Neurotoxicological Outcomes of Gestational Methylmercury Exposure in Rodents

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ABSTRACT

Developmental origin of Health and Disease (DOHAD) hypothesis suggests that early life influences appear as the roots for placing the offspring at a high risk of perinatal mortality. Methylmercury (MeHg) is ubiquitous and persistent environmental pollutant and food contaminant. It's neurotoxic, especially for the developing nervous system. Contrasting studies on the toxic effects of methymercury (MeHg) during developmental stages of Wistar rats, lead us to investigate the neurofunctional effects caused by its gestational exposure, devoid of any overt sign of toxicity and / or gross malformation. The present neurofunctional/behavioral effects in the offspring on postnatal day (PND) (1-28) were investigated following maternal exposure to methylmercury (0.5, 1.0 and 1.5 mg.kg/day) by oral gavages from gestation day (GD) 15 to till parturition. Slowness of surface righting reflex and pivoting was gender specific with significantly observed in male offspring only. The offspring's motor ability was investigated using rota rod shows dose dependent significant reduction in latency to fall (sec.) or spent shorter time on rotating rod (10 RPM; cut-off time: 60s) in male offspring. There were significant reductions in forelimb hanging time, significant increases in hindlimb foot splay in female offspring and reductions in forelimb hanging time with MeHg treatment groups was observed. Significantly increases in CNS activity and excitability by measuring numbers of rear in either sex with high dose MeHg treatment group. These results, combined with those of our earlier study, suggest that gestational exposure would enhance the MeHg-induced maternal and embryo/fetal toxicity, confirmed the high-teratogenic potential along with postnatal neurofunctional/behavioral measurements revealed gender and dose specific impairment of neurofunctional/behavioral performance in offspring of mothers suggest to pay increased attention to MeHg concerning its exogenous use during pregnancy.

Keywords: methylmercury, gestational exposure, neurofunctional/behavioral assessment, rat.

INTRODUCTION

Developmental origin of Health and Disease (DOHAD) hypothesis suggests that early life influences appear as the roots for placing the offspring at a high risk of perinatal mortality. Such early life exposures cause long lasting health effects worsening the quality of life. Due to more hazardous contaminants in our environment, human beings have higher probabilities to be exposed to toxic agents starting at the fertilization throughout the whole life. During the gestational period, mammal's embryo/foetus can be exposed through the maternal intake of toxic compounds. In animal studies, it has been shown that both persistent and non-persistent xenobiotics can induce disruption of brain development when administrated during a period of rapid brain growth in the neonatal rat. During this period it has been established that xenobiotics can cause persistent changes in behavior and brain receptors. It is the presence of the chemicals during a defined period of neonatal brain development that induces persistent brain disorders. Methylmercury (MeHg), an organic methylated form of mercury, exists in aquatics receiving industrial wastes containing mercury, is a global pollutant with known effects on the CNS, especially when the exposure occurs at early developmental stages, as demonstrated in the Minamata astud thtive outcomes of MeHg exposure on fish-eating populations and non-fish-eating populations[5,6], without conclusive results. Mercury (Hg2+) compounds including methylmercury (MeHg) induce neurodegeneration, oxidative stress, alterations in gene expression and declines in immune function, processes that are often associated with the aging process in aquatic animals and humans[7-10]. It is widely recognized that the nature and severity of responses to toxic exposure are age-dependent. Animal studies show that early life history stage, especially embryonic, exposures to toxic chemicals are extremely deleterious due to the high sensitivity of actively developing organ systems[11-15]. Developmental exposure to environmental pollutants has been associated with the onset of cognitive disturbances, due to the sensitivity of the immature central nervous system (CNS) to external insults. Data from experimental studies suggests that neonatal exposure to low-doses of MeHg is associated with visual, memory, and social alterations in nonhuman primates[16-18], memory deficits, and depressive-like behaviour in mice[19] and at higher doses (>3,000 µg/kg/day) severe motor dysfunction and cognitive deficits[20]. Rats, due to their toxicokinetics, must consume 10-fold higher doses of MeHg than humans, nonhuman primates, and mice to achieve similar brain Hg levels and present neurotoxic effects[21,22]. Experimental evidence from pregnant rats exposed to daily doses of 500 µg/kg of MeHg showed that MeHg levels in the brain of pups reached concentrations in the range of MeHg levels found in the brains of infants from populations exposed through fish consumption[22,23]. Very few animal studies have been examined the potential adverse effects of MeHg on the developing offspring taking into account the human exposure scenario of chronic ingestion of MeHg through the consumption of contaminated fish. Numerous experiments using laboratory

