THE CONSEQUENCES OF EXPOSURE TO LEAD ALONG THREE GENERATIONS ON MORPHOLOGICAL BIOMARKERS OF MALE REPRODUCTIVE FUNCTION: GENITAL ORGANS AND ACCESSORY SEXUAL GLANDS WEIGHT

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The study carried out on rats exposed to lead acetate along three generation emphasized: general tendency of testis weight increase comparative to control group, indirect correlation between testis weight, exposure level and duration; significant decrease of epididymis weight (excepting, not significant, in F_0 generation at 1000 and 2000 ppm exposure level, F_1 at 3000 ppm exposure level and significant increase in F_1 generation at 1000 ppm exposure level) comparative to control group, indirect correlated, significantly, to exposure level and duration (excepting, not significant, F_1/F_0); significant decrease of seminal vesicles weight (excepting, increase in F_1 generation) comparative to control group and indirect correlated, significant, to exposure level (not significant in F_0 generation and in F_2 generation in case of tripling of exposure level); direct correlated, significantly in F_1 generation at tripling of exposure level; significant decrease of prostatis weight (excepting F_0 generation, not significant at 2000 and 3000 ppm exposure level; F_0 generation at 1000 ppm and F_1 generation – significant increase) comparative to control group and indirect correlated to exposure level and duration (excepting, direct correlation, in F_2 generation); significant decrease of bulbo-urethral weight (not significant in F_0 generation and F_1 generation at 3000 ppm) comparative to control groups and indirectly correlated, significantly to exposure level (excepting, not significant in F_0 generation, direct correlated, significantly, in F_1 generation) and exposure duration.

Key words: lead, male rats, reproductive function, biomarkers

Lead is a heavy metal, undesirable for animals and humans. Lead is known as an enzymatic toxicant (7, 12, 14, 21), is neurotoxic (14, 16), hemato and cardiovascular toxic (12, 16), nephrotoxic (16), immunotoxic (6, 19), carcinogenic (16), teratogenic (16) and mutagenic (18).

Concerning the impact on the morphological biomarkers of male reproductive function, the opinions are divided. The negative impact on genital organs weight was emphasized by CHOWDHURY et al., 1987 quoted by APOSTOLI et al. (1), PINON-LATAILADE et al. (10), BATAINEH et al. (3) and denied by FOWLER et al, 1980; MARCHLEWICZ et al. 1993 and KEMPINAS et al. 1994, quoted by APOSTOLI et al. (1), WADI et al. (15). Lead is responsible for prostate (23, WILDT et al. 1983, quoted by *** - 17).

Material and methods

The study was carried out in three steps.

Step I. A group of 36 white Wistar male rats, ageing 28 days (immediately after weaning) were exposed during three month to dietary lead acetate (as briquettes) as follows:

- C group: not exposed to lead acetate;
- E_1 group: 1000 ppm lead acetate – value representing LOAEL – AYAR et al, 1973, quoted by *** (6);
- E_2 group: 2000 ppm lead acetate – double LOAEL;
- E_3 group: 3000 ppm lead acetate – triple LOAEL.

Step II. Two males from each E groups were mated with female unexposed to lead (ratio: 1 male/2 female) to obtain F_1 generation. During pregnancy the females were exposed to the same lead acetate levels; the males from F_1 generation were exposed to lead *in utero,* during lactation until weaning and than, three month to 1000 ppm (E'_1), 2000 ppm (E'_2) and, respectively, 3000 ppm (E'_3) lead acetate.

Step III. Two males from each E' groups were mated with female unexposed to lead (ratio: 1 male/2 female) to obtain F_2 generation. During pregnancy the females were exposed to the same lead acetate levels; The males from F_2 generation were exposed to lead *in utero,* during lactation until weaning and than, three month to 1000 ppm (E''_1), 2000 ppm (E''_2) and, respectively, 3000 ppm (E''_3) lead acetate.

After three month of exposure to lead acetate, seven males from each group and from each generation were euthanatized and genital organs (testis, epididymis) and sexual accessory glands (seminal vesicles, prostate and bulbo-urethral glands) were drown for evaluation of morphological biomarkers – weight.

The weight was determined with analytic balance Shimadzu AY 220.

The results were statistically analyzed by ANOVA method and Student test.

