



## Lead accumulation and metallothionein content in female rats of different ages and generations after daily intake of Pb-contaminated food



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### ABSTRACT

Female Wistar rats of different ages (45, 90 and 140 days) and generations (mothers and offspring) were fed a feed containing 2.0 mg of Pb kg<sup>-1</sup> daily from weaning and the Pb accumulation was determined in different organs and in maternal milk, in addition metallothioneins (MTs) content was determined in the liver and kidneys. The results showed that Pb accumulation exhibited the following pattern: bone > liver > kidney > gut > blood cells > muscle > brain > ovary. Bones accumulated the most Pb in all animals, with its concentration increasing with age and prenatal exposure. Pb accumulation in the liver, kidney and blood cells, did not follow a consistent pattern with increasing age and our data did not indicate a relationship between the presence of MTs in liver and kidney and metal accumulation in these organs. However, in the offspring and with increasing age, Pb accumulated in more organs. Mothers fed with Pb produced contaminated milk, exposing their offspring to the metal via nursing. Thus, increasing age and prenatal exposure increases susceptibility to Pb toxicity-induced damage.

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### 1. Introduction

Lead (Pb) poisoning remains a major public health hazard, particularly in developing countries, by causing various deleterious effects on renal, hematopoietic, and reproductive functions as well as on the central nervous system (Flora et al., 2012; Beier et al., 2015). Pb is a metal that, mostly through anthropogenic action, can be found in soil, water, and the atmosphere; humans can be exposed via ingestion of contaminated water or food sources, among other routes. Nevertheless, the dietary intake of Pb is the main source of human exposure to this metal (EFSA, 2012).

Lead is one of the most studied metal elements, and its accumulation in organisms may cause harmful effects over time (Flora et al., 2012). Lead is absorbed into the bloodstream via the gastrointestinal tract and can be deposited in organs such as the liver, kidneys, lungs, brain, spleen, heart, muscles and bones; approximately 94% and 73% of the total Pb in the body is found in the bones in adults and children, respectively (ATSDR, 2007). Younger organisms tend to accumulate higher amounts of lead compared with adults, as

their mucous membranes are more permeable and their defenses are not fully effective (Winiarska-Mieczan and Kwiecień, 2016).

Lead accumulated in bones may be gradually released into the bloodstream even after exposure has ceased, particularly during physiologic or pathologic bone demineralization periods, such as pregnancy, lactation and osteoporosis (EFSA, 2012). Thus, prenatal exposure to Pb may harm maternal health and fetal and infant development even if maternal exposure levels are low. Furthermore, as bones constitute the main Pb reservoir over decades, women and their offspring may be at risk of continuous exposure well after exposure has ceased (CDC, 2010).

Metallothioneins (MTs) are low-molecular-weight intracellular proteins that are rich in cysteine residues and have high affinity for essential and non-essential metals (Park et al., 2001). These proteins contribute to the homeostasis of some essential metals and act as a protective mechanism against metal toxicity (Klaassen et al., 1999). Although some studies have linked MTs to Pb detoxification, information on Pb-driven MTs induction mechanisms remains scarce (Dai et al., 2013).

Thus, the aim of the present study was to evaluate how age and maternal transfer influence lead accumulation in different organs. Furthermore, MTs content in kidney and liver Pb was also assessed. To accomplish these objectives, female Wistar rats of different ages

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and generations were fed a feed containing 2.0 mg of Pb kg<sup>-1</sup> daily from weaning and were used to evaluate the accumulation of Pb in different organs and in maternal milk, in addition to the MTs content in the liver and kidneys.

## 2. Material and methods

### 2.1. Chemicals

All chemicals and reagents used were of analytical grade. Lead nitrate was purchased from Vete (Brazil), grade >99% pure. All other analytical laboratory chemicals and reagents were purchased from Merck (Germany), Sigma (USA) or Fmaia (Brazil), unless indicated otherwise.

### 2.2. Animals

Female Wistar rats (*Rattus norvegicus*) were provided by the central animal house of the State University of Londrina. Immediately after weaning (22 days), animals weighing  $37 \pm 5.58$  g ( $n = 80$ ) were transferred to the animal house of the Department of Physiological Sciences, where they were kept in individual cages at  $23 \pm 2$  °C with a 12 h/12 h photoperiod. Female rats were fed commercial feed with and without added lead (Pb and control groups, respectively). Feed and water were available *ad libitum*. The animals were weighed, and feed consumption was assessed three times per week by comparing the weight of the feed provided with the weight of the feed remaining (including waste feed in the under-cage collecting tray). Two female rat groups 78 days of age were subjected to mating and kept under the same conditions described previously throughout pregnancy and nursing. For mating purposes, male Wistar rats (from the UEL central animal house) were placed in cages with females at a 1:3 or 1:4 (male:female) ratio. Assessment of weight and feed consumption was discontinued during the mating period. The ethics committee for animal experiments of the State University of Londrina, Brazil approved the present study (CEUA/UEL – Process: 34715.2011.16).

