http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 3 (9): 1519-1524, 2000
© Copyright by the Capricorn Publications, 2000

Effects of Chromium Compounds on Incidence of Social Aggression and Fertility in Prepubertal Male Mice

H.H. Hussain[1], Merza H. Homady[2] and Khaled A. Tarawneh[2]
[1]Department of Chemistry, Ottawa University, 10 Marie Curie, Ottawa, Ontario KIN 6N5 Canada
[2]Department of Biological Sciences, Mu'tah University, P.O. Box (7), Al-Karak-Jordan

Abstract: The effects of ingestion of trivalent (chromium chloride) and hexavalent (potassium dichromate) chromium compounds were investigated on social aggression and fertility in male mice. Prepubertal male mice were exposed to these salts in drinking water at concentrations of 1000 and 5000 ppm for 90 days. The exposure of male mice to chromium chloride at 1000 or 5000 ppm significantly augmented social aggression. Fertility was significantly reduced in males exposed to the hexavalent chromium compound. The number of implantation sites and the number of viable fetuses in females impregnated by males exposed to this compound were significantly reduced. The exposure of male mice to the trivalent chromium compound had, however, no effect on fertility. Body, testes, preputial gland and seminal vesicle weights were significantly suppressed in males exposed to the hexavalent compound but no such effects were evident in mice given the trivalent chromium compound. The results show that the ingestion of trivalent and hexavalent chromium compounds by male mice in prepubertal life have very different effects on both social aggression and fertility. Only potassium dichromate produced a pattern of responses clearly indicative of suppressed gonadal function.

Key words: Chromium, aggression, fertility, fetus, testes, pheromone, mice, preputial gland

Introduction

It is generally accepted that significant quantities of toxic metallic elements, such as cadmium, lead, mercury and nickel are being introduced into our environment from man-made and natural sources (Fishbein, 1981). Since projections for the near future indicate increased utilization for all of these metallic pollutants. Continued study of their biological effects is important and timely due in part to the possibility of human infertility (Clarkson *et al*., 1985; Zelikoff *et al*., 1995). Metals are omnipresent in the environment occuring in varying concentrations in air, bedrock, soil, water and all biological matters. Environmental exposure to heavy metals is associated with a wide range of toxic effects. Elements such as arsenic, cadmium, lead, mercury are notorious developmental toxicants that have profound effects upon embryonic and fetal development. These elements are responsible for certain malformations in mammalian embryos (Ferm, 1971).

Chromium is found in nature in several valence states. In general the metallic and trivalent forms are nontoxic while hexavalent chromium compounds produce toxic effects in various tissues of humans and experimental animals because of their mutagenic and carcinogenic actions (Langards, 1982). Research on the effects of chromium on living organisms involves two general areas: 1-at physiological levels chromium in the form of an organic complex is considered as "glucose tolerance factor" which is involved in the regulation of normal carbohydrate metabolism in mammals (Mertz, 1975). Upon the basis of this research chromium is considered as an essential trace elements (National Academy of Sciences, 1974; Hambridge, 1981) but at higher exposure levels chromium is a toxic substance. It is important to note that this toxicity is directly related to the valence state of this element. Both acute and chronic toxicity of chromium are mainly caused by hexavalent compounds while the metallic and trivalent forms exhibit a low degree of toxicity. Human exposure to hexavalent chromium compounds produces a variety of deleterious effects including chronic irritation of the respiratory system, ulceration and perforation of the nasal septum, cutaneous ulcers and allergic eczematous contact dermatitis, bronchial carcinomas, gastroenteritis, hepatocellular deficiency and renal oligoanuric deficiency (Baruthio, 1992). 2-the second area of research concerns the effect of varying the time of administration on the incidence and characteristics of the chromium-induced embryotoxicity.

