Effect of Hexavalent Chromium on Egg Laying Capacity, Hatchability of Eggs, Thickness of Egg Shell and Post-Hatching Development of *Gallus domesticus*

Asmatullah, A. Asma, A. Latif and A. R. Shakoori*
Department of Zoology, University of the Punjab, Quaid-e-Azam Campus, Lahore 54590, Pakistan

ABSTRACT : Hexavalent chromium (CrVI) was fed to one day old chicks of *Gallus domesticus* in the form of different concentrations (250 and 500 mg/kg feed) of potassium dichromate mixed with the feed, *ad libitum*, for 32 weeks. After 20 weeks of feeding, the total body weight was higher in the low dose (260 mg/kg (feed) group and lower in the high dose (500 mg/kg feed) group, as compared with the control chicks. After 32 weeks of feeding, however, the total body weight was significantly decreased in both the treated groups. Egg laying was enhanced. Fertility remained unaffected, whereas hatchability was considerably decreased after CrVI-treatment. The egg shell thickness increased significantly (13%). Cr was deposited in a dose dependent manner in the liver and lungs. Some structural derangements in liver were also noted in treated chicks. The results of this study i.e., rapid ageing, excessive Cr deposition, decreased hatchability and hepatotoxicity indicate toxic effects of CrVI. (*Asian-Aus. J. Anim. Sci. 1999. Vol. 12, No. 6 : 944-950*)

Key Words : *Gallus domesticus*, Hexavalent Chromium, Egg Shell Thickness, Hepatotoxicity, Egg Hatching

INTRODUCTION

Chromium (Cr) is one of the heavy metals in industrial effluents, specialty from tanning industries, which have been increasingly documented to be toxic, carcinogenic, mutagenic and teratogenic in high concentrations (Petrilli and DeFlora, 1977; Luli et al., 1983; Nair and Krishnamurthi, 1991; Asmatullah et al., 1998a, b). It exists in two valence states i.e., trivalent state (CrIII) and hexavalent state (CrVI) which differ markedly in a number of their biological properties (Levis and Bianchi, 1982). Derivatives of CrIII are water soluble at neutral pH and can be removed from medium in the form of Cr hydroxide, while CrVI are highly insoluble (Cary, 1982; Levis and Bianchi, 1982; Ohtake et al., 1990; Yamamoto et al., 1993; Vishnyakov et al., 1992). CrVI is more toxic and mutagenic than trivalent form.

CrVI compounds in contrast to CrIII, are easily taken up by cells via the sulfate anion transport system (DeFlora and Wetterhahn, 1989), and are reduced through reactive intermediates such as CrV and CrIV to the more stable CrIII by cellular reductants including glutathione, vitamins C and B_2 as well as flavoenzymes (DeFlora et al., 1990; DeFlora and Wetterhahn, 1989; Sudgen and Wetterhahn, 1996). Some *in vitro* studies have shown that this reduction process also causes the generation of active oxygen species (Kawanishi et al., 1986; Kawanishi and Hiraku, 1996). For instance, CrV complex has been reported to react with H_2O_2, in a Fenton type manner, to produce hydroxyl radical, resulting in the induction of DNA damage (Aiyar et al., 1990; Coudray et al., 1992; Shi and Dalal, 1990).

CrVI compounds are potent toxic (Laborda et al., 1986; Goyer, 1990; Hojo and Satomi, 1991), carcinogenic (Shivas, 1980; DeFlora et al., 1990), genotoxic *in vivo* (Sunderman, 1978; Bianchi et al., 1983; Coogan et al., 1991) and in cultured cells (Sugiyama et al., 1986), and selectively inhibit the activity of enzymes such as glutathione reductase in mammalian cells (Sugiyama et al., 1986).

The embryotoxic effects of Cr have been reported in hamster (Gale, 1982) and mice (Trivedi et al., 1989), whereas Junaid et al. (1995, 1996) and Asmatullah et al. (1998a, b) have described several developmental abnormalities in mice and chicks, respectively during organogenesis. Heavy metals in industrial wastes caused significant reduction in embryonic growth in externally exposed eggs of mallards (Hoffman and Eastin, 1981). Embryo lethality, developmental abnormalities, delayed developmental stage and mortality after hatching in the Japanese Medaka, *Oryzias latipes* is known to be because of heavy medals (Cooper and McGeorge, 1991). Heavy metals also induced gross malformations in chick embryos such as reduced body size, micromelia, twisted neck, everted viscera, haemorrhage and microphthalmia (Gilani and Alibhai, 1990; Asmatullah et al., 1998a, b).

