

## Effect of potassium dichromate intake on water consumption and toxic amount intake, in female rats, *Rattus norvegicus* (exposure on three generations)

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**Abstract.** Material and methods: The study was carried out on three generations of female rats, each generation comprising 28 white Wistar adult female rats, divided in three experimental (E) groups, exposed to 25 ppm Cr – LOAEL (E<sub>1</sub>), 50 ppm Cr (E<sub>2</sub>), 75 ppm Cr (E<sub>3</sub>) and one control (C) group - tap water. Results: It pointed out significant decrease of water daily mean consumption in experimental groups comparative to C group, inversely, significantly correlated with the exposure level, in F<sub>0</sub>, F<sub>1</sub> and F<sub>2</sub> generations, the mean daily water intake decreasing with the increase of the number of generations exposed, the lowest being found in the case of F<sub>2</sub> generation, followed by F<sub>1</sub> generation and F<sub>0</sub>, the differences being insignificant. Also hexavalent chromium intake was calculated, F<sub>2</sub> generation having the poorest appetite for water, received the smallest amount of toxic, but yet not undermining the gravity of exposure in F<sub>2</sub> generation, due to parental exposure factors.

**Key words:** chromium, females, rat, water.

**Rezumat.** Material și metodă: Studiul a fost efectuat pe trei generații de femele de șobolan, fiecare generație având 28 de femele de șobolan adulte din rasa White Wistar, împărțită în trei grupuri experimentale (E), expuse la 25 ppm Cr – LOAEL (E<sub>1</sub>), 50 ppm Cr (E<sub>2</sub>), 75 ppm Cr (E<sub>3</sub>) și un grup martor (C) – apă de robinet. Rezultate: au dezvăluit scăderea semnificativă a consumului mediu zilnic de apă la loturile experimentale comparativ cu grupul martor, în corelație inversă, semnificativ, cu nivelul de expunere, la generațiile F<sub>0</sub>, F<sub>1</sub> și F<sub>2</sub>, media zilnică a aportului de apă scăzând odată cu creșterea numărului de generații expuse, cea mai scăzută fiind întâlnită în cazul generației F<sub>2</sub>, urmată de F<sub>1</sub> și F<sub>0</sub>, cu diferențe ne semnificative. De asemenea, a mai fost calculat aportul de crom hexavalent, generația F<sub>2</sub> având cel mai scăzut apetit pentru apă, primind astfel cel mai puțin toxic, nesubestimând însă cu toate acestea gravitatea expunerii în cazul generației F<sub>2</sub>, datorată factorilor parentali de expunere.

**Cuvinte cheie:** crom, femele, șobolan, apă.

**Introduction.** Chromium compounds are found in the environment, due to erosion of chromium containing rocks and can be distributed by volcanic eruptions in food and water. Metals being non-biodegradable, persist in the environment for a long period, causing serious ecotoxicological problems (Mclachlan et al 2008; Coșier & Petrescu-Mag 2008; Petrovici et al 2010; Trif et al 2010).

Hexavalent chromium is an important reproductive and developmental toxicant, having major side effects in humans and animals. It develops important irreversible perturbances (in short and in long term exposure), covering both the structural and functional levels of the organism (Toxicology Profile for Chromium, U.S. EPA, 2001; Oliveira et al 2010).

**Material and Method.** The present study was carried out on three generations of female rats (White Wistar) as follows: 28 adult female rats, exposed three months to potassium dichromate, mated with males exposed also three months to potassium dichromate represented the F<sub>0</sub> generation. The exposure of F<sub>0</sub> female rats continued during

pregnancy and lactation period. The F<sub>1</sub> generation females were exposed *in utero*, in suckling period, and after that via drinking water until sexual maturity. They were mated with other males exposed to the same doses of potassium dichromate. The exposure followed during pregnancy and lactation, the resulting female offspring, belonging to F<sub>2</sub> generation was exposed until sexual maturity. So, the F<sub>2</sub> generation was exposed *in utero*, during suckling period and until sexual maturity. In F<sub>0</sub>, F<sub>1</sub> and the F<sub>2</sub> generation, water consumption (in milliliters) was evaluated at sexual maturity in each case of exposure, after being calculated the daily intake, presented as a mean, by dividing the period to the number of days.

