin. Exposure to chromium trioxide by inhalation or skin contact may lead to sensitization. Chromium trioxide has exhibited teratogenic activity in animal tests. The differential toxicological relevance of these chromium species mainly depends on the fact that Cr VI, in the form of chromate anion, which predominates the dichromate anion at physiological pH, is easily taken up via the general anion is chemical protein or band 3 protein. In contrast, cellular membranes are normally impermeable to Cr III cation, which can penetrate into cells only under particular condition (DeFlora *et al.*, 1989). Toxic effects of chromium VI to algae vary between 20 and 10000 $\mu g\ l^{-1}$ (Wong and Trevors, 1988).

Bacterial cells incorporate CrO_4^{2-} via their active transport system (Nies and Silver, 1989; Ohtake *et al.*, 1987). Some *in vitro* studies have shown that Cr VI is reduced through one-electron loss by intracellular reductants such as NAD(P)H to Cr V. This one-electron reduction from Cr VI to Cr V has been observed in various cases *in vitro*. O'Brien *et al.* (1985) showed that the reduced form of glutathione reacted with Cr VI, to generate Cr V (O'Brien *et al.*, 1985; Goodgame and Joy, 1986). Many compounds containing a diol residue, such as NAD(P)H, FAD, ascorbate, ethylene glycol, sugars (glucose, fructose, ribose, cellobiose, lactose, etc), glyceraldehyde, and oxalate, are also known to be able to reduce Cr VI to Cr V, resulting in formation of the respective complex with Cr V (Branca *et al.*, 1988, 1990; Shi and Dalal, 1990a,b; Goodgame and Joy, 1987a,b). Suzuki *et al.* (1992) reported that Cr V was formed during the reduction of Cr VI to Cr III by NAD(P)H-dependent Cr VI reductase of *Pseudomonas ambigua* G-1. Kawanishi *et al.* (1986) showed that double-strand DNA was cleaved randomly when DNA reacted with Cr VI and H_2O_2, or with Cr V. In addition, their results revealed the formation of OH and sing let oxygen during the cleavage of the DNA strand.

In deeper soils, S^{2-} and Fe^{2+} will reduce chromium VI to chromium III in the presence of anaerobic conditions. But the general rule of reduction is the presence of organic matter in the soil, which accelerates the reduction of chromium VI at any pH (Bartlett and James, 1988). In addition, various studies indicate possible reduction of chromium VI to chromium III by activated carbon under acidic conditions (Huang and Bowers, 1978; Neufeld *et al.*, 1990).

Once in touch with cells, the chromate anion easily crosses cell membranes (DeFlora and Wetterhan, 1989), after which chromate VI tends to be reduced inside the cell. Thus, chromium VI functions as a sort of Trojan horse, allowing penetration of chromium into the cell. Other types of cellular uptake, such as internalization of insoluble particles, have also been shown to occur *in vitro* (Singh *et al.*, 1998).

Chromium VI is reduced in different cell compartments. Reduction in the cell cytosol is greater than in the nucleus (DeFlora *et al.*, 1985). Chromium VI reduction is a composite process due to a network of mechanisms, which involve the contribution of reducing molecules, such as

ascorbate, glutathione (GSH), cysteine, hydrogen peroxide and riboflavin, as well as enzyme-catalyzed reactions *e.g.*, by cytochrome P450, aldehyde oxidase and DT diaphorase (DeFlora and Wetterhahn, 1989).

The explanation for the lack of carcinogenecity of chromium VI at a distance from the penetration site is that, in case chromium VI escapes detoxification in the respiratory tract, it will be released into the blood stream. Therefore, chromium can be transported to any organ via the blood circulation. In particular, while chromium III is transported in the blood plasma, bound to proteins, such as transferring (DeFlora and Wetterhahn, 1989), chromium VI is selectively accumulated in RBC. This is so typical that, for half a century, radioactive chromium (^{51}Cr) has been used to tag RBC (Gray and Sterling, 1950). After penetration into RBC, chromium VI is reduced to chromium III, especially by GSH, and bound to low molecular weight compounds and chiefly to hemoglobin (DeFlora and Wetterhahn, 1989; Kerger *et al.*, 1996, 1997; Aaseth *et al.*, 1982; Paustenbach *et al.*, 1996).