The present study describes the effect of hexavalent Cr on egg laying capacity, egg hatchability, thickness of egg shell and the post-hatching development of *Gallus domesticus*.

MATERIALS AND METHODS

Chicks

Forty five chicks, one day old Golden puff variety

* Address reprint request to A. R. Shakoori. Tel: 92-42-588-4989, Fax: 92-42-5868376, E-mail: info@zoolpu.brain.net.pk.
Received July 16, 1998; Accepted October 17, 1998

of *Gallus domesticus* were purchased from a local hatchery in Lahore. These chicks were divided into three groups, each of 15 and were kept in separate pen with a layer of saw dust on the floor which was replaced on alternate days. Room temperature was maintained at 35℃ during first week and then was kept constant at 27℃. The chicks were given commercial feed and water *ad libitum*.

Administration of chromium

CrVI was administered orally to chicks along with the feed in the form of potassium dichromate ($K_2Cr_2O_7$). Five and 10 ml of 5%, $K_2Cr_2O_7$ were sprayed separately on one kg feed, mixed thoroughly for homogenous distribution, to prepare feed containing 250 and 500 mg $K_2Cr_2O_7$/kg feed, respectively.

Procedure adopted

Two experimental groups, each of 15 chicks, were fed on feed containing 250 and 500 mg/kg feed for 32 weeks. A control group of 15 chicks was also maintained on normal Cr-free feed. All birds were weighed every week and a record of feed consumed was also maintained. At the end of 140 d (20th week) and 224d (32nd week), 6 and 4 female birds, respectively, from each group were weighed and sacrificed. The males were not sacrificed, but were maintained in the pen for mating purposes. The blood was drained off. Different body organs including liver, heart, kidney and lungs were dissected out and weighed. Small pieces of liver were fixed in Bouin's fixative for histology. Pieces of liver and lung were also stored in the freezer for Cr estimation. Data were also collected on egg laying, fertility, hatchability and egg shell thickness. The behaviour of male chicken was recorded.

Determination of feed intake

The daily feed intake was recorded by offering a weighed amount of feed to the chicks in a pen and then weighing the left-over in the bowl at the time of next replenishment. The feed intake/bird was calculated by dividing the total feed intake with the number of chicken in that particular pen.

Egg laying, fertility and hatchability

Every morning, after 224 d (32nd week) of experimentation, the pens were checked for egg laying. The eggs were collected from the cages, counted and incubated at 38.0±0.5℃ to check the effect of Cr-contaminated feed on fertility. Humidity was provided by putting water filled beaker in the incubator. The eggs were rotated twice a day. At 7th day of incubation the development of embryo was observed with the help of candling. The fertile eggs were incubated till hatching. Small pieces of shells from middle parts of eggs from each experimental group were used for determination of egg shell thickness.

Determination of egg shell thickness

After termination of egg incubation, at least 3 shell pieces from 20 eggs of each experimental group were taken. The pieces of shell from middle part of the egg were cleaned, shell membranes were removed and thickness of the shell measured with the help of Vernier calipers.

Histological studies

The liver pieces already fixed in Bouin's fluid were washed in 70% alcohol, dehydrated and embedded in molten paraffin wax. The sections (4-6 μm) were cut and stained with haematoxylin and eosin. The sections were studied for hepatic histological changes.

Chromium estimation

For chromium estimation 500 mg of each tissue (liver, lung) was homogenized in 3 ml distilled water, and digested by adding 2 ml concentrated H_2SO_4 and 5 ml HNO_3. These homogenates were made upto 100 ml with distilled water and boiled till approximately half the solution was evaporated. The samples were cooled, filtered and made upto 50 ml by adding distilled water. The pH of each sample was adjusted at 0.8 with 0.2 N H_2SO_4. The samples were made upto 100 ml with distilled water. In each sample 2 ml of colour reagent, 0.5% diphenylcarbazide solution, was added, mixed well and left for 10 minutes for colour development. Optical density of each sample was measured at 540 nm.

Statistical analysis

The various parameters of Cr-treated experimental groups were compared with the controls using Student's 't' test and the significant differences indicated in tables and figures.

