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HEXAVALANT CHROMIUM IN DRINKING WATER INDUCED DEVELOPMENTAL TOXICITY IN THE RAT

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ABSTRACT

This study examines the developmental toxicity of hexavalant chromium (Cr (VI)) in rats following oral exposure to Cr (VI) in drinking water. Pregnant Fischer 344 rats ingested deionized water containing concentration levels of 100, 300 and 500 ppm Cr (VI) during the organogenesis period (days 6 through 15 of gestation). Maternal effects in the 500 ppm Cr (VI) treatment group included decreased body weight, water consumption, feed consumption, and absolute organ weights. No maternal toxicity was apparent in the 100, and 300 ppm Cr (VI) treatment groups. Maternal exposure to Cr (VI) during organogenesis significantly affected the number of live fetuses, number of early and late resorptions, number of dead fetuses, mean fetal weight, and incidence of skeletal abnormalities in the 500 ppm Cr (VI) treatment group. No external and visceral malformations were observed in any of the treated groups. On the basis of the present results hexavalant chromium showed maternal and developmental toxicity at 500 ppm Cr (VI) treatment group compared to the control and the other treated groups. Neither maternal toxicity nor embryo toxicity was apparent at the treatment groups of 100 and 300 ppm Cr (VI).

Key words: developmental toxicity, chromium hexavalent, malformations, fetotoxicity, rats.

INTRODUCTION

Chromium, like other heavy metals, is a persistent environmental contaminant with the potential for exposure through the air and water. In the United States, chromium is a major contaminant at approximately 33% of the toxic sites designated as Superfund sites (ASTDR, 1989). In Egypt, Alexandria city has more than 40 % of the Egyptian industries. Several industries in Alexandria are discharging waste water containing chromium into the surrounding watercourses either in the Mediterranean Sea or to Lake Mariut (El Mahdy, 1996). Occupational exposure to Cr (VI) generally occurs through inhalation and dermal contact, whereas the general population is exposed by ingestion through chromium contact in soil, food, and water (IPCS, 1988). The toxic effects of chromium are related to its valence state, with the hexavalent chromium compounds being much more toxic than the trivalent species as a result of its higher cellular uptake than the trivalent chromium (O'Brien *et al*., 2003; ATSDR, 2000a). Therefore, it poses a serious occupational health hazard in terms of both their potential respiratory tract carcinogenicity (ASTDR, 2000b; Crump *et al*., 2003; Wise *et al*., 2003), and their toxic effect on other organs (ASTDR, 2000a; Dartsch *et al*., 1998). In animals, several studies have reported the induction of tumors by chromium (VI) compounds (Juturu and Komorowski, 2003; Liu *et al*., 2001; Tsapakos *et al*., 1993). Cr (VI) has been shown to induce chromosomal aberration, mutations, and transformation in cultured mammalian cells (De Flora *et al*., 1990; De Flora and Wetterhahn, 1989; Sugiyama *et al*., 1992), and a variety of DNA lesions such as streaks, DNA protein cross-links, and DNA base modification (Juturu and Komorowski, 2003; Shi *et al*., 1999a; Shi *et al*., 1999b; Shi *et al*., 1992; Shi *et al*., 1994). Furthermore, Cr (VI) has been shown to produce ovarian dysfunction in mice at concentration levels 500 and 750 ppm (Murthy *et al*., 1996), impair reproductive functions and fertility in male and female mice at 1000 ppm (Al- Hamood *et al*., 1998; Elbetieha and Al-Hamoode, 1997). Indeed, Cr (VI) induced embryo lethal effects in mice in dose dependent manner at the levels 500 and 1000 ppm (Trivedi *et al*., 1989). Administration of hexavalent chromium (500, 750 ppm as potassium dichromate) via drinking water during organogenesis (day 6-14 of gestation) in mice reduced fetal weight, number of live fetuses, and induced high incidences of dead and resorption fetuses (Junaid *et al*., 1996). The same results were obtained when mice exposed to Cr (VI) at concentration levels 500 and 750 ppm during late pregnancy (days 14-19 of gestation) (Junaid *et al*., 1995). Other studies have been focused on the short and long-term exposure of female rats prior to mating and recorded a slight decrease in weight gain in mothers, a slight decrease in number of live fetuses, an increase in number of resorption and post-implantation loss in the exposed rats (pre-gestational) to 250 ppm Cr (VI), and fetal retardation and skeletal abnormalities at concentration levels 500 and 750 ppm (Kanojia *et al*., 1996; Kanojia *et al*., 1998). On the other hand, the long-term

