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Multi-biological defects caused by lead exposure exhibit transferable properties from exposed parents to their progeny in *Caenorhabditis elegans*

WANG Da-yong[1,2,*], YANG Peng[1,2]

1. Department of Genetics and Developmental Biology, Southeast University, Nanjing 210009, China. E-mail: dayongw@seu.edu.cn
2. Key Laboratory of Developmental Genes and Human Disease, Ministry of Education of China, Nanjing 210009, China

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Abstract

Whether the multi-biological toxicity from lead exposure could be transferred to progeny has not been clarified. In the present study, we explored the *Caenorhabditis elegans* to analyze the multiple toxicities from lead exposure and their possibly transferable properties. The lead exposure could cause series of severe multi-biological defects with a concentration-dependent manner by affecting the endpoints of life span, development, reproduction and locomotion behaviors in nematodes. Moreover, most of these toxicities could be transferred to progeny from lead exposed animals and some of the defects in progeny appeared even more severe than in their parents, such as the body sizes and mean life spans. We summarized the defects caused by lead exposure into three groups according to their transferable properties or rescue patterns. That is, the defects caused by lead exposure could be largely, or partially, or became even more severe in progeny animals. Therefore, our results suggest that lead exposure can cause severely multi-biological defects, and most of these multiple toxicities can be considered as transferable for exposed animals in *C. elegans*.

Key words: lead exposure; toxicity; transferable; phenotype; behavior; *Caenorhabditis elegans*

Introduction

Lead (Pb) is a dangerous heavy metal widely used in industries and our daily life. It can be absorbed through the lungs by respiration and the intestines. Although we are chronically exposed to Pb from environment, it would still be dangerous to our health (Garza *et al*., 2006). Such as, Pb continuation is already a leading public health problem in Mexico (Romieu *et al*., 1995). The most common sources of Pb exposure are lead paint in older, dilapidated housing, the Pb content of the glazed ceramics used to prepare food, and contaminated dust and soil produced by accumulated residue of leaded gasoline (Romieu *et al*., 1995; Gasana *et al*., 2006). A significant success of the public health system has been achieved in Bombay, India by removal of Pb from gasoline (Nichani *et al*., 2006).

Pb exposure is a severe risk for humans and animals. It has been found that Pb toxicity is involved in the carcinogenesis through damage to DNA and inhibiting DNA repair machine (Silbergeld, 2003; Barciszewskaa *et al*., 2005). Hemopoietic system and immuno-system would be affected while exposed to Pb (Dietert *et al*., 2004). Reproductive defect is another importantly toxic effect from Pb exposure. Pb exposure can reduce sperm count and motility, as well as the formation of abnormal sperms (Gidlow, 2004; Bonde and Apostoli, 2005; Pace *et al*., 2005). Especially at high levels of Pb exposure, spontaneous abortion is increased and the offspring birth weight and neurobehavioral development are reduced (Bellinger, 2005; Papanikolaou *et al*., 2005). Moreover, blood lead levels as low as 10 μg/dl in children are closely associated with impaired cognitive function, behavior difficulties, and reduced intelligence (Gasana *et al*., 2006). Therefore, the Pb exposure has multiple biological toxicities. However, whether these multiple biological toxicities from Pb exposure could be transferred to progeny has not been clarified yet.

Nematodes, small soil-dwelling worms, play an important ecological role in the cycling of key nutrients and organic matter degradation (Freeman *et al*., 1998). *Caenorhabditis elegans*, a species of nematode, is often used within the laboratory setting because it has the properties of short life cycle, small size, simple cell lineage, and ease of cultivation (Riddel *et al*., 1997). Because worms can detect trace metals or compounds and can exhibit different phenotypes, heavy metal contamination in artificial soil and river system can be successfully assessed using wild-type and stress-inducible transgenic nematodes as biomarkers (Mutwakil *et al*., 1997; Peredney and Williams, 2000; David *et al*., 2003). Heavy metal

Project supported by the Southeast University Foundation for Excellent Young Scholars (No. 4023001013). *Corresponding author.
E-mail: dayongw@seu.du.cn.

exposure can affect the endpoints of reproductive speed, life cycle, development, and other properties in *C. elegans* (Swain *et al*., 2004). In addition, a standardized method for conducting laboratory soil toxicity tests using *C. elegans* was also published in the America Society for Testing and Materials (ASTM) Guide E2172-01 (2002). However, the transferable properties of multi-biological toxicities have never been investigated for heavy metals till now. In the current work, we selected the *C. elegans* organism to analyze the multiple toxicities from Pb exposure. Moreover, we investigated whether these multi-biological toxicities could be transferred from parental animals to their progeny. Our results suggest that the multi-biological toxicities can be transferred from Pb exposed animals to their progeny and some specific defects in progeny appeared even more severe than in parental generations.

