Effects of prenatal and neonatal exposure to lead on white blood cells in Swiss mice

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Abstract
Lead exposure is one of the major environmental issues for children and women of child bearing age. It crosses the placental barrier and its greater intestinal absorption in fetus results in developmental defects. Lead, as one of the environmental pollutants, can threat the lives of animals and human beings in many ways; especially during developing stages. The present study was carried out to study the alterations in different types of white blood cells (WBC) due to chronic lead acetate toxicity in neonates, which passes from adult pregnant female during gestation and lactation. Lead acetate was administered orally at 8, 16, 32 mg /kg/BW to pregnant Swiss mice from 10th day of gestation to 21th day of lactation. Hematopathological and numerical alterations in the WBCs were examined in the neonates after birth at postnatal days 1, 7, 14 and 21. Blood smears examined illustrate that lead induces disturbances in the development of different types of WBCs during postnatal development and lead to an abrupt neutrophilic degeneration, immature cells, abnormal neutrophils, reactive and plasmacytoid lymphocytes. The results of the present study emphasize that prenatal lead exposure is extremely dangerous to developing fetus.

Keywords: Lead acetate, Swiss albino mice, prenatal, neonatal, white blood cells.

Swiss farelerde prenatal ve yenidoğan kurşun maruziyetinin beyaz kan hücreleri üzerine etkileri

Özet

Anahtar kelimeler: Kurşun asetat, Swiss albino fare, prenatal, yenidoğan, lüksosit.
Introduction

Lead has been recognized as a biological toxicant and different doses have been used to study lead-induced alterations. Prenatal exposure to lead produces toxic effects in the human fetus, including increased risk of preterm delivery, low birth weight, and impaired mental development; because during the period of early organogenesis the onset of greatest susceptibility to teratogenesis occurs (Falcon et al., 2003). This highly sensitive or critical period is the time during which a small dose of a teratogen produces high percentage of fetuses that exhibit malformations of the organ in question (Wilson, 1973; Desesso et al., 1996).

Pregnancy and breastfeeding can cause a state of physiological stress that increases bone turnover of lead. Lead stored in the bone moves into the blood, increasing the mother’s blood lead level and passing to the fetus, affecting fetal development. Lead is tightly bound to red blood cells, enhancing transfer from maternal circulation through the placenta to the fetus. Fetus is more sensitive to lead because the fetal blood-brain barrier is more permeable. The toxic effects of lead on blood indices are well known.

Lead potentially induces oxidative stress and evidence is accumulating to support the role of oxidative stress in the pathophysiology of lead toxicity. Lead is capable of inducing oxidative damage to brain, heart, kidneys, and reproductive organs. The mechanisms for lead-induced oxidative stress include the effects of lead on membranes, DNA, and antioxidant defense systems of cells (Ahamed and Siddiqui, 2007). Lead interferes with a variety of body processes and is toxic to the body systems including cardiovascular, reproductive, hematopoietic, gastrointestinal and nervous systems (Kosnett, 2006), renal functions (Patocka and Cerny, 2003) and release of glutamate (Xu et al., 2006). It affects the hematological system even at concentrations below 10μg/dl (ATSDR, 2005).

Many reports are available regarding lead toxicity and its deleterious effects in various species of animals and there has been lot of work carried out on pharmacokinetics and genotoxicity but very few researchers tried to correlate haematopathological alterations of lead acetate in different white blood cells at different dose levels in laboratory animals, especially in mice.

Therefore the current study was performed to clarify the lead induced hematological changes, especially those related to white blood cells, during gestational and lactational exposure to lead in Swiss mice.