Treatment of experimental animals with hexavalent chromium results in pathological changes in the respiratory system, gastrointestinal tract irritation and corrosion and kidney lesions (National Academy of Sciences, 1974). It also causes chromosomal aberrations and sister-chromatid exchanges (Sarto *et al*., 1982; Hamamy *et al*., 1987). Considerable interest has been given to chromium compounds including their embryotoxicity (Gale and Bunch, 1979) teratogenicity (Shepard, 1980) and postnatal development (Al-Hamood and Al-Bayati, 1994) in laboratory animals. It has also been determined that chromium trioxide is embryolethal and produces cleft palates and skeletal abnormalities in surviving fetuses when this hexavalent chromium compound is administrated to pregnant LVG hamsters on day 8 of gestation (Gale, 1978).

A significant factor in teratologic research involves the interaction between the genetic composition of an organism and the teratogenic agent to which that organism is exposed. This interrelationship is demonstrated by the fact that different species do not exhibit the same degree of susceptibility to environmental factors which produce congenital malformations. This is exemplified by such diverse teratogenic agents as cortisone, thalidomide (Wilson, 1973) and lead (Jessup, 1967).

The present work was aimed to examine the effects of exposure of adult male mice after prepuberty to trivalent (chromium chloride) and hexavalent (potassium dichromate) on social aggression and fertility.

Materials and Methods

Animals: Tuck Ordinary (TO) strain albino mice were bred and maintained in the animal house unit in the Faculty of Sciences at Mu'tah University under controlled temperature 21 + 1°C in 12 h light:12 h darkness schedule (white lights on 06.00-18.00 h local time). Subjects were housed in type M1 plastic cages (North Kent plastics, Erith Kent, U.K.) measuring

30 × 12 × 11 cm with wire grid tops. Sawdust bedding was used and food and water were available *ad-libitum*.

Forty intact male "resident" mice were individually-housed at 15 weeks of age for three days before behavioral tests to induce a moderate level of aggressiveness (Goldsmith *et al.,* 1976).

A further 40 group-housed (N = 8) intact male mice were allocated to 5 categories at 3 weeks of age as follows with:

1 Category (1) received chromium chloride 1000 ppm (trivalent)
2 Category (2) received chromium chloride 5000 ppm (trivalent)
3 Category (3) received potassium dichromate 1000 ppm (hexavalent)
4 Category (4) received potassium dichromate 5000 ppm (hexavalent)
5 Category (5) received tap water and served as control

These mice served as "intruders" were left for 90 days (period of ingestion of chromium compounds) before being individually introduced for 10 minutes into the home cages of residents. Another 48 untreated female mice of comparable age were allocated to 3 categories (N = 16) to test the fertility of males exposed to chromium compounds.

Administration of chromium compounds: Chromium chloride (Trivalent chromium compound: Janssen Chimica, B-2440 Geel, Belgium), potassium dichromate (hexavalent chromium compound: Fluke AG, Chemische Fabrika CH-9470, Buchs, Switzerland) were dissolved in drinking tap water, each at a concentration of 1 gm and 5 gm/liter. Male mice were provided access to drinking water containing the test substances for 12 weeks. Control male mice were given tap water for the same period. It is worth mentioning that all male mice were healthy and continued to ingest their respective drinking solutions with or without chromium throughout the experimental period.

Aggression tests: Treated mice were individually introduced for 10 minutes into the home cages of residents. Tests were conducted under dim red light and encounters were repeated over three consecutive days (McKinney and Christian, 1970). A series of electromechanical counters were employed to obtain routine measures of attack (Goldsmith *et al.,* 1976). The behaviors of the aggressive resident mouse was observed and the following
parameters were monitored:

1 The number of animals eliciting over attack
2 The latency of attack (in seconds) from the time of the introduction of the opponent
3 The number of bouts of biting attacks directed towards the opponent
4 The total time spent attacking the intruder or accumulated attacking time (AAT)

Fertility: Fertility was estimated in male mice exposed for 90 days to 5000 ppm concentration of chromium chloride; potassium dichromate or tap-water. 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 estrous cycles should have elapsed. One week after the removal of the exposed males, females were killed by cervical dislocation under light ether anaesthesia and the number of pregnant females, number of implantation sites and number of viable fetuses were recorded.

