



Lead harmfulness and a few subsets of motor aptitude: Comparative assessment of grown-up and prenatally uncovered rodents

H. B. Akpan, O. O. Olubiyi

Department of Anatomy, Obafemi Awolowo College of Health Sciences Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Nigeria.

Abstract

Research has shown that even low levels of lead (Pb) exposure are dangerous. Thus, Pb exposure/toxicity continues to be a leading environmental health issue for children and women of childbearing age. A variety of toxic effects caused by Pb exposure during gestation and early childhood have been reported in both human and animal studies. Adult wistar rats of both sexes were used for this study. They were housed in individual improvised cages and were given food and water *ad libitum*.

The animal room temperature was maintained at approximately 27 C with a 12 light/dark cycle. After the period of acclimatization, adult rats were randomly assigned into four groups of eight rats each (4 males and 4 females) viz; Adult exposed and control; prenatally exposed and control. The adult and prenatally groups received 2n mM lead acetate solution as sole drinking fluid for the duration of the experiment. The study showed significant ($p < 0.05$) results in pups negative geotaxis and cliff avoidance and sex by measure interaction response to weight changes in the adult groups, while the adult negative geotaxis, cliff avoidance, pups' righting reflex showed no significant value ($p > 0.05$). The lead exposed group hastens the eruption of upper incisors and eye opening and delayed the ear unfolding. The present results suggest that the prenatal exposure to Pb, influences the rate of physical maturation and sensory motor reflexes in pups.

Keywords: Lead acetate, toxicity, physical maturation, motor reflexes, anomalies.

INTRODUCTION

Research has shown that even low levels of lead (Pb) exposure are dangerous (Silergeld, 1990). Thus, Pb exposure/toxicity continues to be a leading environmental health issue for children and women of childbearing age (Mushak, 1992). A variety of toxic effects caused by Pb exposure during gestation and early childhood have been reported in both human and animal studies. Many neurological and behavioral anomalies have been attributed to pre- and post-natal Pb exposure, such as aggressiveness, decreased IQ, learning disabilities, hyperactivity,

and impulsiveness, as well as aberrant neuromotor coordination function (Kishi et al., 1983; Needleman, 1987, 1993). In both humans and experimental animals, Pb readily crosses the placental- fetal barrier (Donald et al., 1986; Goyer, 1990; Lataillade et al., 1995), causing a direct relation between the Pb exposed mother and the possibility of irreversible developmental damage to her offspring.

Keller and Doherty (1980) reported that Pb-exposed mouse dams transmitted a significantly greater amount of Pb to their offspring through their milk than by *in utero*

exposure (transplacentally). Earlier studies in humans and experimental animals have shown that not only does Pb accumulate in the fetus from the second trimester onwards (Bhattacharayya, 1983), but during lactation, it is excreted into the milk, which continues the risk to nursing offspring (Namihiro et al., 1993; Hallen et al., 1995a; b; 1996). The detrimental effects of Pb occur regardless of the developmental time exposure, although gestational exposure appears more sensitive to the Pb effects (Dearth et al., 2002). The developmental toxicity of lead has become a significant area of research since children are more sensitive than adults to learning impairment following low-level lead exposure (Davis and Svendsgaard, 1987). Regarding this point, animal and human studies have reached similar results, since behavioral effects induced by lead are seen in rats at approximately the same blood levels that cause deficits in humans (Davis et al., 1990; Annau, 1990; Needleman et al., 1990).

Administration of diets with a high lead content to rodents during suckling causes growth impairment, hema-tological alterations, breakdown of the blood-brain barrier, and higher pup mortality (McClain and Becker, 1972; Rosenblum and Johnson, 1968). Moreover, significant effects on neuromotor development of rats have also been described. Reiter and colleagues (Reiter et al., 1975) reported delay in development of righting reflex and eye opening in animals exposed to lead acetate in the drinking water through gestation and lactation. These data were confirmed by Luthman and colleagues (Luthman et al., 1992), who described delayed eye opening and impairment in negative geotaxis. However, a statistically significant effect of lead treatment on body weight was evidenced in these studies. Since it is well known that undernutrition is causally related to neuro-behavioral development retardation (Smart and Dobbing, 1971), it becomes difficult to attribute these deficits solely to lead exposure. Studies using milder forms of lead intoxication have revealed no differences in weight gain, but significant effects on adult rat behavior (Rodrigues et al., 1993; 1996). Therefore, in the present study we comparatively evaluated the effects of chronic lead exposure on adult and prenatal rats starting from day one of gestation to post natal day 30 on body weight and some subset of motor skills and physical maturation.

