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# Effect of Low-level Arsenic Exposure on the Development of Neurobehavioral Toxicity in Rats

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## ABSTRACT

Arsenic has been widely studied for its effects as a human carcinogenic agent but few studies have dealt with neurobehavioral effects. Gestation exposure is a time of high sensitivity to chemical, associated with an important risk factor for development of newborns of neurobehavioral dysfunctions. In the present study, we investigated *in utero* exposure studies to characterize developmental and behavioural alterations induced by arsenic exposure. Pregnant female Albino rats were exposed to inorganic arsenic (0, 4.5, 6.0 and 7.5 mg $kg^{-1}$ $day^{-1}$ by gavages) from gestation day 8 to till parturition and the offspring were observed over the first 3 postnatal weeks, until they were weaned on PND21. The offspring were examined for physical development, reflex development, strength and motor coordination through battery of test in order to evaluate their developmental neurotoxicity. The result of the study has not confirmed the high behaviours-teretrogenic potential of arsenic at given dose levels. Prenatal administration of arsenic failed to have physical development, neuromotor and reflex development, decreased exploration in the open field and delayed onset of negative geotaxis and startle reflex in arsenic-exposed animals in comparison to the control group. Present study demonstrated that, arsenic intoxication caused no significant alterations in the development of pups. Fetal effects such as mortality rate were observed but no maternal mortality was observed during the study with given doses. No statistically significant increases were observed for *in utero* deaths, resorptions and skeletal malformations during gestation day 8 to up to parturition with gavages. Prenatal administration of arsenic failed to induce neurobehavioral end points in rat suggest to pay increased attention to low level of arsenic concerning its exogenous use during pregnancy and further studies of dose-effect relationship are needed to assess the possible risk associated with arsenic exposure during gestation period.

**Key words:** Prenatal exposure, heavy metal intoxication, arsenic, neurobehavioral development, rat

## INTRODUCTION

Arsenic is an environmental contaminant found in soil, water and air in some zones of the world. It has been widely studied for its effects as a human carcinogenic agent but few studies have

dealt with neurobehavioral effects. Data from animal studies by Vahidnia *et al.* (2007), Hass (2006), Huot *et al.* (2004) and Vahter (2008) demonstrate that arsenic can produce developmental toxicity, including malformation, growth retardation and even death after a single oral, gavage or intraperitoneal exposure. On postnatal weight or maturational indices was reported but survival through 30 days of age was reduced in mice treated with sodium arsenite by gavage (5 mg $kg^{-1}$) or IP injection (2 or 4 mg $kg^{-1}$) on gestation day (GD1-17) (Golub *et al.*, 1998). Most of the studies (Hood, 1972; Earnest and Hood, 1981) conducted to compare single dose gavages and IP administration of sodium arsenite showed fetal effects (malformation and embryo lethality) were higher by injection than by gavages. In contrast, maternal mortality was higher by the oral route than by injection. Also compared doses of sodium arsenate considered to produce similar levels of maternal toxicity in mice by gavages (120 mg $kg^{-1}$) or IP injection (40 mg $kg^{-1}$) on single day between GD7 and GD15 (Baxley *et al.*, 1981). With gavages, statistically significant increases were reported for *in utero* death and resorptions on GD11 and for skeletal malformations on GD9. There was no statistically significant increase in gross malformation on any day. For chronic administration, most studies have used oral route. However, several studies (Hood *et al.*, 1978; Ferm and Hanlon, 1985) described a large dose response relationships study for developmental endpoints. Therefore, the current investigation was undertaken to quantify the risk on early physical and neurobehavioral outcomes. Primarily, the physical and behavioural development were explore related to the prenatal exposure of arsenic.

## MATERIALS AND METHODS

**Animal:** Female Wister albino rats, (90-120 days old, weighing 120-150 g) were selected. One male and two female were kept together for mating. The animals were maintained in a temperature-controlled environment (22±2°C) at 70% humidity and on a 12 h light/dark cycle. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and was conducted according to the Indian National Science Academy (INSA) guidelines for the use and care of experimental animals, chemical, dose and treatment schedule.

**Chemical:** Exposure to arsenic was given in the form of sodium arsenite and it was purchased from M/S Thomas Baker Chemicals Ltd., Mumbai. India.

**Treatment:** After a one-week acclimation period, 78-mated female rats were examined for vaginal smears. The day on which a vaginal plug was found or spermatozoa were found in the vaginal smears was considered gestational day 0 (GD0). Sperm-positive females were randomised in weight to receive arsenic. Controls were treated with 0.9% saline solution. The females were exposed to 0, 4.5, 6.0 and 7.5 mg sodium arsenite/kg/day by gavages on GD8 to till parturition.

**Neurobehavioral development:** Beginning on GD21, dams were inspected frequently between 0800 and 2000h for birth until delivery. The day of parturition was defined as postnatal day (PND1), meaning the maximum resolution for gestational length was one half day. The pups were counted, examined for gross malformation and weighed individually. Pups body weight and maternal behaviour was recorded daily during nursing. After parturition, the neonates were

observed for mortality and signs of toxicity. The offspring were evaluated for survival, growth, development and behaviours. When parturition complete, the numbers of stillborn and live pups in each litter were recorded.