animals have confirmed, especially earlier ones[24-28], the toxic effect of MeHg on reproduction and offspring neurobehavioral functions, for only a brief period during gestation. In addition, the endpoints evaluated were often limited in scope. The selection of dose levels, manner of administration and duration of exposure will directly impact on the outcomes being measured. MeHg is toxic to embryotoxic and fetal tissues and can induce embryonal and teratogenic effects in golden hamsters[29], cats[30], rats[31] and mice[32,33]. Several animal studies indicates a strong correlation between exposure to toxic elements such as arsenic[34-36] or mercury[21, 22, 37-41]*in utero* and increased incidence of adverse effects on the development of fetuses and neurobehavioral outcomes. Despite knowledge of the adverse effects on the CNS caused by exposure to high doses of MeHg, there is a gap in knowledge of the detrimental effects of low and continual MeHg exposure during gestation and early postnatal life. The majority of studies evaluated the outcomes at one developmental stage, in some cases finding effects that were not assessed later in life, leaving as an open possibility the amelioration or worsening of the observed effects. Therefore, it is important to determine whether gestational exposure to MeHg produce dose-dependent effects on developmental and neurobehavioral, to support studies in humans and evaluate actions to reduce exposure to MeHg and to apply early interventions to decrease the long-term harmful effects of MeHg. We used a rat model exposed to MeHg from gestational day (GD) 15 to till parturition; the offspring were exposed through the mother, by gavages. The exposure period, doses, and administration routes were chosen to resemble the occurrence of exposures in humans during development. ; in addition, we assess the effect of prenatal exposure of MeHg on 15th day of gestation (GD15), it's a period of timing of histogenesis and synaptogenesis has a detrimental impact on fetal brain development and subsequently early physical and neurobehavioral outcomes

MATERIALS AND METHODS

Animals

Mature male and female Wistar Albino rat weighing 180200g were obtained from National Institute of occupational health (NIOH) breeding colony. After oneweek acclimation in the laboratory, female rats were mated with males (2:1) overnight and examined the following morning for vaginal smears. Vaginal smears were taken daily between 9 a.m. and 10 rats a.m. from mated females. On the day when spermatozoa in the vaginal smear were found, the female was weighted and this day was regarded as the first day of gestation (GD0). During all the experimental period, rats were placed in an animal room (temperature 22 · 2·c, relative humidity of 65 ± 5%, and 12h light/dark cycle), with free access to food (Purina lab chow) and tap water. The study was approved by the Institutional Animal Ethics Committee (IAEC) and the experiments were performed in accordance the guidelines of the Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA), India.

Study Design

Mated female Wistar albino rats were dosed daily with MeHg from GD15 to till parturition. Developmental and Behavioral parameters were assessed in pups from PND1 to PND35 *Chemicals and dosing*

Pregnant animals were treated with MeHg (Sigma Aldrich, USA) at doses of 0.5, 1.0 and 1.5 mg/kg/day by gavages from GD15 till parturition. Control group received 0.9% saline water throughout treatment period.

Exposure and exposure control

In all experimental groups the exposure was carried out from the 15 day of gestation (GD15) until the parturition. All experiments were performed on Wister albino rats and the maternal cohort in experiment (exposure to MeHg) consisted 22 pregnant females separated into four groups; a control group (n=6), 0.5 mg.kg/day MeHg group (n=5); 1.0mg/kg/day MeHg group (n=5) and 1.5mg/kg/day MeHg group (n=6). The level of the dose was based on data showing that at this exposure level, the mercury concentration in newborn rats was comparable to that of found in human infants from populations with high dietary fish consumption[22,42].

Observation made during pregnancy

Details of distribution and fate of all mated rats were given in Table: 1. Beginning on GD20, dams were inspected frequently between 0800 and 2000h for birth until delivery, each presumably pregnant female was checked twice daily for completion of or difficulties in parturition. The day of parturition was defined as postnatal day (PND0), 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 behavior was recorded daily during nursing. The offspring was considered the experimental unit. After parturition, the neonates were observed for mortality and signs of toxicity. Assessment of the reproduction success; offspring's morphological development and maternal behaviors were assessed as earlier reported[37,38].

Neurobehavioral evaluation of pups

Forty pups of either sex (twenty each) were randomly chosen from each treatment groups to perform functional and behavioral development measurements on pre and post-weaning observations and testing on certain postnatal days. Pre-weaning observations and testing such as on set of reflexes or reflexological Tests such as righting reflex on PND4, 6, 8, Cliff Avoidance Reflex (PND10) , Auditory Startle (PND7, 15), Negative Geotaxis (PND10), Forelimb and Hindlimb Grasp (PND11), and Mid-Air Righting Reflex (PND13); sensory development test such as startle response on PND11,13; spontaneous movements (Motor activity) test such as pivoting onPND7, 9, 11; motor and coordination/neuromuscular maturation test such as swimming performance or ontology on PND6. Post-weaning observations and Testing such as rotarod test (PND20) Motor coordination and balance were assessed by using a computerized software base controlled system (Columbus Instruments-Rotamex-5 System, Columbus, OH, USA), consisting of a four-lane rotating drums (7.0 cm x 9.5 cm in diameter for rat), suspended 44.5 cm above the stainless steel floor grid, whose surface was manufactured

from grey PVC with a knurled finished to provide grip for the animals. The rotational speed of rotarod spindle is controlled by system software. Animal falls were detected by Infrared beams sensors in to each compartment and with the unit equipped with an escape preventing transparent cover when a subject has fallen from the rotarod. When the subject has fallen from the rotarod, the system logs this as the end of the experiment for that particular subject. Information such as the total time running on the rotarod (Time Running), and the Rotarod's current speed at the time of the subjects fall are recorded. Rats were acclimated to the moving rod for 3 min on the day before the test. A single session at constant rotation speed mode (10 r.p.m.) was performed: In this procedure (constant speed session), rats were placed on the rotating rod at 10 rotations per minute (r.p.m.) and five trials were conducted monitoring the latency to fall up to 60 sec. Inter trial interval for each animal was 10 min. Grip test for neuromuscular function (PND28), the open field test (PND28), functional observation battery on PND28 and Landing Foot Splay On PND28 were assessed as previously described[38,39].

