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DEVELOPMENTAL AND MATERNAL TOXICITY IN PROGENY OF RAT MOTHERS EXPOSED TO METHYL MERCURY DURING GESTATION

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ABSTRACT

Methylmercury (MeHg) is ubiquitous and persistent environmental pollutant and food contaminant. It's neurotoxic, especially for the developing nervous system. The main source of human exposure to MeHg is seafood. It is well established that exposure to toxic elements such as mercury or arsenic during gestation and lactation may potentially cause adverse effects on the development of fetuses and neonates. The aim of the present work was to find out whether and how exposure at different gestational periods to MeHg affects early developmental millstones and neurobehavioral functions in maturity. Therefore, the earlier developmental and neurobehavioral effects in the offspring on postnatal day (PND) (1-28) were studied following maternal exposure to methylmercury (0.5, 1.0 and 1.5mg.kg/day) by oral gavages from gestation day (GD) 8 to till parturition. It's a period of timing of neurulation and organogenesis. Neither maternal toxicity nor any noticeable signs or symptoms were observed with all exposure/treatment groups. Maternal weight gain (%) during gestation was reduced significantly only in those animals that had received 1.0 and 1.5mg/kg/dayMeHg treatment groups on day 8 of gestation. There were no absorption or early deliveries observed in all dose levels MeHg exposures, except two pups of deaths in each MeHg-treated groups, In contrast, dam treated with 1.5mg/kg/day MeHg-treatment

group alone caused reduction in pups, dead fetuses, as well as the percentage of post implantation loss were significantly affected. The length of gestation was increased and/or delays in delivery at 1.5mg/kg/day MeHg treatment group. The number of dams that delivered viable litters reduced in dose dependant manner. The number of live pups per dam treated with 1.5 mg/kg/day MeHg treatment group affected significantly. There was a significant increased in percentages of pups' mortality with 1.5 mg/kg/day MeHg group on PND1-4. However, the values of the viability index (i.e. percentage of pups surviving beyond PND4) as well as resorption per litter were significantly affected with 1.5-mg/kg/day MeHg dose 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 of MeHg suggest to pay increased attention to MeHg concerning its exogenous use during pregnancy.

KEYWORDS: *methylmercury, prenatal exposure, postnatal development, maternal toxicity, fetotoxicity, rat.*

INTRODUCTION

Methyl mercury, an organic methylated form of mercury, exists in aquatics receiving industrial wastes containing mercury. Fish and other aquatic organisms are important source of dietary proteins for many human populations. Fish meat, however, is contaminated with methylmercury (MeHg), a potent neurotoxin. The accumulation of MeHg in fish tissue (10–40 ppm) and subsequent consumption of this contaminated fish tissue by humans pose the greatest health risk. The health impact of water contamination of methyl mercury continues to draw concern of researchers since accidental poisoning that occurred in Minamata, Japan, Niigata and Iraq[1,2] Faroe Islands cohort study reported, MeHg related deficits in some neurological and cognitive functions in school-age children.[3] The principal sources of exposure to mercury in the general population are ingestion and inhalation of mercury compounds from dental amalgams, and ingestion of fish (fresh water and marine) and seafood, which contain mercury, primarily as methyl mercury. Due to its ubiquitous presence in the environment, health concerns are increasing. The epidemiological and animal studies demonstrate that the fetuses are more vulnerable to MMC neurotoxicity than mothers, as the sensitivity of the nervous system to MeHg toxicity is the highest during developmental stages.[4, 5] The major target site of MeHg intoxication was nervous system specifically on brain functions involved in sensory and coordination skills.

In order to better understand the potential risk of MeHg exposure and to provide necessary control, an extensive experimental study on the effect of prenatal, postnatal and perinatal MeHg exposures were conducted in last few decades.[4-9] However, the detail mechanisms of MeHg induced developmental neurotoxicity are still not fully understood. It is well established that exposure to toxic elements such as mercury[10] or arsenic[11-12] during gestation and lactation potentially cause adverse effects on the development of fetuses and neonates.[8] Thus, it is likely that maternal fish intake-related MeHg exposure during pregnancy at levels safe for mothers may affect adversely the developing nervous system of the foetus. This possibility is supported by data from studies of the victims of the mass MeHg poisonings in Japan.[1-2] Although, no evidence of maternal and embryo/foetal toxicity when high doses of MeHg were given by gavages during GD8 till parturition to pregnant rats; signs of maternal and developmental toxicity in rat were observed when MeHg was given concurrently. So it further worsened MeHg toxicity even before birth, adding up the impact throughout life. However, the test procedure, the route of administration, the dose, the duration of exposure, the period of exposure and the age, play important role in the laboratory outcomes of methyl mercury induced developmental neurotoxicity. It is essential to establish an ideal study design, which more closely resembles that of humans. However, till date, no such study design is established that may conquer all these lacunas. Therefore, the objective of present study was to establish the experimental design that can be better representative of possible human exposures, to investigate at which developmental stage, MeHg cause neurotoxicity to the rat's fetuses and to assess whether in utero/gestational methyl mercury (MeHg) exposure, a well-known teratogen, has a detrimental impact on early physical and neurodevelopment outcomes.

