Potassium Dichromate Impact on Sexual Cycle Duration and Regularity in Female Rats (F_1 Generation)

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Abstract
The study was carried out on 28 white Wistar adult female rats from F_1 generation (derived from females and males exposed for three months before mating to potassium dichromate, 25, 50 and 75 ppm CrVI). F_1 generation was exposed *in utero* and until sexual maturity to the same Cr VI levels. The study pointed out: significant increase of sexual cycles duration comparative to control group and over the physiological limits, directly correlated to exposure level ($p<0.01$); changes in regularity of sexual cycles, respectively: significant ($p<0.01$) decrease of proestrus, estrus and diestrus percentages in physiological limits as duration and inversely correlated to exposure level, appearance of sexual cycles with absent proestrus and estrus, directly correlated with the exposure level ($p<0.01$) and of prolonged diestrus, directly correlated to exposure level, ($p<0.01$).

Keywords: *female, F_1, sexual cycle, rat*

1. Introduction

Chromium is a widely used industrial chemical. Industrial employments, burning of fuels and waste incineration are sources of chromium in air and water. Chromium VI occurs mostly due to anthropogenic origin and is considered a human carcinogen [1, 2]. The National Toxicology Program (NTP) recently completed chronic toxicity and carcinogenesis of Cr VI administered in drinking water [3].

2. Materials and methods

The study was carried out on 28 white Wistar adult female rats, divided in three experimental (E) groups, exposed from *in utero* until sexual maturity to 25 ppm Cr – LOAEL (E_1) [4], 50ppm Cr – 2 X LOAEL (E_2), 75ppm Cr – 3 X LOAEL (E_3) and one control (C) group not exposed to chromium. They represent the **F_1 generation**, being mature offspring derived from mothers exposed for three months to the same Cr (VI) levels, mated with similar exposed males.
Cr VI was administered as potassium dichromate in drinking water.
The female rats were fed with standard diets, corresponding to species and age.
Forages and water were *ad libitum*.
Duration of sexual cycle and of sexual cycle stages were appreciated by examination of vaginal smear cytological characteristics (Diff-Quick colouring, examination by optic microscope, X 20).
The results had been processed by ANOVA method and Student test.
All assays with animals were conducted in accordance with present laws regarding animal welfare and ethics in animal experiments [5, 6, 7, 8, 9, 10].

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3. Results and discussions

The results are presented in table 1 and 2, figures 1 and 2.

Table 1. Mean sexual cycle length (days) after Cr VI exposure: *F_1 generation*

Group	X±Sx	S.D.	C. L. 95%
C	4.47±0.16	0.50	0.55
E_1	5.35±0.26	0.82	0.55
E_2	6.15±0.24	0.75	0.55
E_3	7.40±0.37	1.17	0.55

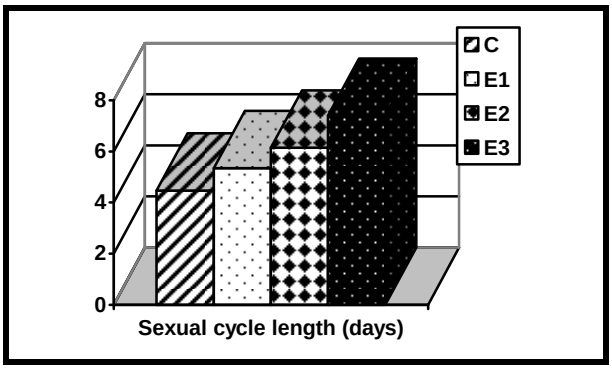

Figure. 1. Mean sexual cycle duration (days) dynamics

In C group, sexual cycle was in physiological limits (4-5 days) [11], but in exposed groups, sexual cycle duration was significant ($p<0.01$) higher than physiological limits, directly correlated with the exposure level (E_1/C: +19.68%, E_2/C: +37.58%, E_3/C: +65.54%; E_2/E_1: +14.95%, E_3/E_2: +38.31%, E_3/E_1: +20.32%).