1 Materials and methods

1.1 Chemicals and strains

Three concentrations of $Pb(NO_3)_2$ solution were used in the current work and they are 2.5, 75 and 200 μmol/L, respectively. All the chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA).

All nematodes used were wild-type N^2, originally obtained from the *Caenorhabditis* Genetics Center (CGC). They were cultivated on nematode growth medium (NGM) agar plates with *Escherichia coli* strain OP50 (Brenner, 1974). Gravid animals were washed off the plates into centrifuge tubes and were lysed with a bleaching mixture (0.45 mol/L NaOH, 2% HClO). Age synchronous populations of N^2 (L4-larva stage) were obtained by the collection as described by Donkin and Williams (1995). The L4-larva stage animals were washed with double-distilled water twice, followed by washing with K medium once (50 mmol/L NaCl, 30 mmol/L KCl, 10 mmol/L NaOAc, pH 5.5). Exposures were performed in 12-well sterile tissue culture plates. Approximately 100 animals were transferred in 5 μl to each exposure solution using micropipetter. All exposures were 3-d long and were carried out in 20°C incubator in the absence of food. To evaluate the Pb toxicity in progeny, eggs were obtained from animals subjecting to the Pb exposure with the bleaching mixture and then transferred to a normal NGM plates without addition of Pb solution. Endpoints of life span, body size, brood size, generation time, body bends, and head thrashes were used for toxicity testing in *C. elegans*. Animals were all maintained at 20°C.

1.2 Body size

The exposed and progeny animals were picked out directly for body size measure. Body size was determined by measuring the flat surface area of nematodes using the Image-Pro® Express software. For each test, at least 15 animals were picked for assay.

1.3 Brood size, generation time and life span

The methods were performed as described by Swain *et al*. (2004). Brood size was assayed by placing single tested animal onto individual well of tissue culture plates. The parental (P0) animals were transferred to a new well every 1.5 d. Progeny were counted the day following transfer. The generation time refers to the time from P0 egg to the first generation (F1) egg. For both the brood size and the generation time test, at least 10 replicates were performed for statistical purposes. For life span assay, the exposed and progeny animals were picked onto the assay plates and the time was recorded as t=0. About thirty animals were placed onto a single plate and adult animals were transferred every 2 d to fresh plates during the brood period. The numbers of survivors were scored every day. Animals that failed to respond to repeated touch stimulation were considered as dead. Life span graphs are representative of at least three trials.

1.4 Thrash assay

The thrashes were assayed as described in the literature (Tsalik and Hobert, 2003). To assay the head thrashes, animals were washed with the double-distilled water, followed by washing with K medium. Every animal was transferred into a microtiter well containing 60 μl of K medium on the top of agar. After a 1-min recovery period, the head thrashes were counted for 1 min. A thrash was defined as a change in the direction of bending at the mid body. Fifteen animals were examined per treatment.

1.5 Body bend frequency

The method was performed as described by Tsalik and Hobert (2003). To assay the body bends frequency, animals were picked onto a second plate and scored for the number of body bends in an interval of 1 min. A body bend was counted as a change in the direction of the part of the animals corresponding to the posterior bulb of the pharynx along the y axis, assuming that the animal was traveling along the x axis. Fifteen animals were examined per treatment.

1.6 Statistical analysis

All data in this article were expressed as means ± SD and analyzed by SPSS 13.0 software. Graphs were generated using Microsoft Excel (Microsoft Corp., Redmond, WA). Paired-sample t-test were performed between control and animals exposed to Pb or their progeny. The probability levels of 0.05 and 0.01 were considered statistically significant.