MATERIALS AND METHODS

Animal care

Adult wistar rats of both sexes were obtained from animal holding of the department of Anatomy, Olabisi Onabanjo University, and Remo campus, Ikenne, Ogun State, Nigeria. They were housed in individual standard cages. They were given food and water *ad libitum*. The animal room was maintained at approximately 22°C with relative humidity of between 40 - 60% a 12 h off light/dark cycle was maintained. The animal beddings were changed thrice weekly. The present study was approved by the Animal Ethics Committee of the Faculty of Basic Medical Sciences of the

University in line with the National Animal Care Standard Operating Procedures.

Feed composition

Rats pellets was purchased from Grand Cereals and Oil mills limited depot, was given to the rat throughout the period of experiment *ad libitum*. The feed composition includes; crude protein - 14.50%, fat - 7.00%, crude fibre - 7.20%, calcium - 0.80%, available phosphorus - 0.40%

Experimental design and duration

After the period of acclimatization, adult rats were randomly assigned into four groups of eight rats each (4 males and 4 females) viz; Adult exposed and control; prenatally exposed and control. The adult and prenatally groups received 2mM lead acetate solution as sole drinking fluid. The adult rats were exposed for a period of 4weeks. Prenatally rats were exposed rats were placed 1 male to 2 females in a cage containing wood shavings which was regularly replaced. After the confirmation of pregnancy they were separated into individual cages for a period of 21 - 22 days (gestation period). Reflex tests and observations of the maturation of physical characteristics (The date of appearance of ear unfolding, eruption of upper incisors and eye opening) were carried out daily at the appropriate ages by one experimenter that was not aware of the subject treatment in accordance with previous criteria (Kruck and Pycock, 1979; Lee, 1980; Kellog et al., 1980; Jett and Guilarte, 1995; Soufir, 1995. Testing was carried out between 9:00 and 11:00 am, and progress of the same individuals from each litter was followed throughout the experiment. Animals in the adult group were weighed every 3 days while Cliff avoidance was carried out every 5 days and Negative geotaxis every 3 days. Pups from this group were subjected to reflex analysis, observation of behavioral pattern and physical parameters at appropriate ages within 30 days of birth. These include body weight at postnatal day 0, 5, 10, 15, 20, 25, 30. Date of appearance of ear unfolding, eruption of upper incisors and eye opening was also observed. Reflexes assessed include Cliff avoidance, equilibration on 2 L beaker, Negative geotaxis and righting reflex

Sample collection and chemical analysis

After 4 weeks of administration, adult rats were deeply anaesthetized, while the Pups were sacrificed after postnatal day 30. Brain was dissected out and 5 g weight and 5 mm thick section of the prefrontal taken and homogenized. This was used for neurotransmitters assessment.

Reflex analyses

The reflex analyses assessed in the adult group are cliff avoidance every 5days, negative geotaxis every 3 days. Reflex analyses for the pups were taken at appropriate ages such as

- (i) Righting reflex: Postnatal day 1 - 5
- (ii) Equilibration on beaker rim: Postnatal day 19

Righting reflex

This was assessed by measuring the time required for the animal to turn ventrally after being placed in a dorsal position from postnatal day 1 - 5.

Table 1. Physical parameters evaluated with dates or percentage. Values shown are the group of mean \pm SEM (n=5).

Variable	Control	Exposed
Percentage of stillbirth	0	16.13
Percentage of unilateral Amelia in pups	0	3.23
Date of eye opening	Postnatal day 17	Postnatal day 13
Date of ear unfolding	Postnatal day 3	Postnatal day 4
Date of eruption of upper incisors	Postnatal day 10	Postnatal day 8
Balance and movement along beaker rim	10.29% fell	18% fell
Lead serum level	19.60 \pm 2.93	40.00 \pm 2.86

Cliff avoidance

This was assessed by placing the animal on the edge of a bench with nose and forepaws just over the edge and the number of times the animal moves away in 3 consecutive 30 s trial recorded.

