Potassium Dichromate Impact on some Biomarkers of Physiological Development (Vaginal Opening and Weight at Sexual Maturity) in Female Rats Related to Exposure Moment

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Abstract
The study was carried out on white Wistar female rats exposed to potassium dichromate (Cr VI) in different periods: *in utero* (28 female rats, derived from 20 exposed pregnant females, divided in four groups, one control - C and three experimental – E), in suckling and prepubertal period (in each stage, 28 offspring were divided in four groups: one C and three E).
The levels of exposure were: E_1 – 25 - LOAEL, E_2 - 50 - 2XLOAEL, E_3 - 75 -3XLOAEL ppm Cr VI in drinking water or through milk in suckling period.
Exposure to Cr VI determined significant delay of vaginal opening (onset of puberty) in direct correlation with the exposure level and significant decrease of body weight in the moment of vaginal opening under the optimum weight and inversely correlated to exposure level.
The most critical period of exposure for both biomarkers was *in utero.*

Keywords: *female, hexavalent chromium, rat, vaginal opening*

1. Introduction

The population at large is exposed to risk of suffering health problems caused by contaminating wastes inadequately treated for their safe disposal. Hexavalent chromium is found in a large world ground surfaces, being responsible of important reproductive perturbances both in animals as in humans [1, 2, 3].
Excess exposure to Cr VI is known to cause hepatotoxicity, nephrotoxicity, carcinogenicity in humans and experimental animals, and recognised reproductive health perturbances (both physical as well as functional) [4, 5, 6, 7].

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2. Materials and methods

The experiment was conducted on three sets of four groups (one control – C, and three experimental – E) of rats, differing by the exposure moment: *in utero*, in suckling and prepubertal period.
The each set of 28 female rats exposed in utero, in suckling or on prepubertal period derived from different 20 White Wistar adult female rats.
Potassium dichromate (Cr VI) was administered in drinking water as follows: E_1: 25 mg Cr (VI)-LOAEL [1], E_2: 50 mg Cr (VI) (2 x LOAEL), E_3: 75 mg Cr (VI) (3 x LOAEL), C: tap water not containing chromium.

All offspring were evaluated for pubertal onset (vaginal opening).
At the vaginal opening moment, the weight (by technical balance) and age of each female were evaluated.
All results were statistically analyzed by Anova method and Student test.
Food and water were *ad libitum*.

All assays with animals were conducted in accordance with present laws regarding animal welfare and ethics in animal experiments [8, 9, 10, 11, 12, 13].

3. Results and discussions

The results are presented in table 1, figure 1.

Table 1. Age and weight at vaginal opening moment depending on exposure moment

***In utero* exposure**						
	age			**weight**		
	X±Sx	**S.D.**	**C.L.95%**	**X±Sx**	**S.D.**	**C.L.95%**
C	40.50±0.34	1.08	0.71	101.60±0.37	1.17	0.68
E_1	69.70±0.42	1.34	0.71	78.00±0.26	0.82	0.68
E_2	79.40±0.34	1.07	0.71	74.50±0.34	1.08	0.68
E_3	86.00±0.26	0.82	0.71	64.50±0.34	1.08	0.68
X_E	**78.37±4.73**	**8.20**	**12.41**	**72.33±4.04**	**7.01**	**11.82**
Lactational exposure						
C	39.20±0.01	0.01	0.04	100.60±0.27	0.84	0.66
E_1	67.20±0.02	0.06	0.04	97.20±0.39	1.23	0.66
E_2	75.30±0.03	0.08	0.04	86.10±0.23	0.74	0.66
E_3	85.30±0.01	0.01	0.04	79.10±0.38	1.20	0.66
X_E	**75.93±5.23**	**9.07**	**12.41**	**87.47±5.27**	**9.13**	**11.82**
Prepubertal exposure						
C	40±0.03	0.08	0.03	102.70±0.37	1.16	0.77
E_1	65.20±0.01	0.01	0.03	99.10±0.35	1.10	0.77
E_2	72.40±0.01	0.01	0.03	98.60±0.40	1.26	0.77
E_3	83.20±0.01	0.01	0.03	83.60±0.40	1.26	0.77
X_E	**73.60±5.23**	**9.06**	**12.41**	**93.77±5.09**	**8.81**	**11.82**