**Following variables were observed:** *Birth measures:* Litters were examined on PND1 for obvious morphological anomalies (e.g., missing digits, facial malformations etc.), sexed by relative no genital distance and culled pseudo-randomly to 20 pups and keeping an equal number of male and female pups per litter when possible. Gestation length was calculated at birth and the following litter data were collected on PND1: litter size, sex ratio, body weight for each pup and the number of malformed offspring. Neonatal death was noted from PND1 through PND5. Postnatal biobehavioral maturation of the pups was assessed over the first 3 postnatal weeks, until they were weaned on PND23.

**Developmental and behavioural milestones:** Pups from each litter were weighed on PND1-21 and the emergence of physical maturation landmarks were noted each day, including physical developmental parameters such as pinna detachment; Incisor irruption; eye opening; development of fur; ear unfolding; tastes descendent; Vaginal opening were carried out daily at the appropriate ages by one experimenter that was not aware of the subject treatment. The dam was first removed from the home cage and the individual tests within each testing category proceeded in the following order: (1) reflex development-righting reflex, (2) motor coordination-the negative geotaxis test, (3) muscle strength test, (4) Open field activity and (5) spontaneous or non-behaviour tests. The day of appearance of ear unfolding, eruption of upper and lower incisor and eye opening was recorded using previous criteria (Ferm and Hanlon, 1986; Tilson and Moser, 1992).

**Neuromotor and reflex development:** The righting reflex (PND1-5), the pup's ability to turn over from supine position and the time required (latency(s) for the animal to turn ventrally itself recorded. Pups that did not successfully right themselves were assigned the maximum of 60 sec. Other reflexes assessed were testing, cliff avoidance (PND1-4); auditory startle (PND7)-the presence or absence of sensorimotor reaction (jerks) to auditory stimulus; negative geotaxis (PND9-11)-the pup's ability to turn180° on a 25° and 45° incline placed head down; palmer grasps (PND11)-the pup's was evaluated by stroking gently the palm of forepaw with a forceps and observing the digital flexing response; and free-fall righting (PND12-14)-the pup's turned in mid-air to land vertically after being dropped, back downwards, from 35 cm height on to a cotton-wool pad in three consecutive trials. We also assessed forelimb placing (PND4-5); hind limb placing (PND6); Surface righting (PND9-11); ear twitch (PND18); tail pinch and limb withdrawal reflexes.

**Strength:** Forelimb grip strength (PND20)-ability to hold on to a thin wire, performances on rotating rod by rotarod (Columbus Instrument, Columbus, Ohio, USA). On day 20, the ability to balance and move along the drum of variable diameter (10-20 cm) is rotated at 7, 8 and 9 rpm. The drum rotated at particular speed until the rat falls of the drum in a 30 sec session. Latency to escape from the drum and percentage of animals that fell from the drum were calculated and compared between groups.

**Activity and emotional reactivity:** Exploratory behaviour in an Open field-Head elevation is generally first observed on the second postnatal day consists of keeping the head raised above the surface, the activity decreases and then increases at about day 10. This behaviour during 30 sec observation time is generally recorded. Intensity of motor activity and rearing, defecation and urination rate, hindlimb elevation, Pivoting (Circular movement, PND2-12), grooming, sniffing, gait abnormality and auditory startle (PND7) were recorded as the day animal responded with a sudden and brief extension of the hindlimbs to the sound of a clicker or snapping.

**Spontaneous or non-behaviour tests:** On postnatal day 17, animals were tested for spontaneous alteration in T-maze (a 16.5" base and two arms each 16.5" (Total 33.0") long along with central arena 10.8" wide with transparent lid. Two emitter detector infrared beam pairs per arm to monitor animal behavior (Columbus Instruments, Columbus, Ohio, USA). Latency to enter one arm of the maze and arm entered recorded. The animals were tested in consecutive trails (intertribal interval 20 sec) until they entered the contra lateral arm, with a maximum of 5 trials. When the latency to enter one of the sides was longer than 300 sec, the animal was excluded then subsequent testing. Percentage of failure to alternate was also calculated for each litter and compared between groups.

**Statistical analysis:** Data were analysed by one-way Analysis of Variance (ANOVA) followed by Duncan test. The level of statistical significance was set at $p \leq 0.05$. All data are expressed as Means±SEM.

## RESULTS

The pregnant rats exposed at 4.5. 6.0 and 7.5 mg As $kg^{-1}$ $day^{-1}$ from GD8 to till parturition, produced neither maternal toxicity nor any noticeable signs or symptoms of any of the treatment groups exposure to arsenic given by gavages The behaviour of the treated rats was almost similar to that of the control rats. On day 4 of gestation, the maternal body weight remained within the control range. The respective control values (g) were 187.94±2.12; 172.55±3.55; 175.55±3.67 and 188.08±1.89. On day 20 of gestation, the maternal body weight gain (g) of control, 4.5, 6.0 and 7.5 mg $kg^{-1}$ arsenic exposed dams were 64.35±1.56; 41.22±2.32; 54.28±1.30 and 44.32±2.12. Arsenic exposure induced maternal and litters characteristics analysis as is evident from the data presented in Table 1 and 2. Prenatal administration of arsenic (As) resulted in increased mortality of pups during the first day of postnatal life 12, 22 and 58% with respective dose levels. Also have significant effect on viability of arsenic-treated rats (89.91, 83.20 and 65.30%) with respective dose of treatment.