MATERIALS AND METHOD

Animals

Mature male and female rat weighing 180-200g were obtained from National Institute of occupational health (NIOH) breeding colony. After one-week 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.

Chemical

Exposure to methylmercury (MeHg) was given by oral gavages and it was purchased from Sigma Aldrich, USA.

Exposure and exposure control

In all experimental groups the exposure was carried out from the 8 day of gestation (GD8) until the parturition. All experiments were performed on Wister albino rats and the maternal cohort in experiment (exposure to MeHg) consisted 24 pregnant females separated into four groups; a control group (n=6), 0.5mg.kg/day MeHg group (n=6); 1.0mg/kg/day MeHg group (n=6) 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.[13-14]

Observation made during pregnancy

Details of distribution and fate of all mated rats were given in Table: 1. Beginning on GD20.

Table 1: The distribution and fate of all mated rats in the (GD8 to till parturition) study.

Dose	Control	0.5mg/ kg/day MeHg	1.0mg/ kg/day MeHg	1.5mg/ kg/day MeHg
No. Of Vaginal smear Positive Females (GD0)	6	6	6	6
No. Of pregnant Female (day10)	6	6	6	6
Deaths	0	0	0	0
Absorption or early or delay in deliveries	0	3*	3*	2*
No delivery	0	0	1	1
Evaluated at term	6	6	5	5
Resorbed litters	0	0	0	0
No of litters	6	6	5	5
Live pups (Male)	27	21	23	23
Live pups (Female)	28	26	23	22
Total Live pups	55	47	46	45

***Delay in delivery**

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

The offspring were evaluated for survival, growth, development and behaviors. When parturition complete, the numbers of stillborn, implantation, post implantation, resorption and live pups in each litter were recorded. Following variables were observed: Birth measures: Offspring were examined on PND1 for obvious morphological anomalies (e.g., missing digits, facial malformations etc.), sexed by relative anogenital distance and culled pseudo-randomly to forty animals each and balanced for sex (20 females and 20 males) to the extent possible.

Assessment of the offspring's morphological development

Gestation length was calculated at birth and the following offspring data were collected on PND1: Pups size, sex ratio (as percent males), body weight for each pup and the number of malformed offspring. Neonatal death was noted from PND1 through PND5. On PND1, the pups were identified within each groups of treatment and were assessed. Pups from each litter were weighed on PND1, 7, 14, and 21. Offspring remained with their biological mother and postnatal bio-behavioural maturation of the pups was assessed over the first 3 postnatal weeks, until they were weaned on PND23.

Maternal behaviours

A maternal behaviour was observed daily in the home cage of each dam and her litter between gestational day 21 and post-delivery (PND) 1 till 14. The time of observation was during the light phase of light/dark cycle, between 08.00 and 09.00h. The following behaviours were recorded: number of pups nourishes; number of pups not nourishes; number of pups with mother; number of pups alone and number of pups with others.

Statistical analysis

Data were analysed by one-way analysis of variance (ANOVA) followed by Duncan test. The level of statistical significance was set at $p< 0.05$. All data are expressed as means ± S.E.M.

RESULTS

Effects of exposure to methylmercury

Maternal health status and reproduction outcome

During pregnancy, the treatment groups did not differ in water and food intake, and in the rate of the body mass increase. No statistical difference in body weight was observed between control and MeHg exposed dams. The pregnant rats from GD8 to till parturition at 0.5, 1.0 and 1.5mg MeHg /kg/day produced neither maternal toxicity nor any noticeable signs or symptoms. The behaviour of the treated rats was 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 167.71±6.22; 191.54±13.32; 196.02±6.25 and 197.30±12.09. On day 20 of gestation, the maternal body weight gain (g) of control, 0.5, 1.0 and 1.5mg/kg MeHg exposed dams were 86.54±11.98; 72.47±6.26; 47.54±12.12 and 52.85±13.34. Maternal weight gain (%) of dams during gestation and weight gain during treatment was significantly reduced at 1.5mg/kg/day MeHg treatment group. The dams of 1.5mg/kg/day MeHg treatment group showed no sign of anxiety, hindlimb ataxia or gait alterations at the end of the treatment period. The 0.5 and 1.0mg/kg/day MeHg treatment groups did not differ from the control group in the level of food and water consumption and body weight gain, whereas maternal body weight during gestational period (GD0-20) significantly reduced in 1.5 mg/kg/day MeHg treatment group.(Table: 1a;Table: 2; Fig.1)

Table 2. Effect of Methylmercury on maternal weight (gm) on different gestational days.