Petrilli and DeFlora (1978) demonstrated that chromium VI mutagenicity is lost in the presence of human RBC lysates. Whole blood has an overall sequestering capacity of 234 and 187 mg chromium VI/individual, and that RBC reduce at least 138 and 100 mg chromium VI/individual in males and females, respectively. The lifespan (mean±SD) of human RBC is 107±12 days (Lentner *et al.*, 1986). Therefore, every day a population of RBC in 19 ml in males and 14 ml in females will be renewed, thus accounting for at least 1.3 and 0.9 mg chromium VI reduced just by the proportion of RBC, which is, renewed daily. Also in 'old' RBC, however, there is a continuous replenishment of GSH, which is imported from the liver (DeFlora *et al.*, 1989).

Reduction of chromium VI results in the formation of chromium III, the stable reduced form, which binds DNA more efficiently than chromium VI (DeFlora *et al.*, 1990). Moreover, intermediate reduced products, *i.e.*, chromium V and chromium IV, are also suspected to play a role in chromium genotoxicity and carcinogenicity, either through reaction of reactive oxygen species, and in particular of the hydroxyl radical (OH) (DeFlora and Wetterhahn, 1989).

Oxidative DNA damage is detectable after exposure of cells to chromium VI *in vitro* (DeFlora and Wetterhahn, 1989; Singh *et al.*, 1987, 1998; Stearns *et al.*, 1995; Kasprazak, 1995), whereas Izzotti *et al.* (1998) reported its occurrence *in vivo* in lung cells but not in liver cells of rats.

De Flora *et al.* (1989) reported that the intracellular chromium VI reduction, leading to generation of reactive species, might be viewed as an activation process, when it occurs in the proximity of DNA. Alternatively, reduction is a detoxification process when it occurs far away from a DNA, and the reactive species can be trapped by a large number of ligands, nucleophiles and antioxidants, which are present in the intracellular environment. Therefore, the cellular site of reduction is crucial in affecting the fate of the cell taking up chromium VI. It is also noteworthy that Cr VI exposed cells can undergo apoptosis (Singh *et al.*, 1998) as a consequence of DNA damage, and therefore are eliminated from the organism.

Chromium mutagenicity can be induced by the generation of reactive oxygen intermediates during reduction of Cr VI by glutathione (Liu and Dixon, 1996). Cr VI also causes point mutation by inducing 4 hot spots in 104 bp domain of hprt exon 3 (Chen and Thilly, 1994). Anjum and Shakoori (1997) reported that the hexavalent chromium also causes genotoxic effects. They studied hepatotoxic effects of chromium on the liver function enzymes of male *Oryctolagus cuniculus* (white rabbit), with and without phenobarbitone and promethazine treatments. $K_2Cr_2O_7$ administered at a dose of 8 mg/kg body weight/day for 5 days, decreased serum AST (44%), LDH (63%) and AP (44%) activities. The hepatic AST, ALT and AP activities were likewise decreased (88%, 51% and 45%, respectively). Tezuka *et al.* (1991) reported that pretreated with Cr III relieves the acute lethal toxicity induced by carbon tetrachloride (CCl_4) in mice and rats, and reported that Cr III pretreatment of mouse suppressed both a decrease in blood sugar levels and an increase of AST and ALT activities which had been induced by CCl_4 exposure, whereas hepatic LDH and ICDH activities and AP activities were increased. Tezuka *et al.* (1995) reported *in vitro* protective effect of pretreatment with Cr VI to CCl_4 induced hepatotoxicity due to a rapid reduction

of Cr VI to Cr III. Gautam and Gupta (1989) have reported increase in the number of circulatory erythrocytes, hematocrit value, clotting time, leukopenia due to reduction in the number of small lymphocytes, thrombocytopenia and significant decrease in the ESR of fresh water fish after Cr administration.

