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EFFECT OF ORAL ADMINISTRATION OF HEXAVALENT CHROMIUM ON TOTAL BODY WEIGHT, CHROMIUM UPTAKE AND HISTOLOGICAL STRUCTURE OF MOUSE LIVER

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Abstract: Administration of different concentrations (500, 750, 1000, 1500 and 2000 ppm) of potassium dichromate ($K_2Cr_2O_7$) mixed in drinking water to adult mice, *Mus musculus ad libitum* for 8 weeks resulted in dose-related decreased body growth and reduced feed intake, during the first 4 weeks of administration. Chromium was accumulated in the liver in a dose dependent manner, accompanied by some hepatolobular derangements in the liver. Excessive chromium deposition, retarded body growth and hepatotoxicity as indicated by histological abnormalities indicate dichromate toxicity.

Key words: Potassium dichromate toxicity, tannery effluent, *Mus musculus*, total body growth rate, heavy metal toxicity, metal induced pathological changes in liver.

INTRODUCTION

Chromium is one of the heavy metals which in the ambient air, originates from industrial sources particularly ferrochrome production, ore refining, chemical and refractory processing and combustion of fossil fuels (Jworski, 1980). A controllable source of chromium is waste water from chrome plating and metal finishing industries, textile plants and tanneries (Cheremisinoff and Habib, 1972).

High concentration of chromium are toxic, carcinogenic, mutagenic and teratogenic (Petrilli and Deflora, 1977; Luli *et al.*, 1983; Nair and Krishnamurthi, 1991). Chromium (Cr) exists in two valance states *i.e.*, trivalent state (Cr^{+3}) and hexavalent state (Cr^{+6}) and these two forms differ markedly in a number of their biological properties (Levis and Bianchi, 1982). Hexavalent forms Cr(VI) are more toxic and mutagenic than the more common trivalent forms, Cr(III). Chromate and dichromate ions are strong oxidizing agents. Derivatives of Cr(III) are water soluble at neutral pH and can be removed from medium in the form of Cr hydroxide, while Cr(VI) are highly insoluble (Cary, 1982; Levis and Bianchi, 1982; Ohtake *et al.*, 1990; Yamamoto *et al.*, 1993; Vishnyakov *et al.*, 1992).

Chromium (III) is considered to be an essential trace nutrient serving as a component of the glucose tolerance factor (Mertz, 1969). It is a cofactor for insulin

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action and has a role in the peripheral activities of this hormone by forming a tertiary complex with insulin receptors, facilitating the attachment of insulin to these sites.

Trivalent chromium salts have little or no mutagenic activity in bacterial system. Since there is preferred uptake of hexavalent form by cells and it is the trivalent form that is metabolically active and binds with nucleic acids within the cell, it has been suggested that the causative agent in chromium mutagenesis is trivalent chromium bound to genetic material after reduction of the hexavalent form.

The major lesions caused by acute and generally accidental poisoning with chromium (VI) involve the kidneys and liver (Franchini and Mutti, 1985). In workers which were exposed to chromium (VI), a mild severe rhinopathy was the most common ailment. It has also been shown that most affected organs are the digestive system, respiratory tract and the skin. In case of skin lesions, chronic ulcers and irritative dermatitis is attributable to the oxidizing properties of chromium (VI) and eczematous dermatitis caused by cutaneous sensitization.

The present study aims at determining the toxic effects of hexavalent chromium on the total body growth rate and relative growth rate of liver, heart and kidney of male albino mice *Mus musculus*, administered with $K_2Cr_2O_7$ in drinking water. Histopathological effects of hexavalent chromium have also been assessed in liver of mouse.

MATERIALS AND METHODS

Mice and their maintenance

One hundred mature male albino mice, *Mus musculus*, Swiss strain maintained in the Animal House of Department of Zoology (temperature 25±2°C) were used for this study. The animals kept in iron cages in groups of three per cage, were fed on chick feed No.3 (Punjab Feeds, Lahore) and provided with drinking water *ad libitum*. Saw dust was used for bedding which was replaced on alternate days.