MeHg treatment Group	GD0	GD5	GD7	GD14	GD21
Control (n=6)	167.71 ± 6.22	175.22 ± 7.43	178.19 ± 8.39	200.08 ± 9.28	254.25 ± 16.81
0.5mg/kg (n=6)	191.54 ± 13.32	194.57 ±13.71	196.46 ±12.95	214.28 ±13.32	264.02 ± 17.52
1.0mg/kg (n=5)	196.02 ± 6.25	198.91 ± 6.00	202.88 ± 6.24	217.44 ± 8.24	253.06 ± 16.47
1.5mg/kg (n=5)	197.30 ± 12.09	201.73 ± 2.49	203.64 ± 1.77	221.53 ± 9.87	260.73 ± 7.75

Data are expressed as Mean ± S.E.M, n = number of dams in respective groups of the treatment.

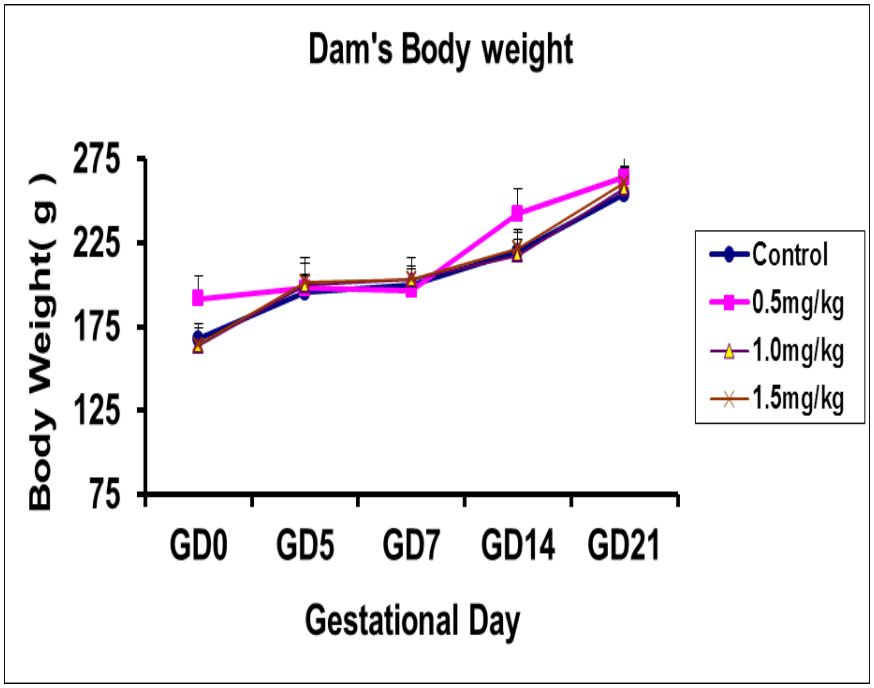

Figure 1: Effects on maternal body weight of rat (Dam) exposed to MeHg on gestational day 8 to till parturition. Data are presented as mean ± S.E.M.

In addition body weight gain (%) during gestation significantly decreased in dams given MeHg at 1.5mg/kg/day [F (3, 76) = 3.43, $p < 0.05$]; (Fig. 2). However, there were non-significant difference in litter size [F (3, 21) =0.3, $p < 0.825$] in either sex were noted. There were no deaths, absorption or early deliveries due to MeHg 0.5, 1.0 and 1.5mg/kg/day exposures. In contrast, treatment of dam with 1.5mg/kg/day alone caused reduction in percentages of litter's size (Table: 1a; Fig.3).