### 2.3. Feed composition

Nuvilab® commercial feed for rats was ground, and Pb was added in the form of lead nitrate ( $\text{Pb}(\text{NO}_3)_2$ ) at a ratio of 2.0 mg Pb per kg feed (dissolved in 800 mL of water). New pellets were formed and dried at 60 °C for 24 h. The feed for the control group underwent the same process but without the addition of  $\text{Pb}(\text{NO}_3)_2$ . The Pb concentration of 2.0 mg kg<sup>-1</sup> was defined based on the maximum Pb concentration for fish meat established by the Brazilian Health Surveillance Agency ([ANVISA/685, 1998](#)).

### 2.4. Experimental design

Female rats were divided into different treatment groups ( $n = 9$  to 10 rats per treatment) as follows:

- Rats 45d: female rats fed commercial feed without lead (CTR 45d) or with added lead (Pb 45d) shortly after weaning at 22 days of age until they were 45 days of age;
- Rats 90d: female rats fed commercial feed without lead (CTR 90d) or with added lead (Pb 90d) shortly after weaning at 22 days of age until they were 90 days of age;
- Mothers 140d: female rats, which became pregnant and nursed, fed commercial feed without lead (CTR 140d) or with added lead (Pb 140d) shortly after weaning at 22 days of age until they were 140 days of age. These rats were also fed the manipulated feed (CTR or Pb) during pregnancy and lactation;

- Offspring 45d: female offspring from the Mothers 140d (CTR and Pb groups), fed commercial feed without lead (OffCTR 45d) or with added lead (OffPb 45d), respectively, shortly after weaning at 22 days of age until they were 45 days of age; and
- Offspring 90d: female offspring from the Mothers 140d (CTR and Pb groups), fed commercial feed without lead (OffCTR 90d) or with added lead (OffPb 90d), respectively, shortly after weaning at 22 days of age until they were 90 days of age.

In female rats, 45 days of age corresponds to puberty, while 90 and 140 days of age correspond to adulthood, when mating is possible ([Anderson et al., 2004](#)).

### 2.5. Sampling

After the experimental treatments, the rats were weighed, anesthetized with sodium thiopental (40 mg kg<sup>-1</sup>), and euthanized by exsanguination without previous fasting. Blood was collected from the inferior vena cava and centrifuged (1870g, 15 min, Hsiangtai centrifuge, model MCD-2000, Taiwan); blood cells were used to evaluate Pb accumulation. One section of the small intestine (1 cm of the first duodenal portion), one small portion of the left lateral lobe of the liver, brain, ovaries, right kidney, left tibia, and one muscle from the left leg were also collected for an evaluation of Pb accumulation. Samples were stored in nitric acid-washed (for metal decontamination) cryogenic tubes. One small section from the right kidney and left lateral liver lobe were taken for a separate analysis of MTs.

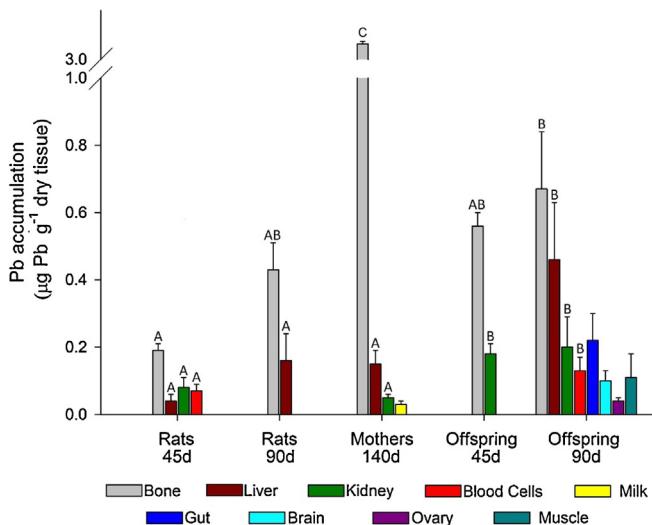
One hour prior to milk sampling, the offspring were separated from their mothers. Then, the female rats were anesthetized intraperitoneally with ketamine (60 mg kg<sup>-1</sup>) and xylazine 2% (10 mg kg<sup>-1</sup>); following anesthesia, 0.125 mL of oxytocin (125 UI) was injected intraperitoneally. Milk was collected by manual milking with the help of a micropipette and stored in cryogenic tubes pre-washed with nitric acid, for Pb accumulation analysis.