The significant effect of treatment on somatic growth such as reduction of percentage maternal weight gain (23.56%) at higher dose level where as reduction in percentage of live birth (89.9, 83.20 and 65.30%) with respective dose treatment groups. Percentage of affected pups per litter (8.32, 4.54 and 1.71%) as well as affected pups per affected litter (16.66, 18.18 and 24.13%) was markedly declined in the arsenic treated groups respectively (Table 1, 2). However, no significant delayed effect of arsenic treatment on maturation of pups (PND1-35) such as pinnadetechment, unfold of external ear, incisor eruption, fur development, opening of eyes and ears, testes descent and vaginal lumen opening (Table 3) was observed.

Table 1: Maternal and litter characteristics (Mean±SEM)[d]

| Dose of Arsenic (As) | Control | 4.5 mg $kg^{-1}$ | 6.0 mg $kg^{-1}$ | 7.5 mg $kg^{-1}$ | p-value |
|---|---|---|---|---|---|
| Dams (N = 78)[a] | 17 | 18 | 18 | 25 | |
| Live litters (N = 457)[b] | 132 | 107 | 109 | 109 | |
| Maternal weight on GD4 (g) | 187.94±2.12 | 172.55±3.55 | 175.55±3.67 | 188.08±1.89 | n.s |
| Maternal weight gain to GD20 (g)[c] | 64.35±1.56 | 41.22±2.32 | 54.28±1.30 | 44.32±2.12 | * |
| Maternal weight gain (%) | 34.23 | 23.88 | 30.91 | 23.56 | * |
| Live births (%) | 100.00 | 89.9 | 83.20 | 65.30 | * |
| Gestational age (days) | 21.93±0.45 | 22.00±0.23 | 22.05±0.16 | 22.27±0.14 | n.s |
| Litter size | 7.76±0.45 | 8.44±0.24 | 8.94±0.17 | 6.48±1.54 | n.s. |
| Males | 3.88±0.33 | 4.72±0.26 | 3.77±0.75 | 3.20±1.25 | n.s. |
| Females | 3.88±0.78 | 3.72±0.89 | 5.16±0.12 | 3.28±1.45 | n.s |
| Sex ratio (% males) | 50.00 | 55.90 | 42.23 | 49.38 | |
| Mean weight on PND1 (g) | 44.96±0.56 | 39.11±1.34 | 38.50±2.47 | 34.20±3.87 | n.s. |
| Dead pups on PND1 to PND5 | 0 | 12 | 22 | 58 | * |
| Affected litters (%) | 0 | 10.1 | 16.8 | 34.7 | * |
| No. of affected pups | 0 | 12 | 22 | 58 | * |
| No. of affected pups per litter | 0 | 9.91±0.34 | 5.95±0.23 | 2.87±0.12 | * |
| Affected pups per litter (%) | 0 | 8.32 | 4.54 | 1.71 | * |
| Affected pups per affected litters | 0 | 12/2 | 22/4 | 58/14 | * |
| Affected pups per affected litters (%) | 0 | 16.66 | 18.18 | 24.13 | * |

a. of the total of 78 dams received in to the experiment.; total number of pups delivered from respective dams of each group of treatment. Two dams in the 4.5 mg $kg^{-1}$, one dam in the 6.0 mg $kg^{-1}$ and three dams were not pregnant in 7.5 mg $kg^{-1}$. Mean±SEM. maternal weight gain from GD4-GD20. Maternal weight on GD4 subtracted from maternal weight on GD20. Mean±SEM. maternal litter characteristics of the 65 confirmed pregnant dams and their pups delivered. *p<0.05, compared to control n.s. = Not significant

Table 2: Gestational length (days) and pups viability at birth of arsenic exposed dams

| Group dose (mg $kg^{-1}As^{-1}$) | Gestational length* | Total no. of pups born | No. of pups alive (N) | No. of pups dead | Viability (%)* |
|---|---|---|---|---|---|
| Control (n = 16) | 22.00±0.7 | 132 | 132 | 0.0 | 100.0 |
| 4.5 | 22.00±0.19$^{n.s}$ | 119 | 107 | 12 | 89.91** |
| 6.0 | 22.05±0.14$^{n.s.}$ | 131 | 109 | 22 | 83.20** |
| 7.5 | 22.27±0.15$^{n.s.}$ | 167 | 109 | 58 | 65.30** |

Data are expressed as Mean±SE. n.s.: Not significant (p-value compared to control) (p<0.05). n: Numbers of dams in respective groups of the treatment. **p<0.05 compared to control