Body and organ weight determinations: At the end of the experiment, the treated mice were killed by cervical dislocation. The body, right testes, right preputial glands (with and without sebum) and right seminal vesicles (with and without fluid) were weighed. Organ weights were eventually expressed in relative terms (mg/100 g body wt.). Mean values ± SE were given for each experimental category. The left, testes, preputial and seminal vesicles were used to determine the concentrations of chromium compounds in these organs.

Determination of chromium compounds in male mice tissues by dry Ashing procedure

Sample preparation: In a porcelain crucible 1.0 g of accurately weighed tissue sample was ashed overnight in a muffle furnace at 550°C. The ash was dissolved in 3 ml of 3 N HCl and was transfered quantitatively to a 25 ml, volumetric flask and the volume was completed with 0.36 N HCl.

Analysis: Standard solution was prepared by diluting the stock standard solution with 0.36 N HCl. A 0.36 N HCl solution should be used as a blank. Graphite Furnace analyses of chromium were carried using Perkin Elmer Atomic Absorption spectrometer model Analyst 300 equipeted to Graphite Furnace Model HGA800 and AS-72 autosampler (Germany).

Statistical treatments: Body and organ weights were analyzed using student's t-test and behavioural data were analyzed using Mann Whitney U-test and the analysis of proportions test (Siegel, 1956).

Results

Body and organ weights: Table 1 shows the effect on body, testes, preputial gland and seminal vesicle weights of male mice after long-term exposure to chromium chloride and potassium dichromate. Body, testes, preputial glands (with and without sebum) and seminal vesicles (with and without fluid) weights in chromium chloride (at 1000 or 5000 ppm) and testes and seminal vesicles in potassium dichromate tat 1000 ppm) exposed male mice were not statistically significant. However weights of body ($p < 0.005$), preputial glands ($p < 0.004$), preputial glands minus sebum and seminal vesicle without fluid ($p < 0.02$) were significantly decreased in potassium dichromate (at 1000 ppm) exposed males c.f. controls in category treated with tap-water. Significant reduction (on Student's t-test) were also found between mean weights of body ($p < 0.0001$), testes ($p < 0.001$), preputial glands ($p < 0.002$), preputial glands minus sebum ($p < 0.0001$), seminal vesicles ($p < 0.001$) and seminal vesicles without fluid ($p < 0.002$) in potassium dichromate (at 5000 ppm) exposed males. c.f. controls.

Aggression test data: Table 2 shows the effects of long-term exposure of male mice to 1000 and 5000 ppm of each of chromium chloride and potassium dichromate on social aggression. Exposure of male mice to potassium dichroniate (at 1000 or 5000 ppm) did not significantly alter the aggression compared to control group. Male mice exposed to chromium chloride (at 1000 or 5000 ppm) significantly augmented social aggression. Intruders exposed to chromium chloride (at 1000 ppm) significantly reduced the latency to the first attack toward standard opponents ($p < 0.05$ on Mann

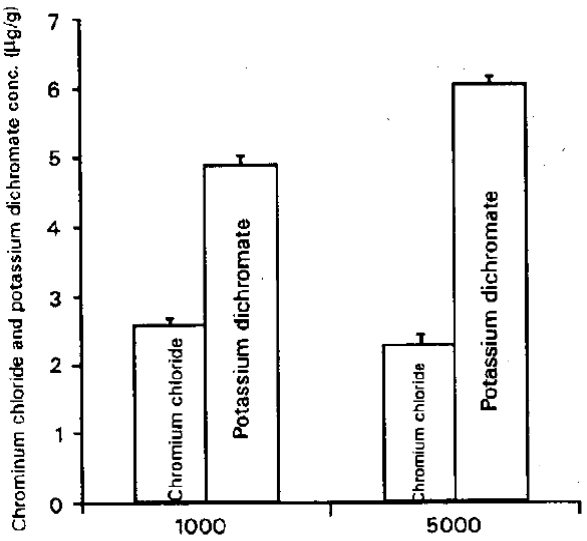

Fig. 1: Concentrations of chromium compounds in testes after long-term ingestion of male mice to different dosages of chromium chloride and potassium dichromate. Values represent means ±S.E. of eight mice/group

