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# Histomorphological evaluation of mice testis after co-exposure to lead and cadmium

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ABSTRACT

**Objective:** The present study investigates the effects of co-exposure to lead and cadmium on mice testis using histomorphological approach. **Methods:** Male mice were subcutaneously injected with lead chloride on day 1 and cadmium chloride on day 2 (74 and 1 mg/kg body weight, respectively), and kept for 24 h. Vehicle control group was also considered. Mice were then sacrificed and testis were collected and weighed. Samples were fixed on Bouin´s solution and processed for histology. The diameter of seminiferous tubules in both groups was calculated using software based on deformable models (SNAKE). **Results:** The combined exposure of lead and cadmium induced degenerative changes in testis, namely, wavy contour of seminiferous tulules, germ cell loss, and release of immature cells into the lumen. Atrophy of seminiferous tubules was seen in this group, confirmed by a significant ($P<0.001$) decrease in the diameter. **Conclusions:** Cumulative effects of lead and cadmium may have disrupted the blood-testis barrier, then causing the histopathological lesions within testis.

## 1. Introduction

Exposure to the myriad of hazardous substances at both environmental and occupational settings may severely disrupt semen quality and fecundity. Elegant studies evidenced the deleterious effects of some chemicals such as heavy metals, solvents, and pesticides on male reproductive function[1-6]. Attention was focussed on spermatogenesis, a complex process which is easily adversely affected by pollutants. Among the innumerable environmental contaminants, lead and cadmium compounds deserve special concern, since they target multiple organs and systems in both human and wildlife[7]. In fact, cadmium and lead were classified as human carcinogens by the International Agency for Research on Cancer[8-9]. In addition, reprotoxic effects of these compounds were largely reported in the literature. For example, adverse effects of lead chloride on sperm parameters of mice were described[10]. Although sperm physiological parameters such as motility, morphology and acrosome status were altered, no significant genotoxic effect was detected in this report. Recently, Shafai and co-workers evidenced severe testis alterations on albino rats after prolonged lead exposure, using light and electron microscopy studies[11].

Cadmium is also a known endocrine disruptor compound, interfering on the synthesis and regulation of some hormones in both females and males[12]. The toxic effects of cadmium on the reproductive system and underlying mechanisms were reported[13]. In those papers, testicular changes have been described on animal models at different stages of growth and maturity. Disruption of the blood-testis barrier by cadmium due to adverse effects on cell adhesion, oxidative stress, and necrosis at higher experimental doses was also underlined. Poor semen quality such as low sperm count and motility was also associated with cadmium exposure

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in heavy smokers.

In similar context, there is a great interest on the investigation of complexes mixtures on human and environmental health, since persistent pollutants may contaminate water, food, and air. For example, the complexity of biological and chemical interactions among three metals/metalloids (lead, cadmium, and arsenic) was reviewed by Wang and Fowler[14], in both experimental animal and human exposures. In this review, several common mechanisms underlying their toxicities, including production of oxidative stress, reaction with sulfhydryl groups, and interference with essential metals, were described.

Previous reports from our laboratory revealed that mice exposed per se to lead chloride and cadmium chloride (74 and 1 mg/kg body weight, respectively) for a short period of time (48 and 24 h, respectively) did not impair spermatogenesis[15-16]. Then, the current study aims to explore if the co-exposure to lead and cadmium for the same periods have cumulative effects on mice testis morphology.

## 2. Materials and methods

### 2.1. Animals, housing conditions, and experimental design

Eight-week-old male ICR-CD-1 mice weighing (30±2) g purchased from Harlan (Spain) were randomly divided in two groups (five animals each) and housed in polypropylene cages with stainless steel grills under the following laboratory conditions: temperature (22±2) °C, relative humidity 50%-60%, and 12 h dark-light period. Water and food were given *ad libitum*. Mice were allowed to acclimate to the vivarium environment for 5 d. Solutions of lead chloride (Merck, Darmstadt, Germany) and cadmium chloride (Sigma-Aldrich, Dorset, UK) were prepared in saline (74 and 1 mg/kg body weight, respectively) as previously reported[15-16]. For the co-exposure treatment, mice were subcutaneously injected with lead chloride on day 1 and cadmium chloride on day 2, and kept for 24 h. Control animals were administered with 9 g/L sodium chloride as a vehicle at the same time. The behaviour of animals was monitored during the study. After sacrifice, body and testis weights were recorded. Animal experiments were performed according to procedures for ethics in animal experimentation (Rule number 86/609/CEE- 24/11/92).

