Potassium Dichromate (Cr VI) Impact on Fundamental Biomarkers of Reproductive Functionality in Female Rats (Six Months Exposure)

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Abstract
The study carried out on 28 white Wistar adult female rats, divided in four groups: one control and three experimental, exposed in drinking water for six months to 25 ppm Cr VI – LOAEL (E₁), 50 ppm Cr VI - 2XLOAEL (E₂), 75 ppm CR VI - 3XLOAEL (E₃), pointed out: significant (p<0.01) increase of sexual cycle duration comparative to control group and over the physiological limits, in direct correlation to exposure level; modification of sexual stages regularity: significant decrease of sexual cycles percentage with proestrus, estrus and diestrus in physiological limits as duration comparative to control group and inversely correlated to exposure level; appearance of sexual cycles with absent proestrus and estrus directly correlated to exposure level and of prolonged diestrus, directly, significantly (p<0.01) correlated to exposure level.

Keywords: female, hexavalent chromium, rat, sexual cycle

1. Introduction
Chromium (Cr) has been used in wide-range of industries for more than a century. Millions of people, and majority of industrial workers, worldwide are exposed to Cr VI during their life time via metallurgy, chrome plating, spray paints, textile manufacture, welded or cut metal, photography and photoengraving, stainless steel industries and cooling systems [1, 2, 3, 4]. Hexavalent chromium is an important reproductive and developmental toxicant as Office of Environmental Health Hazard Assessment (OEHHA) and the Developmental and Reproductive Toxicant Identification Committee (DART IC) mentioned in 2007 [5, 3].

2. Material and methods
The study was carried out on 28 white Wistar adult female rats, divided in three experimental (E) groups, exposed for six months via drinking water to 25 ppm Cr – LOAEL (E₁) [3], 50 ppm Cr – 2 X LOAEL (E₂), 75 ppm Cr – 3 X LOAEL (E₃) and one control (C) group which received tap water not containing chromium.

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 [6, 7, 8, 9, 10, 11].

3. Results and discussions

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

Table 1. Mean sexual cycle duration (days) after six months of exposure to Cr VI

<table>
<thead>
<tr>
<th>Group</th>
<th>X±Sx</th>
<th>S.D.</th>
<th>C. L. 95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>4.78±0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>E1</td>
<td>5.50±0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>E2</td>
<td>6.40±0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>E3</td>
<td>7.70±0.01</td>
<td>0.01</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Exposure to Cr VI determined the appearance of sexual cycles with absent proestrus, increasing significantly (p>0.01), in direct correlation with the exposure level: E2/E1: +14.28%, E3/E2: +12.5%, E3/E1: +28.57%.

No sexual cycles with prolonged proestrus were reported.

The percent of sexual cycles with estrus in physiological limits was in E group significantly (p<0.01) lower than in C group, inversely, significantly (p<0.01) correlated with the exposure level (E2/C: -25%, E3/C: -35%, E3/E1: -6.66%, E3/E2: -7.14%, E3/E1: -13.33%).

Appearance of sexual cycles with absent estrus was recorded, in experimental groups. The percent of sexual cycles with absent estrus was directly, significantly (p<0.01) correlated with the exposure level (E2/E1: +14.28%, E3/E2: +12.5%, E3/E1: +28.57%).

No sexual cycles with prolonged estrus were reported.

The percent of sexual cycles with diestrus in physiological limits was in E group significantly (p<0.01) higher than in C group: E1/C: +15.06%, E2/C: +33.89%, E3/C: +61.08%, E2/E1: +16.36%, E3/E2: +20.31%, E3/E1: +40%.

In C group, sexual cycle was in physiological limits – 4-5 zile [12], but in exposed groups, the duration was significantly (p<0.01) higher than physiological limits, directly correlated with the exposure level: E2/C: -15.06%, E3/C: -33.89%, E3/C: -61.08%, E2/E1: -16.36%, E3/E2: -20.31%, E3/E1: -40%.

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

Percentage of proestrus in physiological limits was significantly (p<0.01) lower comparative to C group: E1/C: -15.06%, E2/C: -33.89%, E3/C: -61.08%, E2/E1: -16.36%, E3/E2: -20.31%, E3/E1: -40%.

Appearance of sexual cycles with absent diestrus was recorded, in experimental groups. The percent of sexual cycles with absent diestrus was directly, significantly (p<0.01) correlated with the exposure level: E2/E1: +20%, E3/E2: +16.66%, E3/E1: +40%.

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

The percent of sexual cycles with prolonged diestrus was significantly (p<0.01) higher in Cr VI exposed groups than in C group: E1/C: +1500%, E3/C: +1700%, E3/C: +1700%, directly, significantly (p<0.01) correlated with exposure level: E2/E1: +6.25%, E3/E2: +5.88%, E3/E1: +12.5%.

Other authors also emphasized the increasing of sexual cycle duration in female rat that were pregestational exposed to 250, 500 and 750 ppm potassium dichromate [13], and in female mouse, exposed to 750 ppm potassium dichromate via drinking water [4].

Prolonged estrus after potassium dichromate administration was also reported by other authors in the context of irregular sexual cycle appearance [13].
Table 2. Sexual cycle stages consecutive six months exposure to Cr VI (% of total sexual cycles)
NB: 70 supervised sexual cycles/group (7 individuals/group x 10 supervised sexual cycles)

<table>
<thead>
<tr>
<th>Sexual cycle stage</th>
<th>N</th>
<th>X ± Sx</th>
<th>C</th>
<th>E1</th>
<th>E2</th>
<th>E3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proestrus</td>
<td>N</td>
<td>0.00±0.00</td>
<td>100±0.31</td>
<td>65.00±0.31</td>
<td>60.00±0.31</td>
<td>55.00±0.31</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>35.00±0.31</td>
<td>40.00±0.31</td>
<td>45.00±0.31</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Estrus</td>
<td>N</td>
<td>0.00±0.00</td>
<td>100±0.31</td>
<td>75.00±0.31</td>
<td>70.00±0.31</td>
<td>65.0±0.31</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>25.00±0.31</td>
<td>30.00±0.31</td>
<td>35.0±0.31</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Diestrus</td>
<td>N</td>
<td>0.00±0.00</td>
<td>95.0±0.31</td>
<td>20.00±0.38</td>
<td>15.0±0.31</td>
<td>10.0±0.31</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td></td>
<td>P</td>
<td>5.00±0.31</td>
<td>80.00±0.38</td>
<td>85.0±0.31</td>
<td>90.0±0.31</td>
<td>1.00±0.31</td>
</tr>
</tbody>
</table>

- E1: 25 ppm Cr VI
- E2: 50 ppm Cr VI
- E3: 75 ppm Cr VI

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

Figure 2. Sexual cycle stages dynamics after six month exposure to potassium dichromate
4. Conclusions:
Exposure to potassium dichromate (Cr VI) for six months determined:

- significant increase of sexual cycle duration comparative to control group and over the physiological limits, in direct correlation to exposure level;
- modification of sexual stages regularity:
  - significant decrease of sexual cycles percentage with proestrus, estrus and diestrus in physiological limits as duration comparative to control group and inversely correlated with the exposure level;
  - appearance of sexual cycles with absent proestrus and estrus, directly correlated with the exposure level;
  - appearance of sexual cycles with prolonged diestrus, directly, significantly correlated with exposure level.

References

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