Materials and methods

Sexually mature random bred Swiss mice with the age of 5-6 weeks, weighing 25-30 gm was used for this study. During the entire experimental period, the animals were fed on a standard diet and water ad libitum. Mice were kept in the ratio of 1:4 males and females, respectively, and females showing vaginal plugs were separated in the control and lead treated group. Lead acetate solution was prepared by dissolving 4gm lead acetate in 12ml distilled water. Pregnant Swiss mice were given lead acetate at a concentration of 8, 16 and 32 mg (266.66, 533.33, and 1066.66 mg/kg/bodyweight) from 10th day of gestation to 21st day of lactation. Blood samples were obtained from the tail of pups from each litter at days 1,7,14 and 21 day after birth. The tip of the tail was cleaned with spirit before being cut with a sharp blade and was not squeezed to avoid dilution of blood by tissue fluid.

Blood cells were studied in smears prepared by spreading a drop of blood thinly over a clean and sterilized microscopic slide with the help of another slide moved over the first at the angle of 45° after discarding first drop of blood. These blood films were air-dried and fixed in absolute methanol for 15 minutes by dipping the film briefly in a Coplin jar containing absolute methanol. After fixation the slides were removed and air-dried. Afterward blood smears were stained with freshly made Giemsa stain diluted with water buffered to pH 6.8 or 7.0 (1:9) stain and buffer respectively. The slides were washed by briefly dipping the slide in and out of a Coplin jar of buffered water and air dried again for taking observations. The erythrocytes appear pink to purple, whereas leukocytes turned blue black in color. All the experimental work was approved by the Institutional Animal Ethics Committee. No./CS/Res/07/759.

Group 1- Control (distilled water only).

Group 2- Exposure to 8 mg lead acetate (266.66 mg/kg BW) from 10th day of gestation up to 21st day of lactation.

Group 3- Exposure to 16 mg lead acetate (533.33 mg/kg BW) from 10th day of gestation up to 21st day of lactation.

Group 4- Exposure to 32 mg lead acetate (1066.66 mg/kg BW) from 10th day of gestation up to 21st day of lactation.

The statistical analysis was performed following t-test for the comparison of data between different experimental groups. The data was calculated using...
Results

In the control group all the WBCs showed normal appearance. The neutrophils in control group were examined by a very characteristic nucleus with condensed chromatin. It is divided into 3-5 lobes (Fig.1A, 1, 2, 5 and 6) at birth which was observed with an increase by 5 to 6 lobes (Fig.1B, 1 and 3) at the termination of lactation, connected by thin strands of chromatin. Lymphocytes were round or ovoid at the time of birth (Fig. 1A, 3 and 4) but further on they were found notched or slightly indented (Fig. 1B, 5 and 6). The chromatin was generally diffusely dense. Ordinarily, nucleoli were not visible. A perinuclear clear zone surrounding the nucleus was visible after first week of lactation in some cells. The cytoplasm stained light blue and ranges from sparse to moderately abundant in amount. The monocyte in control group were round with smooth margins, the nucleus was oval, indented and slightly folded (Fig.1B, 4). The chromatin material was moderately clumped and relatively less dense compared to that of neutrophils or lymphocytes. There was no visible nucleolus with abundant cytoplasm.

The administration of lead acetate altered the appearance and caused structural changes. The following hematological observations were taken during postnatal period from birth till the termination of the lactation period upon exposure of different doses of lead acetate:

1. At the time of birth (PND1)

Abnormal neutrophils: In lead treated groups the neutrophils showed structural abnormalities in their nucleus including improper segmentation and lesser condensation of nucleus. At a lower dose the chromatin material was condensed, all the lobes were interconnected with each other and form a nodule like structure at one side (Fig.1C, 1).

Degeneration: In lead treated group most of the neutrophils appeared in degenerating state in which the chromatin material was very less condensed, fused and there was no sign of clear lobulization and segmentation (Fig. 1C, 2).

Immature cells: In lead treated group the number of immature cells was increased (Fig. 1C, 3).

Ring shaped: In lead treated groups, some neutrophils showed abnormal ring like appearance and diffuse chromatin material, with unclear cytoplasm. In 32 mg lead treated group vacuolization in chromatin material was also observed (Fig. 1C, 4).