Statistical analysis

The data are expressed as means ±S.E.M. and statistical significance was assessed by one-way analysis of variance (ANOVA) followed by post hoc Duncan's tests where the treatment effects were detected. Values of probability level $p < 0.05$ were considered as significant.

RESULTS

Effects of exposure to methylmercury

A summary of the distribution and fate of all mated rats of the study is given in Table 1. Pregnant (GD0) females were divided into 4 groups: control (n=6) (with free access to fresh tap water), 0.5mg/kg/day MeHg (n=5); 1.0mg/kg/day MeHg (n=5) and 1.5mg/kg/day MeHg (n=6) by oral gavages. Dam's body weight was noted everyday during gestation. After birth the number of pups for each group was as follows: control ($N = 45$), MeHg 0.5mg/kg/day ($N = 31$); MeHg 1.0mg/kg/day ($N = 35$) and MeHg 1.5mg/kg/day ($N = 47$). We have randomly selected either sex of twenty offspring per litter to achieve/assess the behavioural test (20 males and 20 females). The observer was blind to the treatment as well as evaluation for all sensorimotor test used in this work.

Neurobehavioral assessment

Regarding neurobehavioural parameters, there were nonsignificant adverse effect of MeHg exposure on cliff avoidance (%) in male [$F(3, 76) = 2.36$, $p < 0.078$] and in female [$F(3, 76) = 3.45$, $p < 0.029$] offspring at PND10 (Table 2, Fig.1); and also the post hoc *Tukey* test was significant with 1.0mg/kg/day MeHg treatment group ($p < 0.05$) in female offspring; percentage swimming ontogeny (PND6) in male [$F(3,76) = 0.0$, $p < 1.000$] and in female [$F(3,76) = 0.0$, $p < 1.000$] offsprings as well as assess by score [$F(3,76) = 0.30$, $p < 0.825$] and [$F(3,76) = 0.33$, $p < 0.803$] in male and female offspring respectively (Data not shown). Swimming behavior results show that most of the offspring from each MeHg treatment groups, swims circling, used hind limbs with nose and top of head out of water; and mid-air righting reflex or Arial righting reflex

Table 1: The distribution and fate of all mated rats in the (GD15 to till

Dose MeHg	Control
No. of vaginal smear positive females (GD 0)	6
No. of pregnant female (Day 10)	6
Death	0
Absorption and/or early delivery	0
Evaluated at term	6
Resorbed litters	0
No. of litters	6
Live pups (Male)	17
Live pups (Female)	28
Live pups	45

Table 2: Neurobehavioral data in the offspring of rats exposed to methy

Data are presented as mean · S.E.M. Significantly different from the co

[a] Score: 2= righting in or before the third frame; 1= righting other fr behavior was rated for direction: 3=straight; 2=circular; 1=floating used; 2=all hind limb is used Head angle: 4=ears out of water; 3=ear

did not affected significantly as measured by seconds and score in male [F (3,76) = 0.46, p < 0.711] offspring as well as in female [F (3,76) = 0.52, p < 0.669] with all MeHg treatment groups (Table 2). Surface righting reflex was observed in either sex on PND4. The gender specific variation in spending time to turn over to restore their normal prone position when they were placed in supine position in the animals of MeHg treatment groups as of control. However, this slowness of righting reflex was significantly observed in male offspring [F (3, 76) = 3.90, p < 0.011], whereas non-significant affects in female offspring [F (3, 76) = 3.5, p < 0.029] with MeHg treatment groups (Table 2; Fig.2). There were no differences in the percentage between MeHg treated and control groups of pups eliciting a fully response to startle reflex on PND 7 and 15. Pivoting was observed in sex on PND7, 9 and 11. On PND7, all MeHg treatment groups, male offspring show slowness of pivoting [F (3, 76) = 6.88, p < 0.0003] and also post hoc *Tukey* test [p < 0.05] with 1.5 mg/kg/day MeHg treatment group, whereas non-significantly slowness of pivoting [F (3,76) = 0.8, p < 0.497] was observed with female offspring (Table 2; Fig.3). The animal's ability to hang with forelimb, the length of time it does hang, and its activity while hanging was observed on PND 11 in either sex. There were significant reductions in forelimb hanging time (Seconds) in male offspring [F (3,76) = 42.36, p < 0.001] and also post hoc *Tukey* test [p < 0.01] with 0.5, 1.0 and 1.5 mg/kg/day MeHg treatment groups as well as in female offspring [F (3,76) = 39.89, p < 0.001] and also post hoc *Tukey* test [p < 0.01] with 0.5, 1.0 and 1.5 mg/kg/day MeHg treatment groups (Table 2; Fig.4). There were non-significant reductions in hindlimb hanging time (Sec.) in male offspring [F (3, 76) = 2.96, p < 0.037] and also post hoc *Tukey* test [p < 0.01] with 1.5 mg/kg/day MeHg treatment group. However, female offspring fail to cause reduction in hindlimb hanging time [F (3, 76) = 1.42, p < 0.243] and also post hoc *Tukey* test [p < 0.05] with 0.5 mg/kg/day MeHg treatment group (Table 2). Negative geotaxis reflex as measured by latency in seconds as well as by score. There was non-significant change in time (Sec.) differences between control and MeHg treated groups of male offspring [F (3, 76) = 3.18, *water; 1=unable to hold head up.*