Table 3: Physical developmental milestones in pups of dams exposed to arsenic (Values are Mean±SE from 20 pups/treatment groups)

| Dose (mg $kg^{-1}$) | Pinna detachment | Incisor eruptions | Fur development | Eye opening | Ear opening | Testes descent | Vaginal opening |
|---|---|---|---|---|---|---|---|
| Control | 2.10±0.06 | 10.85±0.08 | 17.00±0.07 | 09.95±0.05 | 19.00±0.07 | 24.95±0.05 | 29.95±0.55 |
| 4.5 | 2.05±0.05$^{n.s.}$ | 11.00±0.07$^{n.s.}$ | 17.02±0.07$^{n.s.}$ | 09.55±0.45$^{n.s.}$ | 18.95±0.05$^{n.s.}$ | 25.05±0.05$^{n.s.}$ | 30.10±0.06$^{n.s.}$ |
| 6.0 | 2.11±0.06$^{n.s.}$ | 11.00±0.10$^{n.s.}$ | 17.04±0.10$^{n.s.}$ | 10.00±0.10$^{n.s.}$ | 19.05±0.05$^{n.s.}$ | 25.10±0.07$^{n.s.}$ | 30.25±0.02$^{n.s.}$ |
| 7.5 | 2.20±0.09$^{n.s.}$ | 11.00±0.13$^{n.s.}$ | 17.03±0.13$^{n.s.}$ | 10.10±0.07$^{n.s.}$ | 18.95±0.05$^{n.s.}$ | 24.85±0.08$^{n.s.}$ | 31.00±0.42$^{n.s.}$ |

The litter was considered as the experimental unit. The values correspond to the days of the life required for the entire litter to show each milestone. Data are expressed as Mean±SE.; N: 20 whole litter/treatment. n.s.: Not significant. (p-value compared to control).

There was a significant increase in reaction time for negative geotaxis at 6.0 mg $kg^{-1}$ (13.00±0.58 25° incline); 17.00±0.68 (45° incline) and 7.5 mg $kg^{-1}$ (12.00±0.57 25° incline); 18.00±0.67 (45°) incline) arsenic treated groups at 25° as well as 45°C incline (Table 4) However, postnatal day of appearance of reflexes such as startle reflex, cliff avoidance, free fall righting, palmer grasp, tail pinch, ear twitch hind limb withdrawal reflexes were mot significantly change in arsenic treated groups (Table 5).

Table 4: Effect of Arsenic on the negative geotaxis (Sec/30 sec) at 25 and 45° (Values are Mean±SE from 20 pups/treatment groups)

| | Arsenic (As) orally negative geotaxis (sec/30 sec) (Mean±SE time 30 sec) | | | |
|---|---|---|---|---|
| Negative geotaxis degree | Control (n = 131) | 4.5 mg $kg^{-1}$ (n = 105) | 6.0 mg $kg^{-1}$ (n = 106) | 7.5 mg $kg^{-1}$ (n = 109) |
| 25 | 6.00±1.39 | 6.00±0.39$^{n.s}$ | 13.00±0.58** | 12.00±0.57** |
| 45 | 12.00±0.52 | 13.00±0.68$^{n.s}$ | 17.00±0.68** | 18.00±0.67** |

Data are reported as Means±SE of pups. n: No. of pups in respective groups; the mean of values determined on number of pups in treatment groups was considered the litter performance of dams exposed to 4.5,6.0 and 7.5 mg $kg^{-1}$ arsenic or control solution. ** $p<0.01$ value compared to control. n.s.: Not significant

Table 5: Postnatal day of appearance of some reflexes in Arsenic exposed rats (Values are Mean±SE from 20 pups/treatment groups)

| | Arsenic (As) orally | | | |
|---|---|---|---|---|
| Reflexes Parameters (n = 20) Mean±SE | Control | 4.5 mg $kg^{-1}$ | 6.0 mg $kg^{-1}$ | 7.5 mg $kg^{-1}$ |
| Cliff avoidance | 11.00±0.07 | 11.00±0.10$^{n.s.}$ | 10.35±0.49$^{n.s.}$ | 11.15±0.10$^{n.s.}$ |
| Palmar grasp | 17.00±0.10 | 16.70±0.11$^{n.s.}$ | 17.3±0.11$^{n.s.}$ | 17.1±0.16$^{n.s.}$ |
| Startle Reflex | 24.95±0.05 | 24.60±0.16$^{n.s.}$ | 24.75±0.09$^{n.s.}$ | 24.4±0.18$^{n.s.}$ |
| Free fall righting | 24.05±0.08 | 23.95±0.11$^{n.s.}$ | 24.15±0.17$^{n.s.}$ | 23.90±0.18$^{n.s.}$ |
| Tail pinch | 23.50±0.15 | 23.25±0.12$^{n.s.}$ | 22.75±0.09$^{n.s.}$ | 23.40±0.11$^{n.s.}$ |
| Ear twitch | 24.00±0.10 | 23.70±0.11$^{n.s.}$ | 24.25±0.09$^{n.s.}$ | 24.00±0.16$^{n.s.}$ |
| Limb withdrawal reflexes | 23.05±0.11 | 22.75±0.09$^{n.s.}$ | 22.20±1.06$^{n.s.}$ | 21.95±1.06$^{n.s.}$ |