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ingestion of Cr (VI) in rats at the 1000 ppm treated group had adverse effects on sexual behavior and territorial aggression in adult male rats (Bataineh *et al*., 1997), and induced disruption of normal testicular physiology leading to reproductive impairment in male rats (Murthy *et al*., 1991). There is no information regarding the potential developmental toxicology of Cr (VI) in rats during organogenesis period at the low concentration levels. The present study was conducted to evaluate the fetal and maternal toxicity in rats during the organogenesis period at the concentration levels considerably lower than those reported to cause reproductive toxicity in rats (Kanojia *et al*., 1996; Kanojia *et al*., 1998; Bataineh *et al*., 1997; Murthy *et al*., 1991).

MATERIAL AND METHODS

Test Species and Husbandry:

Male and female Fischer 344 rats, approximately 10 weeks old, were obtained from the high institute of public health, Alexandria University, Alexandria, Egypt. Rats were examined for health status and acclimated to the laboratory environment for 2 weeks prior to use. The animal room was designed to maintain temperature at 23 ± 2 ºC relative humidity at approximately 50% and a 12 hr light: 12 hr dark photoperiod. All animals were housed in stainless- steel cages and given standard diet, which was analyzed and shown to contain no detectable level of chromium (Mateos *et al*., 2003), and water *ad libitum* throughout the study. Adult virgin female rats were mated with adult males (one male /two females). Sperm- positive females were considered to be in Day 0 of gestation.

Cr (VI) drinking water preparation:

Potassium dichromate ($K_2Cr_2O_7$; Aldrich Chemicals) was used as a source of soluble Cr (VI). All drinking water samples were prepared in 1- liter deionized water and divided into ten separate 100- ml portions using a graduated cylinder. Each portion was placed in the labeled 100 – ml white glass bottles and *ad libitum* to each rat. The deionzed water used in the preparation of the solution was analyzed and shown to contain no detectable levels of chromium (Sun *et al*., 2003). Potassium dichromate ($K_2Cr_2O_7$) was used to make three solution concentrations; 100, 300, and 500 ppm Cr (VI). Analytical center laboratory (high institute of public health, Alexandria, Egypt) verified the concentrations of each solution using USEPA METHOD 218.6 (Arar *et al*., 1991). Water containing potassium dichromate exhibited a distinct bright yellow color, while the deionized water was used as the control exhibited a transparent colour.

Maternal effects:

Animals were examined daily throughout the experimental period to record any signs of toxicity. Body weight was recorded on days 0, 6, 9, 12, 16 and 21 of gestation. Feed consumption was recorded for each rat at days 6, 9, 12, 16 and 20 of gestation. Water consumption was measured during the treatment period. Weights of maternal organs (liver and kidneys) were recorded at the time of Cesarean section on day 21 of gestation.

Fetal effects:

Test animals were anaesthetized by diethyl ether on day 21 of gestation. The uterine horns were exteriorized through a midline abdominal incision. The following data were recorded: number of implantation sites, post implantation loss, number of live and dead fetuses, number of resorption sites, fetal sex ratio, fetal body weight, and gross external fetal abnormalitilies. The uteri of apparently nonpregnant mice were stained with a 10% sodium sulfide (Salewski, 1964), and examined for evidence of implantation sites. One-third of the fetus was fixed in Bouin's solution and then were examined by the serial sectioning technique (Wilson and Wurkany, 1965; Staples, 1974). The remaining fetus was fixed in alcohol, double stained with Alizarin Red S for ossified bone and alcian blue for cartilage, and cleared in 2% KOH and glycerin (Wilson and Wurkany, 1965).