p < 0.028] as well as in female offspring MeHg treatment groups [F (3, 76) = 1.15, p < 0.334] at 30 degree observed (Data not shown). However, there was significant change in time (Sec.) differences between control and MeHg treated groups of male offspring [F (3,76) = 6.94, p < 0.003] and also post hoc *Tukey* test [p < 0.05] with 0.5 mg/kg/day MeHg treatment group as well as in female offspring MeHg treatment groups [F (3,76) = 6.77, p <
0.004] at 45 degree followed by post hoc *Tukey* test [p < 0.01] with 0.5 mg/kg/day MeHg treatment group (Table 3, Fig.5). The offspring's motor ability was investigated using rota rod on PND20. Male offspring [F (3, 76) = 6.14, p < 0.0008] shows significant reduction (sec.) in latency to fall on rotating rod (10 RPM; cut-off time: 60s), whereas

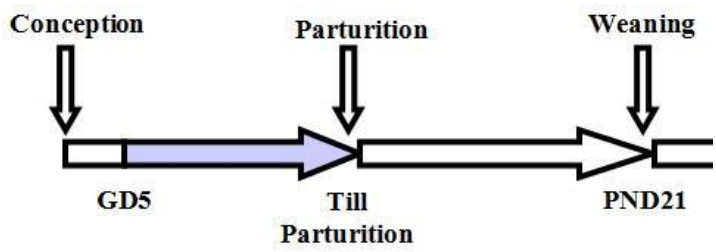

Table 3: Effects on negative geotaxis (latency in seconds, 45 Degree) in the offspring of rats exposed to methyl mercury on gestational day 15 to till parturition. Negative geotaxis on PND 10 (latency in seconds)

	Male	Female
Control	10.04 · 1.55	8.22 · 1.58
0.5 mg/kg	16.94 · 1.66	19.27 · 2.35
1.0 mg/kg	10.16 · 1.97	9.26 · 2.06
1.5 mg/kg	6.03 · 0.63**	15.76 · 2.33**

*Data are presented as mean · S.E.M. Significantly different from the control groups: *p < 0.05; **p < 0.01, respectively.*

Table 4: Effects on rotarod test on PND 20 (cutoff time: 60s; rpm: 10) in the offspring of rats exposed to methyl mercury on gestational day 15 to till parturition.

Rotarod test on PND 20

	Male	Female
Control	12.95 · 0.24	9.23 · 0.25
0.5 mg/kg	10.82 · 0.25	9.15 · 0.22
1.0 mg/kg	9.77 · 0.27**	8.91 · 0.31
1.5 mg/kg	9.59 · 0.31**	8.58 · 0.24

*Data are presented as mean · S.E.M. Significantly different from the control groups: **p < 0.01, respectively.*

female offspring [F (3, 76) = 0.39, p < 0.760] did not show any changes in latency to fall on rotating rod than control. However, male offspring significantly spent shorter time on rotating rod indicated by the post hoc *Tukey* test [p < 0.01] with 1.0 and 1.5mg/kg/day MeHg treatment groups (Table 4; Fig.6). Exploratory activity in an open field in offspring on PND28 was tested. Following parameters, considered to be indicative of spontaneous locomotion, were evaluated: distance travels (Cms), immobility or resting time (Sec.) and rearing (no. of rear). Nonsignificant changes in distance travel in male offspring [F (3, 76) = 0.57, p < 0.636] and in female [F (3, 76) = 1.11, p < 0.350] offspring, whereas significant increase in rear [F (3, 76) = 4.27, p < 0.007] in male and [F (3, 76) = 7.04, p < 0.0003] in female offspring observed respectively (Fig.7). However, significantly increases in numbers of rear indicated by the post hoc *Tukey* test in male offspring [p < 0.01] and in decrease female offspring [p < 0.01] with 1.5 mg/kg/day MeHg treatment groups (Table 5). Nonsignificant decreases in neuromuscular

function/measures such as forelimb grip strength (g) with MeHg treatment groups in male offspring [F (3, 76) = 3.48, p < 0.02]. However, significantly decreases in neuromuscular function/measures indicated by the post hoc *Tukey* test [p < 0.01] in male offspring with 1.5 mg/kg/day MeHg treatment groups; whereas no such effect was observed [F (3,76) = 2.93, p < 0.038] in female offspring (Table 6; Fig.8) but followed by post hoc *Tukey* test significant [p < 0.01] with1.5 mg/kg/day MeHg treatment group; similarly no change in neuromuscular function/measures such as hindlimb grip strength (g) with MeHg treatment groups in male offspring [F (3,76) = 1.82, p < 0.150] and in female offspring [F (3,76) = 2.93, p < 0.038] was observed with 1.5mg/kg/day MeHg treatment group; no changes in hindlimb foot splay (Cms) with MeHg treatment group [F (3,76) = 0.05, p < 0.985] in male offspring, whereas significant increases in hindlimb foot splay (Cms) [F (3,76) = 38.27, p < 0.001] in female offspring was observed and also post hoc *Tukey* HSD test significant[p < 0.01] with 1.5 mg/kg/day MeHg treatment group (Table 6;Fig.9). All together FOB test revealed, sex and dose dependent effect on rearing, forelimb grip strength, hindlimb grip strength and hindlimb foot splay in MeHg exposed offspring.

