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Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: effect on fertility

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Abstract

Sexually mature male and female mice at 50 days of age were exposed to trivalent (Chromium chloride) or hexavalent (potassium dichromate) chromium compounds in drinking water for 12 weeks. The effects of the direct chromium exposure on fertility was assessed at day 140 of age.

Fertility was significantly reduced in males exposed to the trivalent chromium compound. The number of implantation sites and the number of viable fetuses was significantly reduced in females impregnated by males exposed to the hexavalent chromium compound. The number of resorptions and dead fetuses was increased in females impregnated by males exposed to trivalent and hexavalent chromium compounds. The exposure of female mice to trivalent and hexavalent chromium compounds significantly reduced the number of implantation sites and the number of viable fetuses. The number of females with resorptions was significantly increased in hexavalent chromium exposed females. The number of resorptions was increased in trivalent and hexavalent exposed females.

Body, seminal vesicles and preputial gland weights were significantly reduced in males exposed to trivalent and hexavalent chromium, whereas testes weight was significantly increased in males exposed to these compounds. Furthermore, ovarian weight was significantly increased in females exposed to trivalent and hexavalent chromium, whereas uterine weight was significantly decreased in trivalent chromium exposed females.

In conclusion, the ingestion of trivalent and hexavalent chromium compounds by adult male and female mice would cause adverse effects on fertility and reproduction. Copyright © 1997 Elsevier Science Ireland Ltd.

Keywords: Chromium chloride; Potassium dichromate; Fertility; Mice

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1. Introduction

There is growing interest in toxicological studies on chromium compounds because of their potential use in modern industries and the consequences of human exposure to these compounds (Al-Hamamy et al., 1987; Nriagu and Nieboer, 1988; Bonde and Christensen, 1991; Domingo, 1994). The toxic effects of chromium and its compounds have been reviewed recently (Baruthio, 1992). Occupational exposure to hexavalent chromium is of concern because of its mutagenic and carcinogenic actions (Stern, 1981; Langard, 1982). Chromium compounds are being used in ceramics, catalysts, pigments, metal finishing, corrosion control, the tanning industry, wood preservatives, fungicides, printing and dyeing textiles and manufacturing magnetic tapes (Nriagu, 1988).

Extensive work has been conducted on the biological effects of chromium including its role in regulating normal carbohydrate metabolism (Mertz, 1969), embryotoxicity (Gale, 1978; Gale and Bunch, 1979; Iijima et al., 1979; Danielsson et al., 1982), teratogenicity (Matsumoto et al., 1976; Iijima et al., 1979) and postnatal development (Al-Hamood and Al-Bayati, 1994).

In the light of the shortage of published data on the effects of chromium compounds on reproductive capacity in adult laboratory animals, the present work was planned to monitor the effects of long-term ingestion of trivalent and hexavalent chromium compounds on the fertility of male and female mice. It is well known that the biochemical actions of chromium are dependent on its chemical form (Baruthio, 1992).

2. Materials and methods

2.1. Animals

Sexually mature male and female Swiss mice (day 50 of age) were used in these experiments. They were raised in the animal house unit in the Medical Faculty at the Jordan University of Science and Technology in controlled temperature 21°C ± 1°C under a 12 h light: 12 h darkness schedule (lights 06.00–18.00 h). Food and water were available ad libitum.

2.2. Administration of chromium compounds

Trivalent chromium (Chromium chloride: Janssen Chimica, B-2440 Geel, Belgium) or hexavalent chromium (potassium dichromate: Fluka AG, Chemische Fabrik CH-9470, Bucks) were dissolved in drinking tap water at various concentrations. The duration of exposure of mice was 12 weeks. Control mice were given tap water. It is worth mentioning that at higher concentrations of chromium chloride and potassium dichromate, the experimental animals consumed less water per day compared to the control group.

