Nature Environment and Pollution Technology
An International Quarterly Scientific Journal

p-ISSN: 0972-6268
e-ISSN: 2395-3454

Vol. 16 | No. 1 | pp. 213-218 | 2017

Original Research Paper | Open Access

Chronic Toxic Effect of Lead on Male Testis Tissue in Adult *Pelophylax nigromaculata*

Min-yi Huang*(**)(***)†, Qiu-lei Men*(**), Xiao-gao Meng*(***), Xiang-xuan Fang*(**) and Man-zhi Tao*
*College of Life Sciences, Anqing Normal University, Anqing 246011, Anhui, China
**Anhui Key Laboratory for Research and Ecological Conservation on Anhui Southwest of Biodiversity, Anqing 246011, Anhui, China
***Anhui Research Center of Aquatic Organism Conservation and Water Ecosystem Restoration, Anqing 246011, Anhui, China
†Corresponding Author: Min-yi Huang

Nat. Env. & Poll. Tech.
Website: www.neptjournal.com

Received: 31-01-2016
Accepted: 18-03-2016

Key Words:
Lead
Testis
Pelophylax nigromaculata
Androgen receptor

ABSTRACT

Lead (Pb) is one of the most common heavy metals in nature which has an adverse effect on aquatic ecosystems and affects animal health. Animal survival depends on its reproductive ability. To analyse the influence of Pb on the male testis tissues in the spot frog (*Pelophylax nigromaculata*), the male adult individuals were exposed to Pb (10, 100 and 1000 µg/L) in water for 30 days. We observed the spermatic histopathologic structure and detected the expression and location of androgen receptors (AR) on the germ cells by immunohistochemical method. Compared to the control, the testis histopathologic structure and the expression of AR at Pb treatment were different. At relatively higher concentration (100 and 1000 µg/L), the changes of spermatic pathological mainly included germ cell necrosis, congestion and fibrosis congestion. The expressions of AR on the gonad cells were affected by Pb. With the increase of Pb concentration, AR expressions were decreased.

INTRODUCTION

Lead (Pb) is one of heavy metals found naturally existing in our living environment. It is obviously toxic, estrogenic and persistent contaminant, which accumulates in food chains and water in nature (Isidori et al. 2010). In recent years, Pb is found in elevated concentrations in the environment, for it can be anthropogenically released into the environment from a wide range of battery production Pb mines, smelters and industrial applications. Excessive Pb may lead to water contamination and poor water quality, and even be absorbed by animals through atmosphere, soil food and sewage (Shotyk & Le 2005).

There are many studies documenting toxicity of Pb exposure in different species due to its bioaccumulation (Flora 2012, Moorman et al. 1998). Pb causes hypertension, renal disease, chronic neurodegenerative disease, bone, reproductive and developmental impairments (Flora 2012), and even destroys reproduction organs (Moorman et al. 1998). Regarding humans, chronic Pb toxicity remains a major public health problem affecting millions of children and adults (Needleman 1990). Moreover, waterborne Pb exposure can exert a variety of physiological effects on adult learning deficit, behavioural alteration, development and embryonic toxicity (Chen et al. 2012, Huang et al. 2014). Except of toxicity, Pb also can mimic the effect of the hormone *in vivo* to reduce reproductive capacity by disrupting the function of hypothalamic-pituitary-gonadal axis (Mcgivern et al. 1991).

To amphibians, they have been identified as good bioindicators of pollution in their environments. They are constantly exposed to contaminants throughout their entire life cycle with water-permeable skins, which can monitor the changes of contaminant concentrations (Demichelis et al. 2001). The continually decrease in diversity and distribution of amphibian populations attract worldwide attention in the past decades (Houlahan et al. 2000, Alford et al. 2001, Kiesecker 2001). The causes of the drop in amphibian populations include biologic and abiologic factors, e.g. climate change, ultraviolet-B (UV-B) radiation, acid rain, emergent diseases and endocrine disrupting chemicals. The adverse influences may lead to amphibian less fit for survival, which may have significant consequence on population dynamics. It is difficult to measure the effects and assure the drop in population numbers to one particular cause (Houlahan et al. 2000, Hayes et al. 2010a). With the industrial and agriculture development, environmental pollution especially of heavy metal is one of the main threats affecting the conservation of amphibian populations (Blaustein et al. 2003). Pb toxicity depends on several fac-

tors including the exposed animal individuals, gender, age, chemical species, dose, as well as the route of exposure. It has been reported previously on amphibians that Pb exposure can increase embryo malformation (Pérez-Coll & Herkovits 1990), cause larval mortality, developmental retardation and aberrant behaviour (Lefcort et al. 1998, Chen et al. 2006), and affect adult neuromuscular transmission and reproduction (Manalis et al. 1984, Wang & Jia 2009).