Six groups, one control and five experimentals, each of 9 mice, were maintained in separate cages. The control group was provided with simple drinking water, whereas experimental groups were administered with different concentrations (500, 750, 1000, 1500 and 2000 ppm) of aquous solution of potassium dichromate ($K_2Cr_2O_7$) as drinking water for 8 weeks. Total body weight was recorded every week, whereas daily water and feed intake was also recorded for each mice.

After eight weeks of experiment, the mice from each group were weighed and sacrificed. The blood was drained off. Different body organs including liver, heart and kidney, were dissected out, and wet weighed. For determination of their dry weights, these organs were placed in incubator at 120°C for 12 hours until the organs completely dried. Small pieces of liver from each group were fixed in Bouin's fixative for histological sections. Routine procedure for section cutting was adopted. Sections (6-8 μm) were cut and stained with haematoxylin and eosin. The slides were studied under stereomicroscope and the selected sections were microphotographed.

Chromium accumulation in liver

Pieces of liver from control as well as experimental groups of mice were used for determination of Cr. Colorimetric method (Kunicka *et al.*, 1992) was followed for this purpose. One hundred milligrams of each tissue were taken and homogenized in 3 ml distilled water. After this 2 ml H_2SO_2 and 5 ml HNO_3 were added in each sample. The solutions were made 50 ml by adding distilled water and boiled for 15 minutes, cooled, filtered and again made 50 ml each. The pH was adjusted at 0.8 by using 0.2N H_2SO_4. The final volume of each sample was made 100 ml and 2 ml color reagent, diphenyl carbazide solution (0.5% in acetone) was added. After 10 minutes the absorbance was noted at 540 nm and Cr concentration was calculated according to the method.

All the data were analysed by using Student's 't' test.