Results and discussions

The results are summarized in tables 1, 2 and 3.

Table 1

Sexual organs and accessory glands mean weight (g) in parental F_0 (E) generation

Specification		x±Sx	DS	Confidence level 95%
Testis	C	1.21±0.06	0.15	0.16
	E_1	1.4±0.02 *	0.06	0.06
	E_2	1.18±0.02 ^{ns}	0.05	0.05
	E_3	1.26±0.04 ^{ns}	0.11	0.11
Epididymis	C	0.55±0.01	0.02	0.02
	E_1	0.55±0.01 ^{ns}	0.01	0.01
	E_2	0.52±0.02 ^{ns}	0.04	0.04
	E_3	0.48±0.02 *	0.05	0.05

Seminal vesicles	C	1.45±0.09	0.23	0.24
	E_1	1.33±0.1 ^{ns}	0.25	0.26
	E_2	1.31±0.04 ^{ns}	0.1	0.11
	E_3	1.34±0.16 ^{ns}	0.4	0.42
Prostatis	C	0.72±0.04	0.1	0.1
	E_1	0.87±0.05 *	0.13	0.13
	E_2	0.71±0.06 ^{ns}	0.14	0.14
	E_3	0.7±0.09 ^{ns}	0.23	0.24
Bulbo-urethral glands	C	1.27±0.09	0.22	0.23
	E_1	1.23±0.11 ^{ns}	0.26	0.28
	E_2	1.15±0.1 ^{ns}	0.25	0.26
	E_3	0.95±0.11 *	0.26	0.28

E/C: ^{ns} – not significant ; * $p<0.05$

In F_0 generation, testis weight varied in E groups around those of C group (E_1/C: +15.7%, $p<0.05$; E_2/C: -2.47%, $p>0.05$; E_3/C: +4.13%, $p>0.05$) (xE/C: +5.78%). The double and triple doses significantly ($p<0.05$) decreased testis weight (E_2/E_1: -15.71%, E_3/E_1: -10.05%).

Epididymis weight decreased in E group, the differences becoming significant ($p<0.05$) in E_3 group (E_1/C: 0%, E_2/C: -5.45%, E_3/C: -12.72%). Doubling the dose epididymis weight decreased but not significantly ($p>0.05$) (E_2/E_1: -5.45%), but three times higher dose significantly ($p<0.01$) decreased epididymis weight (E_3/E_1: -12.72%).

In all E groups, comparatively to C group, not significant ($p>0.05$) decrease of seminal vesicles was recorded (E_1/C: -8.27%, E_2/C: -9.65%, E_3/C: -7.58%). Double and triple doses determined not significant variation of seminal vesicles weight (E_2/E_1: -1.5%, E_3/E_1: +0.75%).

Prostatis mean weight presented a decrease tendency ($p<0.05$) in E_2 and E_3 group comparative C group (E_2/C: -1.38%, E_3/C: -2.77%) but significantly ($p<0.05$) increased in E_1 group (E_1/C: +23.63%). Double and triple exposure level decreased prostatis weight (E_2/E_1: -18.39%, E_3/E_1: -19.54%) but not significant ($p>0.05$).

Bulbo-urethral glands weight were lower in E group than those from C group (E_1/C: -3.90%, E_2/C: -9.44% - $p>0.05$; E_3/C: -25.19% - $p<0.05$). Doubling and tripling of exposure level decreased bulbo-urethral glands weight but not significantly (E_2/E_1: -6.5%, E_3/E_1: -22.76%) ($p>0.05$).

Table 2

Sexual organs and accessory glands mean weight (g) in parental F_1 (E') generation

Specification		x±Sx	DS	Confidence level 95%
Testis	C'	1.16±0.02	0.05	0.06
	E'_1	1.57±0.08 **	0.19	0.2
	E'_2	1.32±0.01 **	0.01	0.01
	E'_3	1.09±0.16 ^{ns}	0.38	0.4
	C'	0.53±0.01	0.01	0.01
	E'_1	0.63±0.03 **	0.06	0.07