Statistical Evaluation:

Statistical analysis was carried out using the analysis of variance (ANOVA) for body weights and absolute and relative mean organ weights. If the ANOVA was significant, a Tukey-B test was performed. The maternal body weight at day 6 of gestation was used as a covariant (ANCOVA) for comparing weights of the treatment groups with those of the control. Descriptive statistics (means and standard deviation) were reported for water and feed consumption. This was followed by multicovariate analysis (MANCOVA) to compare the entire gestational weight differences. The proportions of maternal deaths and resorbed litters were compared using the Z test for maternal of proportion (Walpole and Meyers, 1978) with a modified Bonferroni correction (Simes, 1986). Fetal weights were analyzed with a nested ANOVA followed by Turkey's multiple comparisons (Winer, 1971). Percentage of resorptions and fetuses with abnormalities were evaluated with pair-wise Mann- Whitney tests with a modified

bonferroni correction to compare each treatment to the control (Lehmann, 1975). Sex ratio and litter size data were compared with the Kruskal – Wallis test (Lehmann, 1975; Norusis, 1994). The statistical tests were conducted using a nominal $\alpha = 0.05$.

RESULTS

Maternal effects:

Sings of toxicity:

There were no deaths or abortions, and signs of toxicity during the course of the present study in any of the treated groups compared to the control.

Table 1. Water consumption (ml/kg/d) and chromium intake (mg/kg/d) in rats after oral ingestion of Cr(VI) via drinking water on gestational dasy 6 -15.

	Cr(VI) in ppm			
	Control	100	300	500
Water consumtion				
Gda				
6.9	76.63+6	75.76+13	76.17+25	76.36+8
9-12	82.67+14	83.61+26	82.12+18	83.08+8
12-15	86.15+8	86.67+6	85.62+17	63.70+5**
6-15	81.82+12	82.01+8	81.30+21	74.38+9*
Chromium intake				
Gda				
6-9	-	8+7	23+18	38+8
9-12	-	8+8	25+20	42+9
12-15	-	9+10	26+14	32+8
6-15	-	8+9	25+22	36+10

Data are presented as means +SD; aDays of gestation; *Significantly different from control at $p<0.05$;
**Significantly different from control at $p<0.01$.

Table 2. Feed consumption (g/kg/d) in rats after oral ingestion of Cr(VI) via drinking water on gestational Days 6-15.

	Days of gestation				
Cr (VI) in ppm	6	9	12	16	20
0	280.74+32	361.84+13	404.73+24	427.81+18	450.45+35
100	284.11+24	366.95+10	411.76+17	430.11+22	466.19+27
300	278.72+14	366.06+25	404.62+27	425.53+19	470.34+23
500	276.21+34	357.25+18	402.30+25	277.78+27**	166.67+16**

Data are presented as means +SD; **Significantly different from control at $p<0.01$

Water consumption and chromium intake:

Water consumption and chromium intake are presented in table 1. The amount of water consumption was reduced in the treated group of 500 ppm compared to the control and the other treated group. This reduction was observed on the gestation days 12-15. Female rats ingested three different concentration of Cr (VI) over the duration of the study (100, 300, and 500 ppm). Approximately, 82, 81, and 74 ml/kg of water of the concentrations 100, 300, 500 ppm (respectively) were consumed during the treatment period, so that the ingested daily doses were approximately equal 8, 25, and 36 mg/kg/d.

Feed consumption:

Feed consumption is given in table 2. Feed consumption was statistically significant decreased in the treatment groups of 500 ppm compared to the control and the other treated groups. These decreases were shown on the day 16 through 20 of gestation.

Table 3. Maternal parameters on rats after oral ingestion of Cr (VI) via drinking water on gestational days 6-15.