DISCUSSION

Alteration in the behavior of the mother is known to affect infant development and several drugs have been shown to disrupt elements of maternal behaviors[43]. Thus, any disturbance to maternal care or the delicate mother-pup relationship may explain different patterns of behaviors in the offspring rather than direct effects of prenatal exposure to a toxicant. The results of the present study suggested that control mothers (dam) spent more time involved in the pup-directed behaviors of nursing and licking and less time in nest-building during the first two postnatal weeks than dams treated with methyl mercury during gestation. Methylmercury (MeHg) has deleterious effects on the development of offspring from intoxicated –mother during pregnancy. However, widely use of MeHg and the present data raise concern about the safety of the use of MeHg during gestation or pregnancy period. In addition, the present findings support the use of developmental and behavioral evaluations in animals when assessing the potential developmental neurotoxicology of this chemical in humans. Present findings are important, since malnutrition during pregnancy and lactation as well as alteration in maternal behavior often results in differences in the maturation of physical features and reflexes[44]. The gestational exposure to MeHg influences the rate of physical maturation, sensory motor reflexes, locomotion and exploratory activity and functional observation battery tests in the offspring at different developmental stages

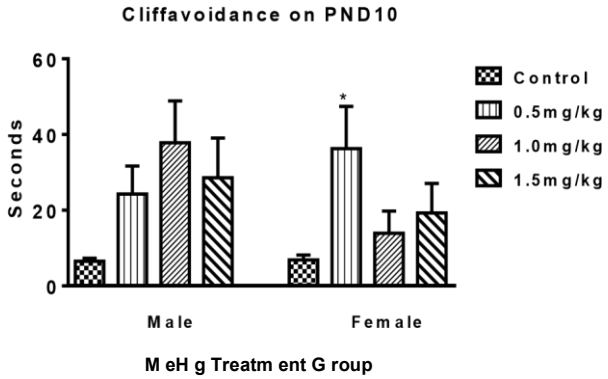

Figure 1: Differences between treated and control groups of male pups eliciting a fully developed cliff avoidance response in seconds. Animals were treated with MeHg (0.5, 1.0.1.5mg/kg/day) or not (Control) during gestation and the test was performed on PND10. Data are expressed as mean ± S.E.M. *(p < 0.05) compared to control group.

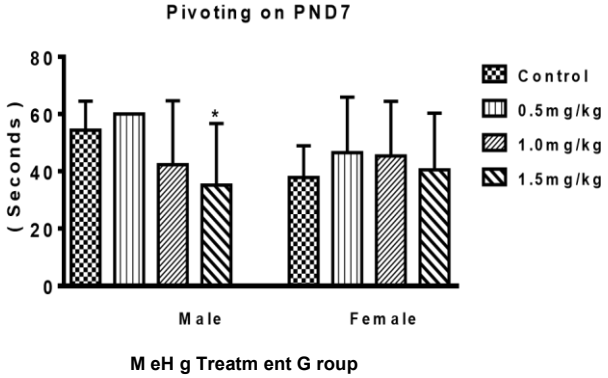

Figure 3: Pivoting (in Seconds) differences between treated and control groups of male and female pups eliciting a fully pivoting. Animals were treated with MeHg (0.5, 1.0, 1.5mg/kg/day) or not (control) during gestation and the test was performed on PND7, 9,11. Data are expressed as mean ± S.E.M. (*p<0.5 compared to control group. n.s.= not significant.

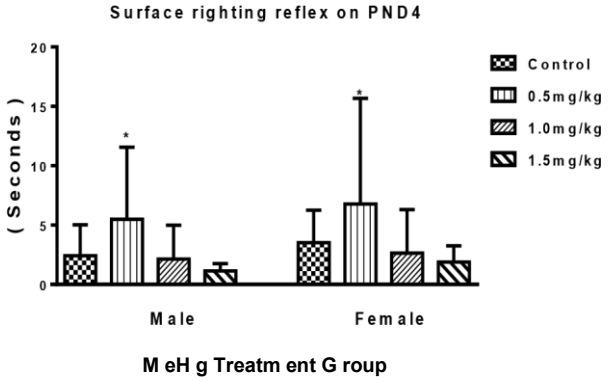

Figure 2: Time differences between treated and control groups of male and female pups eliciting a fully developed surface-righting reflex. Animals were treated with MeHg (0.5, 1.0, 1.5mg/kg/day) or not (Control) during gestation and the test was performed on PND4. Data are expressed as mean ± S.E.M. *(p < 0.05) compared to control group.

Forelim b G rip strength on PN D 11

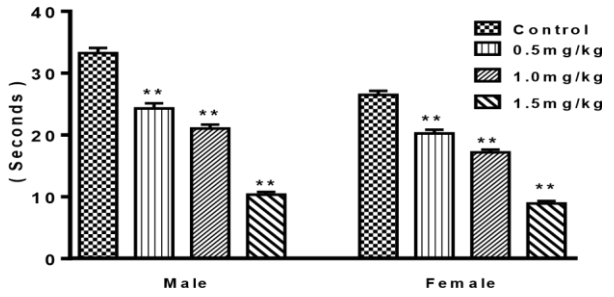

Figure 4: Forelimb grip strength-pup's hanging time (in Seconds) differences between treated and control groups of male and female pups. Animals were treated with MeHg (0.5,1.0.1.5mg/kg/day) or not (control) during gestation and the test was performed on PND11. Data are expressed as mean ± S.E.M. (*p<0.5; **p<0.01; ***p < 0.001) compared to control group.