RESULTS

Body weight and feed consumption

Figure 1 shows effect of Cr contaminated feed on the body weight and feed intake by the chicks. During the first 20 weeks (140d) of treatment the body weight showed an increase in the low dose (250 mg $K_2Cr_2O_7$/kg feed) group, and a decrease in the high dose (500 mg $K_2Cr_2O_7$/kg feed) group, when compared with the control group. At the end of 32 weeks (224d) of feeding the total body weight in both the treatment groups was decreased significantly ($p<0.01$) as compared with the control.

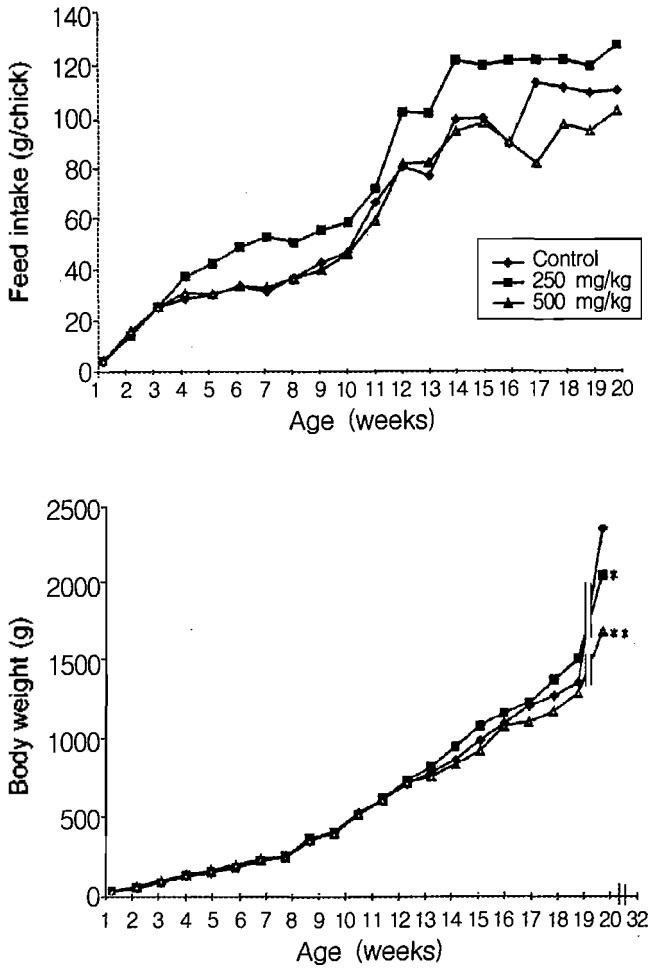

Figure 1. Effects of Cr contaminated feed on the body weight and feed intake by the chicks. Single or double esterics show significant changes with reference to controls * p<0.05; ** p<0.001

The chicks feeding on Cr-contaminated feed at 250 mg/kg feed, consumed more feed from 4th week to 20th week, as compared with the control chicks. The chicks feeding on 500 mg $K_2Cr_2O_7$/kg feed consumed almost the same amount of feed as control chicks for 16 weeks. During the subsequent period, however, they consumed significantly (p<0.01) less feed.

Weight of organs

The different organs follow the same pattern in their weights as described above for total body weight (table 1). In the low dose group the hepatic, renal and cardiac weights increased, 19 (p<0.01), 10 and 20%, respectively at the end of 20 weeks administration, compared with control. At the end of 32 weeks of administration of CrVI, the renal weight showed 6% increase, whereas liver and heart weights decrease 5% and 24%, respectively. In the high dose feeding experiment the hepatic and renal weight decreased (in contrast to that of low dose administration) 3.5% and 8%, respectively, whereas heart showed only 6% increase at the end of 20 weeks of feeding. At the end of 32 weeks of feeding however, all the three organs showed significant decreases viz., 25% in liver (p<0.05), 12% in kidneys and 33% (p<0.001) in heart weight.

The hepato-somatic indices showed a positive allometric increase in hepatic weight (table 1). The reno-somatic indices did not show a considerable difference in renal weight change among experimental groups till 20th week of experiment, but there was an increase in renal weight in treated chicks at 32nd week. Cardio-somatic indices also showed an increase in cardiac weight in treated chicks during 20th week observation, and it became reverse at 32nd week.