### 2.6. Determination of lead in feed and biological samples

Feed, organs, blood cells, and milk samples were oven-dried at 60 °C and digested for 48 h at 60 °C in nitric acid (Suprapur, Merck) 5N (1:5, w:v). The digested material was analyzed for Pb using graphite furnace atomic absorption spectrometry (Perkin Elmer, AAnalyst 700, USA) against a reference Pb standard solution (Specsol, Brazil). The detection limit for Pb using this method is 0.05 µg L<sup>-1</sup>. Two parallel determinations were performed for each sample and the differences in results were on average 3.6%.

#### 2.6.1. Determination of MTs in the liver and kidney

The MTs content was determined according to the method described by [Viarengo et al. \(1997\)](#), with modifications. Liver and kidney samples were homogenized (1:3 and 1:2 w:v, respectively) in specific buffer (0.5 M sucrose, 26 mM Tris, 0.5 mM phenylmethylsulfonyl fluoride, 1.3 mM β-mercaptoethanol) and centrifuged 45 min, 21300g, 4 °C (Hettich, Univesal 320R, Germany). The supernatant was subjected to ethanol/chloroform fractionation to obtain a partially purified metalloprotein fraction. Sulphydryl (-SH) groups in this fraction were quantified spectrophotometrically (Perkin Elmer, Victor<sup>3</sup> Multilabel Reader, USA) with Ellman's reagent (2 M NaCl, 0.43 mM DTNB buffered with 0.2 M Na-phosphate, pH 8) at 412 nm, using glutathione (GSH) as the standard. The MTs content was expressed as nmol MTs mg of protein<sup>-1</sup>. The protein concentration in the homogenate was determined spectrophotometrically (BioTek Instruments, ELx 800 Absorbance Microplate Reader, USA) according to [Bradford \(1976\)](#) at 595 nm, using bovine serum albumin (BSA) as the standard.



**Fig. 1.** Lead accumulation ( $\mu\text{g Pb g}^{-1}$  dry tissue) in tissues of female rats supplied with feed contaminated with Pb (2 mg Pb  $\text{kg}^{-1}$ ) soon after weaning until they are 45, 90 and 140 days (Rats 45d, Rats 90d and Mothers 140d, respectively) and their offspring until they are 45 and 90 days (Offspring 45d and Offspring 90d), in which significant increases in Pb concentrations, relative to respective control (rats supplied with feed without added Pb), were observed. The results are presented as mean  $\pm$  SD (N = 6–9). Different letters indicate significant differences among treatments, for the same tissue ( $P < 0.05$ ).

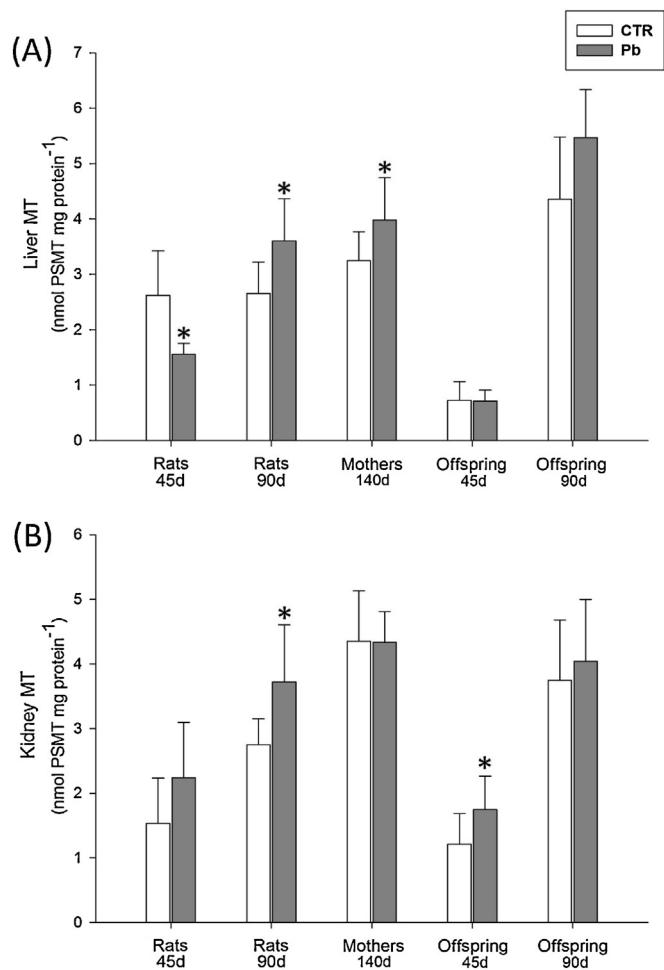
## 2.7. Statistical analysis

The parameters under evaluation in each treatment group (Rats 45d, Rats 90d, Mothers 140d, Offspring 45d, and Offspring 90d) were compared between the CTR and Pb groups using Student's *t* test or the Mann-Whitney test, according to the data distribution (normality and homoscedasticity). The Pb concentrations in a given tissue or organ were compared among the different Pb groups using one-way analysis of variance (ANOVA), and differences were localized using the Student Newman-Keuls (SNK) multiple comparison test. *P* values  $< 0.05$  were considered significant. The results are expressed as the mean  $\pm$  standard deviation.