All studied generations were structured in one control group, C, that received tap water, not containing chromium, and three experimental groups: E<sub>1</sub>: 25 ppm Cr VI – LOAEL for reproductive function (Toxicology Profile for Chromium, U.S. EPA, 2001), E<sub>2</sub>: 50 ppm (2 X LOAEL), and E<sub>3</sub>: 75 ppm (3 X LOAEL).

Also the intake of hexavalent chromium (from the amount of potassium dichromate consumed) was evaluated.

The results were processed by ANOVA and Student's test.

All assays on animals were conducted in accordance with present laws regarding animal welfare and ethics in animal experiments (Directive 86/609 EEC/1986; Romanian Law 205/2004; Romanian Law 206/2004; Romanian Law 471/2002; Romanian Law 9/2008; Romanian Order 143/400).

**Results.** The values of the daily mean water intake in the case of three generations exposed to potassium dichromate are presented in Table 1 and Figure 1.

The dynamics of water consumption for the F<sub>0</sub>, F<sub>1</sub> and F<sub>2</sub> generations was registered from the time when the pups started to consume by their own forages and water. We estimated the mean water daily intake from that moment and until sexual maturity.

Table 1

Mean daily water consumption in the case of three generation female rats exposed to potassium dichromate (25, 50 and 75 ppm CrVI)

		F <sub>0</sub> generation	F <sub>1</sub> generation	F <sub>2</sub> generation
C	(X±Sx)	18.48±0.01	17.87±0.01	17.95±0.01
	S. D.	0.01	0.01	0.01
	C.L.	0.01	0.01	0.01
E <sub>1</sub>	(X±Sx)	15.33±0.01	14.32±0.01	12.41±0.01
	S. D.	0.01	0.01	0.01
	C.L.	0.01	0.01	0.01
E <sub>2</sub>	(X±Sx)	14.98±0.01	13.03±0.01	11.32±0.01
	S. D.	0.02	0.01	0.01
	C.L.	0.01	0.01	0.01
E <sub>3</sub>	(X±Sx)	10.33±0.01	9.90±0.01	8.75±0.01
	S. D.	0.01	0.01	0.01
	C.L.	0.01	0.01	0.01
X <sub>E</sub>	(X±Sx)	13.55±1.61	12.42±1.31	10.83±1.08
	S. D.	2.79	2.27	1.88
	C.L.	3.31	3.31	3.31

SD=standard deviation, CL=limits of confidence, X= mean, Sx= the sample standard deviation of the variable "x", X<sub>E</sub> = mean value for all experimental groups.

### Generation F<sub>0</sub>

Mean water daily consumption decreased, significantly (p<0.01) in the case of exposed groups, comparative to control group (E<sub>1</sub>/C: -17.04%, E<sub>2</sub>/C: -18.93%, E<sub>3</sub>/C: -44.10%), inversely correlated, significantly (p<0.001), with the exposure level: E<sub>2</sub>/E<sub>1</sub>: -2.28%, E<sub>3</sub>/E<sub>2</sub>: -31.04%, E<sub>3</sub>/E<sub>1</sub>: -32.61%.

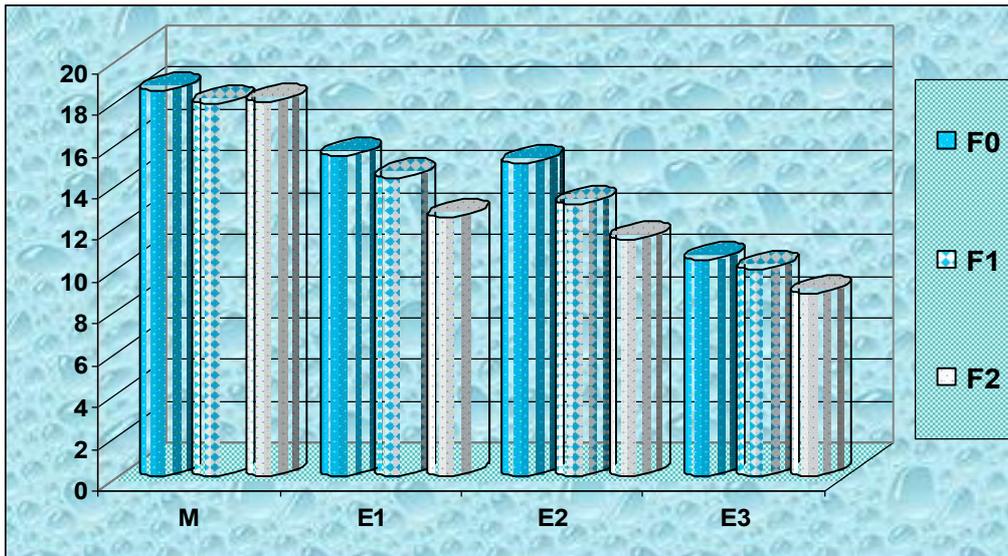


Figure 1. Water main daily consumption in three generations exposed to potassium dichromate.