Egg laying, fertility and hatchability

The hens raised on Cr contaminated feed started laying eggs at the age of 22 weeks as against control chick which started laying after 24 weeks. Table 2 shows some interesting data on total number of eggs

Table 1. The effect of CrVI on weight of different body organs of chicks fed on different concentrations of potassium dichromate ($K_2Cr_2O_7$) (Unit: g)

Parameters[a]	Control (n=6)	$K_2Cr_2O_7$ for 20 weeks		Control (n=4)	$K_2Cr_2O_7$ for 32 weeks	
		250 mg/kg (n=6)	500 mg/kg (n=6)		250 mg/kg (n=4)	500 mg/kg (n=4)
Body wt.	1334 ±144[b]	1483 ±235	1265 ±277	2333 ±294	2020 ±233	1653 ±292**
Total hepatic wt.	45.59 ±3.08	54.11 ±1.67**	44.02 ±1.78	48.18 ±9.69	45.82 ±11.79	36.18 ±6.67
HSI	3.42 ±0.04	3.65 ±0.11	3.48 ±0.34	2.07 ±0.34	2.27 ±0.17	2.19 ±0.13
Total renal wt	12.92 ±2.85	14.16 ±2.16	11.89 ±1.99	12.53 ±0.9	13.32 ±1.46	11.05 ±1.34
RSI	0.97 ±0.18	0.96 ±0.04	0.94 ±0.09	0.54 ±0.01	0.66 ±0.03***	0.67 ±0.01***
Total cardiac wt	6.82 ±1.67	8.19 ±1.23	7.15 ±1.82	13.66 ±2.82	10.44 ±1.46	9.19 ±2.06*
CSI	0.51 ±0.07	0.55 ±0.04	0.57 ±0.06	0.59 ±0.01	0.52 ±0.01***	0.56 ±0.01***

[a] Abbreviations: HSI, Hepato-somatic index; RSI, Reno-somatic index; CSI, cardio-somatic index.

[b] Mean ± SEM, * p<0.05; ** p<0.01; *** p<0.001.

laid, fertility of eggs, hatchability of these eggs and thickness of the shells. The chicks raised on Cr-contaminated feed laid more eggs (29%) in the higher dose group as compared with control. The treated chicks had greater number of fertile eggs (86% in the low and 79% in the high dose groups) out of the total number of eggs laid by respective hens, as against 67% fertile eggs in the control birds. The Cr toxicity was however, obvious during hatching. The control experiment showed 60% hatching, whereas only 6% of the eggs hatched in the low dose group and none in the high dose group. The egg shell thickness was significantly ($p<0.001$) increased (13%) in both the treated groups as compared with control shells (table 2).

Table 2. Effect of CrVI on egg laying, fertility, egg shell thickness and hatching in chicks

Parameters	N	Control	$K_2Cr_2O_7$ (mg/kg feed)	
			250	500
Total Number of eggs	3[a]	45	42	58
(Number of eggs/hen)		(15)	(12)	(19)
Number of fertile eggs	3	30	36	46
(% fertility)		(67%)	(86%)	(79%)
Haching (%)	3	60	5.5	0
Egg shell	20[b]	0.42[c]	0.48***	0.48***
thickness (mm)		±0.07	±0.05	±0.04

[a] No. of egg laying hens. [b] No. of eggs used for this study.
[c] Means±SEM, Student's 't' test. ***$p<0.001$.

Behaviour of males

The present data pertains only to the female chicks. The males were however, kept in the same pen along with the females. In the same age group, the females matured sexually earlier than the males. The males became sexually active not until 26th week of experimentation. After 32 weeks however, the males had grown quite active and particularly aggressive in the treated group as compared with their controls. Not only that they consumed more food, they even ate up eggs, which were laid in the pen. The experimental males would mount the hens much more frequently than the controls which should also explain higher rate of fertilization of eggs as compared with control birds, maintained in the separate pen.

Chromium deposition

The liver of chicks fed on Cr containing feed at a dose of 250 and 500 mg $K_2Cr_2O_7$/kg feed for a period of 20 weeks accumulated 16 and 186% more Cr, respectively in the two treated groups as compared with the control liver. Table 3 shows deposition of Cr in the liver and lungs of chick under both experimental conditions. After 32 weeks of feeding, the low and high dose groups show deposition of Cr 100 and 150% more than the control group, respectively, in the liver. A similar trend of Cr deposition was noted in lungs (table 3). In 20 day treatment group, the Cr deposition in lungs of 250 and 500 mg/kg feed groups was respectively 25 and 88%, whereas after 32 days of treatment this deposition in lungs was, respectively, 122 and 144%, when compared with control group.

