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EFFECT OF LOW-DOSE EXPOSURE TO TOXIC HEAVY METALS ON THE REPRODUCTIVE HEALTH OF RATS A MULTIGENERATIONAL STUDY

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ABSTRACT

The aim of this investigation was to evaluate the effects of the exposure to low doses of lead, mercury and cadmium dissolved in drinking water (200× above maximal permissible dosage) on the reproductive potency of 200 Wistar rats (100 males and 100 females of F1 generation) and their progeny. Ten groups of rats were formed according to their exposure to heavy metals, including one control group without exposure. The females gave births between weeks 13 and 78 of the experiments. Reproduction parameters, such as number of litters, total number of newborns, number of newborns per litter, and number of weanlings were assessed weekly. The results demonstrated that the number of litters and newborns were higher after exposure to mercury and lower after exposure to lead. The number of weanlings and their share from newborns were the highest after exposure to cadmium and the lowest after exposure to mercury. A sex-specific effect of metals was related to the reproductive success.

Key words: low-level exposure; heavy metals; rat; reproduction effect; reprotoxicity; several generations; sex-specific effect

INTRODUCTION

We live in a toxic world [25, 34]. Every day we are exposed to hundreds of toxic metals and chemicals including mercury, lead, cadmium, aluminium, food additives, pesticides, radiation toxins and many more. Heavy metal poisoning and chemical toxicity result in the accumulation of toxins in our tissues and organs, causing nutritional deficiencies [35], hormonal imbalances [26], neurological disorders, and can even lead to autoimmune disorders, cancer [17], and other debilitating chronic conditions [14]. Reproductive hazards from metal exposure is nowadays one of the fastest growing areas of concern in toxicology [12]. Exposure to various heavy metals causes irreversible toxic insult to both male and female reproductive systems [28, 29]. Heavy metals produce cellular impairment of the reproductive system at structural and functional levels.

While exposure to toxic (high-level) doses of heavy metals was examined thoroughly, relatively little data is available with regard to chronic exposure, particularly life-long conditions [24, 30]. There are almost no references in the literature regarding the effects of chronic exposure to very low doses of heavy metals involving multigenerational studies [18]. The problematic issue is the contamination of water and food with very low concentrations of these toxic heavy metals [10]. With chronic exposure to low doses, it is difficult to predict the consequences not only for those who are directly exposed, but also for future generations.

This fact motivated us to conduct multigenerational studies of chronic exposure to sub-toxic (low-level) doses of heavy metals such as in the reproductive experiments presented here. In this study we monitored the reproductive success of rats from the first filial generation whose parents were also exposed to low doses of heavy metals by comparing the number of litters, the number of new-borns, and the number of weanlings.

MATERIALS AND METHODS

Animals

This investigation was carried out on 200 Wistar rats (100 males and 100 females of F1 generation) aged four weeks—(F1) generation of rats and their progeny to age of 28 days. Ten groups of rats were formed according to exposure to heavy metals, including one control group without exposure. The rats were exposed to low doses of heavy metals in the drinking water.

After 28 days, the rats were transferred to another study site as representatives of the second filial (F2) generation. The rats were kept in polyethylene cages, one male and one female with free access to water and food in an air-conditioned animal house at a temperature of 22 ± 2 °C with steady humidity (50 %) and 12 : 12 h light:dark cycle. The experiments were terminated at 78 weeks. The experiments were done at the Central Animal Laboratory of the Faculty of Medicine, Pavel Jozef Šafarik University in Košice, which is accredited for breeding and testing on laboratory animals in compliance with the relevant legislation. The study was approved by the Ethical Committee of the Faculty of Medicine and the State Veterinary and Food Administration of Slovak Republic (No. Ro-7879/04-220/3).