RESULTS

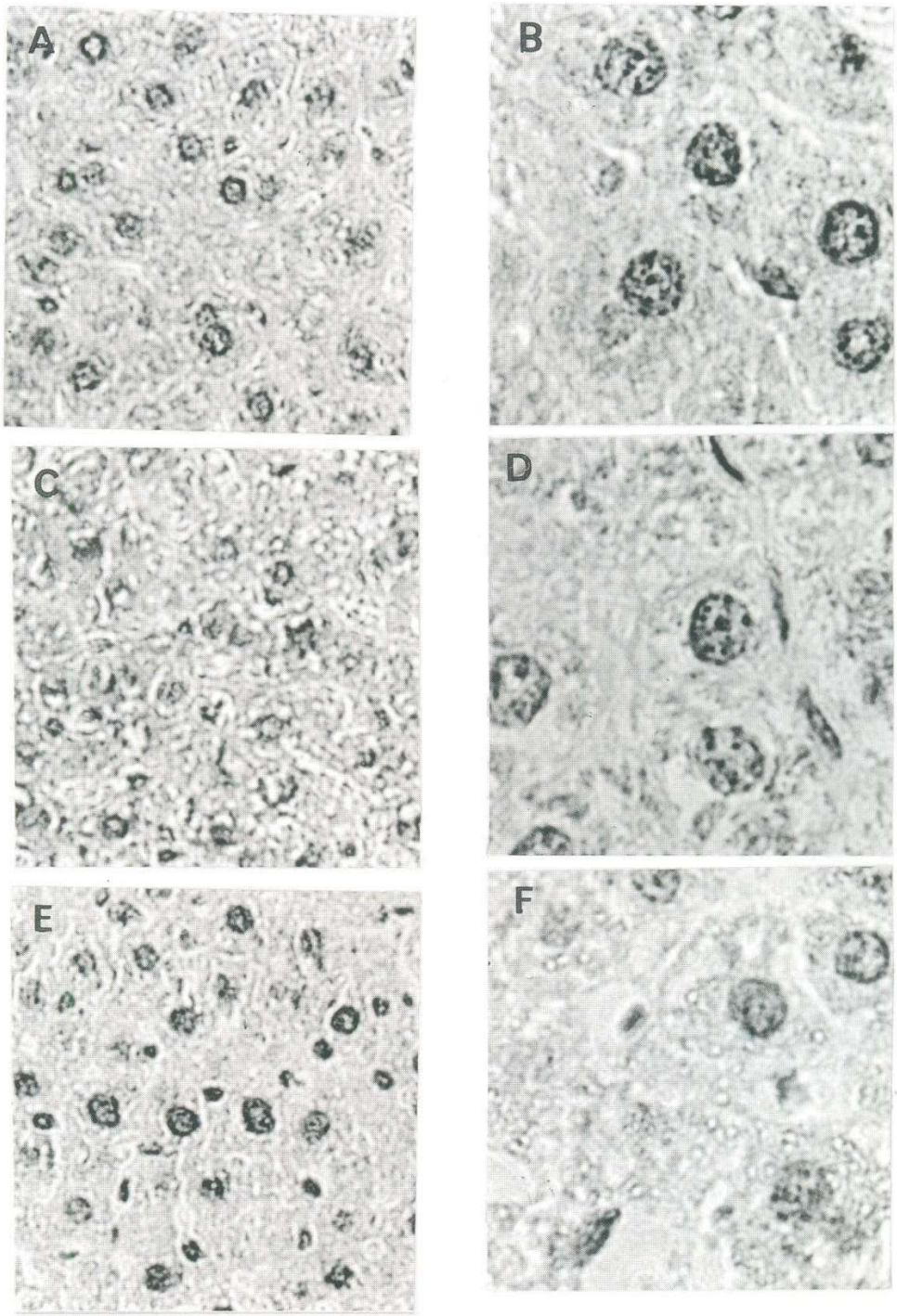
Body weight

Table I shows effect of $K_2Cr_2O_7$ administration on the total body weight of male mice. The mice on the average, weighed 30.66 ± 3.72 g on the first day of the experiment in controls. After two weeks the body weight increased 5% and after 4 weeks the increase was 12.5%, showing thereby an average increase of 0.42% per day (0.128 g/day). Administration of CrVI reduced the total body weight. Within one day $K_2Cr_2O_7$ administration, the body weight decreased 27% and 18% after 1500 and 2000 ppm of CrVI, respectively. After 2 weeks of CrVI administration, the decrease was 5%, 7.25%, 28.5% and 14.5% after 500, 750, 1500 and 2000 ppm. After 4 weeks of CrVI administration at a dose of 500, 750, 1000, 1500 and 2000 ppm, the decrease in body weight was respectively, 8.7%, 10.64%, 15.48%, 28.52% and 20.29%.

The average growth rate in mice administered with 500 ppm, 750 ppm, 1000 ppm, 1500 ppm and 2000 ppm was 0.036% per day (0.011 g/day), 0.170% per day (0.05 g/day), 0.31% per day (-0.022 g/day), 0.348% per day (0.077 g/day), 0.31% per day (0.078 g/day) as against 0.42% per day (0.128 g/day) of control mice.

Liver, kidney and heart weights

Table II shows the effect of $K_2Cr_2O_7$ on the relative weights of liver, kidney and heart. After 8 weeks of CrVI administration the liver of control mice had 0.45 ± 0.05 (wet weight) and 0.15 ± 0.08 gm (dry weight). After hexavalent Cr treatment, both the wet and dry weights of liver increase significantly. After administration of CrVI at a dose of 500, 750, 1000, 1500 and 2000 ppm, the increase in liver weight is respectively, 5.11 fold, 4.4 fold, 3.33 fold, 5.62 fold and 2.6 fold. The increase in dry weight is respectively 7.73 fold, 2.13 fold, 2.06 fold, 2.6 fold and 2.06 fold, respectively. The control liver has 66.66% of water content, whereas in 500, 750, 1000, 1500 and 2000 ppm groups the water content of liver constitute 49.56%, 83.84%, 79.33%, 84.58% and 73.50%, respectively.