Negative geotaxis

This was measured by placing the rat on a slope with the head downward and scored 0, 1, 2, 3, and 4, if it turns to face up the slope 0, 45, 90, 135 and 180°, respectively, in a 90 s session while that of the adult treated group was measured by the time required for the animal to turn 180° that is, to hold the tail in a 30 s trial.

Equilibration on beaker rim

This was assessed by measuring the ability of the rat to balance on a 2 L beaker. Latency to escape the from the rim and percent of animals that fell from the rim was recorded

Statistical analysis

The statistical analysis were carried out by student't' test, p 0.05 was considered significant. All the values are reported as Mean \pm SEM. The diagrams were drawn using Microsoft excel 2007 version.

RESULTS

The average water consumption in the control group was 16 mls per day while the fluid consumed by the treated groups was 12 mls. Table 1 shows the developmental observations of the control and exposed groups. It follows that the mean number of pups at birth is not significantly (p 0.05) different between the control and the treated, likewise the weight of pups from postnatal day 0 - 30. Other developmental observations include unilateral amelia in some exposed pups, hasten eye opening, eruption of upper incisors and delayed ear unfolding. Balance and movement along beaker rim also reveal a more coordinated movement among the pups of the control rat compared to the Pups from exposed group. Figure 1 reveals significant (p < 0.05) increase in weight in the male as compared to the female adult rats, while

Figure 2 shows steady increase in weight between the control and exposed pups although not significant (p 0.05).

The negative geotaxis in adult and pups are presented in Figure 3 and 4. The mean adult values between the control and the exposed was not significant (p 0.05), while the mean values between the control and the exposed pups was significant (p < 0.05). Figure 5 and 6 presented significant (p < 0.05) percentage differential value between the control and exposed groups, in both the adult and pups that score 3 out of 3 in cliff avoidance test. The mean righting reflex values between the control and exposed pups are presented in Figure 7. Lead exposure increased the time for righting reflex when compared to the control. The mean level of acetylcholine was significantly (p < 0.05) reduced in the exposed group (Figure 8).

DISCUSSION

Administration of lead led to higher pup mortality in the present study, this observation is in agreement with previous studies Rosenblum et al., 1968; McClain et al., 1972 and as a result suggested a deleterious role of lead on developing embryo. Lead was also observed to be a teratogenic agent in the present study characterized by unilateral Amelia of the forelimb in the prenatally exposed pups of lead treated rats. Previous study reported congenital anomalies, such as failure of the ventral body wall to close along the midline and leg dysfunction, were observed in treated chicks (Yara et al., 2007). Lead treatment did not affect the number of living pups and body weight of adult and prenatally exposed pups at birth and weight gain, These results are consistent with previous research proving that no gross differential body weight difference in adultsÁrates in normal physiological state (Salinas and Huff, 2002; Cory-Slechta et al., 1989) or during pregnancy and suckling (Trombini et al., 2001). This implies that there is a balance between caloric intake and utilization of caloric in both groups (Figure 2). A significant sex by measure interaction response to weight changes was observed among the individual sexes (Figure 1) which supports previous research by

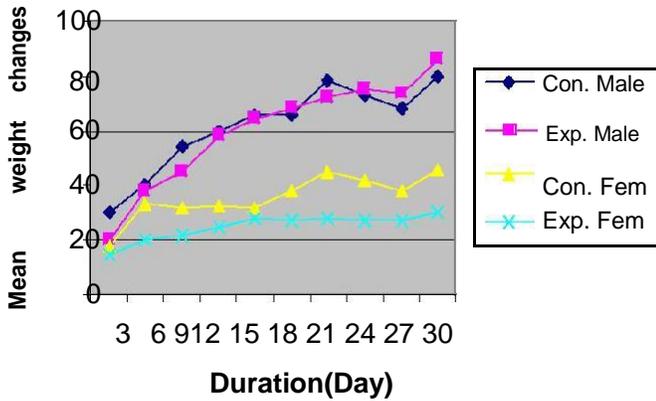


Figure 1. Mean weight changes in adult individual sexes. Values shown are the group of mean \pm SEM (n = 5) p 0.05 between the male and the female and p 0.05 among the individual sexes.