### 2.2. Histological studies

The right testis from each animal was fixed in Boiun´s solution. Six hours later, testis was sliced and kept again in a fresh solution of the fixative for 24 h, dehydrated in graded ethanol series, and embedded in paraffin wax. Sections (5 μm thick) were stained with haematoxylin and eosin and usually prepared for histology. Sections were observed and digital images were taken by a digital camera Olympus Camedia C-5060 coupled to a microscope Olympus BX41 (Olympus, Tokyo, Japan). In addition, the measurement of seminiferous tubules diameter in sections of the two groups of animals (about 30 tubules/histological section) was performed using software based on deformable models (SNAKE) as described by Guevara and co-workers[17].

### 2.3. Statistical analysis

One way ANOVA was carried out to compare body, testis weights, and tubular diameters from the two groups.

## 3. Results

In the present study, a surviving rate of 100% was recorded, although decreased movement was noted after cadmium chloride administration. Body and organ weights of the two groups revealed similar values (data not shown). Macroscopic pathological changes were not apparent. Sections of seminiferous tubules in control animals evidenced normal regular features, namely, compactly organized germinal epithelium (Figure 1). However, histopathological studies revealed considerable damage in lead-cadmium exposed group (Figure 2): shrinkage and atrophy of seminiferous tubules, derangement of cellular organization due to germ cell degeneration, and cell exfoliation was evident. In addition, wavy contour of seminiferous tubules was noted. Seminiferous tubules diameter was significantly reduced ($P<0.001$) in this group (Figure 3).