The spotted frog (*Pelophylax nigromaculata*) belongs to Anura Ranidae. It has a widespread distribution in the farmland of China. Some information has existed on the toxicity of Pb on the oxidative damage and DNA damage of the testis in *P. nigromaculata* (Wang & Jia 2009). However, for this species, the research on the testis tissue is still scarce. It is important to conduct chronic toxicity tests to ensure whether Pb in surface waters is deleterious to their testis tissue. The object of our study is to assess the complex effects of Pb on the testis of *P. nigromaculata* in environmental concentrations.

MATERIALS AND METHODS

$Pb(NO_3)_2$ (Sigma-Aldrich Company, purity: 99.99%) was prepared into 100 mg/L Pb^{2+} mother liquor with demineralized water. *P. nigromaculata* adults (SVL: 65.0 ± 1.5 mm, weight: 28.6 ± 1.6 g) were collected from the suburb of Anqing city in China. After adaptation, *P. nigromaculata* were exposed to three different concentrations of Pb (10, 100 and 1000 μg/L) and control group respectively, and the parallel groups were also set. The animals were handled in accordance with Animal Care and use committee guidelines, and the research was approved by the local government. *P. nigromaculata* were transferred to the tank which held 2 litres dechlorinated tap water (20-30 mm depth). There were 10 frogs in each treatment. The tap water was aerated for 3 days before being used. We recorded the atmosphere and water temperature every day. During the treatment, water was renewed with treated water every 48 hours, and the animals fed earthworms every day. The treated adults were daily checked for activity, and dead individuals were discarded.

The experiments were conducted in a natural photoperiod with light coming through the laboratory windows. We measured the ammonia and dissolved oxygen of tap water, and monitored the water conductance and salinity every week. Quality controls included a certified Pb solution and reagent blanks. The experimental Pb concentration was measured from the aquaria randomly to use an Atomic Absorption Spectrometer (Perkin Elmer PinAAcle 900) to verify the concentration as the primary exposure route during the experiment.

After 30 days treated, *P. nigromaculata* adults were anaesthetized by MS-222, the testis was taken out, put into phosphate buffers (PBS), and then fixed in Bouin's, dehydrated in series ethanol, transparent with dimethyl benzene, embedded in paraffin, 6 μm slice thick. We used the xylene to dewax the sections, descend alcohols to dehydrate. One of the testes was mounted onto the slides coated with polylysine, maintained in 60°C overnight, and the other side of the testes was stained with HE.

We used SABC immunohistochemistry assay to examine the distribution and abundance of AR on the testes in each group. The sections were immersed in 3% H_2O_2 for 5-10 min in order to damage the endogenous inactivate enzymes at room temperature, and then distilled water washed them for 3 times, 0.1 M phosphate-buffered saline (PBS) washed 5 min each for 3 times. The sections were fixed in 0.01 M PBS in the boiled water (92-95 °C) for 2-3 times for 10 min intervals each, and then washed for 1-2 times. The antigen repaired buffer was used to repair the slides for 5-10 min. PBS washed them for 3 times. Goat serum solutions was used to block for 30 min in the room temperature. Rejected the serum, and then added the appropriate dilution of AR to incubate overnight at 4°C. 0.1M PBS washed the sections 5 min each for 3 times, and biotinylytic secondary antibody was added to incubate for 30 min at 37°C. Then, 0.1 M PBS washed the sections again 3 times for 2 min, and the slices were put in horseradish peroxidase conjugate at 37 °C for 30 min and then rinsed with PBS (4 times×5 min), the slices were coloured with diaminobenzidine for 5 times in 30 min. We stopped the colour reaction by immersion in H_2O, and then used haematoxylin to stain. Finally the slices were dehydrated in a graded ethanol, process transparent with xylene and cover with cover slips. We used PBS to replace primary antibody in blank control slices.