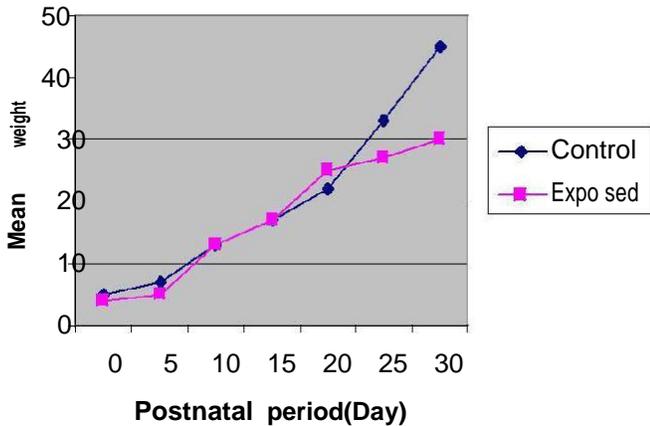


Figure 2. Mean weight of pups. Values shown are the group of mean \pm SEM (n = 5) p 0.05 between control and the exposed.

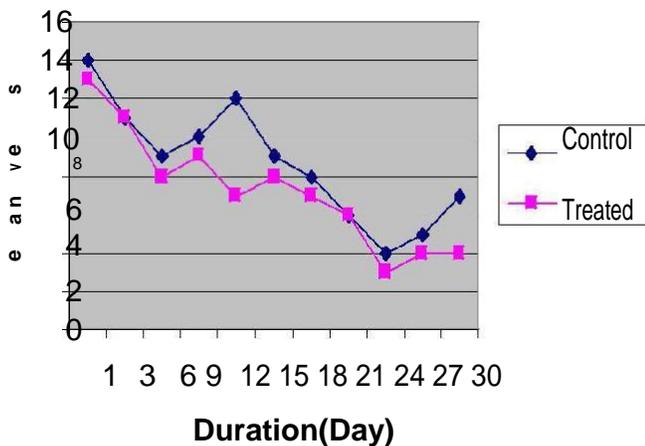


Figure 3. Mean negative geotaxis in adult Wistar rats. Values shown are the group of mean \pm SEM (n = 5) p 0.05 between the control and the treated.

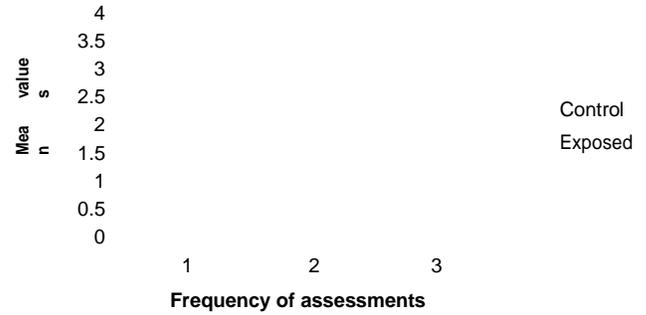


Figure 4. Mean negative geotaxis score in pups. Values shown are the group of mean \pm SEM (n = 5) p 0.05 between the control and exposed.

Mello et al., 1998 that males exhibit a higher weight gain curve compared to females therefore this method of lead intoxication does not affect weight gain, Rodrigues et al., 1993; Rodrigues et al., 1996. The differences in response to weight gain suggest that under the same environmental condition, males exhibit a significantly higher response to weight changes. The present results suggest that the prenatal exposure to Pb, influences the rate of physical maturation, sensory motor reflexes of the pups at different developmental stages. Abnormalities in response to teratogens may be due to several factors that causes alterations in normal cell metabolism, especially in enzymes and their substrates (Wilson, 1973), or due to combination of several factors (Coyle et al., 1976).