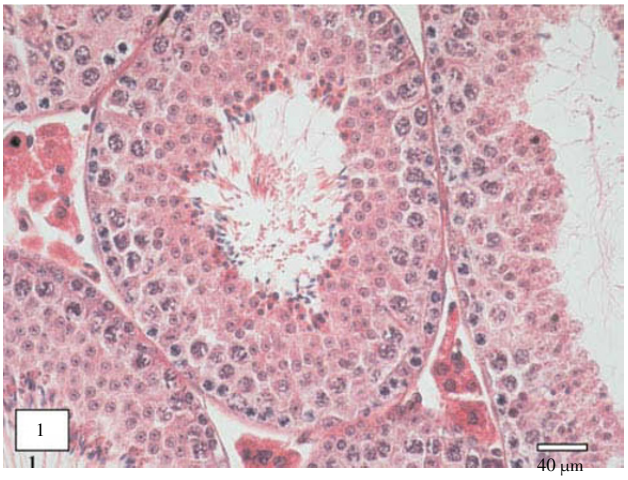


**Figure 1.** Representative microphotograph of the testis in vehicle group displaying normal spermatogenesis.

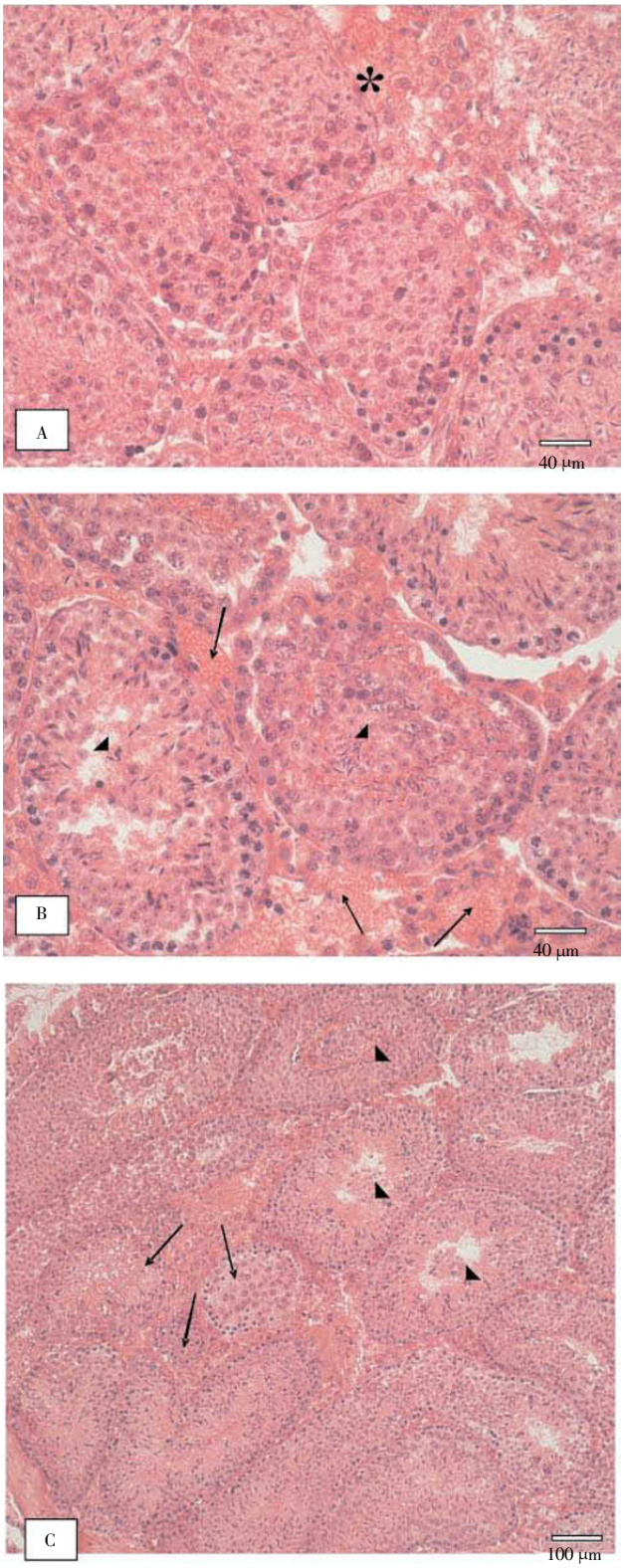


**Figure 2.** Testis sections from lead-cadmium exposed group with several morphological changes being noted.
(A) The organization pattern within distorted seminiferous tubules did not reveal normal orientation of germ cells. Intertubular tissue also presents edema (*). (B) Haemorrhages were seen in intertubular tissue (arrows) and immature cell exfoliation within the lumen was noted (arrowheads). (C) Atrophic seminiferous tubules (arrows) and release of immature germ cells into the lumen (arrowheads) were evident.

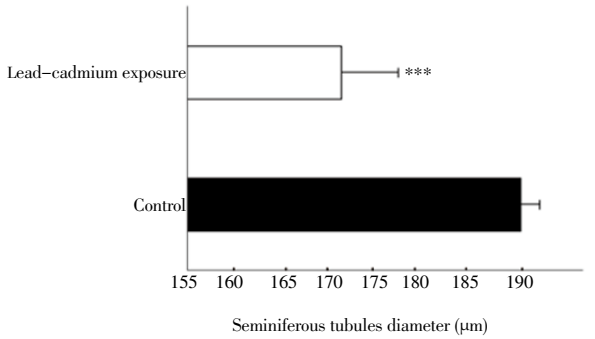


**Figure 3.** Profile of seminiferous tubules diameters from control and lead-cadmium exposed groups.
Data are represented as mean±SD values. $^{***}P<0.001$ exposed versus control group.

## 4. Discussion

Human exposure to hazardous mixtures is still a major public health concern. In fact, antagonistic, additive, and/or synergistic effects on target organs and tissues may occur. The present work clearly demonstrated additive effects of simultaneous administration of lead and cadmium to mice for 72 h. In effect, disturbed spermatogenesis was observed, contrarily to previously reported by our group, where no structural changes within testis was revealed using these doses per se, and time-points[15-16]. Surprisingly, atrophy of seminiferous tubules observed in this group was not accompanied by low testis weight, probably due to haemorraghes within intertubular tissue. Similar additive effects of lead-cadmium mixtures on mitochondrial ultrastructure of Sertoli cells were described by Bizarro and co-workers in the mouse[18]. After 1 week of inhalation exposure of this mixture, significant values were described, whereas the effect of each compound per se became significant after a longer period, *i.e.*, 2 weeks of exposure. However, different result was obtained on male rats co-exposed to cadmium and nickel[19]. In fact, an ameliorating effect induced by nickel on pathological changes caused by cadmium alone in the rat testis was reported by these authors. Investigations undertaken by Elkin and co-workers evidenced disruption of the blood-testis barrier by cadmium chloride, at doses of 1.75-3.00 mg/kg, whereas for lower dose (1 mg/kg) no changes were seen[20]. Then, in the present work, cumulative effects of lead and cadmium may have disrupted the blood-testis barrier, then causing the histopathological lesions above described. However, further studies using a double staining for related proteins and tracer techniques to evaluate blood-testis barrier integrity and function are needed. These approaches added

to mitochondrial function evaluation may clarify the nature of these interactions.