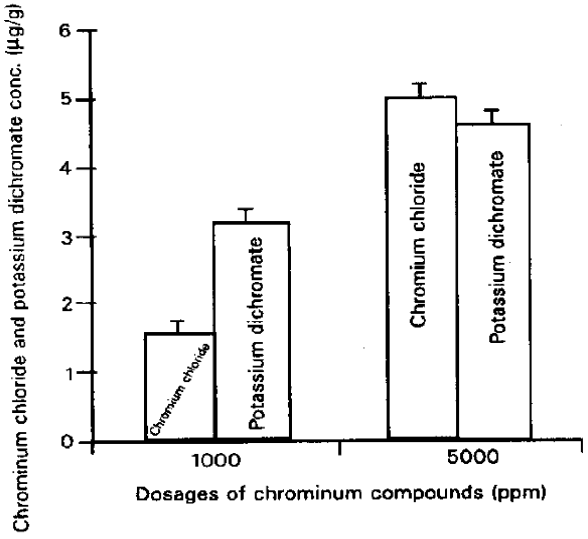

Fig. 2: Concentrations of chromium compounds in preputial glands after long-term ingestion of male mice to different dosages of chromium chloride and potassium dichromate. Values represent means ±S.E. of eight mice/group

Whitney U' test). These subjects were attacked more vigorously in most commonly occurred behavioural elements namely the intensities and number of attacks $p<0.02$ and $p<0.01$ respectively (Mann Whitney U' test) in contrast to control groups. There was a significant declines ($p<0.03$) in the latency of attack as well as a highly significant increases

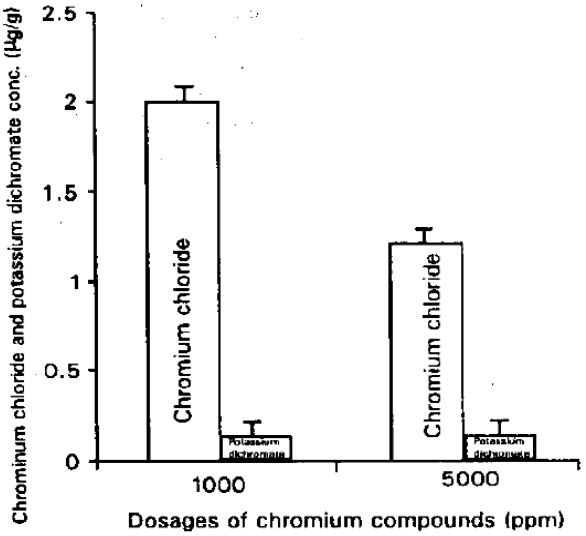

Fig 3: Concentrations of chromium compounds in seminal vesicles after long-term ingestion of male mice to different dosages of chromium chloride and potassium dichromate. Values represent means ±S.E. of eight mice/group

in both the intensity and number of attacks were evident in subjects exposed to 5000 ppm chromium chloride compared with counterparts control groups $p<0.001$ (Mann Whitney U' test).

Effect on Fertility: Table 3 shows that the fertility of male mice exposed to potassium dichromate was significantly reduced. The number of implantations and the number of viable fetuses in females impregnated by such males were significantly reduced ($p<0.05$) as compared to control group values. The fertility of male mice exposed to chromium chloride was unaffected as compared to control group.