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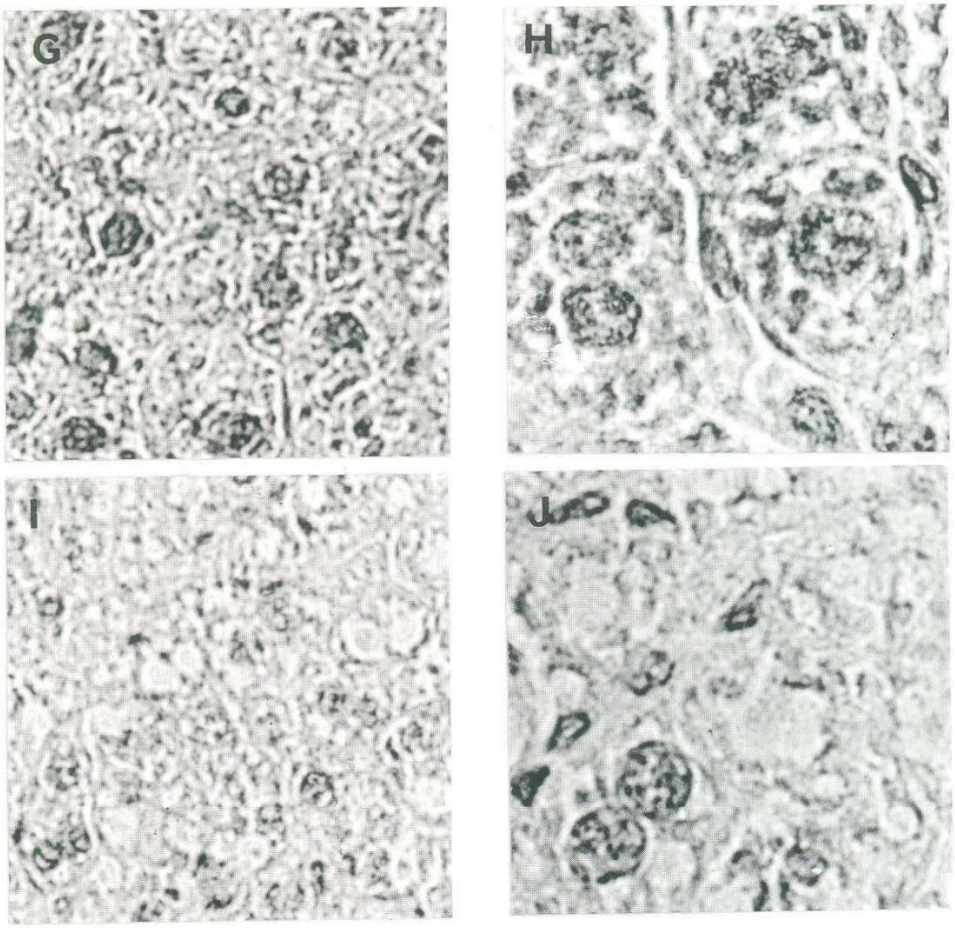

Fig. 1: Histological structure of liver of mouse given different concentrations of hexavalent chromium. A and B: Control group with quite normal hepatic structure. C and D: 750 ppm dose group showing increased sinusoidal space. E and F: 1000 ppm dose group with hepatic fibrosis and cirrhosis. G and H: 1500 ppm dose group with increased sinusoidal space and fibrosis. I and J: 2000 ppm dose group showing increased sinusoidal space, cirrhosis and nuclear pyknosis.

Table I: **Effect of chromium on body weight of mice exposed to different concentrations of $K_2Cr_2O_7$.**

Treatment (day)	DOSE (ppm) $K_2Cr_2O_7$					
	Control	500	750	1000	1500	2000
1st	30.66[a]	31.16	29.33	29.83	22.33**	25.16
	±3.72	±1.67	±5.73	±4.13	±4.26	±4.37**
7th	31.33	30.00*	28.33	28.16	21.83***	24.16*
	±1.88	±1.29	±4.85	±4.21	±3.43	±3.89
15th	32.16	30.66*	29.83	29.83	23.00***	27.50**
	±1.77	±1.37	±4.91	±4.01	±2.76	±3.40
22nd	33.83	29.83**	29.33**	28.00***	23.83***	28.16*
	±2.91	±0.68	±3.90	±3.91	±1.95	±3.23
30th	34.50	31.50**	30.83*	29.16**	24.66***	27.50***
	±2.36	±1.38	±4.17	±3.48	±1.49	±2.21

a = Mean ± S.D. (gm); * = P<0.05; ** = P<0.01; *** = P<0.001.