The litter was considered as the experimental unit. The values correspond to the days of the life required for the entire litter to show each milestone. Data are expressed as Mean±SE N: 20 whole litter/treatment.. n.s.: Not Significant. (p-value compared to control)

Table 6: Effects of Arsenic administration on spontaneous activity of albino rats (T-maze) (Values are Mean±SE from 20 pups/treatment groups)

| | Arsenic (As) orally (Mean Score±SE time 5 min) | | | |
|---|---|---|---|---|
| | Control | 4.5 mg $kg^{-1}$ | 6.0 mg $kg^{-1}$ | 7.5 mg $kg^{-1}$ |
| % Change | 289.20±1.53 | 287.95± 4.05$^{n.s.}$ | 287.88±3.53$^{n.s.}$ | 288.95±3.32$^{n.s.}$ |
| | 100.00% | 99.56%$^{n.s.}$ | 99.54%$^{n.s.}$ | 99.91%$^{n.s.}$ |

The litter was considered as the experimental unit. The values correspond to the days of the life required for the entire litter to show each milestone. Data are expressed as Mean±S.E.; N: 20 whole litter/treatment. n.s.: Not significant (p-value compared to control)

Table 7: Effects of Arsenic exposure on muscular grip strengths of rats (RotaRod) (Values are Mean±SE from 20 pups/treatment groups)

| | Arsenic (As) orally (Mean±SE time 30 sec) | | | |
|---|---|---|---|---|
| Rotation Per Minutes (RPM) | Control | 4.5 mg $kg^{-1}$ | 6.0 mg $kg^{-1}$ | 7.5 mg $kg^{-1}$ |
| 7 | 28.3 ±1.0 | 28.1±2.0$^{n.s.}$ | 28.0± 2.4$^{n.s.}$ | 27.9±2.1$^{n.s.}$ |
| 8 | 27.5±1.1 | 27.4±1.5$^{n.s.}$ | 27.3±1.5$^{n.s.}$ | 27.8±0.1$^{n.s.}$ |
| 9 | 27.8±1.5 | 27.8±2.5$^{n.s.}$ | 27.9±1.1$^{n.s.}$ | 27.5±1.3$^{n.s.}$ |

The values are expressed as time (second) taken by pups to stay on the rod with respective RPM without falling was recorded within 5 min of test observation. ANOVA test, Interaction with p-value. n.s: Not significant

Gestation exposure of arsenic treatment did not have significant effect on percentage of spontaneous activity performances by T-maze (Table 6) as well as forelimb grips strength performances on rotating rod (Rota-rod Test, Columbus Instrument, USA) with different Revolution per Minute (RPM) (Table 7). Exploratory behaviors in open field test such as intensity of motor activity, rearing, defecation-urination rate, grooming, head and hind limb elevation, pivoting and gait abnormality revealed that maternal arsenic treatment in rats was not found to modify open field behaviors of the offspring (Table 8).

Table 8: Effects of Arsenic exposure on behavioural performance in open field activity of rats (Values are Mean±SE from 20 pups/treatment groups)

| | Arsenic (As) orally | | | |
|---|---|---|---|---|
| Open field activity | Control | 4.5 mg $kg^{-1}$ | 6.0 mg $kg^{-1}$ | 7.5 mg $kg^{-1}$ |
| Head elevation (No./30 sec) (D10) | 12.00±0.26 | 11.0±0.29$^{n.s.}$ | 13.20±1.19$^{n.s.}$ | 12.60±0.19$^{n.s.}$ |
| Hind limb elevation (No./30 sec) | 5.00±0.17 | 6.00±0.78$^{n.s.}$ | 5.00±0.09$^{n.s.}$ | 4.90±0.10$^{n.s.}$ |
| Rearing (No. of rear/60 sec) | 7.05±0.16 | 6.85±0.11$^{n.s.}$ | 7.10±0.10$^{n.s.}$ | 6.90±0.14$^{n.s.}$ |
| Fecal boluses (No.) | 46.5±1.1 | 45.8±1.4$^{n.s.}$ | 46.0±1.0$^{n.s.}$ | 45.9±2.5$^{n.s.}$ |
| Urination (No. of pools of urine) | 73.5±0.6 | 72.8±1.0$^{n.s.}$ | 73.0±0.9$^{n.s.}$ | 73.1±1.2$^{n.s.}$ |
| Grooming (D5) | 1.6±0.5 | 1.5±0.3$^{n.s.}$ | 1.6±0.2$^{n.s.}$ | 1.5±0.8$^{n.s.}$ |
| Sniffing | 1.8±0.5 | 1.7±0.7$^{n.s.}$ | 1.6±1.2$^{n.s.}$ | 1.7±1.1$^{n.s.}$ |
| Biting and licking | 0.2±0.2 | 0.4±0.5$^{n.s.}$ | 0.3±0.4$^{n.s.}$ | 0.3±0.6$^{n.s}$ |
| Head bobbing | 0 | 0$^{n.s.}$ | 0$^{n.s.}$ | 0$^{n.s.}$ |
| Auditory startle (D7) (Rank) | 1 | 1$^{n.s.}$ | 1$^{n.s.}$ | 1$^{n.s.}$ |
| Pivoting (360 Degree) (D12) | 100% | 100%$^{n.s.}$ | 100%$^{n.s.}$ | 100%$^{n.s.}$ |
| Gait abnormality (Rank) | 1 | 1$^{n.s.}$ | 1$^{n.s.}$ | 1$^{n.s.}$ |