Table 5: Open Field activity in the offspring of rat exposed to methyl mercury on gestational days PND 15 to till parturition on PND28.

Treatment Groups		DT	RT	V_1C (No. Of rear)
CONTROL (N=20)	Male	103.40 ± 6.31	0.0	3.50 ± 0.58
	Female	97.10 ± 4.14	0.0	2.50 ± 0.77
MeHg0.5mg/kg (N=20)	Male	110.85 ± 9.10	0.0	6.30 ± 1.07
	Female	109.10 ± 5.96	0.0	6.35 ± 0.90
MeHg1.0mg/kg (N=20)	Male	117.50 ± 5.61	0.0	7.40 ± 1.23
	Female	114.05 ± 7.56	0.0	6.95 ± 1.40
Mehg1.5mg/kg (N=20)	Male	121.20 · 7.98	0.0	9.80 · 1.69**
	Female	116.05· 6.94	0.0	12.15 · 2.05**

*Data are presented in mean ± S.E.M. Significantly different from the control group: *p<0.05; **p < 0.01 respectively. DT= Ambulatory distance travelled (Cms); RT= Resting time (Sec.); V_1C= Rearing or jumps.*

during the weaning period as evident. Further, present study with MeHg exposure on GD15 did not reveled significant changed the time course of development of physical landmarks such as pinnadetachment, eye opening auditory startle reflex, incisor eruption, testes descent and vaginal opening. Indeed, it is known that prenatal Hg-treatment, when the neural tube is being formed, resulted in maturational delay of motor responses. Similar findings were observed when pregnant dams were exposed to $HgCl_2$[45] and vanadium[46].

Earlier studies were prompted by the limited epidemiological[47-49] and experimental evidences[37]

Table 6: FOB (CNS excitability and neuromuscular function/measures) in the offspring of rat exposed to methylmercury on gestation day GD15 till parturition on PND28.

		Rearing		Forelimbgrip (g)		Hindlimb grip strength (g)		Hindlimb Splay (cm)	
		Male	Female	Male	Female	Male	Female	Male	Female
Control	Mean	3.50	2.50	170.55	163.40	62.25	59.95	4.93	4.64
	S.E.M.	0.58	0.77	3.29	1.65	0.86	0.57	0.19	0.15
	N	20	20	20	20	20	20	20	20
0.5mg/kg /day MeHg	Mean	6.30	6.35	160.45	155.15	56.85	55.60	3.44**	3.36**
	S.E.M.	1.07	0.90	2.14	4211	1.64	1.01	0.12	0.12
	N	20	20	20	20	20	20	20	20
1.0mg/kg /day MeHg	Mean	7.40	6.95	158.85	152.80	54.95	50.95	3.05**	2.67**
	S.E.M.	1.23	1.40	1.74	1.51	0.71	1.11	0.14	0.09
	N	20	20	20	20	20	20	20	20
1.5mg/kg /day MeHg	Mean	9.80**	12.15**	132.95**	130.15**	52.25	47.95*	2.41**	2.28**
	S.E.M.	1.69	2.05	1.73	1.51	0.80	1.00	0.11	0.11
	N	20	20	20	20	20	20	20	20

*FOB (functional Observation Battery) performed in offspring of rat exposed to MeHg on PND28. Data are presented in mean ± S.E.M. Significantly different from the control group: *p < 0.05 respectively.*