Experimental protocol (Table 1)

All animals received standard food for laboratory animals (Larsen diet, commercially prepared by Velaz Praha, Czech Republic), with the content of heavy metals not exceeding the level of the natural environmental load. The animals were divided to 10 groups (10 females and 10 males, 1 pair in each cage). The first group C (control, n = 20) received pure water only. The second group Pb (n = 20) received drinking water containing basic lead acetate (Lachema, Brno, Czech Republic) in a concentration of 100 $mmol.l^{-1}$ (20.0 $mg.l^{-1}$ of lead in the drinking water), corresponding to 200 times the maximum allowable concentration (MAC) in water. The third group PbF (n = 20) comprised 10 males not exposed to Pb and 10 female offspring of parents exposed to lead that received daily 2.35 $mg.kg^{-1}$ body weight (bw) Pb by gavage, equivalent to the daily dose of their parents. The fourth group PbM (n = 20) comprised 10 females not exposed to Pb and 10 male offspring of parents exposed to lead that received daily 2.35 $mg.kg^{-1}$ bw Pb by gavage, equivalent to the daily dose of their parents. The fifth group Hg (n = 20) received drinking water containing mercuric chloride (Lachema, Brno, Czech Republic) in a concentration of 1 $mmol.l^{-1}$(0.2 $mg.l^{-1}$ of mercury in drinking water), corresponding to 200X the MAC in water. The sixth group HgF (n = 20) comprised 10 males not exposed to Hg and 10 female offspring of parents exposed to mercury that received daily 0.022 $mg.kg^{-1}$ bw of Hg by gavage, equivalent to the daily dose of their parents. The seventh group HgM (n = 20) comprised 10 females not exposed to Hg and 10 male offspring of parents exposed to mercury that received daily 0.022 $mg.kg^{-1}$ bw Hg by gavage, equivalent to the daily dose of their parents. The eighth group Cd (n = 20) received drinking water containing cadmium chloride dehydrate (Lachema, Brno, Czech Republic) in a concentration of 20 $mmol.l^{-1}$ (i. e., 2.0 $mg.l^{-1}$ of cadmium in drinking water), corresponding to 200X the MAC in water. The ninth group CdF (n = 20) comprised 10 males not exposed to Cd and 10 female offspring of parents exposed to cadmium that received daily 0.17 $mg.kg^{-1}$ bw Cd by gavage, equivalent to a daily dose of their parents. The tenth group (CdM; n = 20) comprised 10 females not exposed to Cd and 10 male offspring of parents exposed to cadmium that received daily 0.17 $mg.kg^{-1}$ bw Cd by gavage, equivalent to a daily dose of their parents.

All groups were monitored daily and evaluated for the following parameters: animal weight, food intake, and wa-

Table 1. Subdivision of rats into the groups (200 rats—10 females and 10 males/group)

Group	Mark	Sex	Exposure	Parents
1	C	F, M	Control, not exposed	Unexposed
2	Pb	F, M	Received drinking water containing 20.0 mg.l^{-1} of lead in drinking water	Exposed as their offspring
3	PbF	F	Received daily 2.35 mg.kg^{-1} bw of lead by gavage, equivalent to a daily dose of their parents	Received drinking water containing 20.0 mg/l^{-1} of lead in drinking water
		M	Unexposed	Unexposed
4	PbM	F	Unexposed	Unexposed
		M	Received daily 2.35 mg.kg^{-1} bw of lead by gavage, equivalent to a daily dose of their parents	Received drinking water containing 20.0 mg/l^{-1} of lead in drinking water
5	Hg	F, M	Received drinking water containing 0.2 mg/l^{-1} of mercury in drinking water	Exposed as their offspring
6	HgF	F	Received daily 0.022 mg.kg^{-1} bw of mercury by gavage, equivalent to a daily dose of their parents	Received drinking water containing 0.2 mg/l^{-1} of mercury in drinking water
		M	Unexposed	Unexposed
7	HgM	F	Unexposed	Unexposed
		M	Received daily 0.022 mg.kg^{-1} bw of mercury by gavage, equivalent to a daily dose of their parents	Received drinking water containing 0.2 mg/l^{-1} of mercury in drinking water
8	Cd	F, M	Received drinking water containing 2.0 mg/l^{-1} of cadmium in drinking water	Exposed as their offspring
9	CdF	F	Received daily 0.17 mg.kg^{-1} bw of cadmium by gavage, equivalent to a daily dose of their parents	Received drinking water containing 2.0 mg/l^{-1} of cadmium in drinking water
		M	Unexposed	Unexposed
10	CdM	F	Unexposed	Unexposed
		M	Received daily 0.17 mg.kg^{-1} bw of cadmium by gavage, equivalent to a daily dose of their parents	Received drinking water containing 2.0 mg/l^{-1} of cadmium in drinking water