Histological studies

The histological structure of liver of control chicks was quite normal with binucleated hepatocytes (figure 2A). In case of 250 mg/kg feed group fibrogenesis, pyknotic nuclei, thickening of cell membranes (figure 2B) and signs of liver cirrhosis and excessive vacuolation of hypertrophied hepatocytes were apparent (figure 2C). An increased sinusoidal space and nuclear pyknosis was evident in 500 mg/kg feed group (figure 2D).

DISCUSSION

The presents studies indicate that CrVI 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 Cr in biological system. Although CrIII is a dietary requirement in trace amounts (Starich and Blincoe, 1983), but CrVI has been shown by epidemiological studies to cause respiratory cancers (EPA, 1984; Langard, 1990). Witmer et al. (1989, 1991) reported tissue distribution of the Cr after 14d of oral administration of sodium

Table 3. Deposition of CrVI in body organs of chick exposed to different concentrations of $K_2Cr_2O_7$

Parameters[a]	20 weeks			32 weeks		
	Control (n=6)	250 mg/kg feed (n=6)	500 mg/kg feed (n=6)	Control (n=4)	250 mg/kg feed (n=4)	500 mg/kg feed (n=4)
Liver (Cr μg/g)	235.6±90.6[a]	272.0±36.2	674.7±181.3***	362.7±72.5	725.3± 72.5***	906.7±181.3***
Lungs (Cr μg/g)	145.1± 9.1	181.3±72.5	272.0± 72.5***	408.0±36.3	906.6±181.3***	997.3± 90.7***

[a] Mean±SEM, Student's 't' test, *** $p<0.001$.

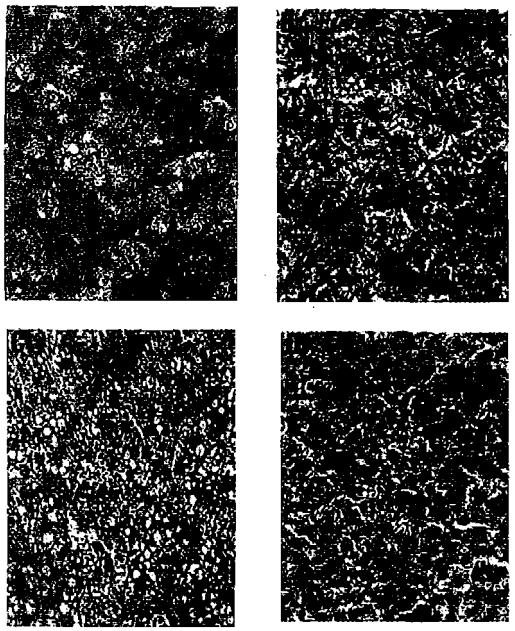

Figure 2. Histological structure of liver of 20 week old *Gallus domesticus*. A, control liver, showing binucleated hepatoytes (large arrow head); B-C, liver of chicks fed on 250 mg/kg feed group with fibrosis (arrow) and cirrhosis (small arrow head); D, liver of chicks fed on 500 mg/kg feed group, showing increased sinusoidal space (ss) and nuclear pyknosis (p).

and calcium chromates to the rats. The Cr level in blood was highest (1800 μM Cr/g) while other body organs including liver, kidney, spleen, lungs, brain, testes and muscles also contained high concentrations of Cr.

It is well known that acute toxicity from chromate compounds occur from accidental exposures and attempts to use the agent for suicidal purposes (Goyer, 1986). Although such cases are rare, the constitutional symptom is often serious and even fatal, due to circulatory collapse in the initial stage, or renal and hepatic malfunction from acute tubular and hepatocellular necrosis respectively (Friestedt et al., 1965; Pedersen and Morch, 1978; Langard and Norseth, 1986; Bader, 1986). Recently, an interesting case of acute poisoning by chromate compounds has been reported by Kurosaki et al. (1995). A 51 year old man committed suicide by ingesting a fatal dose of sodium dichromate solution. He lost consciousness 6 h after ingestion and died 20 h later. An examination of the blood showed noticeable hepatic damage and thrombocytopenia. The postmortem examination revealed extensive bleeding in the alimentary tract and a severe hepatic lesion due to hepatocellular necrosis. A severe renal lesion was observed mainly in the distal tubules. The patient's death was assumed to have been caused by circulatory collapse due to internal bleeding and direct toxicity of chromate compounds with hepatic malfunction and possibly disseminated intravascular coagulation. During some other studies, fatal poisoning was caused by the ingestion of 1-3 g of chromate compounds (Pedersen and Morch, 1978; Kaufmann et al., 1970), This concentration is much higher than the normal Cr concentration, which is known to be 20-30 ng/ml in the blood and 15-40 ng/gm in the liver and kidney (Goyer, 1986; Hyodo et al., 1980).