The value of immunohistochemical records were evaluated by the combination of the percentage of immunoreactive cells and the estimation of the staining intensity. The positive standards: the yellowish to yellowish-brown granules appear in the cytoplasm and (or) the nuclear which are stained significantly above background regarding as positive reaction. Each treated individual was randomly selected 10 different visual fields to analyse the number of positive cells in the testis under the microscope and photoed. Then the pictures were analysed by Image-Pro Plus analysis-based scoring systems. The light density was used to determine the strength of positive expressions. Through detecting light density value of positive expression, we obtained the relative strength of positive reaction, the bigger the light density value was, and more obvious positive expressions were (Kim et al. 2003, Soslow et al. 2000).

We used the Statistica 6.0 (StatSoft, Tulsa, USA) to analyse data. Values are presented as mean ± standard error (SE), and the statistical significance is a *P*-value less than 0.05 ($P < 0.05$).

RESULTS

Influence of Pb on the histology of testis in *P. nigromacula:* In the control group, the testis was oval which was surrounded by a layer of fibrous connective tissue. The testis had regularly arranged seminiferous tubules, in which germ cells closely arranged with all stages. The germ cells included sperm, sperm cells, secondary spermatogonia, primary spermatocytes, and spermatogonia from inside to outside in sequence. Leydig cells distributed in the wall of seminiferous tubules, and sertoli cells distributed between the seminiferous tubules (Fig. 1a).

In the high concentration of Pb treatment (100 and 1000 µg/L), the spermatic pathological changes were more obvious. We observed disordered spermatogenic cells, necrotic sperm cell, decreased sperm numbers, deformed tubes, blood cell infiltration, obvious cell fibrosis and congestion, edema haemorrhage (Fig. 1b,c). We even mainly observed late-developing spermatogenic cells in the seminiferous tubules with the reduction of the number of primary and secondary spermatocytes (Fig. 1d).

Influence of Pb on the expression of AR in the testis of *P. nigromacula:* The androgen receptors mainly positively expressed in the cytoplasm and nuclei of the spermatogenic cells, leydig cells and sertoli cells in the testis. The product of immunohistochemistry was yellowish-brown granule. With Pb concentration increasing, the AR expression became weaker (Fig. 2) and the numbers of immunohistochemical reaction cells including spermatogenic cells, leydig cells and sertoli cells decreased in the testis (Fig. 3). According to the strength of the optical density, compared with the control group, the positive expression of AR was the weakest in 1000 µg/L Pb treatment.

DISCUSSION

Male reproductive system is one of the most sensitive tissue to Pb in animals (Castellanos et al. 2015, Li et al. 2015). Pb has testicular toxicant including oxidative damage, histopathological alteration, spermatogenesis inhibition and steroidogenesis disruption. The studies on Pb toxicity to testis mainly focus on human, rat and crab (Wang et al. 2013b, Li et al. 2015). To our knowledge, until now, the adverse effect of Pb on amphibians testis mainly includes the oxidative damage and DNA damage in *P. nigromaculata* (Wang & Jia 2009). Our research showed that Pb interrupted directly the normal function of male reproductive system at the testicular level, which damaged the testis tissue of *P. nigromaculata* on sperm cells, tissue fibrosis, and inhibited testis development. In other animals, some studies have documented that Pb can alter animal physiological processes. The main reasons on Pb disruptions may be due to the