Lymphocyte: Reactive (Fig. 1C: 5 and 6) and cleaved (Fig. C, 5) types of lymphocytes were observed in lead treated groups.

Monocyte: At postnatal day 1 we cannot identify any structural change in shape and size of monocyte as observed on postnatal day 21.

2. During first and second week of postnatal period (PND7&14)

The following observations were taken at first to second week after birth:

Degenerated neutrophils: In lead treated group overall numbers of neutrophils were increased particularly with degenerated neutrophils, however, their number was less than postnatal day 1. In 16 mg lead group on postnatal day 7 the nuclear material of neutrophil was less condensed and nucleus was divided into 2-3 unequal lobes. The cytoplasm of neutrophil appeared colorless. At the dose of 32mg lead at postnatal day 7, this severity of degeneration was very much increased so that the lobes were broken into many small fragments. No sign of lobulization and appropriate segmentation of neutrophils were found (Fig. 1D, 1).

Ring shaped neutrophils: In contrast to postnatal day 1, ring like nucleus was not observed in lead treated group at postnatal day 7.

Different types of neutrophils: At higher dose 32 mg lead treated groups apoptotic or necrotic neutrophils were more prominent. These neutrophils were characterized by 3-4 separate and equal lobes with less condensed chromatin and diffuse cytoplasmic region (Fig. 1D, 2).

Immature cells: Review of the lead treated smear revealed that most of the leukocytes were myelocytes, bands, myeloblast and other immature and unidentified white blood cells with left shift in leucocytes. A left shift is an increase in the number of band neutrophils and other immature cell of the granulocytic lineage in the peripheral blood (Fig. 1D, 3).

Various lymphocytes: Administration of lead acetate produced great variation in lymphocyte structurally as well as numerically. Various types of lymphocytes such as plasmacytoid, reactive, oval, irregular, binucleated and cleaved lymphocytes were identified, whereas only reactive and cleaved...
Lymphocytes were seen in postnatal day 1, exclusively in lead treated group. Lead treated group with 16 mg lead acetate produced large lymphocytes and most of the lymphocytes were having irregular; clumpy and smudgy chromatin material with very dense nucleus (Fig. 1D, 4). The cytoplasm appeared completely absent as the nucleus reached its largest size and covered all the cytoplasmic area. Overall, number of lymphocytes decreased in most of the groups. At higher dose (32 mg lead) the plasmacytoid lymphocytes (eccentric nucleus and intensely blue / basophilic cytoplasm) (Fig. 1D, 6) and reactive lymphocytes were observed. Reactive lymphocyte was characterized by relatively very large, irregular but flattened nucleus with fine chromatin and agranular light blue stained cytoplasm (Fig. 1D, 5).

3. At the end of lactation period (PND21)

Abnormal nuclear segmentation: It includes abnormal segmentation of nucleus, in which the nuclear lobes were connected with each other. It gave abnormal appearance of nucleus and chromatin condensation in most of the neutrophils (Fig. 1E, 1).

Degeneration: In lower doses of lead diffuse appearance of chromatin material was observed in neutrophils and the lobes were fused with each other as any segmentation was not observed, whereas in higher lead treated group the neutrophils presented fragmented chromatin material and very less condensation of nucleus which finally leads to cell lysis (Fig. 1E, 2). The nuclear arrangement was distorted, as appear that all the lobes were intermingled with each other and in some cases form a nodule at one side known as sessile nodule appeared like hypersegmentation (Fig. 1E, 3).

Immature cells: In lead treated group the numbers of immature cells were increased. A left shift i.e. presence of immature neutrophils, bands, metamyelocytes, myelocytes and other unidentified immature cells were observed (Fig. 1F, 1 to 6).

Lymphocytes: As the dose level increased the number of lymphocytes decreased. In higher dose lead treated group the lymphocyte appeared large in size with higher volume of cytoplasm. The shape of the nucleus also vary from round to elliptical in structure, termed as reactive lymphocyte (Fig. 1E, 4). Some lymphocytes transformed into plasmacytoid lymphocyte in which the lymphocyte contains basophilic cytoplasm and eccentric nucleus (Fig. 1E, 5).