### Generation F<sub>1</sub>

In F<sub>1</sub> generation, the mean water daily consumption decreased significantly ( $p < 0.001$ ) in exposed groups, comparative to control group ( $E_1/C$ : -19.86%,  $E_2/C$ : -27.08%,  $E_3/C$ : -44.59%), inversely correlated, significantly ( $p < 0.001$ ), with the exposure level:  $E_2/E_1$ : -9.00%,  $E_3/E_2$ : -24.02%,  $E_3/E_1$ : -30.86%.

### Generation F<sub>2</sub>

The mean daily water consumption decreased significantly ( $p < 0.001$ ) after the exposure of experimental groups to potassium dichromate, as comparative to control group ( $E_1/C$ : -30.86%,  $E_2/C$ : -36.93%,  $E_3/C$ : -51.25%), inversely correlated, significantly, ( $p < 0.001$ ), with the exposure level:  $E_2/E_1$ : -8.78%,  $E_3/E_2$ : -22.70%,  $E_3/E_1$ : -29.49%.

#### ❖ **Mean daily intake of potassium dichromate**

Based on the mean daily water consumption, we estimated the actual intake of potassium dichromate (Cr VI), respectively the amount (mean/day/experiment) received by the organism in each case of exposure (generation exposed).

Thereby:

- Exposure on F<sub>0</sub> generation: mean water consumption: 13.55 ml;
- Exposure on F<sub>1</sub> generation: mean daily water consumption: 12.42 ml;
- Exposure on F<sub>2</sub> generation: mean daily water intake: 10.83 ml.

Potassium dichromate administration:

- 25 ppm = 0.05 g potassium dichromate;
- 50 ppm = 0.1 g potassium dichromate;
- 75 ppm = 0.15 g potassium dichromate.



$$X_E = 35 \text{ ppm} = 0.1 \text{ g}$$

Example of operation:

35 ppm.....0.1 g.....1ml  
 X (ppm).....Y (g).....16.71 ml.

The intake of potassium dichromate was decreased in the case of F<sub>2</sub> generation because of the progressive decrease of appetite for water in proportion as the exposures continued on following generations.

The low level of potassium dichromate in the case of F<sub>2</sub> generation, yet does not undermine it's negative effect, which is more severe than in the case of the other exposed generations (F<sub>1</sub> and F<sub>0</sub>), because of the generational exposure effect, exposure

during *in utero* period, suckling, added at the parent's intoxication and the one of the previous generation.

Table 2

Potassium dichromate intake

Exposure period	ppm potassium dichromate	potassium dichromate (g)
generația F <sub>0</sub>	474.25	1.355
generația F <sub>1</sub>	434.7	1.242
generația F <sub>2</sub>	379.05	1.083

