

The consequences of lead acetate intake on exposure and integrity biomarkers of reproductive system in female rats at sexual maturity (two generation study)

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Abstract. The aim of the study was the assessment of lead toxic impact on female reproductive system integrity and performances biomarkers. The objectives were: the evaluation of lead levels in ovary, Fallopian tubes and uterus (exposure biomarker) and the structural changes in ovary, Fallopian tubes and uterus (integrity biomarker) at sexual maturity consecutive exposure to lead acetate (50, 100, 150 ppb Pb) in drinking water along two generation (F_0 , F_1). The study pointed out significant increase of lead level in ovaries, Fallopian tubes and uterus comparative to the control group and direct correlation, with different degrees of significance, with the exposure level; severe congestive and degenerative changes in ovary (destruction of parenchymatosa zone, vacuolar epithelial cells, passive vascular congestion, edematous follicle without oocytes, follicle with oocytes) and uterus (necrosis of uterine glands, destruction of uterine lining cells, total detachments of the superficial layers of the epithelia, hypertrophy of the epithelia).

Key words: lead, rats, ovary, uterus, histoarchitectures.

Introduction. Lead is a metal which is naturally found in earth crust in mineral form: blue lead, anglesite, cerussite, linarite, vanadinite. The products that contain lead are: paints, accumulators, ceramics, shots, razors and radiations generally, water pipes, pigments, insecticides, glass, linoleum etc.

The aim of the study was the evaluation of lead toxic impact on the female reproductive system integrity and exposure biomarkers, because of lack of researches and contests related to the opinions regarding lead toxicity on the reproduction function in females and presences in Romania of the pollutant lead industry (Andrews 1993; Nampoothiri & Gupta 2006).

Material and Methods. The evaluation of lead toxic effect on reproductive system exposure and integrity biomarkers was carried out on 28 Wistar female rats, from F_0 and on 28 Wistar female rats from F_1 generation divided into four groups: three experimental (E) and one control (C).

Females and males from F_0 generation were exposed before mating for three months to lead acetate in drinking water as follows: E_1 : 50 ppb Pb (the maximum admitted level in drinking water); E_2 : 100 ppb Pb; E_3 : 150 ppb Pb.

Females from F_0 generation were mated with other males corresponding to the exposure level as mentioned above in a ratio of 1♂:2♀ to obtain F_1 generation. The F_1 offspring were exposed to the same of lead acetate until sexual maturity.

Control group received tap water. The forages and water have been assured *ad libitum*.

All assays with 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).

The females from F_0 generation, after weaning of the F_1 generation offspring and the F_1 female offspring at sexual maturity were sacrificed following protocols and ethical procedures and ovary, Fallopian tubes and uterus were taken of for lead level determination and histological exam.

The lead level was determined in genital organs (ovary, Fallopian tubes and uterus) by atomic absorption spectrometry in the Laboratory of Nutrition and Toxicology from Faculty of Veterinary Medicine Timisoara, with the spectrometer-AAS AA-6650 Shimadzu, with graphite oven, provided by the company Viola Bucharest and the structural changes on histological section trichromic Mallory stained (after fixation in alcohol 80%, sectioned at 5 μ).

The results were statistically processed by the software Anova and the Student test.

Results and Discussion. The results regarding lead level in genital organs (in ovary, Fallopian tubes and uterus) are summarized in Table 1 and Figure 1.

The study emphasized: higher, significant ($p < 0.01$) accumulation in genital organs (E groups) comparative to C group and in direct correlation with exposure level (F_0 : ovary - E_1/C : +597.11%; E_2/C : +697.43%; E_3/C : +750.88%; E_2/E_1 : +14.25%; E_3/E_2 : +6.96%; E_3/E_1 : -22.22%; F_1 : ovary - E_1/C : +58.39%; E_2/C : +654.66%; E_3/C : +701.19%; E_2/E_1 : +10.75%; E_3/E_2 : +6.16%; E_3/E_1 : +17.58%; F_0 : uterus - E_1/C : +18.83%, $p > 0.05$; E_2/C : +83.75%, $p < 0.01$; E_3/C : +113.08%, $p < 0.01$; E_2/E_1 : +54.62%; E_3/E_2 : +15.96%; E_3/E_1 : +79.31%; F_1 : uterus - E_1/C : +16.84%; E_2/C : +76.79%; E_3/C : +102.83%; E_2/E_1 : +51.31%; E_3/E_2 : +14.72%; E_3/E_1 : +73.59%).