Monocytes: In lead treated groups the shape and structure of the monocyte were modified and the shape of the nucleus was also altered from the normal reniform (kidney shaped) nucleus. The indentation of the nucleus became larger and deeper from periphery to center. At higher dose level intensity of the indentation was increased so that the normal range of nucleo-cytoplasmic ratio was disturbed (Fig. 1E, 6). Numerical changes in different types of WBC and percent variations in different types are incorporated in Table 1 and 2 respectively. In present investigation, after evaluating all the cell types, we can conclude that lead acetate at PND 1 and 14 caused significant increase in number of neutrophils and decrease in lymphocytes, while there was no significant difference in the number of neutrophils and lymphocytes at PND 7 and 21.

| Table 1. Various types of WBCs at different postnatal days treated with lead acetate. |
|-----------------|-----------------|-----------------|-----------------|
| Groups          | Neutrophils     | Lymphocytes     | Monocytes       |
| Control at PND 1| 59.25±1.70      | 38.5±1.29       | 2.25±1.70       |
| Lead acetate at PND 1 | 66.00±2.16**  | 28.25±2.06**   | 5.75±1.70*      |
| Control at PND 7 | 57.75±2.21      | 41.75±2.21      | 0.75±0.95       |
| Lead acetate at PND 7 | 61.75±3.5      | 37.5±2.88       | 0.75±0.95       |
| Control at PND 14| 55.25±3.40      | 44.5±3.10       | 0.25±0.5        |
| Lead acetate at PND 14| 61.25±1.70**  | 37.75±1.70**   | 1.00±0.81       |
| Control at PND 21| 47.75±2.5       | 47.75±2.21      | 4.5±2.38        |
| Lead acetate at PND 21| 52.25±4.57    | 44.00±2.26      | 3.75±2.75       |

Values were expressed as means ± S.D.; 4 animals /group; *=p<0.05 and **=p<0.01
**Table 2.** Percent variation in different types of WBCs in lead treated groups

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<thead>
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<th>Neutrophils</th>
<th>Lymphocytes</th>
<th>Monocytes</th>
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<tbody>
<tr>
<td><strong>Lead acetate at PND1</strong></td>
<td>Normal 12.3%</td>
<td>Normal 13%</td>
<td>Normal 2.0%</td>
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<tr>
<td></td>
<td>Degenerated 12.1%</td>
<td>Reactive 17.33%</td>
<td>Abnormal 3.7%</td>
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<td></td>
<td>Ring shaped 8.2%</td>
<td>Cleaved 8.6%</td>
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<td></td>
<td>Immature 4.1%</td>
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<td></td>
<td>Abnormal 10.4%</td>
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<td></td>
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<tr>
<td><strong>Lead acetate at PND7</strong></td>
<td>Normal 12.6%</td>
<td>Normal 12.2%</td>
<td>Abnormal 0.75%</td>
</tr>
<tr>
<td></td>
<td>Degenerated 8.4%</td>
<td>Plasmacytoid 7.4%</td>
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<td>Abnormal 16.8%</td>
<td>Reactive 9.8%</td>
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<tr>
<td></td>
<td>Immature 10.8%</td>
<td>Binucleated 2.4%</td>
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<tr>
<td></td>
<td>Ring shaped 6%</td>
<td>Large 4.2%</td>
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<tr>
<td><strong>Lead acetate at PND14</strong></td>
<td>Normal 19.2%</td>
<td>Normal 4%</td>
<td>Abnormal 1%</td>
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<td></td>
<td>Degenerated 19%</td>
<td>Plasmacytoid 4%</td>
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<td>Abnormal 9.6%</td>
<td>Reactive 12.3%</td>
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<td></td>
<td>Immature 6.4%</td>
<td>Binucleated 8.2%</td>
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<td></td>
<td>Apoptotic 6.4%</td>
<td>Large 4%</td>
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<td><strong>Lead acetate at PND21</strong></td>
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<td>Normal 10%</td>
<td>Normal 2%</td>
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<td>Immature 7.4%</td>
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<td>Irregular 5.86%</td>
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**Figure 1.** A: Peripheral blood smear of control group showing neutrophil (1-2), lymphocytes (3-4), at the time of birth, neutrophils (5-6) during second and third week of lactation. B: Control group showing neutrophil (1), lymphocyte (2), during second and third week of lactation, and, neutrophil (3), lymphocytes (4-5) and monocyte (6) at the termination of lactation. C: Peripheral blood smear of lead treated group showing abnormal neutrophil (1), degenerated neutrophil (2), immature cell (3), ring like neutrophil (4), 5 – cleaved (upper WBC) (5) and reactive (lower WBC) lymphocyte (5 and 6) at the time of birth. D: Lead treated group showing degenerated neutrophil (1), necrotic (2), immature cell (3), large lymphocyte (4), reactive (5) and plasmacytoid lymphocyte (6) During first and second week of postnatal period. E: Lead treated group showing - abnormal neutrophil (1), degenerated neutrophil (2), hypersegmented neutrophil (3), reactive lymphocyte (4), plasmacytoid lymphocyte (5) and reactive monocyte (6) at the termination of lactation. F: Lead treated group showing different immature cells at the termination of lactation (1- 6). (All Giemsa stain, 450x).
Discussion