Wang *et al.* (1994) reported that Cr-induced early change in renal function among ferro chromium producing workers. According to them urinary chromium (U-Cr) levels in the Cr-exposed group was approximately 1.8 times that of control group. They suggest that long-term exposure to water-soluble Cr VI produced chronic renal injury appear to mainly involve the proximal tubules. U-Cr concentration of >15 μg/g creatinine can be proposed as a threshold dosage for nephrotoxicity, significantly increased gamma- GT, NAG, ALP NAG are early sensitive indicators of renal injury.

Bagchi *et al.* (1995) reported that chromium and its salt induced cytotoxicity and mutagenicity. According to them chromium and its salts affect hepatic mitochondrial and microsomal lipid peroxidation and enhanced excretion of urinary lipid metabolism. Ptashekas (1992) monitored effects of hexavalent chromium on Lithuania population. By using electron microscope and immunohisto-chemical study human gastrointestinal endocrine cells revealed changes in the amount of secretory material and intracytoplasmic vacuolization after exposure to Cr VI. The most affected were EC (serotonin, heparin P), D (somatostatin), A (glucagon), B (insulin) and mast (histamine, serotinin, heparin) cells. The results provided ultrastructural evidence of digestive tract epithelial barrier reaction as an expression of environmental distress signal of the organism.

DNA damage

A number of studies evaluated the problem of biological monitoring by assessing cytogenetic end point, such as chromosomal aberrations, sister chromatic exchanges and micronuclei in lymphocytes of chromium exposed workers. In spite of the consistent genotoxicity of chromium VI compounds in *in vitro* test systems, the majority of cytogenetic surveillance studies among chromium platters, ferrochromium workers and stainless steel welders have yielded negative or inconclusive results (Kortenkamp, 1997). Similarly, either positive result was obtained by measuring DNA single-strand breaks in the lymphocytes of chromium-exposed workers (Werfel *et al.*, 1998). No oxidative DNA damage occurred in chromium VI production workers (Gao *et al.*, 1994), and no significant increase of DNA-protein cross-links was observed in chromium platters (Zhithovich *et al.*, 1996).

Chromium VI induces chromosomal aberrations, mutations and transformation in cultured mammalian cells (DeFlora *et al.*, 1989, 1990; Sugiyama, 1992; Venitt and Levy, 1974; Majone and Levis, 1979; DiPaolo and Casto, 1979). Chromium VI compounds produced a variety of DNA lesions such as DNA single-strand breaks, alkali-labile sites, and DNA-protein cross links in kidney, liver, and lung nuclei and Chinese hamster ovary, mouse embryo fibroblast and osteosarcoma cell (Sugiyama *et al.*, 1986). He also reported that Cr VI induced DNA single-strand breaks and cytotoxicity in a H_2O_2-resistant cell line (Sugiyama *et al.*, 1993) as well as selectively inhibiting the activity of enzymes such as glutathione reductase (Sugiyama *et al.*, 1993; Tsapakos *et al.*, 1983).

Chromium VI compounds readily enter the cells by the sulfate transport system (Gray and Sterling, 1950; Sugiyama, 1992). However, after entering the cells, chromium VI is reduced to trivalent form, through chromium V and IV intermediates by cellular reductants such as ascorbic acid, riboflavin, glutathione and flavoenzymes, including cytochrome P-450 reductase and glutathione reductase (DeFlora *et al.*, 1989, 1990; Sugiyama, 1992). Thus, the levels of these biological reductants and paramagnetic chromium species inside cells might be associated with chromium VI-induced damages. Reduction process generates radical species such as active oxygen species, as well as glutathionyl radicals, with concomitant formation of chromium VI (O'Brien *et al.*, 1985; Shi and Dalal, 1988; Sugiyama *et al.*, 1989, 1992; Goodgame, 1986; Aiyar *et al.*, 1990).

Previous studies reported that chromium V complex induced DNA breaks *in vitro,* and mutations in bacterial systems (Kawanishi *et al.*, 1986; Aiyar *et al.*, 1990; Rodney *et al.*, 1989;

Kortenkamp *et al.*, 1989). Biologically generated chromium V complexes react with H_2O_2, in a Fenton-type manner, to produce more hydroxyl radicals than a similar reaction with chromium VI (Aiyar *et al.*, 1990; Shi and Dalal, 1990).