## 3. Results

The concentration of lead was  $2.09 \pm 0.3 \text{ mg Pb kg}^{-1}$  (n = 15) in Pb-contaminated feed and  $0.03 \pm 0.02$  (n = 6) in control feed. The weight of the female rats at the end of each treatment and the total lead intake, based on the mean feed intake by each animal, are summarized in Table 1. Pb accumulation in a given tissue or organ for the CTR and Pb groups of rats subjected to each treatment are shown in Table 2.

The tissues or organs in which significant increases in Pb concentrations, relative to respective CTR groups, were observed are shown in Fig. 1. Animals from the Pb45d group showed increased Pb accumulation in bone ( $P = 0.018$ ), liver ( $P = 0.003$ ), kidney ( $P = 0.004$ ), and blood cells ( $P = < 0.001$ ). Animals from the Pb90d group showed higher accumulations in bone ( $P = < 0.001$ ) and liver ( $P = 0.011$ ); mothers from the Pb140d group showed higher Pb accumulation in bone ( $P = < 0.001$ ), liver ( $P = 0.004$ ), kidney ( $P = < 0.001$ ), and milk ( $P = 0.001$ ). Offspring from the OffPb45d group showed higher Pb accumulation in the kidney ( $P = 0.002$ ) and bone ( $P = < 0.001$ ), while those in the OffPb90d group showed higher accumulation in bone ( $P = 0.001$ ), liver ( $P = < 0.001$ ), kidney ( $P = < 0.001$ ), blood cells ( $P = < 0.001$ ), gut ( $P = < 0.001$ ), brain ( $P = < 0.001$ ), ovaries ( $P = 0.016$ ), and muscle ( $P = < 0.001$ ). Regarding the Mothers 140d group, higher Pb accumulation was found in



**Fig. 2.** Metallothionein content (MT) in the liver (A) and kidney (B) of female rats supplied with feed without added Pb (CTR) and with feed contaminated with Pb (Pb) at the concentration of 2 mg Pb  $\text{kg}^{-1}$ , immediately after weaning until they are 45, 90 and 140 days (Rats 45d, Rats 90d and Mothers 140d, respectively) and their offspring until they are 45 and 90 days (Offspring 45d and Offspring 90d). The values are presented as mean  $\pm$  standard deviation (N = 6–9). \* indicates significant difference from respective CTR ( $P < 0.05$ ).

milk from animals in the Pb140d group than in the CTR140d group ( $P = 0.001$ ) (Table 2).

The Pb concentration in a given tissue or organ was compared among the Pb groups subjected to different treatments (Fig. 1). The results showed that the OffPb90d group showed significantly higher amounts of Pb in liver ( $P < 0.01$ ) and blood cells ( $P = 0.014$ ) than the other groups. In bone tissue, Pb accumulation was significantly higher in the Mothers 140d group, followed by the Offspring 90d, Offspring 45d, Rats 90d, and Rats 45d groups.

The content of MTs in liver (Fig. 2A) was significantly lower ( $P = 0.001$ ) in Pb45d than in the respective CTR group. However, the hepatic MTs content was significantly higher in animals from the Pb90d and Pb140d groups ( $P = 0.007$  and  $P = 0.038$ , respectively) than in the corresponding CTR groups (Fig. 2A). A significantly higher MTs content was found in the kidney of rats from the Pb90d and OffPb45d groups compared with the corresponding control groups ( $P = 0.007$  and  $P = 0.047$ , respectively) (Fig. 2B).

## 4. Discussion

The present work evaluated Pb accumulation in the organs and milk of female rats that consumed Pb-contaminated feed daily. The importance of evaluating the effects of Pb after exposure to contam-

**Table 1**

Weight of the animals at the end of each treatment, total amount of ingested Pb and Pb intake rate in female rats supplied with feed without added Pb (CTR) and with feed contaminated with Pb (Pb) at the concentration of 2 mg Pb kg<sup>-1</sup>, immediately after weaning until they reached 45 (Rats 45d), 90 (Rats 90d) and 140 (Mothers 140d) days old and their offspring until they reached 45 (Offspring 45d) and 90 (Offspring 90d) days old. Pb intake calculations were made based on metal concentration in the feed of CTR group (0.03 mg Pb kg<sup>-1</sup>) and the Pb group (2.09 mg Pb kg<sup>-1</sup>).