Table 1

Lead acetate average concentration ($\mu\text{g/g}$) in ovary, Fallopian and tubes uterus

GROUP		$X \pm S_x F_0$	S.D.	$X \pm S_x F_1$	S.D.	C.L. 95%
Ovary	C	18.71 \pm 0.38	0.85	20.05 \pm 0.17	0.37	0.51
	E1	130.43 \pm 0.67	1.50	136.62 \pm 0.36	0.81	0.51
	E2	149.02 \pm 1.88	4.20	151.31 \pm 0.17	0.38	0.51
	E3	159.40 \pm 1.29	2.89	160.64 \pm 0.22	0.48	0.51
	X_E	146.28		149.52	-	-
Fallopian tubes and uterus	C	12.00 \pm 1.06	2.38	13.06 \pm 0.05	0.11	0.29
	E1	14.26 \pm 0.75	1.68	15.26 \pm 0.16	0.36	0.29
	E2	22.05 \pm 1.08	2.41	23.09 \pm 0.13	0.30	0.29
	E3	25.57 \pm 0.92	2.05	26.49 \pm 0.16	0.36	0.29
	X_E	20.62		21.61	-	-

SD=standard deviation, CL=limits of confidence, X= mean, S_x =the sample standard deviation of the variable "x", X_E = mean for experimental groups.

The data regarding lead presence in ovary, Fallopian tubes and uterus are few. Taupeau et al (2001), Piasek & Kostial (1991), Silberstein et al (2006), mentioned the lead presence in follicular fluid and emphasized that lead level was higher in pregnant woman comparative to the not pregnant one; suggesting that the high lead level induces reproductive disorders.

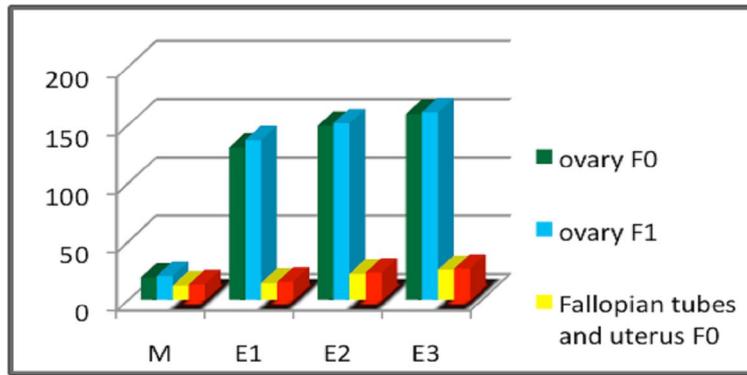


Figure 1. Dynamics of lead acetate levels in ovary, Fallopian tubes and uterus.

Exposure to lead acetate determined severe structural changes in genital organs as: *ovary*: destruction of parenchymatosa zone, vacuolar epithelial cells, passive vascular congestion, edematous follicle without oocytes, follicle with oocytes; *uterus*: necrosis of uterine glands, destruction of uterine lining cells, total detachments of the superficial layers of the epithelia, hypertrophy of the epithelia. The histological images are presented in Figs 2-7.

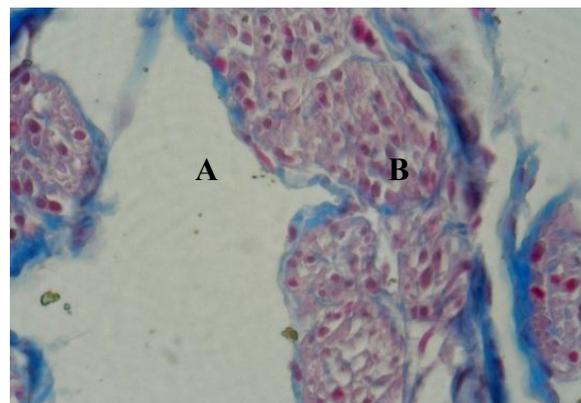


Figure 2. Histological section in rats' ovary after exposure to 150 ppb Pb (F_0), Trichromic Mallory stain, X 100; destruction of zona parenchymatosa (A); vacuolar epithelial cells (B).

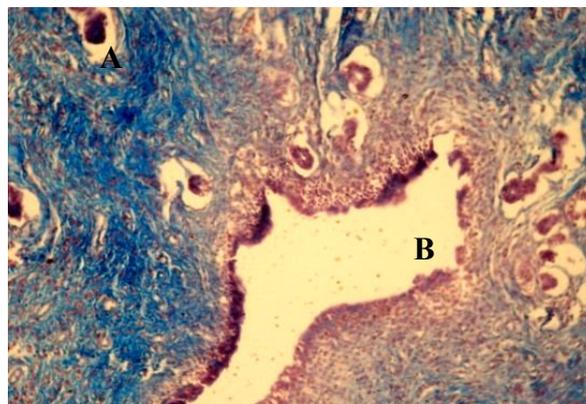


Figure 3. Histological section in rats' uterus after exposure to 100ppb Pb (F_0), Trichromic Mallory stain, X 200; necrosis of uterine glands (A); destruction of uterine lining cells (B).