2 Results and discussion

2.1 Life span defects in Pb exposed animals and their progeny in *C. elegans*

Life span is often used as the main parameter to evaluate the toxicity of a specific metal or compound in mammals, fish and even plants. Here, we first investigated the toxic effects of Pb exposure on life span. In *C. elegans*, high concentrations (75 and 200 μmol/L) of Pb exposure caused more severe maximum life span defects compared to low concentration (2.5 μmol/L) of Pb exposure and controls (Fig.1a). When animals were exposed to 2.5, 75 and 200

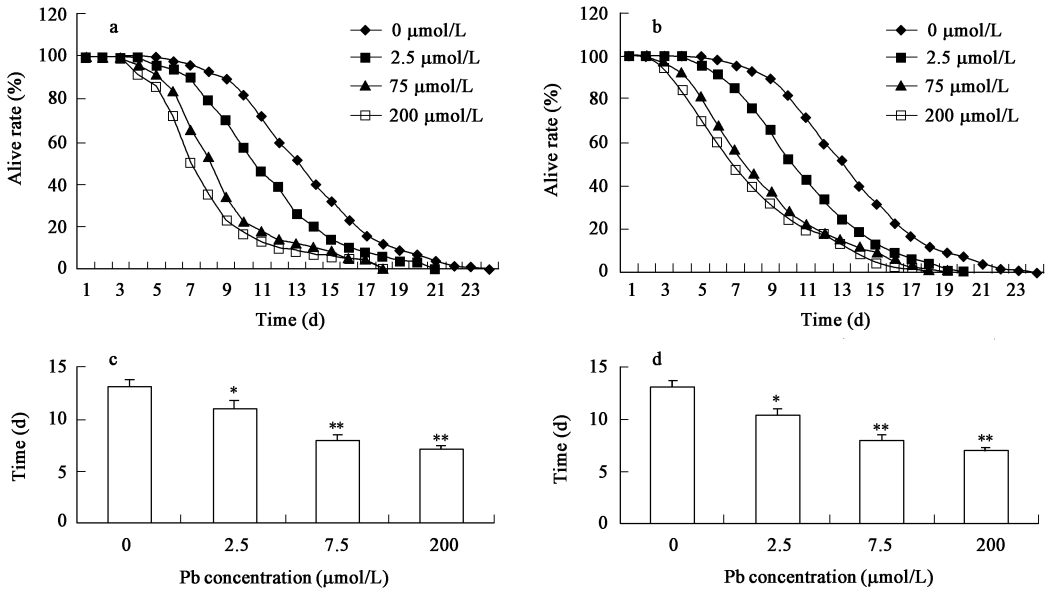

Fig. 1 Life spans of animals exposed to Pb. (a) maximum life spans of animals exposed to Pb; (b) maximum life spans of progeny from animals exposed Pb; (c) comparison of the mean life spans for animals exposed to Pb; (d) comparison of the mean life spans for progeny from animals exposed to Pb. Bars represent means ± SD; * $P < 0.05$; ** $P < 0.01$.

μmol/L concentrations of Pb, their life spans were reduced in the range from 3–6 d, compared to control (Fig.1a). The defects of days corresponding to 50% lethal also magnified a concentration-dependent manner (Fig.1c). The mean life spans of animals exposed to 200 μmol/L Pb was less than 1/2 of those in controls ($P < 0.01$) (Fig.1c).

To study whether the Pb toxicity on life span could be transferred to progeny, we analyzed the changes of life spans in progeny of animals exposed to Pb. We found that the progeny of animals exposed to different concentrations of Pb did not exhibit obvious rescue phenotypes for the life span defects (Fig.1b). Moreover, we found that the progeny animals showed even more severe defects of the mean life spans compared to control and their parents (Fig.1d). Therefore, the Pb toxicity on life span is transferable in Pb exposed animals.

2.2 Developmental defects in Pb exposed animals and their progeny in *C. elegans*

Next, we examined the effects of Pb exposure on nematodes development by observing the body size and morphology (Fig.2). The body sizes of nematodes were markedly reduced after expose to Pb for 3 d compared to controls ($P < 0.01$), with an obvious concentration-dependent manner (Figs.2a and 2b). Furthermore, we found that the body size defects in progeny of animals exposed to different concentrations of Pb appeared even more severe phenotypes ($P < 0.01$) compared to control and their parents (Figs.2a and 2c). Thus, the toxicity on development might be able to be cumulated in progeny of