Group Age	Treatment (n)	Final weight (g)	Total ingested Pb (mg)	Pb intake rate (mg kg <sup>-1</sup> day <sup>-1</sup> )
Rats	CTR (10)	122.70 ± 7.97	0.005 ± 0.000	0.0018 ± 0.0001
45d	Pb (10)	133.70 ± 9.68	0.41 ± 0.04	0.132 ± 0.010
Rats	CTR (10)	226.10 ± 19.97	0.031 ± 0.002	0.0019 ± 0.0002
90d	Pb (10)	218.10 ± 16.49	2.51 ± 0.67	0.169 ± 0.037
Mothers	CTR (10)	156.20 ± 25.17	—	0.0035 ± 0.0006
140d*	Pb (10)	173.20 ± 35.22	—	0.222 ± 0.040
Offspring	CTR (9)	173.67 ± 15.22	0.008 ± 0.001	0.0020 ± 0.0002
45d	Pb (9)	177.33 ± 5.52	0.51 ± 0.08	0.125 ± 0.021
Offspring	CTR (9)	247.56 ± 16.46	0.036 ± 0.004	0.0022 ± 0.0002
90d	Pb (9)	246.78 ± 22.50	2.30 ± 0.23	0.169 ± 0.010

The values are presented as means ± standard deviation (SD). \*The determination of the feed intake was suspended during pregnancy and it was not possible to calculate the total Pb ingested. Consequently, Pb consumption rate was calculated based on the values obtained until the rats had reached the age of 72 days.

**Table 2**

Pb concentration (μg Pb g<sup>-1</sup> dry tissue) in blood cells, organs and in the milk of female rats supplied with feed without added Pb (CTR) and with feed contaminated with Pb (Pb) at the concentration of 2 mg Pb kg<sup>-1</sup> soon after weaning until they are 45 (Rats 45d), 90 (Rats 90d) and 140 days (Mothers 140d) and their offspring until they are 45 (Offspring 45d) and 90 days (Offspring 90d).

	Rats 45d	Rats 90d	Mothers 140d	Offspring 45d	Offspring 90d
Gut	CTR Pb <LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD 0.22* ± 0.08
Blood Cells	CTR Pb <LOD 0.07* ± 0.02	<LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD 0.13* ± 0.04
Liver	CTR Pb <LOD 0.04* ± 0.02	0.02 ± 0.01 0.16* ± 0.08	0.01 ± 0.01 0.15* ± 0.04	<LOD <LOD	0.06 ± 0.02 0.46* ± 0.17
Brain	CTR Pb <LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD 0.10 ± 0.03
Ovary	CTR Pb <LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD 0.04* ± 0.01
Kidney	CTR Pb <LOD 0.08* ± 0.03	<LOD <LOD	<LOD 0.05* ± 0.01	<LOD 0.18* ± 0.03	<LOD 0.20* ± 0.09
Bone	CTR Pb <LOD 0.19* ± 0.02	0.06 ± 0.01 0.43* ± 0.08	0.15 ± 0.22 3.32* ± 0.48	0.10 ± 0.01 0.53* ± 0.04	0.20 ± 0.09 0.67* ± 0.17
Muscle	CTR Pb <LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD <LOD	<LOD 0.11* ± 0.07
Milk	CTR Pb --	--	<LOD 0.03* ± 0.01	--	--

The values are presented as means ± standard deviation (SD). <LOD: below the limit of Pb detection (0.05 μg L<sup>-1</sup>). \*Indicate significant difference compared to the respective CTR group (P < 0.05).

inated food allows a more realistic understanding of the damage to the organism caused by this contaminant, as its absorption via the gastrointestinal tract is dependent on the nutrients present in the food (IARC, 2006). Toxicity studies usually administer high doses of Pb either through injection (Taupeau et al., 2001) or orally via water consumption (Jostina et al., 2012); few studies have addressed Pb administered via ingestion. Recently, Winiarska-Mieczan (2014) and Winiarska-Mieczan and Kwiecień (2016) compared Pb accumulation and distribution in the organs of young rats exposed to the metal via water (7 mg L<sup>-1</sup>) or food (50 mg kg<sup>-1</sup> feed) intake. However, in these studies, the animals were fed for up to 12 weeks with a Pb concentration in food (50 mg kg<sup>-1</sup> feed) far higher than that used in the present study (2 mg kg<sup>-1</sup> feed), which resulted in smaller concentrations of Pb in tissues, although the dose was large enough to cause liver, kidney, and blood cell damage (Nascimento and Martinez, 2016).

In Europe, exposure to Pb in food sources amounts to an average of 0.50, 1.32, and 1.03 μg kg<sup>-1</sup> of body weight per day in adults, infants, and children, respectively (EFSA, 2012). To establish a limit for the weekly intake of Pb, the World Health Organization (WHO) assessed health hazards, such as decrease in the intelligence quotient (IQ) in children and increased blood pressure in adults, and established a maximum value of 25 μg kg<sup>-1</sup> of body weight per week (WHO, 2011). This value was later considered unsafe for consumer health, and this was followed by the conclusion that it is not possible to establish a healthy level of Pb intake (WHO, 2011). In the present study, Pb intake values by adult rats values exceeded previously mentioned.