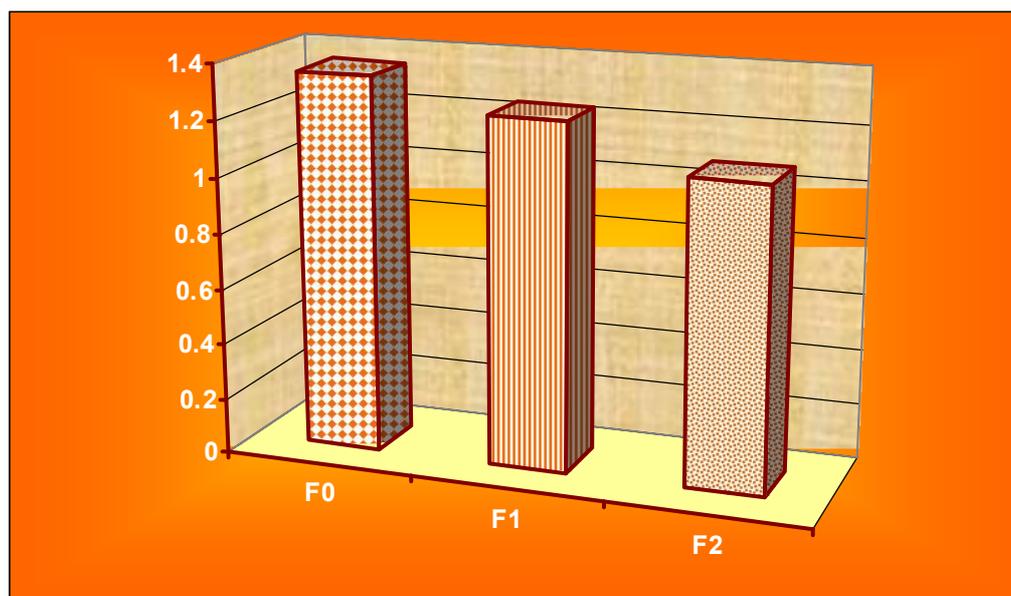


Figure 2. Potassium dichromate intake (g/day/experiment) depending on duration of exposure (three different generations).

**Discussion.** Following generational (F<sub>0</sub>, F<sub>1</sub> and F<sub>2</sub>) exposure to potassium dichromate, the mean daily water intake decreased with the increase of the number of generations exposed, the lowest being found in the case of F<sub>2</sub> generation (X<sub>E</sub>: 10.83 ml), followed by F<sub>1</sub> generation (X<sub>E</sub>: 12.42 ml) and F<sub>0</sub> (X<sub>E</sub>: 13.55 ml), X<sub>E</sub>F<sub>1</sub>/X<sub>E</sub>F<sub>0</sub>: -8.33%, X<sub>E</sub>F<sub>2</sub>/X<sub>E</sub>F<sub>1</sub>: -12.80%, X<sub>E</sub>F<sub>2</sub>/X<sub>E</sub>F<sub>0</sub>: -20.07%, the differences being insignificant (p>0.05).

The results regarding the decrease of water intake/consumption are in accordance with those found by other authors: Oliveira et al (2010), Soudani et al (2010) and Mclachlan et al (2008), in the case of potassium dichromate exposure, but in other doses, respectively 125 and 250 ppm.

Our studies were designed to establish whether there is a negative impact of low doses (as 25 ppm, LOAEL for reproductive function) on water intake, revealing the toxicity of potassium dichromate at low doses, which are accessible to both animals and humans.

The fact that the ingested amount of potassium dichromate was the lowest in F<sub>2</sub> generation is due to the decrease of appetite for water but does not undermine the importance of this exposure, F<sub>2</sub> generation being the most affected generation, followed by F<sub>1</sub> and F<sub>0</sub>, because of the parental factor that is involved.

**Conclusions.** Chromium intake, as potassium dichromate, in drinking water (25, 50 and 75 ppm), determined in female rats from parental generation (F<sub>0</sub>): significant decrease of water daily mean consumption in experimental groups comparative to control group, inversely, significantly correlated with the exposure level.

Chromium intake, as potassium dichromate, in drinking water (25, 50 and 75 ppm), determined in female rats from F<sub>1</sub> generation: significant decrease of daily mean water consumption in experimental groups comparative to control group, inversely, significantly correlated with the level of exposure.

Chromium intake, as potassium dichromate, in drinking water (25, 50 and 75 ppm), determined in female rats from F<sub>2</sub> generation: significant decrease of water daily mean consumption in experimental groups comparative to control group, inversely, significantly correlated with the exposure level.

The mean daily water intake decreased with the increase of the number of generations exposed, the lowest being found in the case of F<sub>2</sub> generation, followed by F<sub>1</sub> generation and F<sub>0</sub>, the differences being insignificant ( $p > 0,05$ ).

The decrease of the appetite for water, pointed out the decrease of potassium dichromate intake, inversely correlated with the exposure level.

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- \*\*\* Romanian Law 205/26.05.2004 regarding animal protection.
- \*\*\* Romanian Law 206/27.05.2004 regarding work in scientific research, technological development and inovation
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- \*\*\* Romanian Law 9/11.01.2008 for modification and addendum of 205/2004 Law regarding animal protection.
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