		Cr (VI) in ppm			
	Days	0	100	300	500
Number of Dams		28	26	26	26
Maternal body weight (g)	6	160.29+0.15	158.39+0.30	161.45+0.22	159.35+0.60
	9	165.82+0.10	163.51+0.90	166.64+0.11	167.95+0.09
	12	172.53+0.9	170.93+0.10	173.93+0.10	174.55+0.16
	16	187.22+0.20	186.83+0.15	188.22+0.21	170.54+0.10*
	21	224.47+0.50	220.30+0.14	225.79+0.89	180.07+0.62**
% weight gain for all damsa	6-21	40.04+5	39.09+9	39.85+11	13.00+13**
Absolute organ weightsb					
Liver		8.11+0.99	7.89+0.25	7.99+0.80	6.38+0.22*
Kidneys		0.42+0.88	0.40+0.20	0.41+0.91	0.28+0.12**
Relative organ weightsc					
Liver		0.04+0.94	0.04+0.33	0.04+0.68	0.04+0.66
Kidneys		0.002+0.71	0.002+0.57	0.002+0.58	0.002+0.49

Data are presented as means +SD; a Average percentage of maternal weight changes from gestation days 6-21; b grams organ weight; c gram organ weight / body weight; *Significantly different from control at p<0.05; ** Significantly different from control at p<0.01.

Table 4. Developmental toxicity in rats after oral ingestion of Cr (VI) via drinking water on gestational days 6-15.

	Cr (VI) in ppm			
	0	100	300	500
Number of females	30	30	30	30
Number of pregnant (%)a	28 (93)	26 (87)	26 (87)	26 (87)
Number of litters	28	26	26	26
Number of implants/litter	10.79+3	9.90+5	10.55+4	10.70+2
Liver fetuses/litter (%)b	10.55+1 (98)	9.79+3 (99)	10.14+4 (96)	6.45+5 (60)**
% of postimplantation lossc	2.22+8	1.11+10	3.89+9	39.72+13**
Dead fetuses/litter (%)b	0.09+0.8 (1)	0.08+0.7 (1)	0.09+0.5 (1)	1.6+0.9 (15)*
Early resorptions/litter (%)b	0.07+0.9 (1)	0.03+0.6 (1)	0.17+0.9 (2)	1.7+3 (16)*
Late resorptions/litter (%)b	0.08+0.4 (1)	0+0.0 (0)	0.15+0.8 (1)	0.95+0.9 (9)*
Litters with resorption (%)b	1 (4)	1 (4)	2 (8)	17 (65)**
Liters totally resorbed (%)	0 (0)	0 (0)	0 (0)	3 (12)*
Sex ratio (M/F) / litter	5.11:5.44	4.80:4.99	5.00:5.14	3.11:3.34
Fetal body weight (g) / litter	5.60+0.09	5.11+0.20	5.75+0.55	3.00+0.22**

Data are presented as means; a Number of females detected as being pregnant by visual inspection of the uterus/total number of females with vaginal plusg x 100. Sodium sulfide staining was performed on all uteri without gross evidence of pregnancy but did not disclose resorption sites in any of the evaluated dams; b % of implantations; c [(No. of implants – No. live fetuses] x 100;
*Significantly different from control at p<0.05; ** Significantly different from control at p<0.01.

Maternal Body and Organ Weights:

Changes of maternal body weight are given in table 3. The analysis revealed statistically significant decreases of maternal body weights in the 500 ppm Cr (VI) treatment group compared to the control and the other treated groups. This decrease was shown at day 16 and 21 of gestation. analysis of the average percentage maternal change from days 6-20 revealed a reduction in the weight gain which, when compared to the control was statistically significant in the 500 ppm treated group. Hexavalent chromium Cr (VI) treatments resulted in changes in absolute weights of liver and kidney. These decreases in organ weights were significantly showed in the 500 ppm treatment group compared to the control and the other

treated groups. There were no statistically significant changes in the relative organ weights in any of the treatment groups compared to the control.