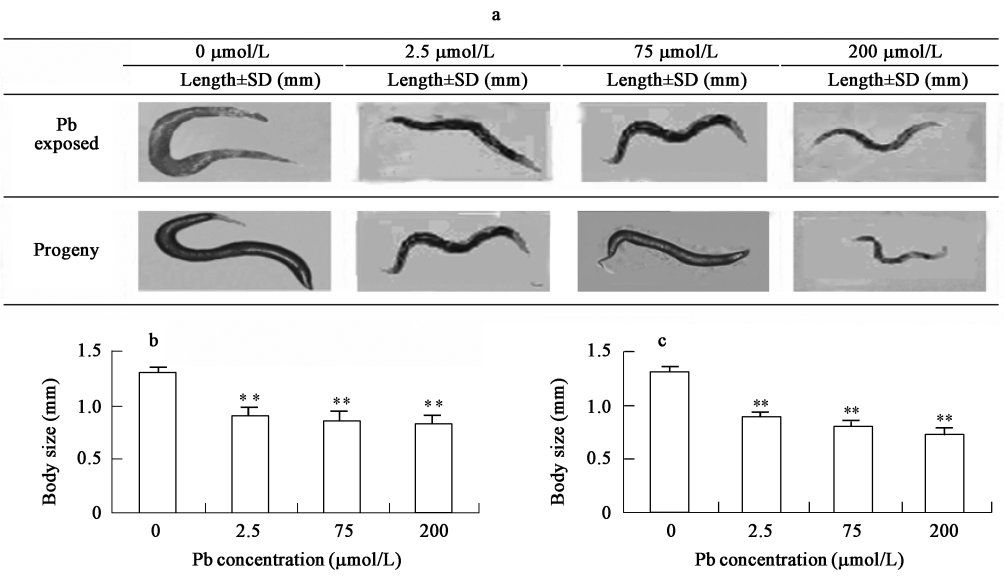

Fig. 2 Body sizes of animals and their progeny exposed to Pb. (a) morphological comparison of animals and their progeny exposed to Pb, all images are representative of 3-d post hatch nematodes; (b) comparison of body sizes of animals exposed to Pb; (c) comparison of body sizes of progeny from animals exposed to Pb. Bars represent means ± SD; ** $P < 0.01$.

Pb exposed nematodes.

In addition, we did not find the toxic effects from Pb exposure on morphology, such as the appearance of vulva abnormality and/or male worm formation as ever found in barium and mercury exposed worms. Therefore, Pb exposure can not be considered as teratogenic in *C. elegans*.

2.3 Reproductive defects in Pb exposed animals and their progeny in *C. elegans*

We investigated the toxic effects of Pb exposure on the reproduction. Usually, longer generation time means low reproductive speed. After Pb exposure, the generation time was prolonged in the range from 46% to 59% and the toxicity magnified a concentration-dependent manner (Fig.3a). Furthermore, we found that the generation time in progeny of animals exposed to Pb could only be slightly rescued. The generation time of progeny was prolonged in the range from 32% to 45% ($P < 0.01$) (Fig.3b).

The reproductive speed is evaluated by generation time; however, the brood size reflects the reproductive capacity. Likewise, Pb exposure resulted in a remarkable toxicity on the brood sizes. The brood sizes of animals exposed to 2.5, 75 and 200 μmol/L Pb were reduced by 52% ($P < 0.01$), 58% ($P < 0.01$) and 65% ($P < 0.01$), respectively, compared to control (Fig.4a). The toxic effects of Pb exposure on the brood sizes are largely consistent with the results from the evaluation in mice and other organisms (Gidlow, 2004; Bonde and Apostoli, 2005; Pace *et al*., 2005). Moreover, the brood size defects were found to be only partially rescued in progeny. The brood sizes in progeny of animals exposed to 2.5, 75 and 200 μmol/L Pb were reduced by 9%, 19% and 31% ($P < 0.01$), respectively, compared to control (Fig.4b). Therefore, Pb exposure can result in severe reproductive defects and the reproductive defects can be considered to have transferable properties in Pb exposed nematodes.

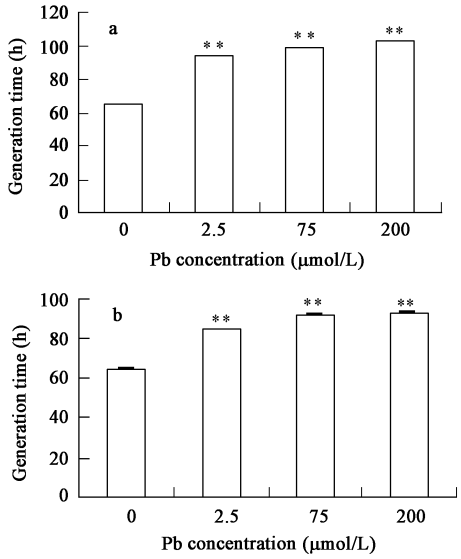

Fig. 3 Generation time of animals (a) and their progeny (b) exposed to Pb. Bars represent means ± SD; ** $P < 0.01$.