F — female; M — male; bw — body weight

ter intake. Every week toxicological parameters were assessed: intake of heavy metal per kg of body weight of a rat, average daily dose (ADD) in the weight of heavy metal/kg bw/d during the experiments. Every week the number of litters, the number of newborns (determined on the date of birth), and the number of weanlings (determined on the 28th day after birth) were assessed.

Statistical methods

The statistical significance was examined by the Student's t-test or one-way analysis of variance (ANOVA) with the Newman-Keuls *post hoc* test. The significance was set to $P < 0.05$.

RESULTS

Our research encountered some problems with the evaluation of its results. It was difficult to determine whether the exposure was low-, medium-, or high-dose exposures or whether it was acute or chronic exposures. According to L u k a č i n o v á et al. [19], exposure to heavy metals in drinking water in our experiment should be classified as a low-dose exposure, as it is at a level normally found in the environment. Toxicological exposure parameters are presented in Table 2. Exposure assessment and the subsequent health risks are the most important steps in environmental toxicology. The basic unit is ADD (average daily dose). The reproductive period (the period with litters) in this inves-

Table 2. Basic toxicological parameters in week 78 of the trial

Parameter	Pb	Hg	Cd
Total dose received during the experiment in $mg.kg^{-1}$ bw	1.23	12.9	93.5
% LD50	26.4	34.8	41.6
ADD in $mg^{-1}.kg^{-1}.d^{-1}$	2.35	0.022	0.17

Pb — exposure to lead (20 mg $Pb.l^{-1}$ in drinking water or daily 2.35 $mg.kg^{-1}$ bw of lead by gavage, respectively); Hg — exposure to mercury (0.2 mg $Hg.l^{-1}$ in drinking water or daily 0.022 $mg.kg^{-1}$ bw of mercury by gavage, resp.); Cd — exposure to cadmium (2.0 mg $Cd.l^{-1}$ in drinking water or daily 0.17 $mg.kg^{-1}$ bw of cadmium by gavage, respectively); LD50 — 50 % lethal dose; ADD — average daily dose; bw — body weight.

Table 3. Reproductive parameters in the week 78 of the trial

Group	Number of litters	Number of newborns	Number of newborns/litter	Number of weanlings	% W
C	99	766	7.73	698	91.1
Pb	82*	725*	8.84*	530*	73.1**
PbF	91*	787$^{*,+}$	8.65	548$^{*,+}$	69.6**
PbM	82*	735^{*,x}	8.96*	653$^{+,x}$	88.8^{*,x}
Hg	101	772	7.64	471*	61.0*
HgF	103	773	7.50	482*	62.4*
HgM	108$^{*,+,x}$	797^{*,x}	7.38	625$^{+,x}$	78.4$^{**,++,xx}$
Cd	95	752	7.92	677	90.0
CdF	101^{+}	762	7.54	717$^{*,+}$	94.1$^{*,+}$
CdM	105$^{*,+,x}$	768	7,31	687^{x}	89.5^{xx}

Pb — exposure to lead (20 mg $Pb.l^{-1}$ in drinking water or daily 2.35 $mg.kg^{-1}$ bw of lead by gavage, respectively); Hg — exposure to mercury (0.2 mg $Hg.l^{-1}$ in drinking water or daily 0.022 mg kg^{-1} bw of mercury by gavage, respectively); Cd — exposure to cadmium (2.0 mg $Cd.l^{-1}$ in drinking water or daily 0.17 mg kg^{-1} bw of cadmium by gavage, respectively); W — percentage of weanlings from the total number of newborns; bw — body weight; * — significance $P < 0.05$ against to C group; ** — significance $P < 0.0001$ against to C group; $^{+}$ — significance $P < 0.05$ against to Pb, Hg, and Cd group, respectively; $^{++}$ — significance $P < 0.0001$ against to Pb, Hg, and Cd group, respectively; ^{x} — significance $P < 0.05$ between F and M in same exposed groups; ^{xx} — significance $P < 0.0001$ between F and M in same exposed groups.

tigation lasted from week 13 to week 78. Reproductive parameters are shown in Table 3.