Epididymis	E'_2	0.37±0.01 **	0.01	0.01
	E'_3	0.48±0.02 ^{ns}	0.08	0.08
Seminal vesicles	C'	1.26±0.02	0.05	0.05
	E'_1	1.51±0.03 **	0.07	0.07
	E'_2	1.2±0.01 *	0.01	0.01
	E'_3	1.86±0.13 **	0.32	0.34
Prostatis	C'	0.70±0.01	0.01	0.01
	E'_1	0.98±0.02 **	0.05	0.05
	E'_2	1.09±0.01 **	0.01	0.01
	E'_3	1.18±0.03 **	0.08	0.09
Bulbo-urethral glands	C'	1.25±0.02	0.04	0.04
	E'_1	1.0±0.05 **	0.12	0.12
	E'_2	1.19±0.01 *	0.01	0.01
	E'_3	1.24±0.07 ^{ns}	0.17	0.18

E'/C': ^{ns} – not significant
* p<0.05
** p<0.01

In F_1 generation, testis weight significantly increased (p<0.01) in groups exposed to 1000 and 2000 ppm lead acetate (E'_1/C': +35.34%, E'_2/C': +13.79%) and not significantly decreased (p>0.05) in the group exposed to 3000 ppm (E'_3/C': -6.03%) (xE'/C': +13.79%). Doubling and tripling of the exposure level significantly decreased testis weight (E'_2/E'_1: -15.92%, E'_3/E'_1: -30.57%).

Not significant differences between the F_1 and F_0 testis weight were recorded (E'/E: -3.03% - p>0.05).

Epididymis weight varied in E' groups around C' group values (E'_1/C': +18.86%, p<0.01; E'_2/C': -30.18%, p<0.01; E'_3/C': -1.88%, p>0.05) (xE'/C': -5.66%). Mean epididymis weight value in E' groups decreased significantly at double exposure level (E'_2/E'_1: -41.26% - p<0.01) and at triple exposure level (E'_3/E'_1: -17.46%).

A decrease tendency in F_1 generation comparative to F_0 generation was registered (xE'/xE: -3.92%) (E'_1/E_1: +14.54%, p<0.05; E'_2/E_2: -28.84%, p<0.01; E'_3/E_3: +8.33%, p>0.05).

In F_1 generation, the weight mean values of seminal vesicles varied around the C' group mean value (E'_1/C': +19.84%, p<0.01; E'_2/C': -4.76%, p<0.05; E'_3/C': +47.61%, p<0.01). The influence of exposure level, even the difference were statistically assured, was not conclusive (E'_2/E'_1: -20.52%, p<0.01; E'_3/E'_1: +23.17%, p<0.05). Comparative to parental generation (F_0) seminal vesicles weight was different, not conclusive, varying, not related to exposure level (E'_1/E_1: +13.53%, p>0.05; E'_2/E_2: -8.39%, p<0.05; E'_3/E_3: +38.8%, p<0.05).

The general tendency in F_1 generation was the increase of seminal vesicles weight.

Prostatis weight significantly increase in E' groups comparative to C' group (E'_1/C': +40%, E'_2/C': +55.71%, E'_3/C': +68.57% - $p<0.01$), direct related to exposure level (E'_2/E'_1: +11.22%, E'_3/E'_1: +20.4%).

In F_1 generation, comparative to F_0 generation, prostatis weight increased, not significantly in E'_1 (E'_1/E_1: +12.64%) and significantly ($p<0.01$) in E'_2 and E'_3 groups (E'_2/E_2: +53.52%, E'_3/E_3: +68.57%).

Bulbo-urethral weight decreased in E' groups comparative to C' groups (E'_1/C': -20%, $p<0.01$; E'_2/C': -4.8%, $p<0.05$; E'_3/C': -0.8%, $p>0.05$) but doubling and tripling of exposure level determined significantly increase of their weight (E'_2/E'_1: +19%, $p<0.05$; E'_3/E'_1: +24%, $p<0.05$). Comparative to parental generation (F_0), not significant ($p>0.05$) variation of bulbo-urethral glands weight was recorded (E'_1/E_1: -18.69%, E'_2/E_2: -3.47%, E'_3/E_3: +30.25%).