Changes in leukocyte parameters are often one of the hallmarks of infection. These include changes in number and in cellular morphology. Review of the peripheral blood smear can provide significant insight into the possible presence of infection. Early changes during infection may include an increase in the number of bands, even before the development of leukocytosis. A great shift to immaturity (left shift) may occur when infection is severe, with metamyelocytes or even earlier forms present on the peripheral blood smear. There are many evidences of studies conducted on adults and RBC concerning lead toxicity, but very few reports are available regarding haematopathological alterations of lead acetate in different white blood cells. Significant decrease in RBC count, hematocrit (Hct) and hemoglobin (Hb) were seen in rats and human with high blood lead levels. (Alexa et al., 2002; Noori et al., 2003; Othman et al., 2004; Toplan et al., 2004)

In our study the control groups showed all the leukocytes in normal appearance. Still some altered types of WBCs were also observed. The administration of lead acetate alters the structure and number of WBCs. The nuclear arrangement was also distorted. In lead treated groups the shape and structure of the monocyte was also altered with reniform (kidney shaped) nucleus. At higher dose level this intensity of indentation was increased so that the normal range of nucleo-cytoplasmic ratio is disturbed and appeared as reactive monocytes. Our findings are also in support of DeNicola et al. (1991) with the evidence of reactive monocytes enclosing the cytoplasm became more intensely basophilic and vacuolated. This usually indicates a chronic inflammatory process or may be seen with hemoplasmas in the cat.

Toxicity in neutrophils is defined by the presence of Döhle bodies (small, basophilic aggregates of RNA in the cytoplasm), diffuse cytoplasmic basophilia etc. In our study each lead treated group in neonatal period, represents increased number of degenerated neutrophils particularly at birth. In the 16 mg lead exposed group, during first week of lactation, the nuclear material of cell was less condensed and nucleus was divided into 2-3 unequal lobes with colorless cytoplasm. At higher dose of 32 mg lead, this severity of degeneration was very much increased with many small fragments of nuclear material and no sign of lobulization and appropriate segmentation of neutrophils were observed.

In a study performed on young dogs, development of anemia, leukocytosis, monocytopenia, polychromato-philia, glycosuria, increased serum urobiligen, and hematuria has been reported (Zook, 1972). Lead suppresses bone marrow hematopoiesis, probably through its interaction with the enteric iron absorption (Klader, 1779; Chnielnika, 1994). In some reports, leukocytosis has been attributed to the lead-induced inflammation (Yagminas et al., 1990).