Shi and Dalal (1990a,b) reported that Cr VI reacted with biological reductants and then generated Cr V, followed by generation of OH in the presence of H_2O_2 (Shi and Dalal, 1989; Goodgame and Joy, 1987a,b; Branca *et al.*, 1988; Suzuki *et al.*, 1992; Kawanishi *et al.*, 1986). They demonstrated by ESR analysis that generation of OH was accompanied by a decrease in Cr V. They proposed that the OH was generated by a Fenton-type mechanism of Cr V (Fig. 1). The OH generated by the reaction of Cr VI with H_2O_2 and glutathione caused damage of DNA strand and formation of 8-hydroxyguanin (Aiyar *et al.*, 1989, 1991). From these reports, it is evident that H_2O_2 may play an important role in the expression of Cr VI toxicity *in vitro*.

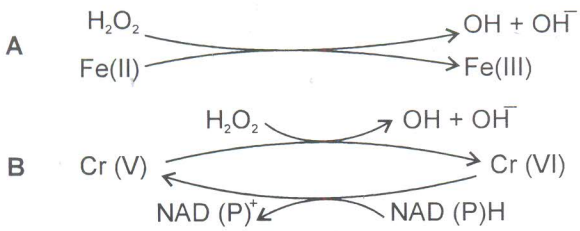

Fig. 1. Mechanism of Cr VI Toxicity in *Escherichia coli.* A, Fenton reaction of Fe (II); B, Fenton-type mechanism of Cr (V).

Chromate genotoxicity may involve two mechanisms. According to Beyersmann (1989) direct DNA damage is caused by an intermediate pentavalent chromium species and trivalent chromium, the end product from the biological reduction of chromates causes DNA-protein cross-links. Bronzetti and Galli (1989) appears to favor a genotoxic mechanism involving the induction of DNA breaks by the oxidizing activity of hexavalent chromium and the subsequent binding of the resulting trivalent chromium (Standeven and Wetterhahn, 1991; Shi and Dalal, 1992; Lefebvre and Pezerat, 1992; Lou *et al.*, 1996; Molyneux and Davies, 1995; Popper and Woldrich, 1991; Tsapakos *et al.*, 1983).

DNA single strand breaks and/or alkali-labile sites induced by chromate were decreased by increasing the levels of (vitamin) α-tocopherol or ascorbic acid, while increased riboflavin content enhanced the levels of both types of DNA damage (Sugiyama *et al.*, 1987, 1989, 1991). Similarly, the elevation of α-tocopherol or ascorbic acid in cells restored the glutathione reductase activity suppressed by enhanced enzyme inhibition.

Cell-permeable metal chelator *o*-phenanthroline suppressed the formation of chromium V intermediates, as evaluated by electron spin resonance spectroscopy at room temperature, resulting in a decrease of chromate-induced DNA breaks and/or alkali-labile sites, as well as in a recovery of glutathione reductase inhibited by this metal in cultured Chinese hamster V-79 cells (Sugiyama *et al.*, 1993a,b). In addition, hydrogen peroxide-resistant Chinese hamster ovary cells were found to have less total chromium V and simultaneously fewer DNA strand breaks than those in the parental cells (Sugiyama *et al.*, 1993a,b). Cellular levels of chromium V were correlated with the levels of chromate-induced DNA single strand breaks, alkali-labile sites, and the enzyme inhibition.

Sugiyama *et al.* (1992) studied the effects of increased levels of α-tocopherol or riboflavin on the induction of chromosomal aberrations and mutations at HGPRT locus by chromium (VI) in V-79 cells. The results showed that an increase of α-tocopherol suppressed the clastogenic and mutagenic action of chromate compounds, while the increased riboflavin resulted in an enhancement of both actions of this metal. These results suggest that chromium V may be associated with the clastogenic and mutagenic activity of chromate.