## Conflict of interest statement

We declare that we have no conflict of interest.

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## References

[1] Jensen TK, Bonde JP, Joffe M. The influence of occupational exposure on male reproductive function. *Occup Med* 2006; **56**(8): 544–553.

[2] Ten J, Mendiola J, Torres-Cantero AM, Moreno-Grau JM, Moreno-Grau S, Roca M, Romero J, et al. Occupational and lifestyle exposures on male infertility: A mini review. *The Open Reprod Sci J* 2008; **1**: 16–21.

[3] Cheng CY, Wong EW, Lie PP, Li MW, Su L, Siu ER, et al. Environmental toxicants and male reproductive function. *Spermatogenesis* 2011; **1**(1): 2–13.

[4] Mruk DD, Cheng CY. Environmental contaminants: Is male reproductive health at risk? *Spermatogenesis* 2011; **1**(4): 283–290.

[5] Wong EW, Cheng CY. Impacts of environmental toxicants on male reproductive dysfunction. *Trends Pharmacol Sci* 2011; **32**(5): 290–299.

[6] Mathur PP, Huang L, Kashou A, Vaithinathan S, Agarwal A. Environmental toxicants and testicular apoptosis. *The Open Reprod Sci J* 2011; **3**: 114–124.

[7] Morais S, Costa FG, Pereira ML. Heavy Metals and Human Health. In: Environmental Health, 1st ed. Oosthuizen, J: InTech Open Sci; 2012, p. 227–246.

[8] IARC. Cadmium and cadmium compounds. IARC Monographs. International Agency for Research on Cancer, Lyon, France; 1993.

[9] IARC. Inorganic and Organic Lead Compounds IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, 87 Lyon, France; 2006.

[10] Oliveira H, Spano M, Santos C, Pereira ML. Adverse effects of cadmium exposure on mouse sperm. *Reprod Toxicol* 2009; **28**(4): 550–555.

[11] Shafai AE, Zohdy N, El Mulla K, Hassan M, Morad N. Light and electron microscopic study of the toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible protective effect of ascorbic acid. *Food Chem Toxicol* 2011; **49**(4): 734–743.

[12] Siu ER, Mruk DD, Porto CS, Cheng CY. Cadmium-induced testicular injury. *Toxicol Appl Pharmacol* 2009; **238**(3): 240–249.

[13] Thompson J, Bannigan J. Cadmium: Toxic effects on the reproductive system and the embryo. *Reprod Toxicol* 2008; **25**(3): 304–315.

[14] Wang G, Fowler BA. Roles of biomarkers in evaluating interactions among mixtures of lead, cadmium and arsenic. *Toxicol Appl Pharmacol* 2008; **233**(1): 92–99.

[15] Graça A, Ramalho-Santos J, Pereira ML. Effect of lead chloride on spermatogenesis and sperm parameters in mice. *Asian J Androl* 2004; **6**: 237–241.

[16] Oliveira H, Loureiro J, Filipe L, Santos C, Santos JR, Sousa M, et al. Flow cytometry evaluation of lead and cadmium effects on mouse spermatogenesis. *Reprod Toxicol* 2006; **22**(3): 529–535.

[17] Guevara MA, Oliverira H, Pereira ML, Morgado FA. Segmentation and morphometry of histological sections using deformable models: a new tool for evaluating testicular histopathology. Progress in Pattern Recognition, Speech and Image Analysis. A. Sanfeliu and J. Ruiz-Shulcloper, Springer Berlin/Heidelberg. *Lecture Notes on Comput Sci* 2003; **2905**: 282–290.

[18] Bizarro P, Acevedo S, Niño-Cabrera G, Mussali-Galante P, Pasos F, Avila-Costa MR, et al. Ultrastructural modifications in the mitochondrion of mouse Sertoli cells after inhalation of lead, cadmium or lead–cadmium mixture. *Reprod Toxicol* 2003; **17**(5): 561–566.

[19] Iscan M, Ada AO, Çoban T, Kapucuoğlu N, Aydin A, Isimer A. Combined effects of cadmium and nickel on testicular xenobiotic metabolizing enzymes in rats. *Biol Trace Elem Res* 2002; **89**(2): 177–190.

[20] Naomi DE, Piner JA, Sharpe RM. Toxicant-induced leakage of germ cell – specific proteins from seminiferous tubules in the rat: Relationship to blood-testis barrier integrity and prospects for biomonitoring. *Toxicol Sci* 2010; **117**(2): 439–448.