2.3. Fertility tests and examination of fetuses

2.3.1. Experiment 1

Fertility was estimated in male mice (day 140 of age) exposed for 12 weeks to 1000 or 5000 mg/l trivalent chromium compound, 1000, 2000, 4000 or 5000 mg/l hexavalent chromium compound and in control male counterparts.

After 12 week chromium exposure each male was placed in an individual cage with two virgin untreated females of the same strain. They were left together for ten days during which two estrus cycles should have elapsed (Rugh, 1968). The chromium exposed males were then removed and 1 week later the females were killed by cervical dislocation under light ether anaesthesia and the following measurements were recorded: number of pregnant females, number of viable fetuses and number of resorptions.

2.3.2. Experiment 2

Fertility was estimated in adult female mice (day 140 of age) exposed to 2000 or 5000 mg/l chromium chloride or potassium dichromate and in control female counterparts.

Treated females were divided into groups of three animals each, and housed with a sexually mature untreated male of proven fertility for ten days. During this period at least two estrus cycles should have elapsed (Rugh, 1968).

Chromium exposed females and control females were killed by cervical dislocation under light ether anaesthesia 1 week after the removal of the males. The following measurements were recorded: number of pregnant females, total implantations, number of viable fetuses and number of resorptions.

2.4. Body and organ weights

In another experiment, male and female mice exposed to chromium chloride and potassium dichromate and their control counterparts were sacrificed after 12 weeks of exposure (day 140 of age). In males, body weight and weights of paired testes, seminal vesicles (stripped of fluid) and preputial glands were recorded. In females, body weight and weights of paired ovaries and uteri were also recorded.

2.5. Statistical analysis

Data are expressed as means ± S.D. Differences between control and chromium exposed groups were analyzed using either Chi-square or Student '*t*' tests (Siegel, 1956)

3. Results

3.1. Effect of trivalent and hexavalent chromium compounds on fertility of male mice

When the adult males exposed to trivalent and hexavalent chromium and unexposed control males were tested with adult untreated females for 5 min, males were sexually interested, as females were pursued by males, their genitalia were examined and mounting occurred.

Table 1 shows that fertility was significantly ($p < 0.005$) reduced in males exposed to 5000 mg/l trivalent chromium compound. The number of implantation sites and the number of viable fetuses were reduced significantly in females impregnated by males exposed to 2000 mg/l ($p < 0.01$) and 4000 mg/l ($p < 0.05$) hexavalent chromium compound.

The numbers of resorptions and dead fetuses were increased in females impregnated by trivalent and hexavalent chromium exposed males.

3.2. Effects of trivalent and hexavalent chromium compounds on fertility of female mice

Table 2 demonstrates that the exposure of adult female mice to trivalent and hexavalent chromium compounds had no effect on female fertility (pregnancy occurred in a similar frequency control and chromium exposed females). The numbers of implantations were significantly reduced in pregnant females exposed to 2000 mg/l ($p < 0.01$) and 5000 mg/l ($p < 0.01$) of the trivalent chromium, 2000 mg/l ($p < 0.01$) and 5000 mg/l ($p < 0.05$) hexavalent chromium compounds. On the other hand, the number of viable fetuses was significantly reduced in pregnant females exposed to 2000 mg/l ($p < 0.01$) and 5000 mg/l ($p < 0.001$) of trivalent chromium compound. The number of viable fetuses was also significantly reduced in pregnant females exposed to 2000 mg/l ($p < 0.05$) and 5000 mg/l ($p < 0.01$) of hexavalent chromium compound.

The number of pregnant female mice with resorptions was significantly increased in females exposed to 2000 mg/l ($p < 0.01$) and 5000 mg/l ($p < 0.005$) hexavalent chromium compound. The number of resorptions was high in pregnant females exposed to trivalent and hexavalent chromium compounds compared to control pregnant females.