Effect on the concentrations of chromium compounds in male mice tissues: Figure 1, 2 and 3, summarizes the concentrations of chromium compounds in the testes, preputial glands and seminal vesicles. The concentration of potassium dichromate in the testes was a dose dependent. Whereas the concentration of chromium chloride- was progressively less pronounced (Fig. 1). A gradual increased tissue concentration of chromium compounds (chromium chloride and potassium dichromate) in the preputial glands was observed with increase in dosage until the highest dosage (5000 ppm) was reached (Fig. 2). Concentrations of chromium chloride in seminal vesicles (Fig. 3) did not exhibit the same degree of variability and that the pattern of this variability was different in the different dosages used. Both the lowest and highest dosages (1000 or 5000 ppm) of potassium dichromate were uneffective.

Discussion

The aim of this work was to study the effects of trivalent (chromium chloride) and hexavalent (potassium

Table 1: Mean (±S.E.) body (g) and relative organ weights (mg/100 g) in intact male mice exposed to lon-term chromium compounds via drinking water (N = 8)

Treatment	Body weight	Right testis	Right preputial gland	Right preputial gland minus sebum	Right seminal vesical	Right seminal Vesicle without fluid
Tap water (control)	31.51±1.53	26.86±2.83	12.79±3.15	8.07±0.92	24.24±3.60	12.72±1.70
Chromium chloride 1000 ppm	31.87±0.47	31.99±1.57	14.74±1.52	10.37±1.19	29.54±2.54	16.35±1.001
Chromium chloride 5000 ppm	30.70±1.01	34.10±3.04	11.73±0.99	6.97±0.60	27.54-±2.18	13.46±1.34
Potassium dichromate	+ +		+	*		*
1000 ppm	26.10±0.49	22.92±1.69	7.92±0.89	5.29 ±0.66	18.78 ± 2.09	8.18±1.16
Potassium dichromate	+ + +	**	***	+ + +	**	*****
5000 ppm	18.83±0.69	12.24±0.98	4.72±1.83	1.77 ± 0.19	8.59 ± 0.75	4.48±0.25

* Differs from tap-water treated group ($p<0.021$ on Student's t-test
** Differs from tap-water treated group ($p<0.001$) on Student's t-test
*** Differs from tap-water treated group ($p<0.002$) on Student's t-test
\+ Differs from tap-water treated group ($p<0.004$) on Student's t-test
+ + Differs from tap-water treated group ($p<0.005$) on Student's t-test
+ + + Differs from tap-water treated group ($P<0.0001$) on Student's t-test

Table 2: Summed data (proportions or medians with ranges) over 3 days for individually-housed male mice enco-untering intact male mice "intruders" exposed to long-term chromium compounds via drinking water on aggressive-behaviour

Treatment of intruders	Proportion of animals Attacking in at least 2/3 test	Latency to attack (Secs.)	Median Accumulated Attacking Time (AAT) with range(secs)	Median number of attacks with range
Tap water (control)	6/8	924.5 (62-1800)	47.5 (0-95)	41.5 (0-90)
Chromium chloride 1000 ppm	8/8	129.5* (28-1224)	85** (40-118)	78***(33-115)
Chromium chloride 5000 ppm	8/8	141.52+(34-459)	410++ (81-156)	93.5++(62-137)
Potassium dichromate 1000 ppm	3/8	1213.5 (81-1800)	25 (0-90)	22.5 (0-87)
Potassium dichromate 5000 ppm	6/8	239.5 (88-1442)	66.5 (7-124)	51 (4-96)

* Differs from category treated with tap-water $p<0.05$ (Mann-Whitney U' test)
** Differs from category treated with tap-water $p<0.02$ (Mann-Whitney U' test)
*** Differs from category treated with tap-water $p<0.01$ (Mann-Whitney U' test)
\+ Differs from category treated with tap-water $p<0.03$ (Mann-Whitney U' test)
+ + Differs from category treated with tap-water $p<0.001$ (Mann-Whitney U' test)