Table II: **Effect of $K_2Cr_2O_7$ on different body organs of mice.**

$K_2Cr_2O_7$ (ppm)	Body	LIVER		KIDNEY		HEART	
		Wet	Dry	Wet	Dry	Wet	Dry
Control (9)	30.66[a]	0.45	0.15	0.25	0.07	0.07	0.05
	±3.72	±0.05	±0.08	±0.02	±0.01	±0.02	±0.01
500 (9)	31.50	2.30	1.16	1.16	0.13	1.17	0.02
	±1.38	±0.08	±0.08	±0.08	±0.05	±0.02	±0.01
750(9)	30.83	1.98	0.32	1.18	0.03	1.17	1.02
	±4.18	±0.45	±0.03	±0.02	±0.01	±0.01	±0.01
1000 (9)	29.16	1.50	0.31	1.16	0.02	1.16	0.02
	±3.48	±0.59	±0.02	±0.05	±0.01	±0.01	±0.01
1500 (9)	24.66	2.53	0.39	1.22	0.04	1.17	0.62
	±1.49	±0.27	±0.08	±0.03	±0.01	±0.01	±0.07
2000 (9)	27.50	1.17	0.31	1.16	0.03	1.16	0.02
	±2.21	±1.06	±0.06	±0.02	±0.01	±0.04	±0.003

a = Mean ± S.D. (gm); () = No. of animals.

Table III: Effect of different concentrations of $K_2Cr_2O_7$ on feed intake by mice.

Weeks	$K_2Cr_2O_7$ (ppm)					
	Control (n=9)	500 (n=9)	750 (n=9)	1000 (n=9)	1500 (n=9)	2000 (n=9)
1	5.04[a] ±1.11	4.37* ±0.77	5.42 ±0.54	4.25* ±1.06	3.81** ±0.98	4.34 ±0.95
2	6.20 ±1.02	5.85 ±0.74	5.45** ±0.54	5.30** ±0.52	4.34*** ±0.73	5.58** ±0.40
3	6.61 ±0.29	6.17* ±0.38	5.38*** ±0.57	5.92** ±0.65	5.18*** ±0.30	6.08** ±0.64
4	7.22 ±0.78	6.30** ±0.69	5.75*** ±0.38	5.55*** ±0.51	5.35*** ±0.22	5.10*** ±0.20

a = gm/animal (Mean±S.D.); * = Significantly decreased against controls ($P<0.05$); ** = Significantly decreased against controls ($P<0.01$); *** = Significantly decreased against controls ($P<0.001$).

Table IV: Accumulation of chromium (μg/gm) in liver of mice administered orally different aquous concentrations of $K_2Cr_2O_7$.

Dose (ppm)	$K_2Cr_2O_7$ treatment (weeks)	
	4	8
Control (n=4)	0.0106±0.0005	0.0146±0.0001
500 (n=4)	0.0180±0.0003***	0.0393±0.0011***
750 (n=4)	0.0163±0.0002***	0.1147±0.0027***
1000 (n=4)	0.0225±0.0008***	0.0555±0.0023***
1500 (n=4)	0.0210±0.0008***	0.1155±0.0055***
2000 (n=4)	0.0615±0.0011***	0.1428±0.0015***

After 8th week, the kidney of controls weighed 0.25±0.02 (wet) and 0.07±0.01 (dry) gm. The weight of kidney increased 2.32, 4.72, 2.32, 4.88 and 2.32 fold after administration of CrVI at 500 ppm, 750 ppm, 1000 ppm, 1500 ppm and 2000 ppm, respectively. This increase for the dry weight was respectively 1.85 fold, 0.428 fold, 0.285 fold, 0.571 fold and 0.428 fold. The control kidney had 72% water content, whereas in mice administered with CrVI at 500 ppm, 750 ppm, 1000 ppm, 1500 ppm and 2000 ppm, kidney had water content 88.8%, 94.91%, 98.27%, 96.72% and 97.41%, respectively. The dry and wet weights of heart also follow almost the same pattern. After 8 weeks of experimental period the heart in control mice weighed 0.11±0.01 g (wet weight) and 0.03±0.01 g (dry weight).