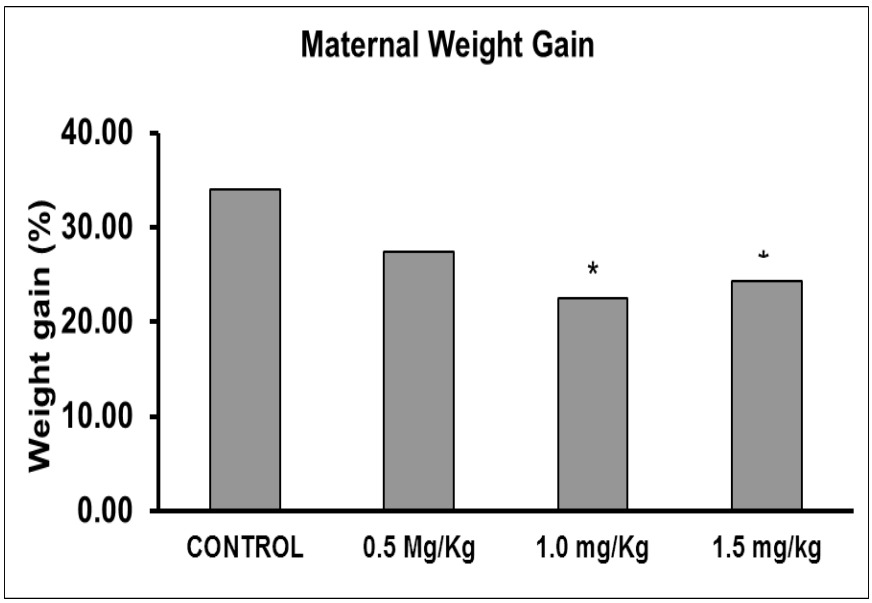

Figure 2: Effects on maternal weight gain (%) of rat (Dam) exposed to MeHg on gestational day 8 to till parturition Data are presented as mean ± S.E.M.

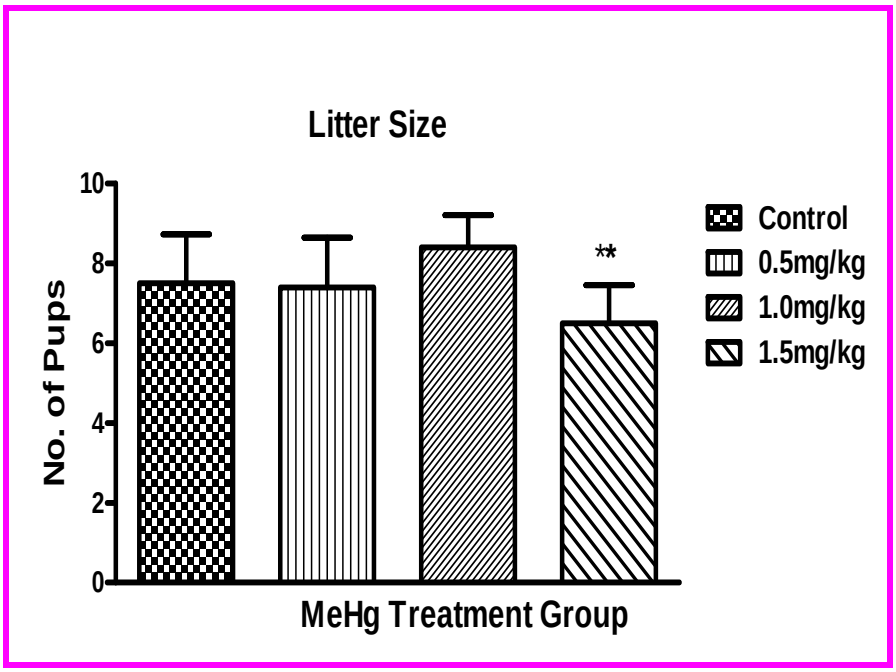

Figure 3: Effects on litter size of rat exposed to MeHg on gestational day 8 to till parturition. Data are presented as mean ± S.E.M. Significantly different from the control group: **p<0.01 compared to control respectively.

Embryo/Fetal Toxicity

A summary of the fetal toxicity finding is presented in Table: 1a. There were no significant differences among groups in the number of total implants per litter or in the sex ratio. The total resorptions and dead fetuses, as well as the percentage of post implantation loss were, however, significantly affected by treatment with 1.5mg/kg/day dose. The average total body weight on PND1 per litter was not affected with 0.5 and 1.0 mg/kg MeHg dose. The number of pups delivered varied from 7 to 10 in both control and MeHg –treated dams (Table: 3).

No significant differences were detected in MeHg-treated female offspring's body weight, as compared to control, neither at birth nor at PND1, 7, 14, and PND21 with 0.5, 1.0 and 1.5mg/kg/day MeHg groups. However, significant differences were detected at PND7, 14 and 21 with all three doses of MeHg-treated male offspring; also significant differences were detected in MeHg-treated male vs female offspring at PND7, 14, and 21 with 1.5mg/kg/day MeHg treated group (Figs. 3A and 3B).