Table 3 : Effect of lone-term exposure to 5000 ppm chromium compounds via drinking water on fertility of adult male mice

Treatment	Number of males	Number of female	Number of pregnant females	Number of implantation	Number of viable fetuses	Total number of resorption
Tap-water (control)	8	16	15/16 (93.75%)	10.48±1.53 (15)	10.16±1.26	2
Chromium chloride	8	16	14/16 187.5%)	9.86±2.41 (14)	9.72±3.31	2
Potassium dichromate	8	16	10/16 162.5%)	*5.76±1.13 (10)	*5.21±2.49	3

Results are expressed as means ±S.D
*Differs from category treated with tap-water ($p<0.05$) on Student's t-test

dichromate) chromium compounds ingested with drinking water by prepubertal male mice on social aggression and fertility. The increasing interest, both from the occupational and environmental viewpoints, in reproductive and developmental toxicity is illustrated by the increasing number of monographs being published in the last ten years.

Chromium compounds to which people are occupationally exposed, have received very little attention despite the fact that animal work suggests the effects might be expected at least in certain times in the life cycle. Chromium is an essential trace mineral nutrient like iron, zinc, selenium, copper which plays a critical role in maintaining normal health and well being. Another area that is gaining more interest lately is the possible effect of chromium on body composition; that is, how chromium affects the relative amounts of lean body mass (mainly muscle) compared to the amount of body fat. Indeed we are still lacking important information on the dose of chromium compounds and the periods of exposure to its compounds which might result in reproductive and developmental effects.

The present investigation demonstrates that long-term exposure of prepubertal male mice to hexavalent chromium compound have adverse effects on male reproductive system and fertility. However, the mating capability of male mice was adversely affected by the exposure of these males to hexavalent (5000 ppm) rather than to trivalent chromium compounds for 12 weeks. These findings confirm that the number of pregnant females impregnated by males exposed to the hexavalent chromium compound was reduced. The number of implantation sites and the number of viable fetuses were also significantly reduced in these subjects. These findings are not in agreement with the findings of Bataineh *et al*. (1997) who reported that the exposure of male rats to trivalent and hexavalent chromium compounds had no effect on fertility. The failure of trivalent exposure to produce the same effect as did the hexavalent compound could be explained by the fact that this compound is not toxic at the used dosage.

Weights of body, testes and sex accessory glands such as preputial and seminal vesicles were significantly reduced in males exposed to hexavalent chromium compounds. The reduction in the weights observed in this study may be likely to be a cause of failure of reproduction or might suggest an alteration in pattern of testosterone secretion. Elbetieha and Al-Hamood (1997) reported that body, seminal vesicle and preputial gland weights were significantly reduced in male mice exposed to trivalent and hexavalent chromium whereas testes weight was significantly increased in males exposed to these compounds. The present data does not agree with the

findings of Al-Hamood *et al.* (1998) who reported that weights of body and sex accessory glands of male mice were reduced in trivalent rather than to hexavalent exposed male mice. The conflicting results are attributed to dosage differences and duration of time used in the present study.

The present findings also show that male mice exposed to chromium chloride had significantly increased the attack to which aggressive conspecific males subjected treated intruders (Table 2). The increases in the aggressiveness of male mice subjected to trivalent chromium compound might be due to the fact that this agent acted on the testes and affected testosterone biosynthesis pathway and produced its effect on the behaviour. This finding was confirmed by the concentrations of these compounds in the testes and other sex accessory glands (Fig. 1, 2 and 3). The concentration of chromium chloride was much lower c.f. the concentration of potassium dichromate in the testes. The failure of hexavalent exposure to produce the same effect noted in male mice exposed to trivalent chromium compound might be due to the effect of hexavalent chromium on the levels of testosterone. It seems however that such effect may alter(s) the amount or component(s) of pheromone released by the preputial glands which is influenced by a variety of steroid hormones (Homady, 1982). This observation was confirmed by the significant reduction of testes and preputial weights as well as the high concentration of hexavalent chromium compound in these tissues. The preputial gland also produces behavior modulating pheromones which alter fighting and other behaviour in mice (Brain *et al.,* 1987; Homady *et al.,* 1992). The present results are not in agreement with the findings of Bataineh *et al.* (1997). These authors reported that long-term exposure of male rats to trivalent and hexavalent chromium compounds suppresses sexual and aggressive behaviour. The differences in these results might be due to species differences which are also exist for chromium metabolism depending on the route of exposure (Kargacin *et al.,* 1993).