3.3. Body and organ weights

Table 3 shows the effects on body and organ weights of male mice after the exposure to 2000 and 5000 mg/l trivalent and hexavalent chromium. As can be seen the body weight significantly decreased in 2000 mg/l ($p < 0.05$), 5000 mg/l ($p < 0.01$) trivalent chromium compound, 2000 mg/l and 5000 mg/l ($p < 0.01$) hexavalent chromium exposed males. Testes weights were significantly increased in 2000 mg/l ($p < 0.01$), 5000 mg/l ($p < 0.05$) trivalent chromium, 2000 mg/l ($p < 0.01$), and 5000 mg/l ($p < 0.05$) hexavalent chrmium exposed males. Seminal vesicles

Table 1
Effect of long-term exposure to trivalent and hexavalent chromium compounds via drinking water on fertility of male mice

Treatment	No. of males	No. of females	No. of pregnant females	No. of implantations[a]	No. of viable fetuses[a]	Total number of resorption or dead fetuses
Control	20	40	33/40 (82.5%)	8.18 ± 1.59 (33)	8.18 ± 1.59 (33)	0
Trivalent chromium (2000 mg/l)	10	20	18/20 (90%)	7.47 ± 2.50 (18)	7.33 ± 2.91 (18)	6 resorptions
Trivalent chromium (5000 mg/l)	9	18	8/18 (44%)***	6.87 ± 2.23 (8)	6.00 ± 3.31 (7)	1 resorption, 12 dead fetuses
Hexavalent chromium (1000 mg/l)	19	38	33/38 (86.8%)	7.84 ± 1.56 (33)	7.75 ± 1.80 (33)	3 resorptions
Hexavalent chromium (2000 mg/l)	11	22	20/22 (90.9%)	6.33 ± 2.79 (20)**	6.33 ± 2.79 (20)**	0
Hexavalent chromium (4000 mg/l)	9	18	16/18 (88.8%)	6.86 ± 1.88 (16)*	6.86 ± 1.88 (16)*	0
Hexavalent chromium (5000 mg/l)	13	26	19/26 (73%)	7.84 ± 2.73 (19)	7.15 ± 2.98 (19)	6 resorptions, 6 dead fetuses

*$p < 0.05$, **$p < 0.01$ significantly different compared to control value (Student's t test).
***$p < 0.005$ significantly different compared to control value (Chi-square test).
[a]Results are expressed as mean ± S.D.

Table 2
Effect of long-term exposure to trivalent and hexavalent chromium compounds via drinking water on fertility of female mice

Treatment	No. of females	No. of pregnant females	No. of implantations[a]	No. of viable fetuses[a]	No. of mice with resorptions	Total number of resorptions
Control	18	17/18	9.00 ± 1.36 (17)	8.76 ± 1.39 (17)	2/18 (11%)	4
Trivalent chromium (2000 mg/l)	14	10/14	6.30 ± 2.71 (10)**	5.55 ± 3.00 (9)**	4/14 (28%)	13
Trivalent chromium (5000 mg/l)	12	10/12	5.70 ± 3.12 (10)**	5.85 ± 1.67 (7)***	4/12 (33%)	16
Hexavalent chromium (2000 mg/l)	15	14/15	7.35 ± 1.54 (14)**	6.55 ± 2.18 (9)*	8/15 (53%)****	37
Hexavalent chromium (5000 mg/l)	11	9/11	7.44 ± 1.50 (9)*	5.88 ± 2.47 (9)**	7/11 (63%)*****	14

*$p<0.05$,
**$p<0.01$, ***$p<0.001$ significantly different compared to control value (Student's 't' test).
****$p<0.01$, *****$p<0.005$ significantly different compared to control value (Chi-square test).
[a]Results are expressed as mean ± S.D.