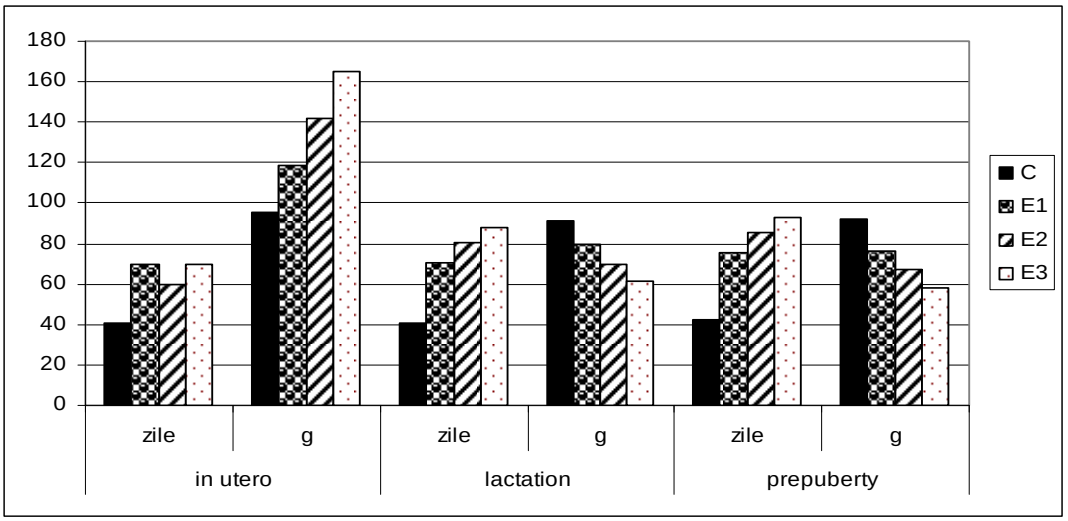

Figure. 1. Age (days) and weight (g) at vaginal opening moment depending on exposure moment

In the case of ***in utero*** exposure, in E individuals, the vaginal opening moment was significantly ($p<0.01$) delayed than in C group (E_1/C: +72.09%, E_2/C: +96.04%, E_3/C: +112.34%) and as weight, under 100 g (normal weight at vaginal opening) [14] (64.5 – 78 g), significantly ($p<0.01$) under C individual's weight (E_1/C: -23.22%, E_2/C: -26.67%, E_3/C: -36.51%).
The delay of the vaginal opening was directly, significantly ($p<0.01$) correlated with the exposure

level (E_2/E_1:+13.91%, E_3/E_2: +8.31%, E_3/E_1: +23.38%).
Between the weight at vaginal opening and the level of exposure a significantly ($p<0.01$) correlation was established (E_2/E_1: -4.48%, E_3/E_2: -13.42%, E_3/E_1: -17.30%).
Potassium dichromate exposure during **suckling** period determined significant ($p<0.01$) increase of the age at vaginal opening comparative to C group (E_1/C: +71.42%, E_2/C: +92.09%, E_3/C: +117.60%), directly, significantly ($p<0.01$) correlated with the exposure level (E_2/E_1:+12.05%, E_3/E_2: +13.28%, E_3/E_1: +26.93%).
The body weight at vaginal opening in E groups was significantly smaller ($p<0.01$) than in C group (E_1/C: -3.37%, E_2/C: -14.41%, E_3/C: -21.37%).
Between the body weight at the vaginal opening and the exposure level an inverse correlation (E_2/E_1: -11.41%, E_3/E_2: -8.13%, E_3/E_1: -18.62%) was established.
In E group individuals, after **prepubertal** exposure to potassium dichromate, the vaginal opening moment was delayed compared to C group (E_1/C: +63%, E_2/C: +81%, E_3/C: +108%, the differences being significant ($p<0.01$) and in direct correlation, significantly ($p<0.01$), with the exposure level (E_2/E_1: +11.04%, E_3/E_2: +14.91%, E_3/E_1: +27.60%).
The weight at the vaginal opening in E groups was significantly ($p<0.01$) lower than in C group (E_1/C: -3.5%, E_2/C: -3.99%, E_3/C: -18.59%) and inverselly, significantly correlated, ($p<0.01$), with the exposure level (E_3/E_2: -15.21%, E_3/E_1: -15.64%), excepting the level increase from 25 to 50 ppm Cr (E_2/E_1: -0.5%, $p>0,05$).
Regarding the moment of exposure, the age at the vaginal opening was the most delayed in the case of *in utero* exposure (X_E: 78.37), followed by the lactational exposure (X_E: 75.93) and prepubertal exposure (X_E: 73.60), the differences being insignificant ($p>0.05$): $X_E$lactation/X_E*in utero*: +3.11%, $X_E$prepubertal/X_E*in utero*: -6.08%, X_E prepubertal/ $X_E$lactation: -3.06%.
The weight at the vaginal opening was the lowest in the case of *in utero* exposure, (X_E: 72.33), followed by lactational (X_E: 87.47) and prepubertal exposure (X_E: 93.77), with different degrees of signification: $X_E$lactational/X_E*in utero*: +20.93% ($p>0.05$), $X_E$prepubertal/X_E*in utero*:+29.64% ($p<0.01$), X_E prepubertal/ $X_E$lactational: +7.20% ($p>0.05$).
The delay of the vaginal opening (puberty onset) consecutive potassium dichromate exposure was also recorded by other authors: Sakhila et al., 2008 [2], Rodriguez R., 2007 [3].
De Lucca et al., 2009 [6], Staniek, H., et al., 2010 [2], Rao, M.V, et al., 2009 [7], Matos, R. C., et al., 2009 [4], are other authors that reported the decrease of the body weight consecutive potassium dichromate exposure.
KEI-ICHIRO et al., 2000 [14], pointed out a correlation between the body weight and the moment of the vaginal opening, the importance of these markers, and the fact that the vaginal opening depends more of the weight than of the age.