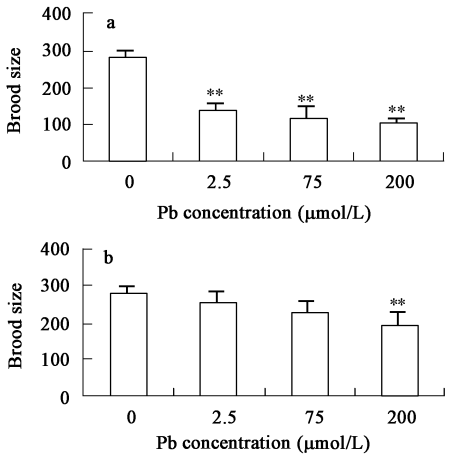

Fig. 4 Brood sizes of animals (a) and their progeny (b) exposed to Pb. Bars represent means ± SD; ** $P < 0.01$.

2.4 Locomotion behavioral defects in Pb exposed animals and their progeny in *C. elegans*

Pb exposure not only influences the reproduction, it might also affect the differentiation and functions of nervous system. In animals, inorganic Pb can affect the neuronal activity and central nerve system functions (Papanikolaou *et al*., 2005). Especially, Pb exhibited a high degree of localization in the nematode, exclusively in the anterior pharynx region (Jackson *et al*., 2005). This region contains a high density of neurons (Jackson *et al*., 2005). To test the influences of Pb exposure on the locomotion behaviors, body bend and head thrash were selected for assay in *C. elegans*. As shown in Figs.5 and 6, both the head thrashes ($P < 0.01$) and the body bends ($P < 0.05$) were severely impaired after exposed to even very low concentration of Pb (2.5 μmol/L). More severe phenotypes were observed while exposed to high concentrations of Pb (75 and 200 μmol/L) for the head thrashes ($P < 0.01$) (Fig.5a) and the body bend frequency ($P < 0.01$) (Fig.6a). Moreover, investigation on their progeny indicates that the defects of body bends could be recovered in progeny of animals exposed to 2.5 and 75 μmol/L Pb (Fig.6b). However, the defects of body bends could be only partially recovered in progeny of animals exposed to 200 μmol/L Pb ($P < 0.05$) (Fig.6b). Different from the rescue patterns of body bends defects, the defects of head thrashes magnified only very limited rescue phenotypes in progeny compared to their Pb exposed parents (2.5 μmol/L, $P < 0.05$; 75 and 200 μmol/L, $P < 0.01$) (Fig.5b). Neurons and muscle cells need to form correct cellular connections and assemble a specific repertoire of signaling proteins into synaptic structures, and disruption of any of these steps would cause defects of locomotion behavior (Loria *et al*., 2004). Therefore, the Pb toxicity might disrupt synaptic function and appropriate contacts between neurons and muscle cells. The data from the investigations in other organisms support our conclusion here. The N-methyl-D-aspartate (NMDA) receptors may serve as targets of Pb toxicity, since Pb exposure can cause a dose-dependent inhibition of NMDA-activated channel activity (Marchetti and Gavazzo, 2005). Pb exposure can also result in presynaptic disruption of

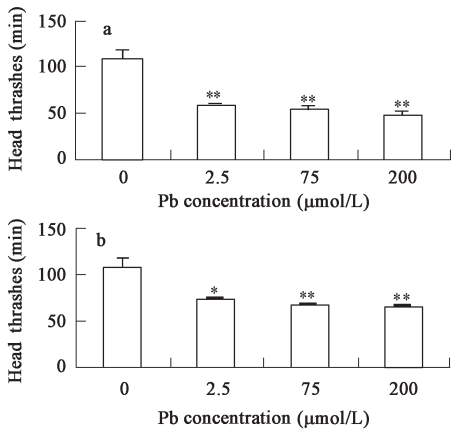

Fig. 5 Head thrashes of animals (a) and their progeny (b) exposed to Pb. Bars represent means ± SD; * $P < 0.05$; ** $P < 0.01$.