Thornalley and Vasak (1985) reported that the metallothionein can scavange free hydroxyl and superoxide radicals. The crucial amounts of Cr in the environment interact with biological life and cause cellular/DNA damage (Lou *et al.*, 1996; Snow, 1994). Dietary Cr supplementation increased proliferation of peripheral blood lymphocytes (PBL) *in vitro,* with or without mitogen concanavalin A (Con A) stimulation, in both beef calves (Chang *et al.*, 1994).

Chromium supplementation was shown to decrease markedly serum cortisol in stressed growing beef calves by 19 to 27% (Chang and Mowat, 1992; Moonsie-Shageer and Mowat, 1993;

Mowat *et al.*, 1993). A linear decrease in serum cortisol was observed with increasing amounts of dietary Cr supplementation (Moonsie-Shageer and Mowat, 1993). These results have been broadly interpreted to show that Cr has an "anti-stress" effect (Mowat, 1997). Similarly, supplemental Cr has also been demonstrated to reduce cortisol in human (McCarty, 1993), guinea pigs (Seaborn *et al.*, 1994), lambs (Ward *et al.*, 1995) and horses (Pagan *et al.*, 1995).

Mutation

Liu and Dixon (1996) reported that in vector containing mammalian cells, treatment with Cr VI also results in a dose dependent increase in mutations in the vector target gene supF. The Cr-induced mutations in supF occurred mostly at G:C base pairs and were widely distributed across the gene, a pattern similar to those observed with ionizing radiation or hydrogen peroxide. These results support the hypothesis that Cr VI induced oxidative-type DNA damage is responsible for Cr mutagenesis in the cell.

Hamilton *et al.* (1998) reported previously that various genotoxic chemical carcinogens, including chromium VI, preferentially altered expression of several inducible genes but had little or no effect on constitutive gene expression. Arsenic III and Cr VI each significantly altered both basal and hormone-inducible expression of a model inducible gene, phosphoenolpyruvate carboxykinase (PEPCK), at non-overtly toxic doses in the chick embryo *in vivo* and rat hepatoma H4 II E cells in culture.

DeFlora (2000) recently quantified in human body compartments, the reduction of chromium VI in body fluids and non-target cells, which result in its detoxification. In target cells Cr VI tends to be metabolized by a network of mechanisms leading to generation of reduced chromium species and reactive oxygen species, which will result either in activation or in detoxification depending on the site of the intracellular reduction and its proximity to DNA. When introduced by the oral route, chromium VI is efficiently detoxified upon reduction by saliva, gastric juice and sequestration, if some chromium VI is absorbed by the intestine it is massively reduced in the blood of portal system and then in the liver.

DeFlora *et al.* (1990) have reviewed approximately 650 results reported in the literature with 32 chromium compounds assayed in 130 short-term tests using different targets and/or genetic end points. The large majority of the positive results were obtained with hexavalent chromium compounds. Trivalent chromium compounds, although more reactive than the hexavalent chromium compounds with purified nucleic acids, did not induce genotoxic effects in the majority of the studies conducted with intact cells. With few exceptions, solutions of hexavalent chromium compounds were consistently positive in cellular systems. Almost 400 out of 450 results showed that soluble hexavalent chromium compounds are mutagenic in bacteria. In addition, the soluble hexavalent chromium compounds induced a broad range of genetic effects in yeasts and in insects. Soluble trivalent chromium compounds were inactive of genetic effects in a cellular or sub cellular target.

The frequency of positive results with hexavalent chromium compounds was related to their solubilities and, hence, to their bioavailabilities to the target cells. Although soluble trivalent chromium compounds appear to be capable of producing genetic effects when directly challenged with purified nucleic acids or with sub cellular targets, this potential genotoxicity is lost in cellular systems.

Carcinogenicity

Out of the studies reviewed by the IARC (International Agency for Research on Cancer, 1990), 12 results obtained with metallic chromium and 20 results obtained with chromium III compounds (chromic acetate, chromic oxide, chromic chloride, chrome tan or basic chromic sulfate, chromic sulfate land chromite) were negative, irrespective of the administration route. Similarly, 27 results available either with chromium containing mixtures and other chromium compounds, one of which is related to chromium VI dioxide, was consistently negative. Only one sample of roasted chromite ore, in which chromium III was likely to have been oxidized to chromium VI, was weakly carcinogenic in rats after intrapleural injection.