Table: 3. Gestational length and pups viability at birth of methyl mercury exposed (GD8 to till parturition) groups of dams.

MeHg-treatment groups	Gestational length (days)	Total no. of pups born	No. of Pups alive (N)	No. of pups dead	Maternal Weight Gain (g)	Maternal Weight Gain(%)
Control (n=6)	21.29±0.00	55	55	0	86.54±11.98	34.04
0.5mg/kg (n=6)	22.00±0.00	47	45	2	72.47±6.26	27.45
1.0mg/kg (n=5)	21.50±0.34	46	44	2	47.54±12.12*	22.54*
1.5mg/kg (n=5)	22.33±0.21	45**	43	2	52.85±13.34*	24.32*

Data are expressed as Mean ± S.E.M, n = numbers of dams in respective groups of the treatment. *p<0.05, and **p<0.01 compared to control respectively.

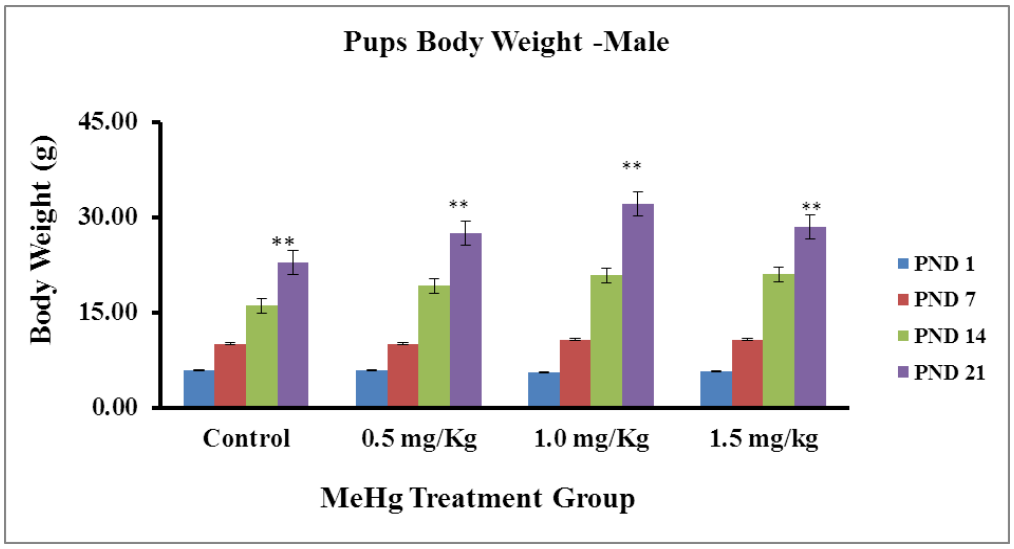

Figure 3A: Effects on body weight in the Male offspring of rat exposed to MeHg on gestational day 8 to till parturition. Data are presented as mean ± S.E.M. Significantly different from the control groups: **p < 0.001.

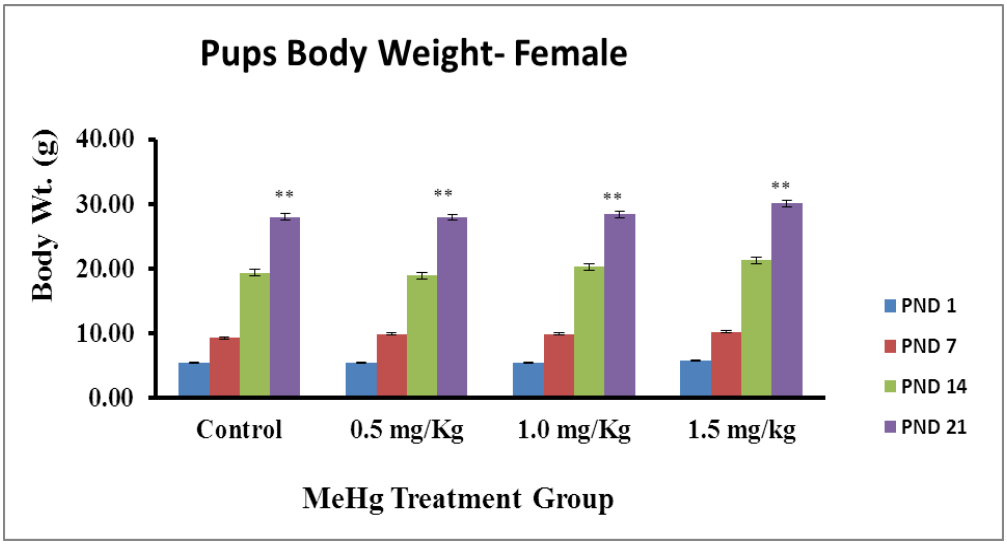

Figure 3B: Effects on body weight in the Female offspring of rat exposed to MeHg on gestational day 8 to till parturition. Data are presented as mean ± S.E.M. Significantly different from the control groups: **p < 0.01.