The number of litters in the groups exposed to mercury and cadmium were higher compared to the control, whereas in the groups exposed to lead, the number of litters were lower. The dynamics of the litters showed that the number of litters in the control group gradually increased up to week 39 and then declined. The number of litters increased up to 26 weeks after birth in the Hg, HgF, HgM, and Pb groups, and in this period the number of litters were greater than in the control group. After week 26, the number of litters declined. The reproductive period in the Pb, PbM, Hg, and HgF groups lasted until week 65. The number of litters in the PbM group peaked on week 13 and then declined. Although the number of litters is itself a little probative, in the context of other reproductive parameters, it is suitable for complex assessment of the reprotoxicity of heavy metals.

The number of newborns was the highest in the groups exposed to mercury and was also significantly higher in the group in which only females were exposed to lead. In the group exposed to cadmium, the number of newborns was similar to that of the control group. Interestingly, in the group with only females exposed to mercury, the number

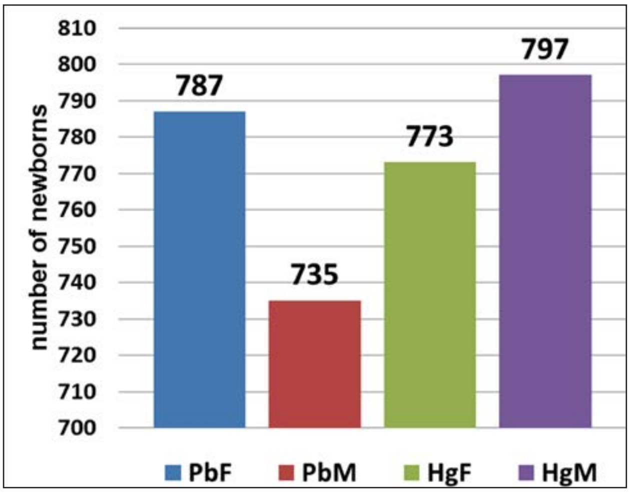

Fig. 1. Number of newborns during the reproductive period (P < 0.05 between PbF, PbM, HgF and HgM)

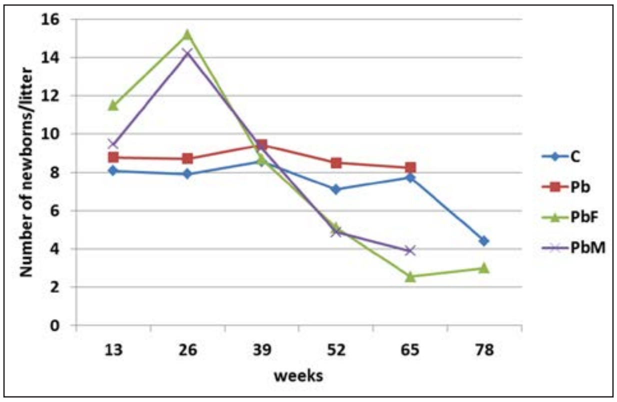

Fig. 2. The newborns/litter during the reproductive period

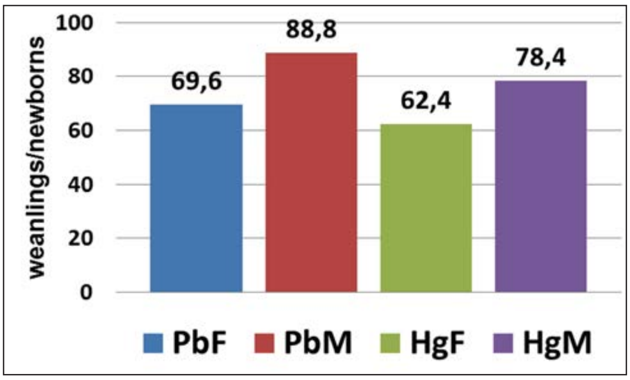

Fig. 3. The percentage of weanlings from newborns during the reproductive period (P < 0.05 between F and M)

of newborns was lower but in the group with only males exposed to mercury, the number of newborns was higher. The opposite was seen in the groups exposed to lead, where in the PbF group the number of newborns was higher in compared to the PbM group, where it was lower (Fig. 1).