The heart wet weight in treated mice is about 17 fold that of control. Likewise the dry weight of heart is increased 0.4 fold, 20.4 fold, 0.4 fold, 12.4 fold and 0.4 fold, respectively in mice group administered with 500, 750, 1000, 1500, and 2000 ppm $K_2Cr_2O_7$.

DISCUSSION

The greater toxicity of hexavalent chromium (VI) may be due to its higher rate of absorption through gastrointestinal tracts (Danielsson *et al.*, 1982; Outridge and Scheuhammer, 1993).

All these observations indicate that hexavalent chromium (VI) has caused toxic effects even at low concentrations, when these were given for a long time. These findings are quite in conformity with some previous studies done to evaluate intoxication of hexavalent chromium (VI) in biological systems.

The trivalent chromium Cr(III) is a dietary requirement in trace amounts (Starich and Blincoe, 1983), but hexavalent chromium has been shown by epidemiological studies to cause respiratory cancers (EPA, 1984; Langard, 1990). Witmer *et al.* (1989, 1991) reported tissue distribution of chromium after 14 days of oral administration of sodium and calcium chromates to the rats. The chromium level in blood was highest (1800 μ mole Cr/gm) while other body organs including liver, kidney, lung, muscle, heart, skin, bone and gonads also contained high concentrations of chromium.

During some other studies, fatal poisoning was caused by the ingestion of 1-3 gm of chromate compounds (Kaufmann *et al.*, 1970; Pedersen and Morch, 1978). This concentration is much more higher than the normal chromium concentration, which is known to be 20-30 mg/ml in the blood and 15-40 mg/gm in the liver and kidney (Hyodo *et al.*, 1980; Goyer, 1986).

Kim and Na (1990) investigated acute toxic effects of sodium dichromate on metabolism of rats. Intraperitoneal injection of sodium dichromate (20 or 40 mg/kg) caused significant increases in serum lactate, pyruvate and creatinine concentrations within 15 min after intoxication. Severe hypoglycemia occurred thereafter, as a result of increased hepatic glycogenolysis. Serum total amino acids were decreased, with a subsequent increase in blood urea nitrogen concentration. In another study, Cr(VI) has been found cytotoxic in osteogenic cells (Puleo and Huh, 1995; Thompson and Puleo, 1995).

The oxidation state of chromium is a critical factor in determining the activities of various chromium compounds in experimental tests (Mathur *et al.*, 1977). Regardless of chromium species, only the hexavalent chromium compounds had an effect on the cellular metabolism. Chromium (VI) compounds, unlike most other metal compounds, are actively transported through cell membranes and thus can reach genetic targets. Consequently, the genotoxic effects of chromium have been studied with a variety of short-term tests covering the entire range of genetic damages (Sunderman, 1978; Bianchi *et al.*, 1983).

Intercellular reduction of chromium occurs either in the cytoplasm or in the nucleus. The intercellular reduction generates the carcinogenic chromium species (DeFlora and Wetterhahn, 1989). Chromium (V) is considered to be a candidate for one of these ultimate carcinogenic species since it has been observed both *in vivo* during the biological reduction of Cr(VI) to the final intercellular stable oxidation state (Cr(III).

Recently, Sugden and Wetterhahn (1996) have suggested formation of Cr(IV) upon disproportionation of Cr(V). Chromium (IV) oxidizes the nucleotide deoxyribose sugar

moiety via a phosphate-bound intermediate. A consequence of this intercellular reduction is the production of a wide array of adverse biological effects including DNA lesions induced by oxidative stress (DNA strand breaks) and chromium directly metalated to the DNA strands (Cr-DNA adducts). At the genetic level, chromium has been shown to affect the expression of some inducible genes and to interact with oxidative stress inducible transcription factors in the signal transduction pathway.

In a recent study on toxicity of chromium (VI) on osteogenesis, chromium appeared to produce a substantial decrease in calcium incorporation, especially at doses between 5 and 10% TC_{50} (Thompson and Puleo, 1995).

It is concluded that the chronic exposure of mice to comparatively high concentrations of potassium dichromate (500, 750, 1000, 1500, 2000 ppm) during the present study found toxic for mice. In spite of chromium in traces being essential for living systems, exposure to high concentrations of hexavalent chromium is dangerous.

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