Figure 4. Histological section in rats' ovary after exposure to 150 ppb Pb (F₀), Trichromic Mallory stain, X 300; follicular edema (A).

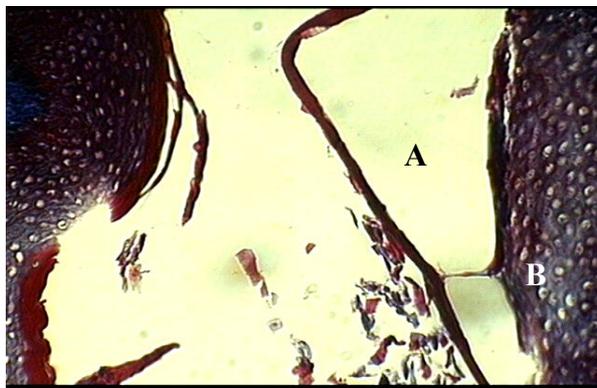


Figure 5. Histological section in rats' cervix uteri after exposure to 150 ppb Pb (F₀), Trichromic Mallory stain, X 300; total detachments of the superficial layers of the epithelia (A); vacuolar epithelial cells (B).

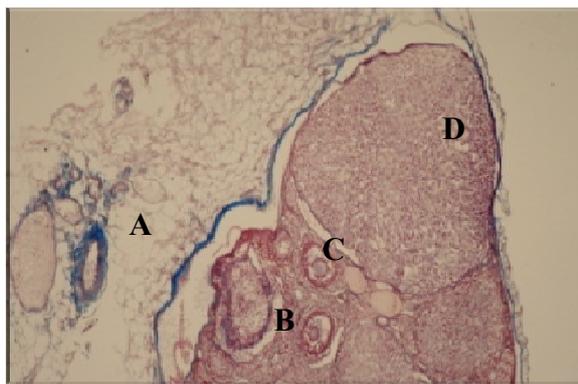


Fig 6. Histological section in rats' ovary after exposure to 150 ppb Pb (F₁), Trichromic Mallory stain X 100; passive vascular congestion (A); edematous follicle without oocyte (B); follicle with oocyte (C); vacuolar epithelial cells (D).

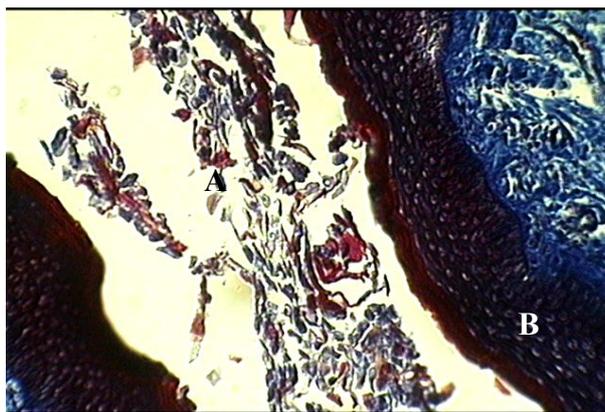


Figure 7. Histological section in rats' cervix uteri after exposure to 150 ppb Pb (F₁), Trichromic Mallory stain, X 300; superficial detachment with cellular deposit formation (A); hypertrophy of the epithelia (B).

Conclusions. Exposure to lead acetate of female rats along two generation determined in adult period at sexual maturity: significant increase of lead level in ovaries, Fallopian tubes and uterus comparative to the control group and in direct correlation, with different degrees of significance, with the exposure level; Higher, significant lead level in ovaries and Fallopian tubes and uterus in F₁ generation comparative to F₀ generation; Severe congestive and degenerative changes in ovary and uterus.

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- *** Romanian Law 206/27.05.2004 regarding work in scientific research, technological development and inovation.
- *** Romanian Law 471/9.07.2002 for O.G. nr. 37/2002 approval for animal protection when used in scientific purposes and other experimental means.
- *** Romanian Law 9/11.01.2008 for modification and addendum of 205/2004 Law regarding animal protection.
- *** Romanian Order 143/400 for approval of instruction for housing and attendance of animals used in scientific purposes and other scientific means.

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