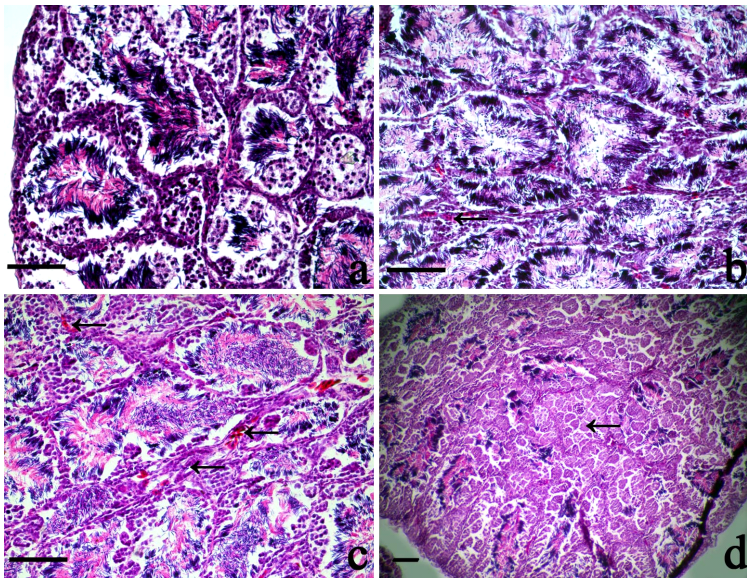

Fig. 1: Effect of Pb on the testis of *P. nigromacula.*
a. Control; b.100 µg/L; c.100 µg/L, → indicating infiltration and fibrosis; d 1000 µg/L, → indicating underdevelopment seminiferous tubule. Scale bar = 100 µm.

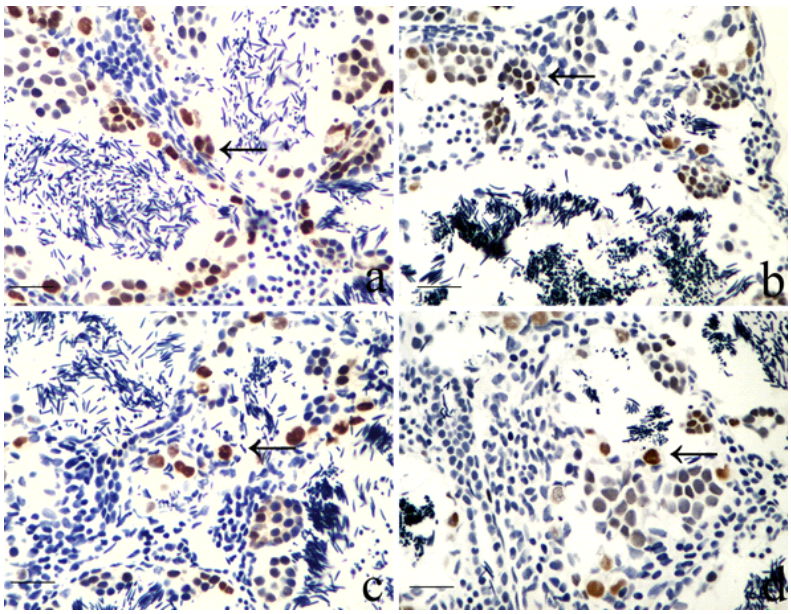

Fig. 2: Positive effect of AR on the testis of *P. nigromacula* exposed to Pb
a. Control; b. 10 μg/L; c. 100 μg/L; d 1000 μg/L. → indicating positive reaction. Scale bar = 25 μm.

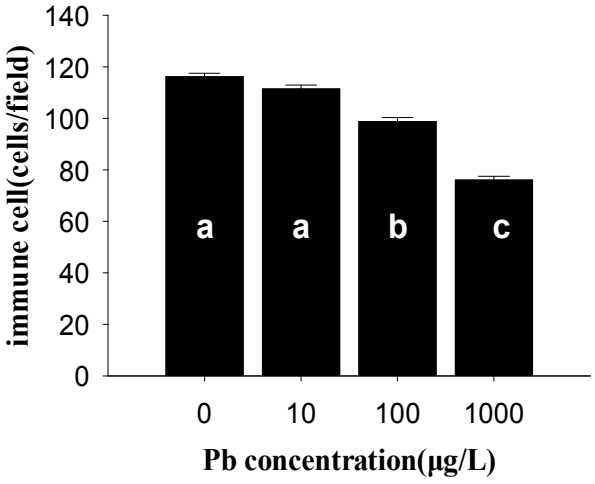

Fig. 3: Influence of Pb on immunohistochemical stain of spermatogenic cells in *P. nigromaculata.*

decrease in the synthesis of testosterone (Wang et al. 2013a), the activity of superoxide dismutase and glutathione peroxidase (Reglero et al. 2009), the expressions of Ddx3y gene expression in testis or spermatozoa (Wang et al. 2013b), or the damage in oxidative stress and DNA in the testis (Wang & Jia 2009). The reduction of androgen inhibits the development of spermatogenic cells, which maybe inhibit its sexual maturity in the breeding season and even the reproductive success (Ketterson & Nolan 1999, Hill et al. 1999).