Table 3
Body and organ weights of male mice exposed to trivalent and hexavalent chromium compounds for 12 weeks via drinking water at 140 days of age

Details	Control	Treatments			
		Trivalent chromium		Hexavalent chromium	
		(2000 mg/l)	(5000 mg/l)	(2000 mg/l)	(5000 mg/l)
No. of animals	10	12	9	13	13
Body weight (g)	35.7 ± 1.40	30.56 ± 6.70*	33.35 ± 2.00**	32.08 ± 3.44**	31.43 ± 4.35**
Testes weights (mg/10 gm B.wt.)[a]	50.72 ± 5.77 (8)	61.78 ± 9.41 (9)**	61.22 ± 8.94 (9)*	59.62 ± 4.87 (9)**	61.64 ± 12.16 (12)*
Seminal vesicles (mg/10 gm B.wt.)[a]	44.50 ± 3.74 (7)	42.16 ± 4.72 (11)	35.44 ± 6.22 (9)**	42.22 ± 6.11 (9)	32.30 ± 10.78 (13)***
Preputial gland (mg/10 gm B.wt.)[a]	18.70 ± 2.57 (9)	10.13 ± 2.08 (11)***	15.00 ± 2.91 (9)*	18.38 ± 1.91 (13)	12.30 ± 4.19 (13)***

Numbers in parentheses indicate number of animals.
Results are expressed as mean ± S.D.
*$p<0.05$, **$p<0.01$, ***$p<0.001$ significantly different compared to control value (Student's t test).
[a]Relative weights.

weights were significantly reduced in 5000 mg/l trivalent chromium ($p < 0.01$) and hexavalent chromium ($p < 0.001$) exposed males. Preputial glands were also significantly reduced in 2000 mg/l ($p < 0.001$) and 5000 mg/l ($p < 0.05$) trivalent chromium and 5000 mg/l ($p < 0.001$) hexavalent chromium exposed males.

Table 4 shows the effects on body weight and organ weights of female mice after exposure to 5000 mg/l trivalent and 5000 mg/l compounds. It is clear that the exposure of female mice to 5000 mg/l trivalent and hexavalent chromium had no significant effect on body weights. However, ovarian weights were significantly increased in trivalent ($p < 0.05$) and hexavalent ($p < 0.05$) exposed females, whereas uterine weights were significantly reduced ($p < 0.01$) in trivalent exposed females only.

4. Discussion

The aim of the present study was to monitor the adverse effects on fertility and reproduction of trivalent (chromium chloride) and hexavalent mice (potassium dichromate) chromium compounds ingested with drinking water by male and female. Generally there were no mortality or clinical signs of toxicity in any group of male or female mice exposed to trivalent or hexavalent chromium compounds at the concentrations used in our experiments.

The animal model used in this work has been used previously by several workers to assess the adverse effects of metals on reproductive functions in laboratory animals (Zerkin et al., 1985; Johansson and Wide, 1986; Llobet et al., 1993; Pinon-Lataillade et al., 1993).

The results presented in this paper show that the exposure of male mice to trivalent and hexavalent chromium compounds for 12 weeks had adverse effects on male reproductive system and fertility. However, the mating capability of male mice was not adversely affected by the exposure of these males to trivalent and hexavalent chromium compounds for 12 weeks.

The results show that the number of pregnant females impregnated by males exposed to the trivalent chromium compound was significantly reduced. The number of implantation sites and the number of viable fetuses were also reduced in females impregnated by males exposed to the hexavalent chromium compound. The number of resorptions and dead fetuses was increased in

Table 4
Body and organ weights of female mice exposed to trivalent and hexavalent chromium compounds for 12 weeks via drinking water at 140 days of age

Details	Treatments		
	Control	Trivalent chromium (5000 mg/l)	Hexavalent chromium (5000 mg/l)
No. of animals	8	11	10
Body weight (g)	34.6 ± 6.08	34.20 ± 4.18	32.73 ± 4.00
Ovarian weights (mg/10 gm B.wt.)[a]	2.21 ± 0.83	3.63 ± 1.62*	3.40 ± 1.26*
Uterine weight (mg/10 gm B.wt.)[a]	23.75 ± 8.64	13.90 ± 4.92**	21.88 ± 4.37

[a]Relative weights.
Results are expressed as mean ± S.D.
*$p<0.05$, **$p<0.01$ significantly different compared to control value (Student's t test).

females impregnated by males exposed to either trivalent or hexavalent chromium compounds (Table 1).