suggesting that developmental exposure to MeHg as contaminants in fish may result in teratogenic effects. Males and females of the each dose exposure groups performed better in the negative geotaxis test than their counterparts from the control group. However, there was a significant change in time (Sec.) differences between control and MeHg treated groups. The earlier studies[26] on motor performance (latency to complete a negative geotaxis response) of rats reported that decreased in negative geotaxis scores, whereas increased in negative geotaxis scores[50] in MeHg-intoxicated animals, showed impaired performance in negative geotaxis test. These differences among results may be attributed to different metals, onset, and duration of exposure and method of imposing heavy metal intoxication. In the present study, pregnant rats were exposed to MeHg dose levels from GD15, pre-weaning tests showed that animals of both genders reached criterion by the time of weaning. However, in a number of observations and tests, significant delay or hastening was observed. A survey of literature shows that subtle changes like the ones observed in the present study did not impair the onset of reflexive behaviour in either sex of offspring screened for mid-air righting reflex(Score), cliff aversion (%) and negative geotaxis(Score), were found. However, several studies[49,51] have reported delay, hastening or no effects following developmental exposure to MeHg or PCBs. Behavioral developmental parameters, Surface righting in either sex showed a significant tendency to be accelerated in 0.5mg/kg MeHg, whereas decelerated in 1.5mg/kg MeHgtreatment groups at PND4 suggesting that the dose levels of MeHg and genders produces influences on surface righting, indicative of development of coordination, in female offspring during the early lactation period (PND4, 6,8). Those newborn rats MeHg-exposed in-utero were slower before reaching the complete upright position on PND 4, 6 and 8. Similarly, the negative geotropism was also impaired dose-dependently by HgCl and this system is responsible for the righting and negative geotaxis reflexes[52] (Roberts, 1967). These results are in agreement with studies that demonstrated a significant retardation in the elicitation of righting reflex in pups exposed during prenatal life to arsenic[53]. Non significant influences on swimming ontogeny at PND6 and startle reflex at PND7 and 15 in either sex of offspring in the present study. The appearance of the startle reflex coincided with ear channel opening, and hearing neuronal circuitry is developed at this age[54,55], suggesting that earlier ear channel opening is a possible explanation for earlier occurrence of the startle reflex. However, the vestibular and proprioceptive systems differentiated by the 14th gestational day[56] and were greatly sensitive to MeHg-exposure during pregnancy. For neurological reflexes, dynamic tests, such as negative geotaxis, cliff avoidance and righting reflexes, were used to evaluate the sensorimotor development[57,58]. The results of the present study revealed delay in surface righting on PND4, with low dose and no delay with higher dose, indicating no impairment in the coordinating movement of the offspring in either sex. Meanwhile, the decrease in forelimb grip strength time measuring on rotating rod on PND20 in male as well as forelimb grip strength in male on PND28 suggests that gender specific effect of MeHg could delay neuromuscular development in the exposed groups. Most of the measured variables did not seem to be impaired at dose levels (0.5mg/kg and 1.0mg/kg) of MeHg probably had minor and/or slight effects on sensorimotor development. With the exception of changes in spontaneous motor activity, surface righting reflex, negative geotaxis and neuromuscular activity, no other effects were observed in either gender, in all treatment groups, with regards to swimming ontogeny and startle reflex. Nevertheless, given the subtle and transient nature of the pre-weaning effects and their lack of gender-and/or compound specificity, their biological significance is unclear, at best. Spontaneous locomotor activity, motor coordination and functional observations battery as postnatal behavioral tests were chosen to assess a variety of behaviors. On PND28 offspring were tested for exploratory activity in an open field. Locomotion frequency measured as distance travels, immobility or

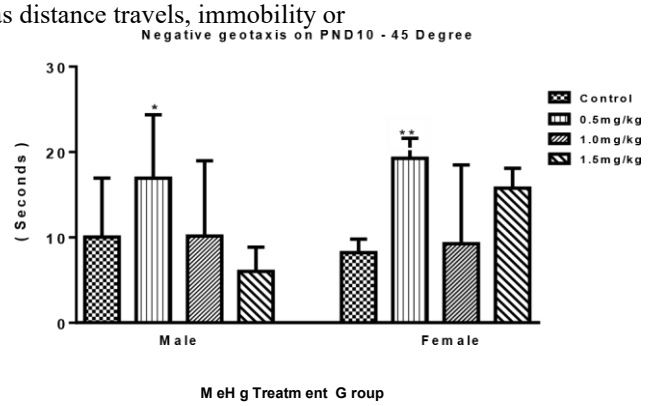

Figure 5: Time differences between treated and control groups of male and female pups (ordinates) eliciting a fully developed negative response geotaxis. Animals were treated with MeHg (0.5, 1.0.1.5mg/kg/day) during gestation and the test was performed on PND10. Data are expressed as mean ± S.E.M. (*P<0.05; **p < 0.01) compared to control group.

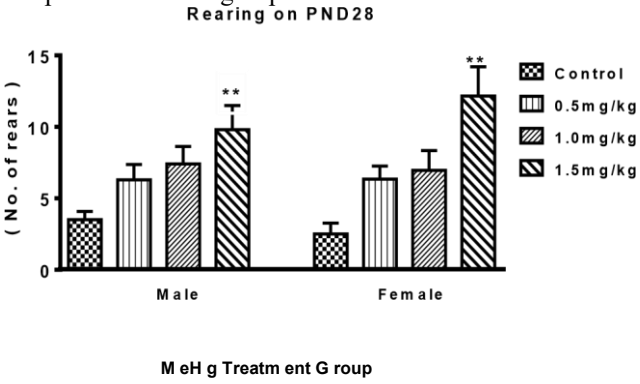

Figure 7: Spontaneous locomotor activity (rearing) in the open field during 60 seconds of recording in rats. Data represents the mean · S.E.M. (either sex n=20). Significantly different from control group: *p<0.05; **p<0.01.

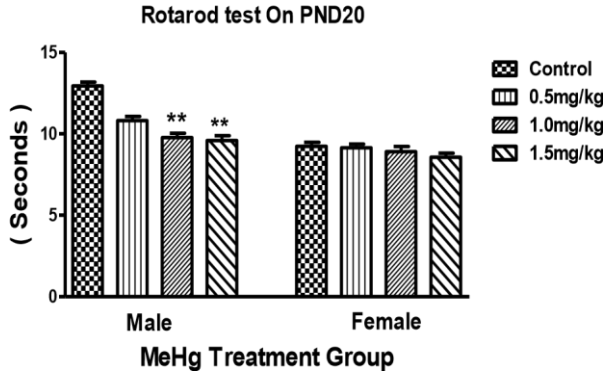

Figure 6: Latency to falling (in seconds) on the rotating rod. Animals were treated with MeHg (0.5, 1.0, 1.5mg/kg/day) during gestation and the test was performed on PND20. Data are expressed as mean ± S.E.M. *($p < 0.05$) and *** ($p < 0.001$) compared to control group.