There was no significant difference between 0.5 and 1.0mg/kg/day MeHg treatment groups, as compared with control in the number of pups per litter, male/female ratio, or the number of stillbirths. In the 1.5 mg/kg/day MeHg treatment group, however, the values of the viability index (i.e. percentage of pups surviving beyond PND4) were notably lower (Tables: 4 and 5).

Table 4: Effect of methylmercury exposure from GD8 to till parturition on pup's mortality.

Treatment groups MeHg	Litter size	Gender ratio (M/M+F)	Pups mortality	
			PND 1-4 (%)	Litters affected
Control	7.50 ± 1.23	0.37	0.0	0/6
0.5 mg/kg/day	7.40 ± 1.25	0.40	0.27	2/6
1.0 mg/kg/day	8.40 ± 0.81	0.38	0.24	2/5
1.5 mg/kg/day	6.50 ± 0.96	0.58	0.31**	2/5

*Mortality rate in the offspring of rat exposed to methyl mercury. Data are presented as mean ± S.E.M. Significantly different from the control group: **p <0.01.*

Table 5: Physical and functional assessment in the offspring of rat exposed to methyl mercury on gestational day 8 to till parturition.

	MeHg- Treatment groups			
	Control	0.5mg/kg/day MeHg	1.0mg/kg/day MeHg	1.5mg/kg/day MeHg
Percentage of live pups at birth	100.00	100.00	100.00	100.00
No. of fetuses/litter	7.50 ± 1.23	7.40 ± 1.25	8.40 ± 0.81	6.50 ± 0.96
Viability (%)	100.0	77.77	71.11	60.00**
Gestational length (day)	21.29 ± 0.18	22.00 ± 0.00	21.50 ± 0.34	22.33 ± 0.21

*Data are presented as mean ± S.E.M. Significantly different from the control group: **p < 0.01.*

Effects of maternal MeHg exposure in the offspring

There were no differences in body weight of male [F (3, 76) = 0.56, p < 0.48] and female [F (3, 76) = 0.19, p < 0.90] offspring at PND1 between the progeny of the control and MeHg exposed groups. In successive days, however, in male and female pups of the 0.5, 1.0 and 1.5mg/kg/day MeHg groups, body weight increases were smaller than in control group. Statistical differences in neither body length in male [F (3, 76) = 0.30, p < 0.825] as well as in

female [F (3, 76) = 0.05, p < 0.985] nor tail length in male [F (3, 76) = 1.94, p < 0.129] and female [F (3, 76) = 0.57, p< 0.636] offspring was noted (Table: 6).

Assessment of the reproduction success

The reproduction success, as measured was unaffected at 0.5, 1.0 and 1.5 mg/kg/day MeHg treatment groups by measuring different gestational parameters. There were no absorption or early deliveries observed in all dose levels MeHg exposures, except two pups of deaths in each MeHg-treated groups, In contrast, dam treated with 1.5mg/kg/day MeHg-treatment group alone caused reduction in pups, dead fetuses, as well as the percentage of post implantation loss were significantly affected (Tables: 3 and 6).

Table 6: Effects of Methylmercury exposure from GD8 to till parturition on gestational parameters in pregnant rat.