The chicks fed low doses of $K_2Cr_2O_7$ consumed more feed, whereas those fed on higher doses took less amount of feed as compared with their controls, which may indicate direct relationship of amount of feed-intake and the total body weight. The amount of Cr in the feed at low doses may increase palatability of feed and stimulate the satiatory centre, while the higher doses may have inhibitory effect, leading to lesser feed-intake. Consequently amount of Cr in the food affects the body growth.

At the end of two observation time points of 140d (20 weeks) and 224 d (32 weeks), 5 chicks, 3 females and 2 males, were left behind. Although the hens had already started laying eggs after 22 (in Cr-fed) and 24 (control) weeks, the egg production in this study was recorded after 32 weeks of experimental period.

The increased number of eggs laid by chicks fed on Cr-containing feed, as compared with the control chick is related to the Cr consumption as substantiated by 6.4 eggs/hen in the Cr-fed chickens as against 5 eggs/hen in the control group. Even the rate of fertilization of these eggs was significantly higher in the experimental birds. The one day old chicks could not be sexually differentiated, but once it was possible to differentiate between the male and the female, only hens were included in the data which is being presented in this study. The males, however, were kept in the same pen along with the females to ensure fertilization of eggs. In the same age group, the females started laying after 24 (control) and 22 weeks (Cr-fed), but males were not yet sexually active. After 32 weeks, however, Cr-fed males had grown aggressive and would mount the hens much more frequently than the controls. That should explain higher rate of fertilization of eggs in the treated chicks. The hatchability of eggs was, however, very low in the experimental birds, which could be (1) because of hardness of egg shells, (2) toxicity of Cr leading to abnormal and interrupted development (Asmatullah et al., 1998a, b).

The increased shell thickness may be because of excessive availability and deposition of Ca^{++} in the egg shell in the presence of Cr. In a recent study on toxicity of CrVI on osteogenesis, Cr appeared to produce a substantial decrease in calcium incorporation,

especially at doses between 5 and 10% TC_{50} (Thompson and Puleo, 1995). This indicates that due to decreased incorporation of calcium during osteogenesis, an excess concentration of calcium may be available for thick shell formation in Cr treated laying hen. Presumably Cr intoxication inducers increased hormonal synthesis and secretion, which accounts for increased egg laying by the hen, higher rate of fertilization of eggs and aggressive behaviour of male chicken.

It is concluded that the chronic exposure of chicks to even low concentrations of potassium dichromate (250 and 500 mg/kg feed), used during present study, has proved negative effect of hexavalent Cr on growth of chicks. In spite of Cr in traces being essential for living systems, exposure to high concentrations of CrVI is hazardous.

ACKNOWLEDGMENTS

The financial support from University of the Punjab and expert assistance of Syed Haider Ali Zaidi in typing this manuscripts is gratefully acknowledged.

REFERENCES

Aiyar, J., H. J. Berkovits, R. A. Floyd and K. E. Wetterhahn. 1990. Reaction of chromium (VI) with hydrogen peroxide in the presence of glutathione; reactive intermediates and resulting DNA damage. Chem. Res. Toxicol. 3:595-603.

Asmatullah, S. N. Qureshi and A. R. Shakoori. 1998a. Hexavalent chromium-induced congenital abnormalities in chick embryos. J. Appl. Toxicol. 18:167-171.

Asmatullah, S. N. Qureshi and A. R. Shakoori. 1998b. Embroyotoxic and teratogenic effects of hexavalent chromium in developing chicks of *Gallus domesticus*. Bull. Environ. Contam. Toxicol. 61:281-288.

Babich, H., M. A. Devanas and G. Stotzky. 1985. The mediation of mutagenicity and clastogenicity of heavy metals by physiochemical factors. Environ. Res. 37:253-286.

Bader, T. F. 1986. Acute renal failure after chromic acid injection. West. J. Med. 144:608-609.

Bianchi, V., L. Celotti, G. Lanfranchi, F. Majone, G. Marin, A. Montaldi, G. Sponza, G. Tamino, J. P. Venier, A. Zantedeschi and A. G. Levis.1983. Genetic affects of chromium compounds. Mut. Res. 117:279.