Animal breeding and mating behaviour are controlled by sex hormone. Environmental disruptors can damage animal endocrine functions in vivo resulting to the change of parameter about reproduction, including gender differentiation, second sexuality, spermatogenesis and sexual maturity (Crisp et al. 1998). Moreover, Environmental disruptors can induce the decrease of the level of the hormone in *vivo* (Hayes et al. 2010b). Androgen is produced by the testis which plays an important role in spermatogenesis and function by combining with AR. Pb exposure can cause male reproductive impairment. It has been reported that the inhibitory effects mainly focus on testicular steroidogenesis including follicle-stimulating hormone, luteinising hormone, prolactin, testosterone and so on. The inhibitory effect of testosterone is obvious, but the mechanism of testicular toxicity of Pb is poorly understood. For example, testosterone supplementation may relieve Pb-induced suppressed reproduction in adult male rats (Reshma & Sreenivasula 2015). Significant decrease in serum testosterone levels and deteriorated sperm quality were observed in Pb treated rats (Anjum et al. 2011). Maternal Pb exposure to male offspring disrupts testicular development and steroidogenesis by inducing the level of serum and testicular testosterone and the number of spermatozoa reduction during lactation persistently (Wang et al. 2013a). The levels of testosterone hormone showed significant decreases in male rats in Pb treated group, which might be induced via the alteration of leydig cells following Pb exposure (Mokhtari

& Zanboori 2011). Moreover, the negative effect of Pb on testosterone production is correlated with the lower expression of the enzymes involved in steroid hormone biosynthesis, as shown by immunohistochemistry (Thoreux-Manlay et al. 1995). However, there are opposite results which Pb exposure is positively associated with testosterone in males (Kresovich et al. 2015). The low or moderate Pb concentrations have no obvious alteration in hypothalamic-pituitary, but higher Pb exposure can increase the level of testosterone, suggesting that Pb may target testicular function (Allouche et al. 2009, Riaz et al. 2011). The results are different for different species maybe because of inter specific sensitivity.

Androgen receptor is an attractive target for the treatment of environment disruptors, and Pb has a similar binding affinity to AR (Liu et al. 2015). Our results showed that the expression of AR weakened with the concentration increasing, which indicated that Pb had influenced the expression of AR and even the level of androgen.

In short, our study suggests that the exposure to Pb affects male reproduction in *P. nigromaculata* by decreasing steroidogenesis and spermatogenesis. A significant decrease in spermatogenesis and androgen receptors was observed in Pb-induced testicular in *P. nigromaculata*. These adverse effects may be attributed to the disruption of Pb on AR signalling, which plays an essential role in mediating spermatogenesis, and then a lethal or sub lethal dose of Pb may decrease sperm activity and disrupt the reproductive organs in *P. nigromaculata* which causes the decrease in amphibian population. Further research is needed to focus on the mechanism of the disruption pathway of Pb on the testes.

ACKNOWLEDGMENTS

This work complies with current laws on animal welfare and research in China and was supported by the National Natural Science Foundation of China (31300342), Anhui Provincial Key Project of Higher Education Institutions for Excellent Visiting Scholar (gxfxZD2016173) and the Startup Project of Doctorial Scientific Research of Anqing Normal University (K150001000013).

REFERENCES

Alford, R.A., Dixon, P.M. and Pechmann, J.H. 2001. Ecology. Global amphibian population declines. Nature, 412: 499-500.

Allouche, L., Hamadouche, M. and Touabti, A. 2009. Chronic effects of low lead levels on sperm quality, gonadotropins and testosterone in albino rats. Exp. Toxicol. Pathol., 61: 503-510.

Anjum, M.R., Sainath, S.B., Suneetha, Y. and Reddy, P.S. 2011. Lead acetate induced reproductive and paternal mediated developmental toxicity in rats. Ecotoxicol. Environ. Saf., 74: 793-799.

Blaustein, A.R., Romansic, J.M., Kiesecker, J.M. and Hatch, A.C. 2003. Ultraviolet radiation, toxic chemicals and amphibian population declines. Divers. Distrib., 9: 123-140.