The lead concentration in the different tissues and organs exhibited the following pattern: bone > liver > kidney > gut > blood cells > muscle > brain > ovary. Bones accumulated the most Pb in all animals undergoing any treatment, thus confirming their role as

the main Pb reservoirs in the body (Monir et al., 2010). Direct or indirect Pb poisoning may alter many functional aspects of bone cells (Pounds et al., 1991) and Pb may replace calcium in cell signaling pathways, resulting in loss of physiologic regulation (Pounds et al., 1991).

There is clear evidence in the present study that Pb accumulation in bone increases with age in female rats, as Pb in the bones of animals in the Pb45d, Pb90d, and Pb140d groups increased in increasing order, reflecting a longer period of Pb poisoning. However, when comparing offspring groups (OffPb45d and OffPb90d) with non-offspring rats of the same age (Pb45d and Pb90d), a higher concentration of lead was found in the bone tissue of offspring born from mothers who consumed contaminated food. Because Pb can cross the placental barrier, these animals were first exposed to the metal while in utero and were further exposed after birth, as Pb is present in the maternal milk, which represents a health hazard for newborns (Dursun et al., 2016). Therefore, contamination of maternal milk is likely a contributing factor for accumulated Pb levels in the bones of offspring, as milk collected from the Mothers 140d group revealed the presence of Pb (0.03 μg g<sup>-1</sup> of milk).

Bone remains a potential endogenous source of Pb during pregnancy and lactation, even after Pb poisoning has ceased, and its contribution towards bloodstream Pb levels in the post-partum period is significantly higher than during pregnancy (Gulson et al., 2003). Plasma and maternal milk Pb levels appear to be similar, and breastfed children are only at risk if the mother is exposed to either elevated concentrations of the contaminant or to an endogenous (e.g., the bone) or exogenous (e.g., diet) source (Gulson et al., 1998). In the present study, the group Mothers Pb 140d exhibited Pb accumulation in bones and milk but not in blood cells, suggesting a flow of Pb toward bones and breast milk (post-partum) that prevents Pb accumulation in erythrocytes.

The results of Pb accumulation in blood cells were mostly focused on erythrocytes, as white blood cells represent a very small fraction of the total blood volume (42–47% erythrocytes, 1% leucocytes) (Pries et al., 1996). The best indicator of Pb accumulation in soft tissue is the blood Pb concentration, which reflects more recent exposure, while the bone concentration reflects long-term exposure (EFSA, 2010). Ahamed and Siddiqui (2007) reported that 99% of blood Pb accumulates in erythrocytes. However, there was no correlation in Pb concentration between the blood and bone and some soft tissues in the present study. In animals of the Pb90d, Pb140d, and OffPb45d groups, the presence of Pb in the liver and kidney was independent of the presence of Pb in erythrocytes. Because plasma is the blood component in which Pb is free to cross cell membranes and cause organ toxicity (Tsaih et al., 1999), we suggest that during some periods of exposure, there may be a higher Pb flow between the plasma and bone and soft tissues that does not cause Pb accumulation in erythrocytes.

Pb accumulation in organs was clearly dependent on the age and generation of the animal. OffPb90d animals accumulated Pb in all analyzed organs – not only in bone, liver, kidney, and blood cells (where all groups showed Pb accumulations) but also in the gut, muscle, brain, and ovaries (the sole group where this occurred). The impact of accumulated Pb on the health of these animals may range from infertility to neurologic alterations. The presence of Pb in the gut of these specimens is not a reflection of its excretion by the gastrointestinal tract, as Pb is eliminated by the bile after absorption (ATSDR, 2005) but may return to the organism via enterohepatic circulation. One other possible consequence of Pb accumulation in these animals is compromised fertility due to accumulation in the ovaries. It is known that women are more vulnerable to infertility by Pb-induced effects on reproductive cells, and if gestation occurs, Pb may cause miscarriage and premature delivery and affect fetal developmental stages during gestation (Pal et al., 2015) and may induce neurologic disorders, especially in children (Xu et al., 2015). Thus, female rats in the OffPb90d group, the older specimens of the second generation, may suffer the consequences of metal accumulation in the brain, leading to disease as age progresses. It has been shown that exposure to Pb increases the occurrence of Alzheimer's disease in elderly brains (Bolin et al., 2006).