The adverse effects of trivalent and hexavalent chromium compounds on the aggressiveness and fertility of male mice noted in the present study 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). Such effects caused a reduction in male steroid production and hormone binding in the testes at the onset of puberty (Wiebe *et al.,* 1982) to impaire hypothalamic-pituitary gonadal axis (McGivern *et al.,* 1991). It was also reported that intraperitoneal administration of chromium nitrate (trivalent compound) for 6 weeks was more effective than potassium dichromate in inducing testicular degeneration and inhibition of both spermatocyte and testicular enzyme production in rabbits (Behari *et al.,* 1978).

One of the questions posed by the results of this study involves the site at which chromium compounds acts to produce its adverse effects. One or a combination of the following sites are possible. (1) There may be a direct action of the chromium on the tissues or cells within the gonadal system. (2) Another potential site of action may involve the disruption of a normal process within either reproductive system or hormonal levels which consequentaly produces such effects.

The experimental work on animals should be used at least to alert us to the possibility of effects on humans. Unfortunately, even for animals, many embryological and histological studies are still lacking.

In conclusion the data presented here confirm that the long-term ingestion of chromium chloride and potassium dichromate of adult male mice after prepubertal exposure had adverse effects on both social aggression and fertility.

References

Al-Hamood, M.H. and Z.F. Al-Bayati, 1994. Effect of hexavalent chromium compound on the development and postnatal survival in mice. Iraqi J. Sci., 35: 23-34.

Al-Hamood, M.H., A. Elbetieha and H. Bataineh, 1998. Sexual maturation and fertility of male and female mice exposed prenatally and postnatally to trivalent and hexavalent chromium compounds. Reprod. Fertil. Dev., 10: 179-184.

Baruthio, F., 1992. Toxic effects of chromium and its compounds. Biol. Trace Element Res., 32: 145-153.

Bataineh, H., M.H. Al-Hamood, A. Elbetieha and I.B. Hani, 1997. Effect of long-term ingestion of chromium compounds on aggression, sex behavior and fertility in adult male rat. Drug Chem. Toxicol., 20: 133-149.

Behari, J., S.V. Chandra and S.K. Tandon, 1978. Comparative toxicity of trivalent and hexavalent chromium to rabbits. III. Biochemical and histological changes in testicular tissue. Acta Biol. Med. Ger., 37: 463-468.

Brain, P.F., M.H. Homady, D. Castano and S. Parmigiani, 1987. <Pheromones> and behaviour of rodents and primates. Ital. J. Zool., 54: 279-288.

Clarkson, T.W., G.F. Nordberg and P.R. Sager, 1985. Reproductive and developmental toxicity of metals. Scand. J. Work Environ. Health, 11: 145-154.

Elbetieha, A. and M.H. Al-Hamood, 1997. Long-term exposure of male and female mice to trivalent and hexavalent chromium compounds: Effect on fertility. Toxicology, 116: 39-47.

Ferm, V.H., 1971. Developmental malformations induced by cadmium. A study of timed injections during embryogenesis. Biol. Neonate, 19: 101-107.

Fishbein, L., 1981. Sources, transport and alterations of metal compounds: An overview. I. Arsenic, beryllium, cadmium, chromium and nickel. Environ. Health Perspect., 40: 43-64.