Castellanos, P., del Olmo, E., Fernández-Santos, M.R., Rodríguez-Estival, J., Garde, J.J. and Mateo, R. 2015. Increased chromatin fragmentation and reduced acrosome integrity in spermatozoa of red deer from lead polluted sites. Sci. Total. Environ., 505: 32-38.

Chen, J., Chen, Y., Liu, W., Bai, C., Liu, X., Liu, K., Li, R., Zhu, J. H. and Huang, C. 2012. Developmental lead acetate exposure induces embryonic toxicity and memory deficit in adult zebrafish. Neurotoxicol. Teratol., 34: 581-586.

Chen, T.H., Gross, J.A. and Karasov, W.H. 2006. Sublethal effects of lead on northern leopard frog (*Rana pipiens*) tadpoles. Environ. Toxicol. Chem., 25: 1383-1389.

Crisp, T.M., Clegg, E.D., Cooper, R.L., Wood, W.P., Anderson, D. G., Baetcke, K.P., Hoffmann, J.L., Morrow, M.S., Rodier, D.J., Schaeffer, J.E., Touart, L.W., Zeeman, M.G. and Patel, Y.M. 1998. Environmental endocrine disruption: an effects assessment and analysis. Environ. Health Persp., 106: 11-56.

Demichelis, S.O., De LaTorre, F.R., Ferrari, L., Garcia, M.E. and Salibian, A. 2001. Tadpoles assay: its application to a water toxicity assessment of a polluted urban river. Environ. Monit. Assess., 68, 63-73.

Flora, G., Gupta, D. and Tiwari, A. 2012. Toxicity of lead: A review with recent updates. Interdiscip. Toxicol., 5: 47-58.

Hayes, T.B., Falso, P., Gallipeau, S. and Stice, M. 2010a. The cause of global amphibian declines: a developmental endocrinologist's perspective. J. Exp. Biol., 213: 921-933.

Hayes, T.B., Khoury, V., Narayan, A., Nazir, M., Park, A., Brown, T., Adame, L., Chan, E., Buchholz, D., Stueve, T. and Gallipeau, S. 2010b. Atrazine induces complete feminization and chemical castration in male African clawed frogs (*Xenopus laevis*). Proc. Natl. Acad. Sci. USA, 107: 4612-4617.

Hill, J.A., Enstrom, D.A., Ketterson, E.D., Nolan, V. and Ziegenfus, C. 1999. Mate choice based on static versus dynamic secondary sexual traits in the dark-eyed junco. Behav. Ecol., 10: 91-96.

Houlahan, J.E., Findlay, C.S., Schmidt, B.R., Meyer, A.H. and Kuzmin, S.L. 2000. Quantitative evidence for global amphibian population declines. Nature, 404: 752-755.

Huang, M.Y., Duan, R.Y. and Ji, X. 2014. Chronic effects of environmentally-relevant concentrations of lead in *Pelophylax nigromaculata* tadpoles: Threshold dose and adverse effects. Ecotoxicol. Environ. Saf., 104: 310-316.

Isidori, M., Cangiano, M., Palermo, F. A. and Parrella, A. 2010. E-screen and vitellogenin assay for the detection of the estrogenic activity of alkylphenols and trace elements. Comp. Biochem. Physiol. C Toxicol. Pharmacol., 152: 51-56.

Ketterson, E.D. and Nolan, V. Jr. 1999. Adaptation, exaptation, and constraint: a hormonal perspective. Am. Nat., 154: 24-25.

Kiesecker, J.M., Blaustein, A.R. and Belden, L.K. 2001. Complex causes of amphibian population declines. Nature, 410: 681-684.

Kim, B.O., Liu, Y., Ruan, Y., Xu, Z.C., Schantz, L. and He, J.J. 2003. Neuropathologies in transgenic mice expressing human immunodeficiency virus type 1 Tat protein under the regulation of the astrocyte-specific glial fibrillary acidic protein promoter and doxycycline. Am. J. Pathol., 162: 1693-1707.

Kresovich, J.K., Argos, M. and Turyk, M.E. 2015. Associations of lead and cadmium with sex hormones in adult males. Environ. Res., 142: 25-33.

Lefcort, H., Meguire, R.A., Wilson, L.H. and Ettinger, W.F. 1998. Heavy metals alter the survival, growth, metamorphosis, and antipredatory behavior of Columbia spotted frog (*Rana luteiventris*) tadpoles. Arch. Environ. Contam. Toxicol., 35: 447-456.