Furst *et al.* (1976) reported that Chromium VI

only induced local tumors at the administration site and not at a distance from the portal of entry into the organism. The only exception was a study in which intramuscular injections of lead chromate in rats resulted in the development of renal carcinomas. However, as commented by the IARC 1980 working Group this effect should be ascribed to the lead moiety, which typically produces kidney tumors in rodents (DeFlora *et al.*, 1997; Costa, 1997; Mancuso, 1997).

Chromium VI can be carcinogenic for the lower respiratory tract. Moreover, a few studies reported an association between exposure to chromium VI and cancer of the sinonasal cavity. An increased mortality for cancers at other sites was sporadically reported, which was counter balanced by several studies in which the risk of developing cancer at various sites was apparently decreased in chromium VI-exposed individuals.

Most occupations involved exposures not only to chromium VI compounds but also to other recognized carcinogens such as other metals (*e.g.* nickel), organic compounds (*e.g.* polycyclic aromatic hydrocarbons), fibers (*e.g.* asbestos) and complex, mixtures (*e.g.* cigarette smoke). It is thus important to discriminate the effects of confounding factors and to pinpoint those findings that are validated by the converging evidence provided by different epidemiological studies.

Based on these considerations, in 1988 the WHO concluded that there is insufficient evidence to implicate chromium as a causative agent of cancer in any organ other than the lung. In 1990, the IARC (International Agency for Research on Cancer, 1990) concluded that for cancers other than that of the lung and Sinonasal cavity, no consistent pattern of cancer risk has been shown among workers exposed to chromium compounds (Cohen *et al.*, 1993).

A recent authoritative article on carcinogenicity of metals in humans (Hayes, 1997) further emphasized the selective carcinogenicity of chromium production, chromate pigment production and chrome plating. In the same year, however, another review article claimed that chromium VI can be involved in the causation of a broad spectrum of additional cancers, including prostate cancer, lymphoma, leukemia, and bone, stomach, brain, kidney and testicle cancers (Costa, 1997).

Chromium is an industrial carcinogen. The potential cancer risk in human extends to all forms of chromium and to total chromium (Mancuso, 1997). Carcinogenic chromium VI is persistent environmental contaminant with potentiality for human exposure through drinking water (Coogan *et al.*, 1995). Exposure of human being to Cr caused chronic hepatitis, liver cirrhosis and there is a potential for increased respiratory cancer risk (Costa, 1997; Itoh and Shimada, 1998; Gurjar *et al.*, 1996). Lung cancer rates and death rates increased by gradient level of exposure to trivalent (insoluble) chromium and to hexavalent (soluble) chromium (Mancuso, 1997). De-Flora *et al.* (1997) reported that hexavalent chromium is not a systemic toxicant and lack carcinogenicity at distance from the portal of entry into the organism. They further explained that chromium VI escaping reduction in the digestive tract would be detoxified in the blood of the portal vein system and then in the liver, having an overall reducing capacity of 3300 mg. These processes give reasons for the poor oral toxicity of Cr VI and its lack of carcinogenicity when introduced by oral route following reflux from the respiratory tract. Costa (1997) reviewed the toxicity and carcinogenicity of hexavalent chromium in animals and human models. The focus of his study was not only based on fact that hexavalent Cr compounds can induce respiratory cancers but rather this review addresses other type of cancer induced by exposure to hexavalent Cr-compounds.

DNA protein cross links

Lin *et al.* (1992) found that both nickel and chromium complex certain amino acids to DNA, histidine and cysteine being among several that are complexed by both these metals. However, the complexing of histidine and cysteine to DNA by Ni^{2+} does not appear to be directly mediated by the metal.

