



PRENATAL EXPOSURE TO AIR POLLUTANTS AFFECTS POSTNATAL DEVELOPMENT AND BEHAVIOR IN MICE

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Abstract

Recent studies suggest that air pollution may affect reproductive outcome. This study in mice aimed to investigate whether exposure to various gases released by air pollution would affect gestation, postnatal development, activity, learning and memory, and biomarkers of trans-placental toxicity. In utero exposure decreased weight gain during lactation. Cognitive function and levels of biomarkers of exposure were generally similar in exposed and control offspring. The chemical composition of the gases and differences in exposure methods may play a significant role on the biological effects observed in this compared to other studies.

Keywords: gestation, postnatal development, biomarkers of trans-placental toxicity



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Back Ground (Greater Noida gets two systems to monitor air quality)

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The Uttar Pradesh Pollution Control Board (UPPCB) has installed two manual ambient air quality monitoring systems in Greater Noida to get exact data on the pollution level in the area. Until now, there is no station in Greater Noida that collects data on air quality. The manual ambient air quality monitoring systems have been set up at New Holland Tractor Limited in Udhyog Kendra and Honda Siel Power products in Kasna. The manual ambient air quality monitoring systems will be operational from December 1. The two locations have been chosen by the UPPCB keeping all the requirements in mind. To set up any such system, the pollution control boards must ensure that there are industries and residences in the area where the system is set up. The UPPCB, on March 31, had floated a tender to set up automatic air monitoring systems in UP cities, including Greater Noida. As per the Central Pollution Control Board (CPCB) guidelines, samples will be taken twice a

week throughout the year to keep a tab on the pollution level. A third party will be monitoring particulate matter (PM) 10, PM_{2.5}, nitrogen and sulphur oxides using the newly installed machines. These are the first monitoring stations to be set up in the Greater Noida region. The UPPCB is also going to install one real-time monitoring system in Greater Noida. “We have purchased one real-time monitoring system which will be operational by December-end. It will help us in getting instant and current levels of pollution. Its server will be connected with the system of CPCB and people will be able to look up data on Greater Noida on the CPCB website along with those of other cities, said Ashok Tiwari, Regional Officer, UPPC. Recently, Delhi-NCR had faced a grave pollution crisis after the air quality dropped to the severe-plus category.

Smog had engulfed the region, mainly due to the burning of crop residue in neighboring states of Punjab and Haryana, vehicular emissions and industrial pollution. “We are taking action against builders and industries which are found not to be following NGT guidelines on pollution control. In the last one month, we have collected over Rs 25 lakh in fines for not following rules and regulations, ” Tiwari said. Until now, the UPPCB was unable to give proper data on Greater Noida owing to the absence of an air quality monitoring system. “We have been using the data from Noida for Greater Noida as well and that was not accurate. Noida and Greater Noida are quite far from each other and there is a noticeable difference in the pollution level at both places, Tiwari said. (Preety Acharya, Hindustan Times)

Ambient Air Quality Monitoring in Greater Noida

Two station points of Greater Noida are chosen for this study for ambient air monitoring. First station point is the construction site of metro station at Pari Chowk and the second one is any residential area in P3. With the help of High Volume Sampler (Respirable Dust Sampler SLE-RDS10), ambient air around the construction site of metro in Pari Chowk was monitored to collect the particulate matter (PM_{2.5} and PM₁₀) for 4 hour a day. Measurement of Sulphur dioxide (SO₂) and Nitrogen dioxide (NO₂) by wet chemical method is fairly simple and can be employed easily in India. High Volume Sampler is being widely used for particulate matter measurement in India.

The Respirable Dust Sampler is meant for monitoring the Total Suspended Particles (TSP) in ambient air conditions. It also simultaneously used for sampling the pollutant gases

like SO₂, NO_X, CL₂ H₂S, and CS₂. These gases are analysed to determine the concentration of specific pollutant.