Li, N., Hou, Y.H., Ma, D.D., Jing, W.X., Dahms, H.U. and Wang, L.

2015. Lead accumulation, oxidative damage and histopathological alteration in testes and accessory glands of freshwater crab, Sinopotamon henanense, induced by acute lead exposure. Ecotoxicol. Environ. Saf., 117: 20-27.

Liu, J., Liu, B., Guo, G., Jing, Y. and Zhao, G. 2015. Discovery of novel androgen receptor antagonists: a hybrid approach of pharmacophore-based and docking-based virtual screening. Anticancer drugs, 26: 747-753.

Manalis, R.S., Cooper, G.P. and Pomeroy, S.L. 1984. Effects of lead on neuromuscular transmission in the frog *Rana pipiens*. Brain Res., 294: 95-110.

Mcgivern, R.F., Sokol, R.Z. and Berman, N.G. 1991. Prenatal lead exposure in the rat during the third week of gestation: Long-term behavioral, physiological, and anatomical effects associated with reproduction. Toxicol. Appl. Pharmacol., 110: 206-215.

Mokhtari, M. and Zanboori, M. 2011. The effects of lead acetate on sexual behavior and the level of testosterone in adult male rats. Int. J. Fertil. Steril., 5: 13-20.

Moorman, W.J., Skaggs, S.R., Clark, J.C., Turner, T.W., Sharpnack, D.D., Murrell, J.A., Simon, S.D., Chapin, R.E. and Schrader, S. M. 1998. Male reproductive effects of lead, including species extrapolarion for the rabbit model. Reprod. Toxicol., 12: 333-346.

Needleman, H.L. 1990. The future challenge of lead toxicity. Environ. Health. Perspect., 89: 85-89.

Pérez-Coll, C. S. and Herkovits, J. 1990. Stage dependent susceptibility to lead in *Bufo arenarum* embryos. Environ. Pollut., 63: 239-245.

Reglero, M.M., Taggart, M.A., Castellanos, P. and Mateo, R. 2009. Reduced sperm quality in relation to oxidative stress in red deer from a lead mining area. Environ. Pollut., 157: 2209-2215.

Reshma, A.M. and Sreenivasula, R.P. 2015. Recovery of lead-induced suppressed reproduction in male rats by testosterone. Andrologia, 47: 560-567.

Riaz, F., Khan, U.A., Ayub, M. and Shaukat, S. 2011. Protective role of ginger on lead induced derangement in plasma testosterone and luteinizing hormone levels of male Sprague Dawley rats. J. Ayub. Med. Coll. Abbottabad., 23: 24-27.

Shotyk, W., Le and Roux. G. 2005. Biogeochemistry and cycling of lead. Met. Ions. Biol. Syst., 43: 239-275.

Soslow, R.A., Dannenberg, A.J., Rush, D., Woerner, B.M., Khan, K. N., Masferrer, J. and Koki, A.T. 2000. COX-2 is expressed in human pulmonary, colonic and mammary tumors. Cancer, 89: 2637-2645.

Thoreux-Manlay, A., Le Goascogne, C., Segretain, D., Jégou, B. and Pinon-Lataillade, G. 1995. Lead affects steroidogenesis in rat Leydig cells in vivo and in vitro. Toxicology, 103: 53-62.

Wang, H., Ji, Y.L., Wang, Q., Zhao, X.F., Ning, H., Liu, P., Zhang, C., Yu, T., Zhang, Y., Meng, X.H. and Xu, D.X. 2013a. Maternal lead exposure during lactation persistently impairs testicular development and steroidogenesis in male offspring. J. Appl. Toxicol., 33: 1384-1394.

Wang, M.Z. and Jia, X.Y. 2009. Low levels of lead exposure induce oxidative damage and DNA damage in the testes of the frog *Rana nigromaculata*. Ecotoxicology, 18: 94-99.

Wang, X., Wang, M., Dong, W., Li, Y., Zheng, X., Piao, F. and Li, S. 2013b. Subchronic exposure to lead acetate inhibits spermatogenesis and downregulates the expression of Ddx3y in testis of mice. Reprod. Toxicol., 42: 242-250.