4. Conclusions

Consecutive exposure to potassium dichromate:

- The age at the vaginal opening was significantly higher than in control group, directly correlated with exposure level, regardless of the exposure moment;
- The weight at the moment of puberty onset was significantly lower than in control group, inversely correlated to exposure level, regardless the exposure moment;
- Puberty onset was the most delayed in the case of *in utero* exposure, followed by the lactational exposure and the prepubertal one, the differences being insignificant;
- The body weight at the vaginal opening was the lowest after *in utero* exposure, followed by lactational and prepubertal exposure, with significant differences between prepubertal and *in utero* exposure.

References

1. Toxicology Profile for Chromium, U.S. EPA, 2001, home page address: http://www.epa.gov;
2. Staniek H., Kostrzewska-Poczekaj Magdalena, Arndt Magdalena , Szyfter K, Genotoxicity assessment of chromium(III) propionate complex in the rat model using the comet assay, Food and Chemical Toxicology 48, 2010, pp. 89–92;
3. Rodriguez, R., Samuel, J., Arosh, J., Lee, J., Aruldas, M., Banu, S., Chromium toxicity induces ovarian follicular developmental arrest, apoptosis, and deregulated steroidogenesis: vitamin C restores

follicular survival and function, Biology of Reproduction 77, 2007, pp. 215-215;
4. Matos, Rita, C., Vieira, Catarina, Morais, Simone, Pereira, Maria de Lourdes, Pedrosa, J., Nephrotoxicity effects of the wood preservative chromium copper arsenate on mice: Histopathological and quantitative approaches, Journal of Trace Elements on Medicine and Biology, 23, 2009, pp. 224-230;
5. Sakhila, K. B., Samuel, J. B., Arosh, J. A., Burghardt, R. C., Aruldhas, M, M., Lactational exposure to hexavalent chromium delays puberty by impairing ovarian development, steroidogenesis and pituitary hormone synthesis in developing Wistar rats, Toxicology and Applied Pharmacology, 232, 2008, pp. 180-189;
6. De Lucca, R. C., Dutrey, P. L., Villarino, M. E., Ubios, A. M., Effect of different doses of hexavalent chromium on mandibular growth and tooth eruption in juvenile Wistar rats, Experimental and Toxicologic Pathology 61, 2009, pp. 347-352;
7. Rao, M. V., Chawla, S. L., Sharma, S. R., Protective role of vitamin E on nickel and/or chromium induced oxidative stress in the mouse ovary, Food and Chemical Toxicology 47, 2009, pp. 1368–1371;
8. Directiva 86/609 din 24.11.1986 privind pretecţia animalelor utilizate în scopuri experimentale şi în alte scopuri ştiinţifice, http://ec.europa.eu/food/fs/aw/aw_legislation/scientific/86-609-eec_en.pdf.;
9. Legea 205/26.05.2004 privind pretecţia animalelor, M. O. nr. 531/14.06.2004;
10. Legea 206/27.05.2004 privind buna conduită în cercetarea ştiinţifică, dezvoltarea tehnologică şi inovare, M. O. nr. 505/4.06.2004;
11. Legea 471/9.07.2002 privind aprobarea O.G. nr. 37/2002 pentru protecţia animalelor folosite în scopuri ştiinţifice sau în alte scopuri experimentale, M. O. nr. 535/23.07.2002;
12. Legea 9/11.01.2008 pentru modificarea şi completarea Legii nr. 205/2004 privind pretecţia animalelor, M. O. nr. 29/15.01.2008;
13. Ordin 143/400 pentru aprobarea instrucţiunilor privind adăpostirea şi îngrijirea animalelor folosite în scopuri ştiinţifice sau în alte scopuri experimentale, M. O. nr. 697/24.09.2002;
14. Kei-Ichiro Maeda., Satoshi Ohkura., Hiroko Tsukamura - Physiology of Reproduction, Academic Press, Japan, 2000, pp. 145-456;