In C group all sexual cycle stages were in pysiological limits as duration.

Proestrous stage in physiological limits as duration was lower in E comparative to C group: E_1/C: -20.00%, E_2/C: -30.00%, E_3/C :-40.00%.

Exposure level determined significant ($p<0.01$) decrease of sexual cycle percent with proestous in physiological limits as duration, inversely correlated with the exposure level (E_2/E_1: -12.5%, E_3/E_2: -14.28%, E_3/E_1: -25.00%).

Cr VI exposure determined the appearance of sexual cycles with absent proestrous, their percent increaseing significantly ($p<0.01$), directly correlated with the exposure level: E_2/E_1: +50.00%, E_3/E_2: +33.33%, E_3/E_1: +100.00%.

No sexual cycles with prolonged proestrous were recorded.

The sexual cycles with proestrous in physiological limits as duration in E groups was significant ($p<0.01$) lower than in C group, inverselly, significantly ($p<0.01$) correlated with the exposure level (E_1/C: -10.00%, E_2/C: -20.00%, E_3/C: -30.00%; E_2/E_1: -11.11%, E_3/E_2: -12.5%, E_3/E_1: -22.22%.

Cr VI exposure determined significant ($p<0.01$) increase of sexual cycle percent with absent estrus comparative to C group: E_1/C: 10/0, E_2/C: 20/0, E_3/C: 30/0.

The percent of sexual cycles with absent estrus significantly ($p<0.01$) increased in E groups, dirrectly correlated with the exposure level: E_2/E_1: +100.00%, E_3/E_2: +50.00%, E_3/E_1: +200.00%.

No sexual cycles with prolonged estrus were recorded.

The percentage of sexual cycles with diestrus in physiological limits as duration significantly ($p<0.01$) decreased in E groups comparative to C group: E_1/C: -90.00%, E_2/C: -95.00%, E_3/C: 0%.

The exposure level to Cr VI determined significant ($p<0.01$) decrease of sexual cycle with diestrous in physylogical limits as duration, inverselly correlated with the exposure level: E_2/E_1: -50%, E_3/E_2 :0/5%, E_3/E_1 :0/10%.

Cr VI exposure did not determined sexual cycles with absent diestrus in E groups.

Sexual cycle percent with prolonged diestrus was significantly ($p<0.01$) higher in E groups comparative to C group: E_1/C: 90/0, E_2/C: 95/0, E_3/C: 100/0.

Also, the exposure to Cr VI determined significant ($p<0.01$) increase of sexual cycles with prolonged diestrus, directly correlated with exposure level: E_2/E_1: +5.55 %, E_3/E_2: +5.26%, E_3/E_1: +11.11%.

The world whide researches regarding chromium exposure influence on the fundamental biomarkers of reproductive functionality are limited. The scientific literature points out the perturbation of sexual cycle lenght (respectively the increase of its duration) in female rats that were pregestational exposed to 250, 500 and 750 ppm potassium dichromate [12], and in female mouse, at 750 ppm potassium dichromate via drinking water [13].

Prolonged estrus after potassium dichromate administration was also signalized by other authors [14], in the context of irregular sexual cycle appearance in this case.

Table 2. Sexual cycle stages consecutive Cr VI exposure: F_1 generation (% of total sexual cycle)