Similar studies have shown that ingestion of lead chloride by male mice reduced their fertility, this was reflected by an increase of the number of mated females without implantations (Johansson and Wide, 1986) and ingestion by females of large doses of lead acetate reduced the pregnancy rate among the females and increased the number of early embryonic deaths (Varma et al., 1974). The increase in the number of resorptions in female mice impregnated by chromium exposed males (Table 1) may be attributed to an increase in perimplantation mortality of fertilized ova. Body weight and weights of sex accessory glands such as seminal vesicles and preputial glands were reduced in males exposed to trivalent and hexavalent chromium compounds, whereas the testicular weights were increased in the same groups (Table 3). The reduction in body weight observed in this study is not likely to be a cause of failure of reproduction, an assumption generally consistent with results of previous reports. Chapin et al. (1993) found that even a 30% decrease in body weight gain had only minimal effects on reproduction in Swiss CD-1 mice. The effect of exposure to chromium compounds on seminal vesicles and preputial gland weights reflects the effect on sex hormones. The size and activity of the preputial gland in rodents are clearly influenced by a variety of steroid hormones (Ebling, 1963). The preputials also produce behavior-modulating pheromones (Brain et al., 1983) which alter fighting and other behaviour in the mouse. The data presented in this paper on the effect of chromium compounds on the weight of the preputial glands suggest that the exposure to chromium might result in a profound effect on the territorial aggression of male mice.

The increase in the testes weight noticed in the present study does not agree with the findings of other workers using the rabbit (Behari et al., 1978). These workers reported that intraperitoneal administration for 6 weeks of chromium nitrate (Trivalent compound) was more effective than potassium dichromate (hexavalent compound) in inducing testicular degeneration and inhibition of both spermatocyte and testicular enzyme production. Species differences for chromium metabolism has been previously indicated (Kargacin et al., 1993).

The results presented in this paper also show that the exposure of female mice to trivalent and hexavalent chromium compounds for 12 weeks had an adverse effect on the female reproductive system and fertility. The number of implantation sites and the number of viable fetuses were reduced in trivalent and hexavalent chromium exposed females and the number of resorptions increased in females exposed to the hexavalent chromium compound (Table 2). The uterine weight was also reduced in females exposed to the trivalent chromium compound (Table 4).

The deleterious effect of trivalent and hexavalent chromium compounds on female mice fertil-

ity observed in this work suggests a disturbance of reproductive endocrine functions (multiple sites of toxicity along the hypothalamic-pituitary-ovarian-uterine axis are possible). Jacquet et al. (1977) and Leonard et al. (1983) also have published evidence suggesting direct ovarian toxicity from lead which decreases the secretion of progesterone responsible for endometrial alteration at the time of implantation. Wide (1980, 1983) confirmed that in addition to possible effects on progesterone secretion, lead also alters uterine estrogen receptors which may have further impact on the maintenance of pregnancy.

In light of these facts, embryonal resorptions noticed in female mice exposed to chromium compounds most probably resulted from modification of the uterine lining function before the arrival of the embryo. Maternal toxicity invariably causes increased early resorptions, reduction in fetal body weight or late fetal death (Khera, 1984, 1985, 1987). Our results show that exposure of female mice to trivalent and hexavalent chromium increased the ovarian weight (Table 4). Histological studies need to be conducted in order to clarify whether the weight increase in testes or ovaries observed in this study is due to hyperplasia or hypertrophy of a specific tissue compartment in these organs.

The adverse effects of trivalent and hexavalent chromium compounds on male and female mice fertility noted in this work suggest that these compounds seriously disturbed the hypothalamic-pituitary-gonadal system. This conclusion is consistent with the evidence that trivalent and hexavalent chromium compounds are embryotoxic and teratogenic in animals (Nieboer and Yassi, 1988).

In conclusion, this work reveals that the ingestion of trivalent and hexavalent chromium compounds by adult male and female mice would cause adverse effects on fertility and reproduction.

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