The motor reflexes evaluated in this study show significant decline in the mean values in pup's negative geotaxis, cliff avoidance and righting reflex while the adult values were insignificant (Figure 3 - 7). These results show that lead toxicity results in hypoactivity, which has been associated with lead intoxication in rats during the early postnatal period, resulting transient as well as persistent dysfunctions in exploratory behavior and motor skills. These observed actions of lead may be related to impaired maturation of sensitive brain regions which develop postnatally (Johan et al., 1992). Lead was reported to induce important deficits on motor behavior of chicks during the first postnatal week and such phenomena are related to lead deposition in the cerebellar tissue during embryonic development (Yara et al., 2007). Implicated in behavior process is ACh (Allikmets, 1974). Changes in the behavior due to toxicants are presumably due to alterations in the availability of neurotransmitters (Kruck and Pycok, 1979; Kellog et al., 1980; Lee, 1980). It is known that a major portion of brain cells (70%) of the closely related rats are formed after birth (Patel, 1983).

In this study a significant (p < 0.05) reduction in the level of ACh was detected in all lead exposed pups (Figure 8). This could possibly explain why the sensory motor reflexes of Pb exposed pups were compromised. Thus, lead could have produced developmental abnormalities in the brain as well as an alteration in the level

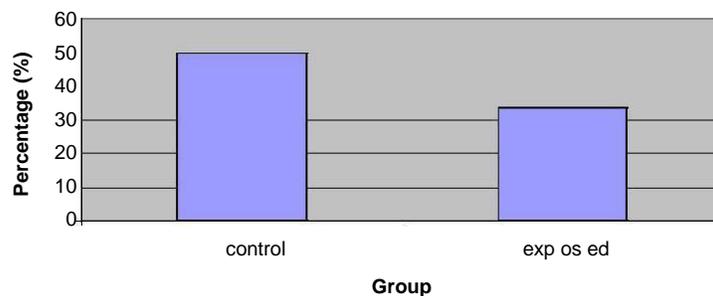


Figure 5. Percentage of adult rats that score 3 out of 3 during cliff avoidance. Values shown are the group of mean \pm SEM (n = 5) p 0.05 between the control and the exposed.

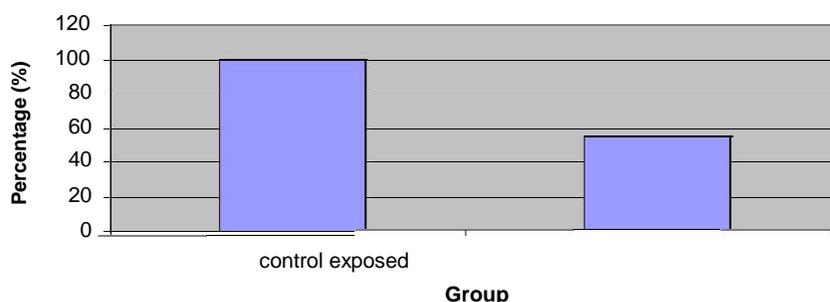


Figure 6. Percentage of pups that score 3 out of 3 during cliff avoidance. Values shown are the group of mean \pm SEM (n = 5) p 0.05 between the control and the exposed.

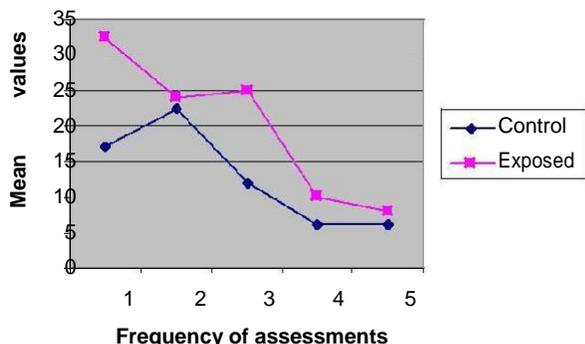


Figure 7. Mean righting reflex score in pups. Values shown are the group of mean \pm SEM (n = 5) p 0.05 between the control and the exposed.

level of ACh that might have brought about the observed effects on the motor reflexes of the developing pups in the present study. It has been reported that alterations in brain enzymes are among the factors responsible for disturbances in behavioral activities of affected animals (Branchey and Friedhoff, 1976; Kellog et al., 1980; Johnson et al., 1981; Ajarem and Ahmad, 1991). Analysis of other physical development parameters in the current study revealed that lead-treated animals presented

accelerated eye opening, as also observed by Mello et al., 1998 and in animals subjected to prenatal methyl-mercury exposure without concomitant undernutrition (Vorhees, 1985), indicating a teratogenic role for lead acetate. The eruption of upper incisors appeared earlier in lead treated animals, while the appearance of ear unfolding was delayed. In contrast to the study, some previous studies (Reiter et al., 1975; Luthman et al., 1992; Mello et al., 1998) reported either a delayed or no difference between groups as regards eye opening in rats exposed to lead.