Dose	Control	0.5 mg/kg MeHg	1.0 mg/kg MeHg	1.5 mg/kg MeHg
No. of dams	6	6	5	5
Implants/litter	7.50 ± 1.23	7.40 ± 1.25	8.40 ± 0.81	6.50 ±0.96
Live fetuses/Litter	7.50 ± 1.23	7.40 ± 1.25	8.40 ± 0.81	6.50 ± 0.96
Dead fetuses/Litter	0	2	2	2
Total resorbed/Litter (%)	0.00	0.00	0.00	0.00
Total resorbed and dead fetuses/Litter (%)	0.00	0.00	0.00	0.00
Postimplantation loss (%)	0.0	0.27	0.24	0.31
Total male pups/dam	2.83 ± 0.46	3.00 ± 0.51	3.20 ± 0.31	4.00 ± 0.59
Total females/dam	4.66 ± 0.76	3.20 ± 0.54	3.80 ± 0.37	3.83 ± 0.57
Sex ratio (M/M+F)	0.37	0.40	0.38	0.58
Average Sex ratio, male (%)	45.94	37.50	34.29	29.31
Males fetal body weight (g; PND 1)	6.05 ± 0.12	5.39 ± 0.10	5.61 ± 0.07	5.65 ± 0.09
Females fetal body weight (g;PND1)	5.54 ± 0.11	5.56 ± 0.09	5.58 ± 0.11	5.82 ± 0.03
Males body length (Cms)	64.45 ± 1.1	65.0 ± 0.7	66.1 ± 0.6	64.5 ± 0.6
Females body length (Cms)	63.5 ± 1.1	64.0 ± 0.7	62.3 ± 0.9	63.1 ± 0.5
Males tail length (Cms)	18.5 ± 0.2	16.2 ± 0.2	15.9 ± 0.4	16.1 ± 0.3
Females tail length (Cms)	17.0 ± 0.2	16.3 ± 0.3	15.9 ± 0.3	15.5 ± 0.2

Gestational parameters in the offspring of rat exposed to methyl mercury. Data are presented as mean ± S.E.M.; significantly different from the control group: $^{**}p$ *<0.01;* $^{***}p$ *< 0.001, respectively.*

Assessment of the offspring's morphological development

The length of gestation was statistically different between the control and 1.0 and 1.5 mg/kg/day MeHg- treated dams. The number of dams that delivered viable litters reduced in dose dependant manner. The number of live pups per dam treated with 1.5 mg/kg/day MeHg treatment group affected significantly. Prenatal administration of MeHg resulted in non-significant change in percentage of live births as well as on viability during the first day of postnatal life with 0.5 and 1.0mg/kg/day MeHg treatment groups (Table: 3). However, there was a significant increased in percentages of pups' mortality with 1.5 mg/kg/day MeHg group on PND1-4(Table:4). The percentages of pups' viability at birth as well as resorption per litter were significantly affected with 1.5 mg/kg/day MeHg dose group. As a result, there was a significant effect of MeHg on post-implantation loss; with the 1.5 mg/kg/day MeHg treated dams, showing a significant change in percentage of resorption of pups whereas no statistical difference was noted in the number of pups per litter, in general appearance, stillbirths, pups weight, mean pup weight, litter size, sex ratio, the viability index and mortality with 0.5 and 1.0 mg/kg/day MeHg treated groups (Table: 4 and 6).

DISCUSSION

In recent years, some attention has been given to consequences of exposure to MeHg during pregnancy and the early postnatal period. Although some attention has been paid to such effects on the foetus development, full evaluation of risk effects has not been undertaken. Few animal studies have examined 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. Many of the animal studies, especially the earlier ones [6, 7, 9], administered MeHg 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 no doubt directly impact the outcomes being measured. Attention has been given to consequences of exposure to methylmercury (MeHg) during pregnancy and the developing foetus. Methylmercury biomagnifies through the food chain and can reach human populations via the consumption of contaminated fish and seafood. MeHg exposure through maternal transfer can induce neurological damage to the developing fetus [13] and such deficits may not manifest themselves until much later.[2] A variety of exposure regimens, therefore, has been used to identify the adverse effects of MeHg on the developing offspring taking into account the human exposure scenario of chronic ingestion of MeHg through the consumption contaminated fish. However, several

experimental studies have confirmed the toxic effect of MeHg on reproduction and offspring neurobehavioral functions, for only a brief period during gestation[4,15-20], perinatal[21] and gestational through postnatal MeHg exposures.[22-24] 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.