Table 3

Sexual organs and accessory glands mean weight (g) in parental F_2 (E") generation

Specification		x±Sx	DS	Confidence level 95%
Testis	C"	1.05±0.02	0.05	0.06
	$E"_1$	1.29±0.01 **	0.03	0.03
	$E"_2$	1.09±0.05 ^{ns}	0.11	0.12
	$E"_3$	0.98±0.01 **	0.01	0.01
Epididymis	C"	0.51±0.01	0.01	0.01
	$E"_1$	0.38±0.01 **	0.02	0.02
	$E"_2$	0.34±0.01 **	0.01	0.01
	$E"_3$	0.28±0.01 **	0.01	0.01
Seminal vesicles	C"	1.17±0.02	0.04	0.04
	$E"_1$	1.07±0.12 ^{ns}	0.3	0.32
	$E"_2$	0.97±0.14 ^{ns}	0.33	0.35
	$E"_3$	0.23±0.01 **	0.01	0.01
Prostatis	C"	0.68±0.01	0.01	0.01
	$E"_1$	0.62±0.04 ^{ns}	0.11	0.11
	$E"_2$	0.45±0.03 **	0.08	0.08
	$E"_3$	0.25±0.01 **	0.01	0.01
Bulbo-urethral glands	C"	1.07±0.03	0.06	0.07
	$E"_1$	0.63±0.01 **	0.04	0.04
	$E"_2$	0.53±0.02 **	0.06	0.06
	$E"_3$	0.32±0.01 **	0.01	0.01

E"/C": ^{ns} – not significant; ** $p<0.01$

In F_2 generation, testis weight in E" groups varied, plus and minus comparative to C" group ($E"_1/C"$: +22.85%, $p<0.01$; $E"_2/C"$: +3.8%, $p>0.05$; $E"_3/C"$: -6.66%, $p<0.01$) (xE"/C": +6.6%). Double and triple exposure level determined the significant decrease ($p<0.01$) of testis weight ($E"_2/E"_1$: -15.5%, $E"_3/E"_1$: -24.03%).

Testis weight in F_2 generation was lower than in F_1 and F_0 generations ($E"_1/E'_1$: -17.83%, $p<0.01$; $E"_2/E'_2$: -17.42%, $p<0.01$; $E"_3/E'_3$: -10.09%, $p>0.05$; $E"_1/E_1$: -7.85%, $p<0.01$; $E"_2/E_2$: -7.62%, $p>0.05$; $E"_3/E_3$: -22.22%, $p<0.01$).

Exposure to lead acetate determined a significant ($p<0.01$) decrease of epididymis weight comparative to C" ($E"_1/C"$: -25.49%, $E"_2/C"$: -33.33%, $E"_3/C"$: -45.09%). An indirect correlation between epididymis weight and exposure level was recorded ($E"_2/E"_1$: -10.52%, $E"_3/E"_1$: -26.31% - $p<0.01$). Epididymis weight in F_2 generation was significantly ($p<0.01$) lower than in F_1 and F_0 generations ($E"_1/E'_1$: -39.68%, $E"_2/E'_2$: -8.10%, $E"_3/E'_3$: -24.32%; $E"_1/E_1$: -30.9%, $E"_2/E_2$: -34.61%, $E"_3/E_3$: -41.66%). The differences were more evident in F_2 generation comparative to F_0 generation.

In F_2 generation, seminal vesicles weight decreased comparative to C" group, not significantly ($p>0.05$) in $E"_1$ and $E"_2$ groups and significantly ($p<0.01$) in $E"_3$ group ($E"_1/C"$: -8.54%, $E"_2/C"$: -17.09%, $E"_3/C"$: -80.34%). Double exposure level decreased seminal vesicles weight, not significantly ($E"_2/E"_1$: -9.34%) but triple exposure level, significantly ($p<0.01$) decreased their weight ($E"_3/E"_1$: -78.5%).

Comparative to F_1 generation, seminal vesicles weight significantly ($p<0.01$) decreased, excepting $E"_1/E'_1$ ($p>0.05$) ($E"_1/E'_1$: -29.13%, $E"_2/E'_2$: -19.16%, $E"_3/E'_3$: -87.63%). Comparative to F_0 generation, the decrease was not significant ($p>0.05$) in $E"_1$ group ($E"_1/E_1$: -19.54%) and significant ($p<0.05$) in $E"_2$ and $E"_3$ groups ($E"_2/E_2$: -25.95%; $E"_3/E_3$: -82.83%).