In contrast to nickel, chromate-induced DNA-protein cross-links appear to involve substantial participation of trivalent chromium in the DNA-protein cross-links (Costa, 1990, 1991). The chemistry of trivalent chromium is such that it forms very tight complexes with DNA and protein, unlike Ni^{2+}, which is primarily catalytic in its ability to

form DNA-protein cross-links. Chromate treatment of intact cells has been shown to complex a number of proteins to DNA. One of the major proteins complexed to DNA by hexavalent chromate has been shown to be actin, and trivalent chromium has been shown to be the ultimate reductant participating in this DNA-protein complex formation (Miller *et al.*, 1991). Lin *et al.* (1992) also reported that cysteine and histidine, as well as serine, threonine and tyrosine, were complexed to DNA by chromium. Residual cysteine seemed to be derived primarily from glutathione, whereas the other amino acids were derived mostly from protein components of DNA-protein complexes. Additional work demonstrated that chromium appeared to bind to the phosphate backbone of DNA rather than to any particular DNA base and, through the binding of trivalent chromium to the phosphate backbone, coordinated to form the DNA-protein complex lesion through certain amino acids (Salnikow *et al.*, 1992).

Hexavalent chromium induces formation of DNA-protein cross-links (Capellmann *et al.*, 1995). The level of DNA-protein cross-links in the peripheral blood mononuclear cells increased (1.3±0.5% -SD) over control (0.8±0.4, $P<0.001$) (Tailoli *et al.*, 1995). The DNA-protein crosslinks are indicative of DNA or protein damage (Yi-Xiong *et al.*, 1995).

Without excessive exposure to chromium blood chromium concentration in man is between 20-30 µg/l and is evenly distributed between erythrocytes and plasma. With occupational exposure, increase in blood chromium is related to increase in blood cells. Urinary excretion in general is less than 10 µg/day in the absence of excessive exposure (Goyer, 1986).

Alexander and Aesth (1995) studied the uptake of chromate in human red blood cells and isolated rat liver cells. According to them uptake in both types could be inhibited by the established anion carrier. The uptake is temperature dependent and could be partly inhibited by high levels of lactate ($mmol^{-1}$) pyruvate or sulphate. The uptake rate was greatly increased at lower pH (6.0 versus 7.4), which could indicate transport of the $HCrO_4$ form or an increased intracellular rate of Cr VI reduction.

Kim and Na (1990) observed acute toxic effect of sodium dichromate on metabolism. According to them sodium dichromate caused significant increase in serum lactate, pyruvate and creatinine concentration within 15 minutes after intoxication. Severe hyperglycemia occurred. Dichromate decreased serum total amino acids with a consequential increase in blood urea nitrogen (BUN) concentration. They also reported that trivalent chromium compounds had no effect at all. Subchronic exposure of chromium for 30 days inhibited the activities of lactate dehydrogenase, succinate dehydrogenase, malate dehydrogenase and isocitrate dehydrogenase (Venugopal and Reddy, 1992). Siwakami *et al.* (1994) reported that the sublethal doses of chromium to fresh water catfish decreased protein, carbohydrate and lipids in tissue of liver and muscles.

Fetotoxicity

Asmatullah and Shakoori (1998) evaluated embryotoxic and teratogenic potential of hexavalent chromium in chick embryos. Different concentrations of aqueous solution of potassium dichromate ranging from 1.00 to 100.00 µg/egg were injected into chick eggs before incubation. A dose-dependent embryonic mortality was observed in all the groups examined on days 7 and 14 of incubation. The following malformations were observed in survivors: reduced body size and organs; microphthalmia; micromelia; everted viscera; abnormal and twisted neck, beak and spinal cord; isolated epicarditis; clubfoot; haemorrhage; and patchy feathers. Total mortality showing embryonic resorption was noted in higher dose groups (25-100 µg/egg) on day 14 of incubation. Itow *et al.* (1998) observed that the heavy metals like Hg, Cd, Cr and Zn inhibits the walking legs regeneration of horseshoe crab (*Limulus polyphemus*) larvae.

Chromium caused fetotoxicity in pregnant rats after exposure to chromium in drinking water (Junaid *et al.*, 1995). Junaid *et al.* (1996) also reported embryotoxic and fetotoxic effects, reduced fetal weight, retarded fetal development, reduced number of fetuses (live and dead) per mother, high incidence of dead fetuses and resorption in treated mother in the highest dosed groups. No significant gross structural abnormalities were observed in any

of the fetuses of chromium VI treated mothers. Significant incidences of reduced ossification were found in the highest dosed group. Chromium level was increased in dose dependent manner in maternal blood, placentae and fetuses.