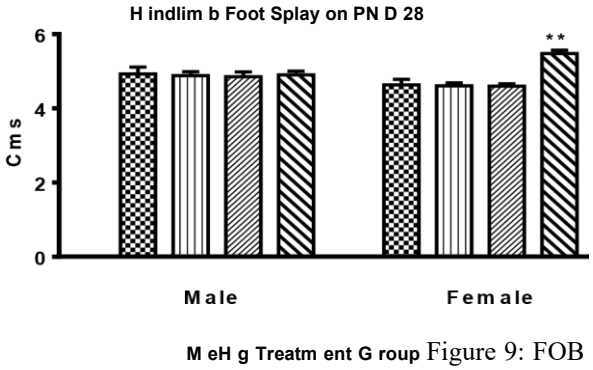

Figure 9: FOB (functional Observation Battery) performed in offspring of rat exposed to MeHg on PND28. Data are presented in mean ± S.E.M.
Significantly different from the control group: **$p < 0.01$ respectively.

resting time and rearing in the open field has been used as an index of both arousal[59,60] and "emotionality"[61]; the decrease or absence of movement within the apparatus normally indicates a reduction in arousal or an increase in the level of emotionality[62]. However, present study spontaneous locomotor activity was assessed in either sex of offspring exposed to MeHg at any dose levels neither played hyperactivity nor hypo-activity as measured by distance travels. In contrast, female offspring exposed to MeHg showed hyperactivity, as previously reported by others[63,64], suggesting impairment in habituation. In light of the present finding, it seems reasonable to suggest that MeHg exposure during gestation did not change arousal and/or emotionality of the offspring. Altogether, the results of the present study show that MeHg causes on age, dose and sex dependent changes in spontaneous locomotor activity suggest that repeated pre-weaning handling of the animals may have subtle effect on spontaneous locomotor performance of the rats. The results of the earlier studies revealed the prenatal exposure of MeHg affected the motor development of offspring. These findings are

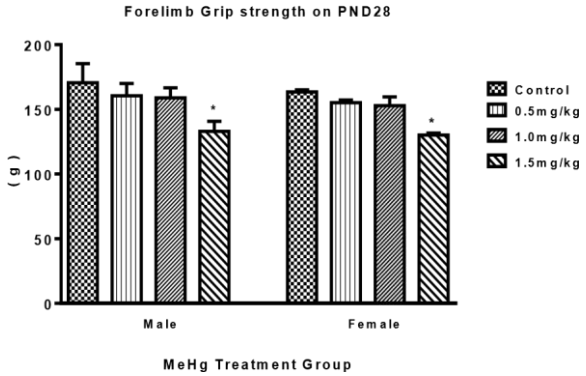

Figure 8: FOB (functional Observation Battery) performed in offspring of rat exposed to MeHg on PND28. Data are presented in mean ± S.E.M.
Significantly different from the control group: *$p < 0.05$; **$p < 0.01$, ***$p < 0.001$ respectively.

consistent with results from high-exposure human studies, which revealed significant delays in aspects of motor development such as crawling, standing, and walking. In fact, offspring of rat exposed to MeHg, were deficient with two muscular tone tasks (rotating rod and forelimb & hindlimb grip strength test) impairment was damaged by MeHg and began to appear on the rotating rod. Shorter latencies before falling during the wire suspension test occurred in MeHg exposure offspring for all doses and were inferior to controls by far. On the contrary, did not find any difference in strength test between offspring exposed prenatally for three consecutive days (PND13, 14, 15) to MeHg and controls[65]. These divergent results might be related to the chemical form of the metal, its doses, the time of treatment during pregnancy, and the method used for behavioral assessment. Excessive MeHg ingestion from a diet high in fish is associated with aberrant central nervous system (CNS) functions[48,66,67]. Recent studies[48,6] in human populations support the earlier findings that maternal exposure to mercury during pregnancy is associated with neurological as well as neuropsychological deficits detectable in the child at 6 to 7 years of age, pointed the selective detrimental effects of MeHg on neurogenesis. However, despite these observations, the issue remains controversial, as exemplified by other studies[70,71] in which no association was noted between MeHg and neurodevelopment outcomes in children at 66 months of age. Several animal studies[38,39,41] also revealed that gender specific impairment of neuromotor performance in offspring of dam exposed to MeHg during different gestational period. However, in the present study we performed Functional Observational Battery on PND28 in offspring of rat exposed to MeHg. Statistically significantly increase in CNS activity and excitability by measuring rearing in either sex; decreases in neuromuscular function/measures such as forelimb in either sex and hind limb grip strength in female offspring with 1.5mg/kg MeHg-treatment groups; decreases in hind limb foot splay measurements revealed dose/gender

specific impairment of neuromotor performance in offspring. Thus, the ultimate effects of MeHg in the human population remain unknown and the risk of teretogenicity, MeHg should be avoided during the period of histogenesis and synaptogenesis and if possible throughout pregnancy, has a detrimental impact on fetal brain development and subsequently early physical and neurobehavioral outcomes. In conclusion, the data reported here raise great concern for the environmental risk to pregnancy, indicating the need to develop more accurate tests for the protection of both the prenatal life and the development of the foetus.

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