Hogan and Adams, (1979) reported a threefold increase in neutrophil and monocyte count along with severe leukocytosis in the young rats that were exposed to lead. The present investigation revealed that administration of lead acetate alters the appearance and cause structural changes. The nuclear arrangement was distorted with intermingled lobes and in some cases formed a sessile nodule.

Controversies exist about monocytes; since in some studies lead-induced monocytopenia (Xintaras, 1992) and in others significant increases in monocyte count have been reported (Yagminas et al., 1990). The reason for such difference is probably due to the extent of lead-induced inflammation.

Mugahi et al. (2003) investigated additional hematotoxic effects of lead on the erythroid cell lineage and leukocytes following long-term exposure in rats. Wahab et al. (2010) showed that lead caused a significant decrease in hematocrit, RBC, WBC, hemoglobin concentration, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration and lymphocyte and monocyte count; and significant increase in neutrophil count. The results of the present study are also parallel to the above findings. In lead exposed pups there was significant increase in the number of neutrophils at different weeks after birth, but decrease in the number of lymphocytes. The shortened life span of erythrocytes is due to increased fragility of the blood cell membrane and reduced hemoglobin production is due to decreased levels of enzymes involved in hemesynthesis (Guidotti et al., 2008). It has long been known that hematopoiesis and heme synthesis affected by lead poisoning (Doull et al., 1980).

In our study reactive and cleaved type of lymphocyte were observed at the time of birth in lead treated groups which were reinstated by increased number of plasmacytoid, reactive, large, oval, irregular, binucleated and cleaved lymphocytes in further days of lactation. In the current investigation at higher dose (32 mg) we
found apoptotic or necrotic neutrophils were more prominent in the first and second week of lactation. These neutrophils were characterized by 3-4 separate and equal lobes with less condensed chromatin and diffuse cytoplasmic region.

Lead treated group at the termination of lactation, include abnormal nuclear segmentation, giving abnormal appearance of nucleus and chromatin condensation in most of the neutrophils and forming a ring like nucleus in some neutrophils. Villagra et al., (1997) also postulates that lead exposure doubles total and segmented neutrophils in both estrogens treated and untreated rats but causes a three-fold increase in band neutrophils in animals without estrogen treatment, but not in animals treated with estrogen. With a disappearance of non-degranulated eosinophils, the decrease in non-degranulated eosinophils was under the effect of lead exposure. He also demonstrates that prepubertal rat exposure to lead affects blood neutrophil and eosinophil leukocyte levels and induces eosinophil degranulation.

Vyskocil et al., (1991) discovered the effect of lead on band neutrophils reveals an increased neutrophilopoiesis rather than release from intravascularly sequestered forms in lead-exposed animals.

In lead treated group from birth till the termination of lactation, the number of immature cells was increased. There was asynchrony of maturation between nucleus and cytoplasm. During normal granulocytopenia the lengthening and pinching of the nucleus were coordinated with progressive condensation of the chromatin with accelerated maturation nuclear division may be skip and cells retain immature features, because toxic changes of lead accompanies a left shift i.e. presence of immature neutrophils, bands, metamyelocytes, myelocytes and other unidentified immature cells. White Blood Cells generally increase as compared to the control level. The increase in WBC count indicates the activation of defense mechanism and immune system of gasoline workers (Whitby, 1980). These findings are also in confirmations, with our results.

In conclusion, lead exposure leads to various hematological disorders in white blood cells including neutrophilic degeneration, immature cells, abnormal neutrophils, reactive and plasmacytoid lymphocyte, reactive monocyte etc. The present study indicates that after administration of 266.66, 533.33 and 1066.66 mg/kg/body weight doses of lead acetate WBCs show structural abnormalities in their nucleus and cytoplasm including improper segmentation and lesser condensation of nucleus. Lead causes fluctuations in the number of various cell types at different stages of postnatal development. The exposure to lead possesses the potentials to induce hazardous biological effects during pre and postnatal development in Swiss mice.

References


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