Human allergies

Metal ions are an important cause of allergies, and evidence is presented to show that the majority of metals or metal compounds can induce allergic changes. Except for chromium and nickel, which are among the most common human allergens, animal models have provided little information. At least cadmium, thorium, lead, chromium, nickel, beryllium, and arsenic and proven or putative carcinogens in animals or humans on the basis of cytological or epidemiological evidence. However only arsenic exhibits a clear predilection for the skin. Other metals such as gold can induce subcutaneous sarcoma following injection, but the relevance of this observation in terms of human occupational risk is discounted.

Nygren and Wahlberg (1998) reported that the wearing chromium tanned leather gloves could provoke a lapse of hand dermatitis in chromium sensitive patient. Various forms of chromium have different biological effects. According to their study chromium tanned leather contained about 3% (m/m) chromium. Chromium can also be found in vegetable dyed tanned leather, probably emanating from the leather dye. Small amount (average 0.08% m/m) of leachable hexavalent chromium were found in both chromium and vegetable tanned leather. Thus, the risk of relapse of chromium dermatitis cannot be disregarded.

Piela and Kiec-Swierczynsk (1998) performed the patch tests with serial dilutions of nickel sulphate, potassium dichromate and cobalt chloride in petrolatum on 124 nickel-sensitive, 64 chromium-sensitive and 72 cobalt-sensitive subjects and reported that the lowest eliciting patch test concentrations were as follows: nickel sulphate-0.005, potassium dichromate-0.0025, and cobalt chloride-0.005.

Liver and kidney enzyme activities and blood glucose and heamoglobin increased after 15 or 30 d of dermal exposure to nickel and chromium. Concentration of nickel and chromium increased in tissues. The changes were related to the duration of exposure (Mathur and Gupta, 1994; Milkovic-Kraus and Macan, 1996; Roto *et al.*, 1996).

Chromium possesses both acute and chronic toxicities mainly associated with hexavalent chromium compounds such as dermatitis, allergic and eczematous skin reaction, skin and mucous ulceration, perforation of the nasal septum, allergic asthmatic, bronchial carcinomas, gastroenteritis, leukemia, Hodgkin's hepatocellular deficiencies and renal oligoanuric deficiencies (Goyer, 1986, Baruthio, 1992; Ptashekas, 1992; Costa, 1997; Gurjar *et al.*, 1996).

Wright *et al.* (1995) measured in transported calves that supplemental Cr reduced serum haptoglobin, whereas only marginally reduced total haemolytic complement activity. In stressed feeder calves, dietary Cr supplementation resulted in an increase in total serum immunoglobulin (Ig), particularly IgM (Chang and Mowat, 1992), an enhanced primary antibody (Ab) response to human red blood cells and increased serum IgG_1 concentration at 14 d post-stimulation (Moonsie-Shageer and Mowat, 1993). There is emerging understanding that because Cr VI is isostructural with phosphate and sulphate, it is readily taken up by the GI tract and penetrates to many tissues and organs throughout the body. Incubation of human lymphocytes with $K_2Cr_2O_7$ (Cr VI) increases DNA strand break without concurrent cytotoxicity. In contrast chromium acetate hydroxide Cr III failed to induce DNA strand breaks at sub cyototoxic concentration in peripheral lymphocytes (Gao *et al.*, 1992). Gao *et al.* (1994) by using molecular epidemiological techniques reported no damage in the lymphocytes DNA of a group of workers exposed to chromium, which may either be due to low chromium exposure or the ability of plasma to detoxify chromium VI to chromium III before it reached the lymphocytes. KortenKamp *et al.* (1996) reported that Cr VI in combination with ascorbate induce single stranded breaks and apurinic/ apyrimidinic sites (AP-sites). The generation of AP-sites and SSB are dependent upon molecular O_2. The Cr V is an intermediate formed during generation of DNA damage but it works only by activation with molecular O_2. The DNA lesions

arising from chromate/ascorbate have potential to cause gene mutation.

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