Fetal effects:

Developmental parameters and fetal body weights are presented in table 4. The number of live fetuses in the 500 ppm treatment group differed significantly from the control and the other treated groups. It was significantly decreased in this treatment group. While no significant difference was observed in the sex ratio of fetuses in any of the treatment groups, Cr (VI) produced significant increases in the embryo lethality in the 500 ppm treated group with approximately 40% of the implants resorbed compared to the control and the other treated groups. Number of the total resorption litters and the litters with resorption in the treated group of 500 ppm was significantly increased up to 58 and 12% (respectively) compared to the control group. There were statistically significant increases in the number of dead, early, and late resportion fetuses in the 500 ppm Cr (VI) group compared to the control and the other treated groups. Fetal growth retardation was observed in the 500 ppm Cr (VI) group compared to the control and the other treated groups.

External, visceral, and skeletal abnormalities are summarized in table 5. Neither external nor visceral abnormalities were observed in the fetuses in any of the treatment groups compared to the control. Skeletal abnormalities shown in the treatment groups of 500 ppm Cr (VI) compared to the control and the other treated groups. Skeletal abnormalities were consisted of absence of sacral vertebrae (arches and, ossified centers), malformation long bones (hypoplastic fibula), and complete absence of phalanges.

Table 5. External and skeleton abnormalities in rats fetuses after maternal oral ingestion of Cr (VI) via drinking water on gestational days 6-15 of gestation.

		Cr (VI) in ppm			
		0	100	300	500
Number of fetuses (Number of litters) examined					
External examinations a		295 (28)	255 (26)	264 (26)	168 (26)
Visceral examinations		98 (28)	85 (26)	88 (26)	56 (26)
Skeletal examinations		197 (28)	170 (26)	176 (26)	112 (26)
Number affected (percentage affected)					
External abnormalities					
Anophthalmia	Fb	2 (0.7)	3 (1)	2 (1)	2 (1)
	L	2 (7)	3 (12)	1 (4)	2 (8)
Kink tail	F	1 (0.3)	1 (0.4)	2 (1)	0 (0)
	L	1 (4)	1 (4)	1 (4)	0 (0)
Polydactyly	F	1 (0.3)	2 (1)	0 (0)	1 (1)
	L	1 (4)	2 (8)	0 (0)	1 (4)
Visceral abnormalities					
Hrmorrhage in the liver	F	1 (1)	1 (4)	1 (4)	1 (2)
	L	1 (4)	1 (1)	1 (1)	1 (4)
Skeletal abnormalities					
Bsence of sacrum	F	0 (0)	0 (0)	0 (0)	10 (9)*
	L	0 (0)	0 (0)	0 (0)	6 (23)*
Hypoplastic fibula	F	0 (0)	0 (0)	0 (0)	11 (10)*
	L	0 (0)	0 (0)	0 (0)	5 (5)*
Absence of phalanges	F	2 (1)	2 (2)	1 (1)	33 (30)**
	L	1 (4)	2 (8)	1 (4)	15 (58)**

A Only live fetuses were examined; b F = fetuses; L = litters;
*Significantly different from control at $p<0.05$; ** Significantly different from control at $p<0.01$.

DISCUSSION

In the present investigation, Cr (VI) had no significant maternal and embryo- / or feto toxicity in the pregnant rats at concentration levels of 100 and 300 ppm compared to the control group. However, Cr (VI) induced maternal and developmental toxicity in female rats at the concentration level of 500 ppm compared to the control group. The major forms of the toxicity evidenced by the appearance of changes of the maternal body weights, feed