The fetus is especially susceptible to MeHg-induced embryo/fetal toxicity found in animals exposed during gestation.[4] Earlier studies have reported that methylmercury-induced embryo/fetal toxicity (including teratrogenesis) in mice and rats.[25-27] Our earlier experimental data from dams exposed to MeHg on GD5 to till parturition also indicate, the number of pups per litter, gender proportion in litters and pup viability were not affected in 0.5 or 1.0mg/kg/day MeHg exposure; where as a high incidence of prenatal mortality, increasing the percentage of postimplantation loss up to 44.83% or resorption (33.33%) in 1.5mg/kg/day MeHg-treatment group.[28] However, in the present study there were no resorptions of pups/offspring with all doses of MeHg treatment groups as well as the pregnancy length (Gestation length) of the animals remarkably increased in high dose of MeHg (1.5 mg/kg/day) exposed during GD8 to till parturition period. Rats treated with MeHg by gavages at 6mg/kg/day from GD 6 to 9 and reported a comparable extension of gestation length, reduced embryonic implantations in the uterus and the number of dams bearing live litters was markedly diminished. Failure to deliver or sustain live pups illustrated the extreme toxicity of this dose.[32] In contrast, the present study with MeHg by gavages at 1.0 and 1.5mg/kg/day from GD8 to till parturition, showed a significantly extension of gestational length, reduced embryonic implantations in the uterus and the number of dams bearing live litters was markedly diminished, failure to deliver or sustain live pups illustrated the extreme toxicity in 1.5mg/kg/day MeHg-treatment group; a reasonable interpretation of which may be that the exposure in the present study was started after implantation of embryo. The pregnant mother, exposed to MeHg at high doses from contaminated fish in Japan reported miscarriages, or had children stillborn or dying shortly after birth.[6] Dietary exposure via drinking water during gestation and lactation at doses of 0.5 and 2.0mg/kg/day was used in some studies.[6.30] Some of the data could be compared with those studies, rats received MeHg at two dosing levels: 2.0 or 6.0mg/kg body weight from GD6 to GD9 as well as 0.5 and 2.0 mg/kg body weight from day 7 of pregnancy (GD7) up to day 21 (PND21) after the delivery to MeHg in drinking water.[31]

Effects of MeHg on reproduction have been studied in nonhuman primates, showing diminished conception rates and increased incidences of abortions and stillbirths in Macaques treated for 4 months at 70mg/kg/day.[29] Our earlier study also confirmed that the prenatal administration of MeHg at 2.0 mg/kg/day from GD5 to till parturition produced adverse effects on developmental outcomes and high-teratogenic potential of MeHg [10, 30] reported altered physical growth of Japanese children exposed to MeHg *in-utero*. In the present study, the body weights of the pups were unaffected at birth and continued to be unaffected throughout the pre-weaning period with respective MeHg-treated groups. The body weight of female offspring increased in postnatal day dependent (PND1-21) whereas bodyweight of male offspring increased only at PND21. In conclusion, prenatal administration of MeHg at dose of 1.5mg/kg/day produced adverse effects on developmental milestones, thereby confirming the high-teratogenic potential of MeHg.

In the present study, MeHg produced significant effects on the female offspring outnumbered the male offspring only in 1.5mg/kg/day MeHg treatment group (Sex ratio: 0.58; 29.31%). However, several report indicates that the females sometimes outnumbered the males in control mice (sex ratio: <0.75).[9, 32] It seems, therefore, that the effect on sex ratio is not caused by MeHg treatment. There was no significant difference between 0.5 and 1.0mg/kg/day MeHg-treatment groups, as compared with control in the number of pups per litter, male/female ratio, or the number of stillbirths. In the 1.5mg/kg/day MeHg-treatment group, however, the values of the viability index (i.e. percentage of pups surviving beyond PND4) were notably lower. However, outcomes from the present offspring's morphological development data indicating the growth was retarded in the progeny of the each exposed groups, possibly due to the poor health of the mothers. The reproduction success, were unaffected in 0.5 and 1.0 mg/kg/day MeHg-treatment groups by measuring different gestational parameters. However, high dose of MeHg-treatment group resulted in 66.56% of resorption of the pups which is one of the most common abnormal signs observed in severe human cases of MeHg poisoning study along with gait abnormalities and ataxia.[33] However, in the present study we could not found gait abnormalities and ataxia in dam treated with 1.5mg/kg/day MeHg throughout the exposure period.

Alteration in the behaviour of the mother is known to affect infant development and several drugs have been shown to disrupt elements of maternal behaviours.[34] Thus, any disturbance to maternal care or the delicate mother-pup relationship may explain different patterns of

behaviours 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 behaviours of nursing and licking and less time in nest-building during the first two postnatal weeks than dams treated with methyl mercury during gestation.

CONCLUSION

It will be interesting to see how the developmental and neurobehavioral effects of early life MeHg exposure are manifested throughout the life span of rodents. Thus, future studies will examine learning and memory of rats at the different stages of the life span, e.g., from early to young to old adulthood, following embryonic MeHg exposure. The results of the study confirmed the high-teratogenic potential of MeHg suggest to pay increased attention to MeHg concerning its exogenous exposure during pregnancy.

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CONFLICT OF INTEREST

No conflict of interest.

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