Prostatis weight decreased in E" groups comparative to C" group ($E"_1/C"$: -8.82%, $p>0.05$; $E"_2/C"$: -33.82%, $p<0.01$; $E"_3/C"$: -63.23%). An indirect correlation between exposure level and prostatis weight was recorded ($E"_2/E"_1$: -27.41%, $p<0.05$; $E"_3/E"_1$: -59.67%, $p<0.01$).

In F_2 generation, prostatis weight were significantly ($p<0.01$) lower at all exposure level, comparative to F_1 and F_0 generations ($E"_1/E'_1$: -36.73%, $E"_2/E'_2$: -58.71%, $E"_3/E'_3$: -78.81%; $E"_1/E_1$: -28.73%, $E"_2/E_2$: -36.61%, $E"_3/E_3$: -64.28%).

Bulbo-urethral glands weight significantly decreased in E" groups comparative to C" group ($E"_1/C"$: -41.12%, $E"_2/C"$: -50.46%, $E"_3/C"$: -70.09%). Double and triple exposure level significantly ($p<0.01$) decreased bulbo-urethral glands weight ($E"_2/E"_1$: -15.87%, $E"_3/E"_1$: -49.2%).

In F_2 generation, at all exposure level, bulbo-urethral weight was significantly ($p<0.01$) lower than of those from F_1 and F_0 generations ($E"_1/E'_1$: -37%, $E"_2/E'_2$: -55.46%, $E"_3/E'_3$: -74.19%; $E"_1/E_1$: -48.78%, $E"_2/E_2$: -53.91%, $E"_3/E_3$: -66.31%).

The results concerning testis weight (decrease of testis weight indirectly correlated to exposure level and generation) are similar to those of CHOWDHURY et al. 1987 quoted by APOSTOLI et al. (1), PINON-LATAILADE et al. (10), BATAINEH et al. (3), BISWAS and GHOSH (4), but opposite (excepting F_2) to those of FOWLER et al. 1980, MARCHLEWICZ et al. 1993, KEMPINAS et al. 1994 quoted by APOSTOLI et al. (1), WADI et al. (15), PINON-LATAILADE et al. (11) which sustain that lead doesn't influence testis weight.

Epididymis weight decreased could be explained by negative impact of lead on sperm count and sperm morphology (1, 5, 8, 9, 15, 24). The results are similar to those of PINON-LATAILADE et al. (10) but different of those obtained by

PINON-LATAILADE et al. (11) which didn't observed the decrease of epididymis weight.

Decrease of seminal vesicles weight (excepting F_1) was emphasized by other authors too (23, WILDT et al. 1983 quoted by *** - 20, 10, 15).

Excepting F_1, prostatis weight decreased in all experimental variants. The same dynamics was observed by SOKOL and BERMAN 1991, quoted by *** (22), PINON-LATAILADE et al. (10), WADI et al. (15), *** (23).

The general decrease tendency of bulbo-urethral glands weight could be a consequence of body weight decrease and of the degenerative phenomenon in bulbo-urethral glands. No data concerning influence of lead exposure on bulbo-urethral glands weight were found in studied references.

Conclusions

Exposure to different lead acetate levels along three generations determined:

- general tendency of testis weight increase comparative to control group, indirect correlation between testis weight, exposure level and duration;
- significant decrease of epididymis weight (excepting, not significant, in F_0 generation at 1000 and 2000 ppm exposure level, F_1 at 3000 ppm exposure level and significant increase in F_1 generation at 1000 ppm exposure level) comparative to control group, indirect correlation to exposure level and duration (excepting, not significant, F_1/F_0);
- significant decrease of seminal vesicles weight (excepting, increase in F_1 generation) comparative to control group and indirect correlation, significant, to exposure level (not significant in F_0 generation and in F_2 generation in case of tripling of exposure level); direct correlation, significant in F_1 generation at tripling of exposure level;
- significant decrease of prostatis weight (excepting F_0 generation, not significant at 2000 and 3000 ppm exposure level; F_0 generation at 1000 ppm and F_1 generation, at all exposure level, significant increase) comparative to control group and in indirect correlation to exposure level and duration (excepting, direct correlation, in F_2 generation);
- significant decrease of bulbo-urethral weight (not significant in F_0 generation and F_1 generation at 3000 ppm) comparative to control groups and in indirect correlation, significant, to exposure level (excepting, not significant, in F_0 generation, and in direct correlation, significant in F_1 generation) and exposure duration.

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