The values (When applicable) correspondence to the pups shows each behavioral performance during 5 min in open field. Measurement of each performance rating is in parenthesis included all pups within the treatment group. ANOVA test, Interaction with p-value. n.s: Not Significant

## DISCUSSION

Most of the early arsenic studies are inappropriate for use in estimating human risk. The potential for single oral exposures to inorganic arsenic to cause developmental toxicity had been investigated in mice (Baxley *et al*., 1981; Hood *et al*., 1978) and hamsters (Hood *et al*., 1982) but not in rats. The intent of most of these studies was to investigate basic phenomena, such as the genesis of neural tube defects. Single oral doses (DeSesso, 2001) as well as Repeated oral or inhalation (Holson *et al*., 2000a) exposures to inorganic arsenic compounds administered to pregnant females early in gestation do not produce neural tube defects or other structural malformations, even at maternally toxic/lethal doses. To that end, in most studies, animals were administered single, high doses of inorganic arsenic by injection (Golub *et al*., 1998; DeSesso *et al*., 1998). Many of the oldest studies did not report how gestational age was calculated (i.e., whether the day of mating was designated day 0 or day 1 of gestation). This leads to uncertainty concerning the exact age of embryos at the time of arsenic exposure which is especially important in rodents because of their brief gestational periods (e.g., hamster, 16 days; mouse, 19-20 days; rat, 21-22 days) (De Sesso, 1997). Many studies did not report clearly whether the administered doses elicited maternal toxicity. (Holmberg and Ferm, 1969). Many older studies did not report the frequencies of specific fetal malformations or the dose and day in gestation of administration that elicited them (Hood and Pike, 1972; Baxley *et al*., 1981; Hood *et al*., 1982) which has been pointed out in our earlier study (Gandhi *et al*., 2011) with arsenate (0, 1.5, 3.0 or 4.5 mg kg $day^{-1}$) by oral gavages on gestational day 8 to till parturition.

Data from animal studies (Vahter, 2008) demonstrate that arsenic can produce developmental toxicity, including malformation, growth retardation and even death. The animal data also revealed that inorganic arsenic caused malformations in offspring only when it was injected (Ricceri *et al*., 2006) into the veins or peritoneal cavity of pregnant animals during early gestation as well as exhibit any propensity to cause neural tube defects, even at maternally toxic dose levels (Holson *et al*, 2000b; DeSesso, 2001). In the present study, arsenic given by gavages to the

pregnant rats from days 8 to up to parturition at 4.5; 6.0 and 7.5 mg kg day$^{-1}$ produced neither maternal toxicity nor any significant alterations in fetal developmental end points. Although, no clinical signs or symptoms of toxicity were observed in arsenic-treated dams, the body weight gain was significantly reduced. The maternal weight loss was expected, as there was a significant *in utero* fetal loss as it evident from data presented in Table 1 and 2. The suppress body weight gain appears to be secondary to fetal loss and may not be considered separate indices of maternal toxicity. These developmental toxic effects were observed at a dosage which did not induce clinical symptoms in dams but significantly affected body weight gain, percentage live births and number of dead pups on PND1-5. However, arsenic exposure did not affected the other malformations such as mean fetal weight of survival pups, the incidence of external malformations and increased resorption after oral exposure were observed only in the absence of maternal toxicity, suggesting that the embryo is more to arsenic then the pregnant female.

Analysis of other physical development parameters revealed that arsenic-treated animals presented non-accelerated eye opening, in contrast to observed in animals subjected to prenatal methyl mercury (Vorhees, 1985) and lead (Mello *et al*., 1998), indicating no teratrogenic role for arsenate. Similarly, the startle reflex appeared earlier in lead-treated animals (Mello *et al*., 1998), whereas, in the present study with different doses of arsenic found no alteration in other physical development parameters such as pinna detachment; Incisor irruption; eye opening; development of fur; ear unfolding; tastes descendent; Vaginal opening at present arsenic dose levels.

Earlier observations (Vorhees, 1985; Mello *et al*., 1998; Grant *et al*., 1980) showed that lead-treated animals present a delay in motor development, including free-fall righting reflex deficits and concomitant body weight gain losses. Similar study with animals exposed to methy lmercury, (which presented weight gain losses) showed impaired performance in the negative geotaxis test (Bull *et al*., 1983), contrasting with data obtained by Vorhees (Vorhees, 1985). Findings of the present study also showed, no increased in scores in the negative geotaxis test comparable to the control group at birth in arsenic treated animals. Negative geotaxis and surface righting are some of the responses reflecting functioning of the cerebellar and vestibular system (Rocha *et al*., 1993). As an unlearned response to gravitational cues, negative geotaxis has been considered diagnostic of vestibular and/or proprioceptive function (Secher *et al*., 2006). These reflex tests are useful in the recognition of developmental disturbances. There was no delay in attaining these that we found in pups of arsenic treated dams is probably indicating no damage or poor development of the motor and vestibular system of the brain.