The present results strongly suggest that lead exposure *in utero* and via mother's milk tend to produced severe effect as compared to adult exposure. Lead produces it deleterious effects probably by causing oxidative stress which ultimately alters the cellular processes. Such effects might be affecting the overall morphological developments and sensory motor reflexes of the developing pups, and behavior of the young adult offspring. In conclusion, the present study demonstrated that lead acetate intoxication caused significant alterations in the development of rats. The majority of the observed alterations pointed to an advance in the day of appearance in physical maturation and decline in some reflexes, Therefore, the lead-induced developmental alterations in rats observed here are important in terms of searching for

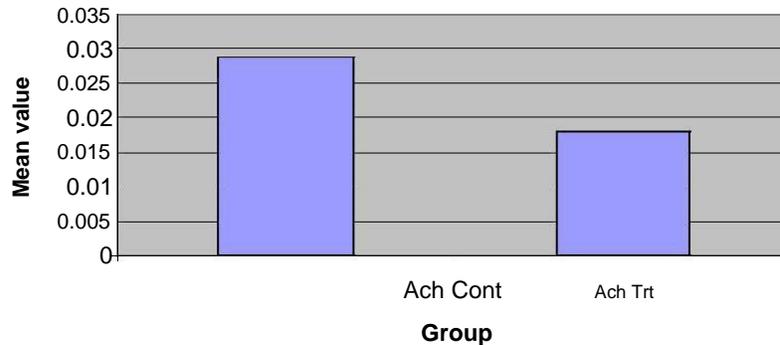


Figure 8. Mean value of a acetylcholine in the prefrontal cortex of prenatally lead exposed pups. Values shown are the group of mean \pm SEM (n = 5) p 0.05 between the control and the treated.

similar developmental alterations in human beings exposed to lead.