Cary, E. W. 1982. Chromium in air, soil and natural waters. In: Biological and environmental aspects of chromium (ed. S. Langard), Elsevier, Amsterdam. p. 49-64.

Coogan, T. P., J. Mots, C. A. Snyder, K. S. Squibb and M. Costa. 1991. Differential DNA-protein crosslinking in lymphocytes and liver following chronic drinking water exposure of rat to potassium dichromate. Toxicol. Appl. Pharmacol. 109:60-72.

Cooper, K. R. and L. McGeorge. 1991. Decreased effluent toxicity in the Japanese medaka, *Oryzias latipes*, embryo larval assay following initiation of the toxicant reduction strategy. Aquat. Toxicol. Risk Assess. 14:67-83.

Coudray, C., P. Faure, S. Rachidi, A. Jeunet, M. J. Richard, A. M. Roussel and A. Favier. 1992. Hydroxyl radical formation and lipid peroxidation enhancement by chromium: *in vitro* study. Biol. Trace Elem. Res. 32:161-170.

DeFlora, S. and K. E. Wetterhahn. 1989. Mechanism of chromium (VI) metabolism and genotoxicity. Life Chem. Rep. 7:169-244.

DeFlora, S., M. Bagnasco, D. Serra and P. Zanacchi. 1990. Genotoxicity of chromium compounds: a review. Mutat. Res. 238:99-172.

EPA.1984. Health Assessment Document for Chromium. EPA-600/8-83-O14F. Environmental criteria and assessments office, Research Triangle Park, NC. pp. 2-10.

Friestedt, B., B. Lindqvist, A. Schultz and P. Ovrum. 1965. Survival in a case of acute oral chromic acid poisoning with acute renal failure treated by hemodialysis. Acta Med. Scand. 177:153-159.

Gale, T. F. 1982. The embryotoxic response to maternal chromium trioxide exposure in different strains of hamster. Environ. Res. 29:196-203.

Gilani, S. H. and Y. Alibhai. 1990. Teratogenicity of metals to chick embryos. J. Toxicol. Environ. Hlth. 30:23-31.

Goyer, R. A. 1986. Toxic effects of metals. In: Toxicology (The Basic Science of Poisons), 3rd Ed. (Ed. C. D. Baassen, M. O. Amdur and J. Doull). MacMillan Publishing Company, New York.

Goyer, R. A. 1990. Environmentally related disease of the urinary tract. Environ. Med. 74:377-389.

Hoffman, D. J. and W. C. Eastin. 1981. Effect of industrial effluents, heavy metals and organic solvents on Mallard embryo development. Toxicol. Lett. 9:35-40.

Hojo, Y. and Y. Satomi. 1991. *In vitro* nephrotoxicity induced in mice by chromium (VI): Involvement of glutathione and chromium (V). Biol. Trace Elem. Res. 31:21-31.

Hyodo, K., S. Suzuki, N. Furuya and K. Meshizuka. 1980. An analysis of chromium, copper and zinc in organs of a chromate worker. Int. Arch. Occup. Environ. Hlth. 46: 141-150.

Junaid, M., R. C. Murphy and D. K. Saxena. 1995. Chromium fetotoxicity in mice during late pregnancy. Vet. Hum. Toxicol. 37:320-323.

Junaid, M., R. C. Murphy and D. K. Saxena. 1996. Embryotoxicity of orally administered chromium in mice: Exposure during the period of organogenesis. Toxicol. Lett. 84:143-148.

Kaufmann, D. B., W. Dinicola and R. McIntosh. 1970. Acute potassium dichromate poisoning treated by peritoneal dialysis. Am. J. Dis. Child. 119:374-376.

Kawanishi, S. and Y. Hiraku. 1995. Mechanism of metal-mediated oxidative DNA damage. Jap. J. Toxicol. Environ. Hlth. 41:399-410.

Kawanishi, S., S. Inoue and S. Sano. 1986. Mechanism of DNA cleavage-induced by sodium chromate (VI) in the presence of hydrogen peroxide. J. Biol. Chem. 261:5952-5958.

Kim, E. and K. J. Na. 1990. Acute toxic effects of sodium dichromate on metabolism. Arch. Toxicol. 64:644-649.

Kurosaki, K., T. Nokamura, T. Mukai and T. Endo. 1995. Unusual findings in a fatal case of poisoning with chromate compounds. Forens. Sci. Intl. 75:57-65.