The dam primarily determined the development of major regulatory systems underlying behaviour and physiology in the neonatal rat (Moser, 1989). In addition, the vulnerability of the developing brain to toxic insults is dependent on exposure and on the stage of development of the potential target organ or damage to particular structure in the circuit or to connecting pathways in the developing nervous system which can produce structural or functional changes that result in behavioural changes (Huot *et al*., 2004; Li, 2005).

Several studies have reported deficits in acquisition of a radial eight-arm maze task, alterations in a spatial learning task (Motz and Alberts, 2005), performing like hippocampally damaged rats (Rodriguez *et al*., 2002) as well as the spontaneous alteration test during adult hood (Alfano and Petit, 1981). In the present study, gestation exposure of arsenic treatment did not have significant effect on percentage of spontaneous activity performances by T-maze. These results are in contrast with the views that lead treatment causes alterations in hippocampus function (Kostas *et al*., 1976). It is reasonable to expect that no hyperactivity observed, at least in part, to the better performance

of arsenic-treated rats in these tasks. However, if hyperactivity were present, one should also expect its influence on other behavioral parameters that depend on motor responses, such as cliff avoidance, drum equilibration task, rotarod task, palmar grasp, ear twitch, tail pinch and limb withdrawal reflex. These behavioral responses were not affected by arsenic treatment and do not corroborate the hypothesis that hyperactivity might be involved in this effect. However, spontaneous activity (Forced behavioral-T-Maze) as well as spontaneous activity (Non-forced behavioral) such as head elevation, rearing, grooming, pivoting, hindlimb elevation, gait abnormality (walking pattern) was not affected in the present study. It is also well known that the appearance of the startle reflex coincides with ear channel opening and that hearing neuronal circuit is already developed at this age. Therefore, earlier ear channel opening is a possible explanation for the earlier occurrence of the startle reflex observed here.

There are several parameters used to characterize motor performance. Of these, the open field seems to be able to provide a good measure of the approach response towards novelty (exploration) and describe influences of chemicals exposure even if exploratory activity and emotional reactivity can interfere with one another. These findings could indicate low doses affect or/and long-term neurotoxicity (Lehmann *et al*., 2000). In addition, motor and/or sensory changes could either mask or exaggerate cognitive dysfunctions. However, the present findings in the open-field test point to no alterations induced by arsenic exposure allow us to suggest that arsenic neither influences the development of motor coordination skills nor general motor activity or muscular strength. No change in appearance of neurodevelopment milestones suggests the possibility that arsenic is not capable of damaging the physiological systems controlling spontaneous motor activity and motor coordination at this dose level.

## CONCLUSIONS

Prenatal administration of arsenic failed to induced neurobehavioral end points in rat suggest to pay increased attention to low level of arsenic concerning its exogenous use during pregnancy and further studies of dose-effect relationship are needed to assess the possible risk associated with arsenic exposure during gestation period.

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## REFERENCES

Alfano, D.P. and T.L. Petit, 1981. Behavioral effects of postnatal lead exposure. Possible relationship to hippocampal dysfunction. Behav. Neural. Biol., 32: 319-333.

Baxley, M.N., R.D. Hood, G.C. Vedel, W.P. Harrison and G.M. Szczech, 1981. Prenatal toxicity of orally administered sodium arsenite in mice. Bull. Environ. Contam. Toxicol., 26: 749-756.

Bull, R.J., P.T. McCauley, D.H. Taylor and K.M. Crofton, 1983. The effects of lead on the developing central nervous system of the rat. Neurotoxicol, 4: 1-18.

De Sesso, J.M., 1997. Comparative Embryology. In: Handbook of Developmental Toxicology, Hood, R.D. (Ed.). CRC Press, Boca Raton, USA., pp: 111-174.

DeSesso, J.M., 2001. Teratogen Update: Inorganic Arsenic. Teratology, 63: 170-173.

Desesso, J.M., C.F. Jacobson, A.R. Scialli, C.H. Farr and J.F. Holson, 1998. An assessment of the developmental toxicity of inorganic arsenic. Reprod. Toxicol., 12: 385-433.

Earnest, N.M. and R.D. Hood, 1981. Effects of chronic prenatal exposure to sodium arsenite on mouse development and behavior. Teratology, 24: 53-53.

Ferm, V.H. and D.P. Hanlon, 1985. Constant rate exposure of pregnant hamsters to arsenate during early gestation. Environ. Res., 37: 425-432.

Ferm, V.H. and D.P. Hanlon, 1986. Arsenate-induced neural tube defects not influenced by constant rate administration of folic acid. Pediatr. Res., 20: 761-762.

Gandhi, D.N., G.M. Panchal and K.G. Patel, 2011. Developmental and neurobehavioral toxicity study of arsenic on rats following gestational exposure. Ind. J. Exp. Biol., (In Press).

Golub, M.S., M.S. Macintosh and N. Baumrind, 1998. Developmental and reproductive toxicity of inorganic arsenic: animal studies and human concerns. J. Toxicol. Environ. Health. B Crit. Rev., 1: 199-241.