Sexual cycle stage						
			C	**E_1**	**E_2**	**E_3**
Proestrus	N	**X ± Sx**	**100±0.31**	**80.00±0.31**	**70.00±0.31**	**60.00±0.31**
		S. D.	0.82	0.82	0.82	0.82
		C.L:	0.47	0.47	0.47	0.47
	A	**X ± Sx**	**0.00±0.00**	**20.00±0.31**	**30.00±0.31**	**40.00±0.31**
		S. D.	0.00	0.82	0.82	0.82
		C.L:	0.47	0.47	0.47	0.47
	P	**X ± Sx**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
		S. D.	0.00	0.00	0.00	0.00
		C.L:	0.47	0.47	0.47	0.47
Estrus	N	**X ± Sx**	**100±0.38**	**90.00±0.38**	**80.00±0.38**	**70.0±0.31**
		S. D.	1.00	1.00	1.00	0.67
		C.L:	0.56	0.56	0.56	0.56
	A	**X ± Sx**	**0.00±0.00**	**10.00±0.38**	**20.00±0.38**	**30.00±0.38**
		S. D.	0.00	1.00	1.00	1.00
		C.L	0.56	0.56	0.56	0.56
	P	**X ± Sx**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**
		S.D.	0.00	0.00	0.00	0.00
		C.L	0.56	0.56	0.56	0.56
Diestrus	N	**X ± Sx**	**100.0±0.31**	**10.00±0.38**	**5.00±0.31**	**0.00±0.00**
		S.D.	0.82	1.00	0.82	0.00
		C.L:	0.47	0.47	0.47	0.47
	A	**X ± Sx**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**	**0.00±0.00**
		S.D.	0.00	0.00	0.00	0.00
		C.L:	0.47	0.47	0.47	0.47
	P	**X ± Sx**	**0.00±0.00**	**90.00±0.31**	**95.00±0.31**	**100.0±0.38**
		S.D.	0.00	0.82	0.82	1.00
		C.L:	0.47	0.47	0.47	0.47

- **E_1: 25 ppm Cr VI**
- **E_2: 50 ppm Cr VI**
- **E_3: 75 ppm Cr VI**

- **N- physiological (as duration) stage**
- **A – absent/reduced stage**
- **P – prolonged (as duration) stage**

NB: 70 supervised sexual cycles /group (7 individuals/group x 10 supervised sexual cycles)

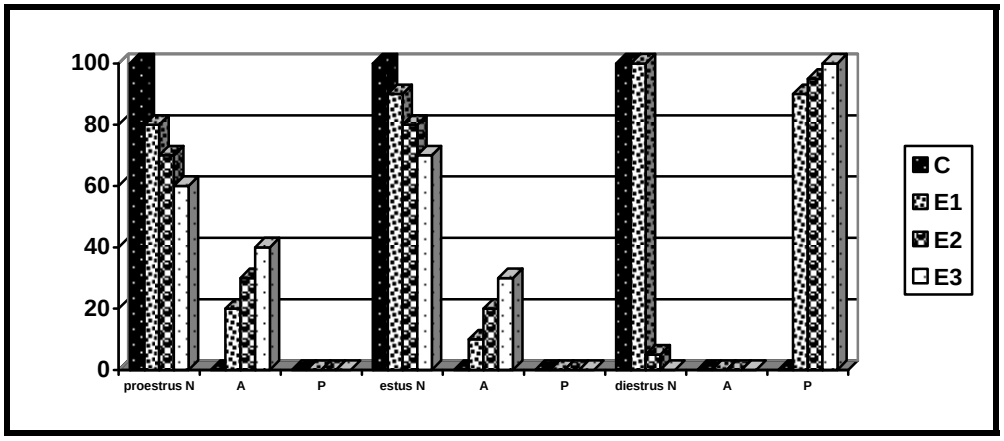

Figure. 2. Sexual cycle stages consecutive Cr VI exposure: F_1 generation

4. Conclusions

The study pointed out: significant increase of sexual cycles duration comparative to control group and over the physiological limits, directly correlated to exposure level ($p<0.01$); changes in regularity of sexual cycles, respectively: significant ($p<0.01$) decrease of proestrus, estrus and diestrus percentages in physiological limits as duration and inversely correlated to exposure level, appearance of sexual cycles with absent proestrus and estrus, directly correlated with the exposure level ($p<0.01$) and of prolonged diestrus, directly correlated to exposure level, ($p<0.01$).

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