Gale, T.F. and J.D. Bunch, 1979. The effect of the time of administration of chromium trioxide on the embryotoxic response in hamsters. Teratology, 19: 81-86.

Gale, T.F., 1978. Embryotoxic effects of chromium trioxide in hamsters. Environ. Res., 16: 101-109.

Goldsmith, J.F., P.F. Brain and D. Benton, 1976. Effects of age at differential housing and the duration of individual housing/grouping on Intermale fighting behavior and adrenocortical activity in TO strain mice. Aggressive Behav., 2: 307-323.

Hamamy, H.A., Z.S. Al-Hakkak and A.F. Hussain, 1987. Chromosome aberrations in workers at a tannery in Iraq. Mutat. Res./Genet. Toxicol., 189: 395-398.

Hambridge, K.M., 1981. Chromium. In: Disorders of Mineral Metabolism, Volume 1. Trace Minerals, Bronner, F. and J.W. Coburn (Eds.). Academic Press, New York, pp: 271-294.

Homady, M.H., 1982. Histological and ultrastructural studies of the mouse preputial gland: Relation of endocrine effects to behavioural influences. Ph.D. Thesis, University of Wales, UK.

Homady, M.H., I. Al-Ani and H. Al-Janabi, 1992. Effects of hormones on the effectiveness of preputial secretions on attack directed towards long-term castrates by isolated male mice. Ibn Al-Haythem J. Sci. Res., 3: 23-31.

Jessup, D.C., 1967. Lead acetate, teratology study-rabbits. US National Technical Information Service Report PB-201, Washington, pp: 139.

Kargacin, B., K.S. Squibb, S. Cosentino, A. Zhitkovich and M. Costa, 1993. Comparison of the uptake and distribution of chromate in rats and mice. Biol. Trace Element Res., 36: 307-318.

Langards, S., 1982. Biological and Environmental Aspects of Chromium. Elsevier, Amsterdam, Netherlands.

McGivern, R.F., R.Z. Sokol and N.G. Berman, 1991. Prenatal lead exposure in the rat during the third week of gestation: Long-term behavioral, physiological and anatomical effects associated with reproduction. Toxicol. Aool. Pharm., 110: 206-215.

McKinney, T.D. and J.J. Christian, 1970. Effect of preputialectomy on fighting behavior in mice. Exp. Biol. Med., 134: 291-293.

Mertz, W., 1975. Effects and metabolism of glucose tolerance factor. Nutr. Rev., 33: 129-135.

National Academy of Sciences, 1974. Chromium. National Academy of Sciences, Washington, DC.

Nieboer, E. and A. Yassi, 1988. Other Health Effects of Chromium Compounds. In: Chromium in the Natural and Human Environment, Nriagu, J.O. and E.E. Nieboer (Eds.). Wiley, New York, pp: 533-550.

Sarto, F., I. Cominato, V. Bianchi and A.G. Levis, 1982. Increased incidence of chromosomal aberrations and sister chromatid exchanges in workers exposed to chromic acid (CrO3) in electroplating factories. Carcinogenesis, 3: 1011-1016.

Shepard, T.H., 1980. Catalog of Teratogenic Agents. 3rd Edn., The Johns Hopkins University Press, Baltimore, USA.

Siegel, S., 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, London, Pages: 312.

Wiebe, J.P., K.J. Barr and K.D. Buckingham, 1982. Lead administration during pregnancy and lactation affects steroidogenesis and hormone receptors in testes of offspring. J. Toxicol. Environ. Health, 10: 653-666.

Wilson, J.G., Wilson, J.. Environment and Birth Defects. Academic Press, New York.

Zelikoff, J.T., J.E. Bertin, T.M. Burbacher, E.S. Hunter and R.K. Miller *et al*., 1995. Health risks associated with prenatal metal exposure. Toxicol. Sci., 25: 161-170.