Laborda, R., J. Diaz-Mayans and A. Nunez. 1986. Nephrotoxic and hepatotoxic effects of chromium compounds in rats. Bull. Environ. Contam. Toxicol. 36: 332-336.

Langard, S. 1990. One hundred years of chromium and cancer: a review of epidemiological evidence and selected case reports. Am. J. Ind. Med. 17:189-215.

Langard, S. and T. Norseth. 1986. Chromium. In: Handbook on the toxicology of metals. 2nd Ed. (Ed. L. Friberg, G. F. Nordberg and V. B. Vouk). Elsevier, North Holland, pp. 185-210.

Levis, A. G. and V. Bianchi. 1982. Mutagenic and cytogenetic effects of chromium compounds. In: Biological and environmental aspects of chromium (Ed. S. Langard), Elsevier, Amsterdam. p. 171.

Lulli, G. W., J. W. Talnagi, W. R. Strohl and R. M. Pfister. 1983. Hexavalent chromium-resistant bacteria isolated from river sediments. Appl. Environ. Microbiol. 46:846-854.

Mathur, A. K., S. V. Chandra and S. K. Tandon. 1977. Comparative toxicity of trivalent and hexavalent chromium to rabbits. II. Morphological changes in some organs. Toxicology 8:53-61.

Nair, S. and V. S. Krishnamurthi. 1991. Effect of chromium on growth of *Pseudomonas aeruginosa*. Ind. J. Exp. Biol. 29:104-144.

Ohtake, H., E. Fujii and K. Toda. 1990. Bacterial reduction of hexavalent chromium. Kinetic aspects of chromate reduction by *E. cloaceae*. Hol. Biocatalysis 4:227-235.

Pedersen, R. S. and P. T. Morch. 1978. Chromic acid poisoning treated with acute hemodialysis. Nephron 22: 592-595.

Petrilli, F. L. and S. DeFlora. 1977. Toxicity and mutagenicity of hexavalent chromium in *Salmonella typhimurium*. Appl. Environ. Microbiol. 33:805-809.

Puleo, D. A. and W. W. Huh. 1995. Acute toxicity of metal ions in cultures of osteogenic cells derived from bone marrow stromal cells. J. Appl. Biomater. 6:109-116.

Shi, X. and N. S. Dalal. 1990. On the hydroxyl radical formation in the reaction between hydrogen peroxide and biologically generated chromium (V) species. Arch. Biochem. Biophys. 277:342-350.

Shivas, S. A. J. 1980. Factors which can influence the oxidation state of chromium in tanning wastes. J. Am. Leather Chem. Assoc. 75:45.

Starich, G. H. and C. Blincoe. 1983. Dietary chromium-forms and availabilities. Sci. Total Environ. 28:443-454.

Sudgen, K. D. and K. E. Wetterhahn. 1996. Identification of the oxidized products formed upon reaction of chromium (Vl) with thymidine nucleotides. J. Am. Chem. Soc. 118: 10811-10818.

Sugiyama, M., S. R. Paterio, O. Cantoni and M. Costa. 1986. Characterization of DNA lesion induced by $CaCrO_4$ in synchronous and asynchronous cultured mammalian cells. Mol. Pharmacol. 29:606-613.

Sunderman, F. W.1978. Carcinogenic effects of metals. Fed. Proc. 37:40.

Thompson, G. J. and D. A. Puleo. 1995. Effects of sublethal metal ion concentrations on osteogenic cells derived from bone marrow stromal cells. J, Appl. Biomater. 6:249-258.

Trivedi, B., D. K. Saxena, R. C. Murphy and S. V. Chandra. 1989. Embryotoxicity and fetotoxicity of orally administered hexavalent chromium in mice. Reprod. Toxicol. 3:275-278.

Vishnyakov, S. I., S. A. Levantovskii and G. F. Rhzhkova. 1992. Biological effect of chromium as a function of its valence. Biol. Nauki (Moscow) 0:105-108.

Witmer, C. M., H. S. Park and S. I. Shupack. 1989. Mutagenicity and disposition of chromium. Sci. Total Environ. 86:131-148.

Witmer, C. M., R. Haris and S. I. Shupack. 1991. Oral bioavailability of chromium from a specific site. Environ. Hlth. Perspec. 92:105-110.

Yamamoto, K. J., T. Y. Kato and H. Ohtake. 1993. Kinetics and modeling of hexavalent chromium reduction in *Enterobacter cloacae*. Biotechnol. Bioeng. 41:129-133.