Grant, L.D., C.A. Kimmel, G.L. West, C.M. Martinez-Vargas and J.L. Howard, 1980. Chronic low-level lead toxicity in the rat, Effects of postnatal physical and behavioral development. Toxicol. Appl. Pharmacol., 56: 42-58.

Hass, U., 2006. The need for developmental neurotoxicity studies in risk assessment for developmental toxicity. Reprod Toxico., 25: 753-757.

Holmberg, R.E. and V.H. Jr Ferm, 1969. Interrelationships of selenium, cadmium and arsenic in mammalian teratogenesis. Arch Environ. Health, 18: 873-877.

Holson, J.F., D.G. Stump, K.J. Clevidence, J.F. Knapp and C.H. Farr, 2000a. Evaluation of the prenatal developmental toxicity of orally administered arsenic trioxide in rats. Food chem. Toxicol., 38: 459-466.

Holson, J.F., J.M. Desesso, C.F. Jacobson and C.H. Farr, 2000b. Appropriate use of animal models in the assessment of risk during prenatal development: An illustration using inorganic arsenic. Teratology, 62: 51-71.

Hood, R.D. and C.T. Pike, 1972. BAL alleviation of arsenate-induced teratogenesis in mice. Teratology, 6: 235-238.

Hood, R.D., 1972. Effects of sodium arsenite on fetal development. Bull. Environ. Contam. Toxicol., 7: 216-222.

Hood, R.D., G.T. Thacker, B.L. Patterson and G.M. Szczech, 1978. Prenatal effects of oral versus intraperitoneal sodium arsenate in mice. J. Environ. Pathol. Toxicol., 1: 857-864.

Hood, R.D., W.P. Harrison and G.C. Vedel, 1982. Evaluation of arsenic metabolites for prenatal effects in the hamster. Bull. Environ. Contam. Toxicol., 29: 679-687.

Huot, R.L., M.E. Gonzalez, C.O. Ladd, K.V. Thrivikraman and P.M. Plotsky, 2004. Foster litters prevent hypothelemic-pitutary-adrenal axis sensitization mediated by neonatal maternal separation. Psycho Neuroendocrinology, 29: 279-289.

Kostas, J., D.J. McFarland and D.G. Drew, 1976. Lead induced hyperactivity: chronic exposure during the neonatal period in the rat. Pharmacology, 14: 435-442.

Lehmann, J., T. Stohr and J. Feldon, 2000. Long-term effects of prenatal stress experiences and postnatal maternal separation on emotionality and attentional processes. Behav. Brain Res., 107: 133-144.

Li, A., 2005. Regulatory developmental neurotoxicology testing: data evaluation for risk assessment purposes. Environ. Toxicol. Pharmacol., 19: 727-733.

Mello, C.F., C.F. Kraewer, A. Filippin, V.M. Morsch and A.L.S. Rodrigues *et al.*, 1998. Effect of lead acetate on neurobehavioral development of rats. Brazil. J. Med. Biol. Res., 31: 943-950.

Moser, V.C., 1989. Screening approaches to neurotoxicology: a functional observational battery. J. Am. Coll. Toxicol., 8: 85-93.

Motz, B. and J. Alberts, 2005. The validity and utility of geotaxis in young rodents. Neurotoxicol. Teratol., 27: 529-533.

Ricceri, L., A. Venerosi, F. Capone, M. Cometa and P. Lorenzini *et al.*, 2006. Developmental neurotoxicity of organophosphorous pesticides:fetal and neonatal exposure to chlorpyrifos alters sex-specific behaviors at adulthood in mice. Toxicol. Sci., 93: 105-113.

Rocha, J.B.T., A.J. Freitas, M.B. Marques, M.E. Pereira, T. Emanuelli and D.O. Souza, 1993. Effects of methyl mercury exposed during the second stage of rapid postnatal brain growth on negative geotaxis and on delta-amino levulinate dehydratase in rats. Brazil. J. Med. Biol. Res., 26: 1077-1083.

Rodriguez, V.M., L. Carrizales, M.S. Mendoza, O.R. Fajardo and M. Giordano, 2002. Effects of sodium arsenite exposure on development and behavior in the rat. Neurotoxicol. Teratol., 24: 743-750.

Secher, T., V. Novitskaia, V. Berezin, E. Bock, B. Glenthqjb and B. Klementiev, 2006. A neural cell adhesion molecule-derived fibroblast growth factor receptor agonist, the fgl-peptide, promotes early postnatal sensorimotor development and enhances social memory retention. Neuroscience, 141: 1289-1299.

Tilson, H.A. and V.C. Moser, 1992. Comparison of screening approaches. Neurotoxicology, 13: 1-13.

Vahidnia, A., G.B. van der Voet and F.A. de Wolf, 2007. Arsenic neurotoxicity-a review. Hum. Exp. Toxicol., 26: 823-832.

Vahter, M., 2008. Health effects of early life exposure to arsenic. Basic Clin. Phatrmacol. Toxicol., 102: 204-211.

Vorhees, C.V., 1985. Behavioral effects of prenatal methyl mercury in rats: A parallel trial to the collaboration behavioural teratrology study. Behav. Toxicol. Teratol., 7: 717-725.