MTs are particularly important for cell protection because they regulate the availability of certain metals, including Cu, Zn, and Cd; other metals, including Fe and Pb, may also induce MTs (Johnson, 1998). However, the data collected in the present study could not indicate whether the presence of MTs in a given organ is associated with increased metal accumulation as MTs content increased in tissues from animals that did not show increased Pb accumulation. By contrast, Pb accumulated in the liver and kidney of OffPb90d animals without a concomitant increase in MTs content, and the relation between MTs and Pb exposure in the present study is unclear. Chidinma et al. (2015) observed fluctuations in MTs expression in mouse liver exposed to different Pb concentrations, but MTs expression was only increased when animals were exposed to industrial effluents, suggesting induction by other metals. Regardless, it is known that Pb is a poor MTs inducer; however, when MTs are already present through prior induction by Cd or Zn, the protein can bind Pb and thus protect against its toxicity (Gonick, 2011). Discrepancies between MTs content and Pb concentration in the organs under study indicate the need for a better understanding of what did or did not cause a change in MTs content.

In summary, the present study showed that the Pb accumulates in bone, with its concentration increasing with age and prenatal exposure. On the other hand, Pb accumulation in the liver, kidney, and blood cells, did not follow a consistent pattern with increasing age of exposure. However, in the second generation and with increasing age, Pb accumulated in more organs (bone, liver, kidney, blood cells, gut, brain, ovary and muscle). Mothers exposed to

Pb daily produced contaminated milk, exposing their offspring to the metal via nursing. Thus, increasing age and prenatal exposure increases susceptibility to Pb toxicity-induced damage.

## Conflict of interest

There is no conflict of interest.

## Acknowledgements

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## References

- ANVISA (Agência Nacional de Vigilância Sanitária), 1998. Portaria no 685, de 27 de agosto de 1998. Aprova o Regulamento Técnico: Princípios Gerais para o Estabelecimento de Níveis Máximos de Contaminantes Químicos em Alimentos e seu Anexo: Limites máximos de tolerância para contaminantes inorgânicos. Secretaria de Vigilância Sanitária. Ministério da Saúde. Brasil.
- ATSDR (Agency for Toxic Substances and Disease Registry), 2005. Toxicological profile for lead. U.S. Department of Health and Human Services, Public Health Services.
- ATSDR (Agency for Toxic Substances and Disease Registry), 2007. Public Health Statement, Lead. U.S. Department of Health and Human Services, Public Health Services.
- Ahamed, M., Siddiqui, M.K.J., 2007. Low level lead exposure and oxidative stress: current opinions. *Clin. Chim. Acta* 383, 57–64.
- Anderson, M.L., D'Almeida, V., Ko, G.M., Kawakami, R., Martins, P.J.F., Magalhães, L.E., Tufik, S., 2004. *Princípios éticos e práticos do uso de animais de experimentação*. São Paulo: UNIFESP—Universidade Federal de São Paulo, São Paulo.
- Beier, E.E., Sheu, T.J., Dang, D., Holz, J.D., Ubayawardena, R., Babij, P., Puzas, J.E., 2015. Heavy metal ion regulation of gene expression: mechanisms by which lead inhibits osteoblastic bone forming activity through modulation of the Wnt/β-catenin signaling pathway. *J. Biol. Chem.* 290, 18216–18226.
- Bolin, C.M., Basha, R., Cox, D., Zawia, N.H., Maloney, B., Lahiri, D.K., Pelaez, F.C., 2006. Exposure to lead and the developmental origin of oxidative DNA damage in the aging brain. *FASEB J.* 20, 788–790.
- Bradford, M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72, 248–254.
- CDC (Centers for Disease Control and Prevention), 2010. Guidelines for the identification and management of lead exposure in pregnant and lactating women. U.S. Department of Health and Human Services.
- Chidinma, N.C., Adewale, A., Chiaka, A., 2015. Differential expression of metallothionein-1 and cytochrome p450-2a5 (cyp2a5) in mice in response to lead acetate exposure and industrial effluents in Ibadan, Nigeria. *Toxicol. Ind. Health*, 1–7, <http://dx.doi.org/10.1177/0748233715594107>.
- Dai, S., Yin, Z., Yuan, G., Lu, H., Jia, R., Xu, J., Song, X., Li, L., Shu, Y., Liang, X., He, C., Lv, C., Zhang, W., 2013. Quantification of metallothionein on the liver and kidney of rats by subchronic lead and cadmium in combination. *Environ. Toxicol. Pharmacol.* 36, 1207–1216.
- Dursun, A., Yurdakok, K., Yalcin, S.S., Tekinalp, G., Aykut, O., Orhan, G., Morgil, G.K., 2016. Maternal risk factors associated with lead, mercury and cadmium levels in umbilical cord blood, breast milk and newborn hair. *J. Matern. Fetal Neonatal. Med.* 29, 954–961.
- EFSA (European Food Safety Authority), 2010. Scientific opinion on lead in food. EFSA J. 8, 1570.
- EFSA (European Food Safety Authority), 2012. Lead dietary exposure in the European population. EFSA J. 10, 2831.
- Flora, G., Gupta, D., Tiwari, A., 2012. Toxicity of lead: a review with recent updates. *Interdiscip. Toxicol.* 5, 47–58.
- Gonick, H.C., 2011. Lead-binding proteins: a review. *J. Toxicol.*, <http://dx.doi.org/10.1155/2011/686050> (Article ID 686050, 10 pages).
- Gulson, B.L., Jameson, C.W., Mahaffey, K.R., Mizon, K.J., Patison, N., Law, A.J., Korsch, M.J., Salter, M.A., 1998. Relationships of lead in breast milk to lead in blood, urine, and diet of the infant and mother. *Environ. Health Persp.* 106, 667–674.
- Gulson, B.L., Mizon, K.J., Korsch, M.J., Palmer, J.M., Donnelly, J.B., 2003. Mobilization of lead from human bone tissue during pregnancy and lactation—a summary of long-term research. *Sci. Total Environ.* 303, 79–104.