consumption, water consumption, number of live fetuses, and evidences of the embryo-lethality. Exposure of rats to 500 ppm of Cr (VI) increased the number of resorptions and dead fetuses, and the incidences of skeleton abnormalities. There were no evidences of external, visceral abnormalities, and changes in the relative organ weights in any of the treated groups compared to the control. Although several studies investigated the potential developmental toxicity of Cr (VI) in mice (Murthy *et al*., 1996; Junaid *et al*., 1996; Junaid *et al*., 1995; Al- Hamood *et al*., 1998; Trivedi *et al*., 1989) only limited studies have addressed Cr (VI) induced the adverse effects in the pregnant rats. In general, most of these studies have been focused on the short and long-term exposure of female rats prior to mating (Kanojia *et al*., 1996; Kanojia *et al*., 1998). The data of those reported studies indicated that a slight decrease in weight gain in mothers, a slight decrease in number of live fetuses, an increase in number of resorption and post-implantation loss in the exposed rats (pre-gestational) to 250 ppm Cr (VI). These results differ from the current study which indicated no developmental effects at 300 ppm treated groups, one possibility that might explain the difference in results is that the exposed period of gestation and the duration of exposure are different and longer than the current study. Regardless of the time and duration of the exposure, Cr (VI) induced significant level of skeletal abnormalities in rats at concentration level 500 ppm, thus the current data are in agreement with those reported (Kanojia *et al*., 1996; Kanojia *et al*., 1998). The present study shows that at the highest concentration group 500 ppm Cr (VI) pregnant females lost their weights in the last period of treatment. A possible explanation as well as that chromium has also a direct weight loss effect (Grante, 1997). On the other hand, Cr (VI) exposure reduced the amount of the water and feed consumption. This reduction is consistent with the data indicated that chromium helps to control appetite (Mertz, 1993). On the other hand, the reduction in the maternal weight gains, food consumption, and weight of organs may have been the trigger for the decreased fetal weights, observed fetal death and resorption. Furthermore, the increase in the number of dead and resorption fetuses' likely accounts for the decrease of live of fetuses. Although Cr (VI) has the ability to penetrate the placental barrier and accumulate in the placenta (Junaid *et al*., 1995; Miller and Thiede, 1981; Clarkson *et al*., 1983), there were no external and visceral abnormalities observed in any of the treatment groups. Skeletal abnormalities were shown in the treatment group of 500 ppm Cr (VI). The fetuses were severely growth retarded at 500 ppm, the skeletal alteration (absence of phalanges) may be due to the developmental delay as opposed to a direct effect of chromium on the bone. Furthermore, these results may be attributed to the slow uptake of chromium from drinking water, thus its modulating physiology resulting in resorptions, skeletal abnormalities, and dead fetuses (Finley *et al*., 1997). Cr (VI) can be reduced by cellular reductants to generate reactive chromium intermediates, such as Cr (VI) and Cr (IV). These reductants include ascorbate, glutathione, and glutathione reductase. During the Cr (VI) reduction processes, molecular oxygen is reduced to super oxide radical (O°`2 ), which then forms H2O2 by dismutation. Both Cr (VI) and Cr (IV) are able to react with H2O2 to generate hydroxyl radical (°OH) through Fenton-like reaction.( O2°`), H2O2 and (°OH) are collectively called reactive oxygen species (ROS). These oxygen species can cause various cellular injuries, including DNA damage, lipid peroxidation, and protein modification (O'Brien, *et al*., 2003; Liu *et al*., 2001; Shi *et al*., 1999a; Pourahmad and O'Brien, 2001; Stohs *et al*., 2001; O'Flaherty, 1996; Debetto and Luciani, 1988). Therefore, it is generally accepted that only Cr6+ penetrates through the plasma membrane, being reducted to Cr3+ inside the cell and firmly bound to cell components, leading to mutagenic effects that may be associated with the maternal and developmental effects. Placental transport plays an active role in the uptake of the chromium hexavalent or metabolites which adds to concern about pregnant exposure to these compounds (Trived *et al*., 1989; Miller and Thiede, 1981; Clarkson *et al*., 1983). In conclusion, the present study data demonstrated that Cr (VI) can produce developmental toxicity in rats at 500 ppm, which were maternally toxic dose (36 mg/kg/d). However no evidence of maternal and developmental toxicity was observed at 100 ppm (8 mg/kg/d), and 300 ppm (25 mg/kg/d). Although 36 mg/kg/d is about 2700 time the RFD (0.02 mg/kg/d) (EPA, 1991), and 0.45 of LD50 for rats (Material Safety Data Sheet, 1999), we are concerned that such an exposure might occur occupationally. There may be a need to more recent information regarding the developmental toxicity of Cr (VI) to re-evaluate its toxicity during the pregnancy.

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