REFERENCES

- Ajarem JS, Ahmad M (1991). Behavioural and biochemical consequences of perinatal exposure of mice to instant coffee: a correlative evaluation. *Pharmacol. Biochem. Behav.*, 40: 847-852.
- Allikmets LH (1974). Cholinergic mechanisms in aggressive behavior. *Med. Biol.*, 52: 19-30.
- Annau Z (1990). Behavioral toxicology and risk assessment. *Neurotoxicol. Teratol.*, 12: 547-551.
- Bhattacharayya MH (1983). Bioavailability of orally administered cadmium and lead to the mother, fetus, and neonate during pregnancy and lactation. *Sci. Total Environ.*, 28: 327-342.
- Branchey L, Friedhoff AJ (1976). Biochemical and behavioural changes in rats exposed to ethanol in utero. *Ann. N.Y. Acad. Sci.*, 273: 328-330.
- Cory-Slechta DA, Weiss B, Cox C (1989). Tissue distribution of Pb in adult vs. old rats: A pilot study. *Toxicol.*, 59: 139-150.
- Coyle I, Wayner MJ, Singer C (1976). Behavioural teratogenesis: A critical evaluation. *Pharmacol. Biochem. Behav.*, 4: 191-200.
- Davis JM, Svendsgaard DJ (1987). Lead and child development. *Nature*, 329: 297-300.
- Dearth RK, Hiney JK, Srivastava V, Burdick SB, Bratton GR, Dees WL (2002). Effects of lead (Pb) exposure during gestation and lactation on female pubertal development in the rat. *Reprod. Toxicol.*, 16: 343-352.
- Donald JM, Cutler MG, Moore MR (1986). Effects of lead in the laboratory mouse. I. Influence of pregnancy upon absorption, retention, and tissue distribution of radiolabelled lead. *Environ. Res.*, 41: 420-431.
- Goyer RA (1990). Transplacental transport of lead. *Environ. Health Perspect.* 89: 101-105.
- Guilarte TR, Miceli RC, Jett DA (1995). Biochemical evidence of an interaction of lead at the zinc allosteric sites of the NMDA receptor complex: effects of neuronal development. *Neurotoxicol.*, 16: 63-72.
- Hallen PI, Jonsson S, Karlsson MO, Oskarsson A (1996). Toxicokinetics of lead in lactating and nonlactating mice. *Toxicol. Appl. Pharmacol.*, 136: 342-347.
- Hallen PI, Jorhem L, Lagerkvist B, Oskarsson A (1995b). Lead levels in human milk and blood. *Sci. Total Environ.* 166: 149-155.
- Hallen PI, Jorhem L, Oskarsson A (1995a). Placental and lactational transfer of lead in rats: a study of the lactational process and effects on offspring. *Arch. Toxicol.*, 69: 596-602.
- Jett DA, Guilarte TR (1995). Developmental lead exposure alters N-methyl-D-aspartate and muscarinic cholinergic receptors in the rat hippocampus, an autoradiographic study. *Neurotoxicol.* 16: 7-18.
- Johan L, Agneta O, Lars O, Barry H (1992). Postnatal lead exposure affects motor skills and exploratory behavior in rats. *Environ Res.*, 58(1-2): 236-252.
- Johnson JWC, Mitzner W, Beck JC, London WI, Sly DL, Lee PA, Khonzami VA, Calvalieri RL (1981). Longterm effects of betamethasone on fetal development. *Am. J. Obstet. Gynecol.*, 141: 1053-1064.
- Kellog C, Tervo D, Ison J, Parisi T, Miller RK (1980). Prenatal exposure to diazepam alters behavioral development in rats. *Science* 207: 205-207.
- Kishi R, Ikeda T, Miyake H, Uchino E, Tsuzuki T Inoue K (1983). Effects of low lead exposure on neurobehavioral function in the rat. *Arch. Environ. Health.* 38: 25-33. NOT CITED
- Kruck ZL, Pycock CJ (1979). *Neurotransmitters and Drugs*. Croom-Helm, London.
- Lataillade GP, Thoreux-Manlay A, Coffigny H, Masse R, Soufir JC (1995). Reproductive toxicity of chronic lead exposure in male and female mice. *Hum. Exp. Toxicol.*, 14: 872-878.
- Lee ML (1980). Aminoacidopathy and mental retardation and other developmental disabilities. In: McCormack, M.K. (ed.). *Marcel Dekker*, New York, pp. 135-150.
- Livesey DJ, Livesey RGD, Barrett J, Spickett TJ (1986). Lead retention in blood and brain after preweaning low-level lead exposure in the rat. *Pharmacol. Biochem. Behav.*, 25: 1089-1094.
- Luthman J, Oskarsson A, Olson L, Hoffer B (1992). Postnatal lead exposure affects motor skills and exploratory behavior in rats. *Environ. Res.*, 58: 236-252.
- McClain RM, Becker BA (1972). Effects of organolead compounds on rat embryonic and fetal development. *Toxicol. Appl. Pharmacol.*, 21: 265-274.
- Mello CF, Kraemaer CK, Filipin A, Morsch VM, Rodrigues ALS, Martins AF Rubin MA (1998). Effect of Lead Acetate on Neurobehavioural Development of Rats Brazilian J. Med. Bio. Res., 31(7): 943-950.
- Mushak P (1992). Defining lead as the premier environmental health issue for children in America: Effect of prenatal lead in the cross-fostered mice offspring criteria and their quantitative application. *Environ. Res.*, 59: 281-309.
- Namihira D, Saldivar L, Pustilnik N, Carreon GJ, Salinas ME (1993). Lead in human blood and milk from nursing women living near a smelter in Mexico City. *J. Toxicol. Environ. Health.* 38(3): 225-232.
- Needleman HL (1987). Low level lead exposure in the fetus and young child. *Neurotoxicol.*, 8: 389-393.
- Needleman HL (1993). The current status of childhood low-level lead toxicity. *Neurotoxicol.*, 14: 161-166.
- Needleman HL, Schell AS, Bellinger D, Leviton A and Allred EA (1990). The long-term effects of exposure to low levels of lead in childhood. *New Engl. J. Med.*, 322: 83-88.
- Patel AJ (1983). Undernutrition and brain development. *Trends Nat.*