- IARC (International Agency for Research on Cancer), 2006. WORLD HEALTH ORGANIZATION: Summaries & evaluations: Inorganic and organic lead compounds. Lyon, International Agency for Research on Cancer (IARC Monographs for the Evaluation of Carcinogenic Risks to Humans), Vol. 87.
- Johnson, F.M., 1998. The genetic effects of environmental lead. *Mutat. Res.* 410, 123–140.
- Josthna, P., Geetharathan, T., Sujatha, P., Deepika, G., 2012. Accumulation of lead and cadmium in the organs and tissues of albino rat. *Int. J. Pharm. Life Sci.* 3, 2186–2189.
- Klaassen, C.D., Liu, J., Choudhuri, S., 1999. Metallothionein: an intracellular protein to protect against cadmium toxicity. *Annu. Rev. Pharmacol. Toxicol.* 39, 267–294.
- Monir, A.U., Gundberg, C.M., Yagerman, S.E., van der Meulen, M.C.H., Budell, W., Boskey, A.L., Dowd, T.L., 2010. The effect of lead on bone mineral properties from female adult C57/BL6 mice. *Bone* 47, 888–894.
- Nascimento, C.R.B., Martinez, C.B.R., 2016. Daily intake of lead in Wistar rats at different ages Biochemical, genotoxic and physiological effects. *Environ. Toxicol. Pharmacol.* 41, 132–141.
- Pal, M., Sachdeva, M., Gupta, N., Mishra, P., 2015. Lead exposure in different organs of mammals and prevention by Curcumin-Nanocurcumin: a review. *Biol. Trace Elem. Res.* 168, 380–391.
- Park, J.D., Liu, Y., Klaassen, C.D., 2001. Protective effect of metallothionein against the toxicity of cadmium and other metals. *Toxicology* 163, 93–100.
- Pounds, J.G., Long, G.J., Rosen, J.F., 1991. Cellular and molecular toxicity of lead in bone. *Environ. Health Persp.* 91, 17–32.
- Pries, A.R., Secomb, T.W., Gaehrtgens, P., 1996. Biophysical aspects of blood flow in the microvasculature. *Cardiovasc. Res.* 32, 654–667.
- Taupeau, C., Taupeau, K., Nomé, F., Lefèvre, B., 2001. Lead Accumulation in the mouse ovary after treatment-induced follicular atresia. *Reprod. Toxicol.* 15, 385–391.
- Tsaih, S.W., Schwartz, J., Lee, M.L.T., Amarasiriwardena, C., Aro, A., Sparrow, D., Hu, H., 1999. The independent contribution of bone and erythrocyte lead to urinary lead among middle-aged and elderly men: the normative aging study. *Environ. Health Persp.* 107, 391–396.
- Viarengo, A., Ponzano, E., Donderob, F., Fabbri, R., 1997. A simple spectrophotometric method for metallothionein evaluation in marine organisms: an application to Mediterranean and Antarctic mollusks. *Mar. Environ. Res.* 44, 69–84.
- WHO (World Health Organization), 2011. Evaluation of certain food additives and contaminants. Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives.
- Winiarska-Mieczan, A., Kwiecień, M., 2016. The effect of exposure to Cd and Pb in the form of a drinking water or feed on the accumulation and distribution of these metals in the organs of growing Wistar rats. *Biol. Trace Elem. Res.* 169, 230–236.
- Winiarska-Mieczan, A., 2014. Cumulative rate and distribution of Cd and Pb in the organs of adult male Wistar rats during oral exposure. *Environ. Toxicol. Pharmacol.* 38, 751–760.
- Xu, J., Hu, H., Wright, R., Sanchez, B.N., Schnaas, L., Bellinger, D.C., Park, S.K., Martinez, S., Hernández-Avila, M., Téllez-Rojo, M.M., Wright, R.O., 2015. Prenatal lead exposure modifies the impact of maternal self-esteem on children's inattention behavior. *J. Pediatr.* 167, 435–441.