The number of newborns per litter and the percentage of weanlings from newborns during the reproductive period is shown in Figs. 2 and 3.

DISCUSSION

The data indicated a difference in the sensitivity to various toxic heavy metals between the sexes [11, 31, 33]. The higher numbers of litters and newborns have led us to believe that in this case there is some adaptive response [7, 23] to the increased background of toxic heavy metals in the environment in environmentally compromised populations. These adaptive responses are well known in bacteria [8], plants and lower animals [3, 4, 22]. Other authors also observed an increased number of offspring followed by a high mortality during the first two weeks of their life after chronic exposure to high doses of cadmium [24, 30].

We assume that exposure to low concentrations of heavy metals activate biological mechanisms leading to maintenance of the species (particularly the activation of reproductive function), and the negative effects begin to occur after exceeding a certain exposure (dose) to the heavy metal.

The number of newborns per litter is one of the most commonly used indicators of reprotoxicity [9]. It was surprising that the highest number of newborns per litter was observed in the group exposed to lead, even though the number of litters was the lowest. Compared to the control group, only in Pb and PbM groups the difference was significantly (P < 0.05) higher; the other groups did not differ significantly (Fig. 2). In our opinion, this is related to a hormetic effects after exposure to low doses of heavy metals [5, 15, 16]. The nature of hormesis was described in detail by C a l a b r e s e [6].

A very important parameter of reproductive toxicity is the number of weanlings (individuals who live up to day 28 of life) and especially the percentage of weanlings from the number of newborns in the litter. At the end of the experiment, the lowest numbers of weanlings were in groups exposed to mercury, followed by those in groups exposed to lead. The number of weanlings in groups exposed to cadmium was comparable to that in the control group.

The highest percentage of weanlings was found after exposure to cadmium, which was comparable to the control group. The lowest percentages of weanling were found after exposure to mercury and lead ($P < 0.05$). After exposure to mercury and lead in groups with only male exposure, unexposed females took care of the offspring and may account for the significantly higher percentage of weanlings ($P < 0.05$) compared to groups with only female exposure or with both male and female exposure. The exposed females are less effective at taking care of the offspring (Fig. 3). This phenomenon could involve epigenetic and neurobiological mechanisms [2]. In contrast, after exposure to cadmium, there was a higher percentage of weanlings, which may be the result of the epigenetic phenomena of adaptation [20, 21, 32]. The high percentage of weanlings after exposure to cadmium can be attributed to the fact that low doses of cadmium may have the character of essential elements [1, 13, 27].

CONCLUSIONS

The knowledge obtained during our study allows us to state that the reproductive success after chronic (lifetime) exposure to low doses of toxic heavy metals in drinking water may involve a number of biological mechanisms:

1. Adaptation to a toxic environmental background results in an increase in the number of litters and newborns (vulnerable populations increase reproductive activity).
2. There exist sex differences in the reproductive success of exposed individuals as certain toxic metals act more on the reproductive capabilities of males while other affect the females more.
3. Unexposed mothers take better care of their offspring than the exposed ones.
4. Low doses of certain toxic heavy metals, including mercury and lead, may have hormetic effects.
5. Some heavy metals, including cadmium, may exhibit essential characteristics related to reproductive success in animals.
6. There is an indication that epigenetic mechanisms are involved in the adaptation to a background with low levels of toxic heavy metals.

Further studies are required to support the above conclusions. Obtaining detailed knowledge of the relevant processes and their precise mechanisms can significantly contribute to the reproductive success of vulnerable populations.

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