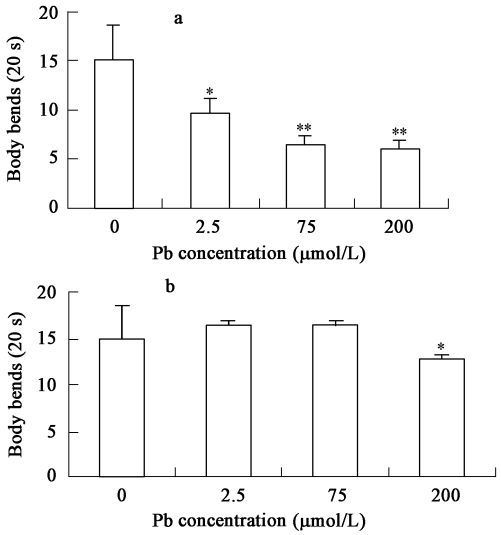

Fig. 6 Body bends of animals (a) and their progeny (b) exposed to Pb. Bars represent means ± SD; * $P < 0.05$; ** $P < 0.01$.

neurotransmission by causing aberrant augmentation of spontaneous and suppression of evoked release (Suszkiw, 2004). In addition, defects of memory, cognitive functions and sensory motor function can be found in Pb exposed people (Lanphear *et al*., 2005; Toscano and Guilarte, 2005).

In the current work, we analyzed the phenotypic and behavioral defects induced by Pb exposure *in vivo*. First, Pb exposure could cause reduced life spans (Fig.1). Second, Pb exposure made the body size become severely smaller than controls (Fig.2). Third, the brood sizes were significantly reduced after exposing to Pb even at very low concentration of 2.5 μmol/L (Fig.4). Fourth, the generation times were markedly prolonged in Pb exposed animals (Fig.3). Fifth, the locomotion behaviors were dramatically decreased in animals undergoing the Pb toxicity (Figs.5 and 6). Our results indicate that the Pb toxicity can cause multi-biological defects with a concentration-dependent manner in *C. elegans*, which are largely consistent with the conclusions drawn from other organisms. The lethal concentration (LC_{50}) value and the corresponding 95% confidence levels of Pb were ever assessed in *C. elegans* (Chu and Chow, 2002). Our work here provides new endpoints to evaluate the toxicity from Pb exposure using wild-type *C. elegans* as biomarker. In addition, it seems likely that the skin of the nematodes should protect these animals well for the exposure of heavy metals. Thus, oral exposure might be the main pathway to induce the multi-biological toxicities, since the Pb was given in the liquid where the nematodes are forced to swim and drink the toxin continuously. Nevertheless, we can not exclude the possibility that Pb exposure can induce multi-biological defects though through the lungs by respiration or the skins for human beings.

According to the analysis above, the defects caused by Pb exposure can be classified into three groups. First, the defects caused by Pb exposure can be largely recovered in progeny. Only the body bends defects of animals exposed to 2.5 and 75 μmol/L Pb exhibited this type of rescue phenotypes. Second, the defects caused by Pb exposure can only be partially rescued in progeny, such as the generation times, the brood sizes and the head thrashes. Most of the multi-biological effects caused by Pb exposure exhibit this type of rescue patterns. Third, the defects caused by Pb toxicity became more severe in progeny than those in Pb exposed parents, such as the body sizes and mean life spans. Thus, most of the multi-biological defects caused by Pb exposure can be considered to have transferable properties for exposed nematodes. However, the transferable properties of Pb exposure revealed in the current work still can not be understood as a kind of heredity in genetics, because some of the defects can be partially rescued and some of the defects can show even more severe phenotypes in progeny animals. Therefore, we suppose that gain of the transferable properties for animals exposed to Pb might be largely due to the deposition of Pb toxicity in their eggs or embryos in the next generation. Hence, considering that Pb exposure can result in the severe defects of brood sizes and generation times and more severe developmental defects in progeny, we should pay more attention to its toxic effects on the progeny of animals and humans while facing up the threaten of lead exposure from environments.

3 Conclusions

Overall, the results showed that the Pb exposure can result in multi-toxicities and these multi-biological defects can be transferred to progeny from Pb exposed animals. However, the mechanisms causing these multi-toxicities and the transferable properties still need to be further elucidated.

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