This high volume sampler separates the particles larger than 10 microns that are present in air stream. These coarse particles are separated before filtering the air on 0.5 micron size filter and allow the measurement of TSP and Respirable fraction of the Suspended Particulate Matter (SPM). The sampler draws the air with the help of high flow rate blower at a nominal flow rate of 1.4 cubic meters per minute. The air passes through the cyclone inside the sampler; the coarse and non-respirable dust is separated from the air stream using the centrifugal force and is collected inside a sampling bottle. This dust size varies from 10 to 100 microns. The fine dust with a diameter of less than 10 microns will pass through the filter paper. The sampler serves as particle collector that collects the particles less than 10 microns on filter paper and bigger than 10 microns in a sampling bottle with the help of a cyclone separator. The sampler features bower motor instead of high speed blower. This is a brushless and noiseless motor with no carbon brushes.

Construction site near pari chowk (metro)

CITIES		PM 10	SO2 µg/m ³	NO2µg/m ³	AQI	S NO.
18/04/17	0.9m ³ /min	1m ³ /min	6.06590g	6.08002g	11.52530g	11.54894g
20/04/17	1.0m ³ /min	1.1m ³ /min	6.05914g	6.09024g	11.52442g	11.57420g

Calculation for pm2.5 & pm10 at construction site

S.NO.		DAY 1	DAY 2
1	PM2.5	0.01412g	0.03110g
2	PM10	0.02364g	0.04978g
3	Flow rate	0.95m ³ /min	1.05m ³ /min
4	Time	4hrs	4hrs
5	Volume of air	228m ³	252m ³
6	For 1m ³ of air	PM2.5=61.92	PM2.5=123.4
7		PM10=103.68	PM10=98.76

Methods

Animals and Exposure

Many time-mated, nulliparous, young adult mice were supplied at GD 3. The animals were, upon arrival, randomly distributed in groups of five and housed in white polypropylene cages with bedding and enrichment under controlled environmental conditions. Food and tap water were provided ad libitum. The day after arrival, i.e. GD 4, the animals were weighed and assigned to two groups of 20 animals each, with similar weight distributions.

Exposure to polluted air was performed in an 18 L inhalation chamber with walls of glass and stainless steel. The airflow in the chamber was dynamic with a flow of 20 L/min and the exposure atmosphere was evenly distributed, with a slightly negative inside pressure. The two groups of mice were exposed to either filtered clean air on GDs 7–19 for one hour/day. The dose level and exposure period were chosen as not to induce marked maternal toxicity or pup mortality based on published studies of developmental toxicity of diesel exhaust (Tsukue N et al., 2002, Fujimoto A et al., 2005, Watanabe N et al., 2001).

Exposure took place between 8.00 am and 3:00 p.m. During exposure, the animals were placed singly in the rooms of a cylindrical wire mesh cage with radical partitions, holding ten mice at a time. The females were observed during exposure for signs of toxicity and returned to their cages less than 10 minutes after the end of the one hour exposure period. Body weight was recorded on GD 4, and before exposure on GD 5, 7, 10, 14, 18, and 19.

Parturition and lactation

After termination of exposure on (Gestation Day) GD 19, the females were housed alone and were monitored for birth each day, early in the light cycle. The expected day of delivery, GD 20, was designated PND 0 for the pups. Weights of dams and individual pups were recorded on (Postnatal Day) PND 2 and the pups were counted and sexed. On this day one pup with median body weight was removed from litters with at least 5 pups, and the lungs and liver were dissected, weighed, and snap frozen in cryotubes (NUNC, Nunc® Cryo Tubes®) in liquid N₂. Pup weights were also recorded on PND 9 and at weaning on PND 22. At weaning, one male and one female with median body weights from each litter were kept for behavioral testing and housed in groups of 5 to 6 mice of the same gender and prenatal exposure status. Thus, the same male and female from each litter took part in all behavioral testing. One male from each litter was allocated to other studies. The females who had not given birth, the dams, and remaining offspring were anaesthetized with a mixture of 1.5 mg/kg hypnorm and 1.5 mg/kg dormico and sacrificed by exanguination on PND 23 or 24. Blood from dams and offspring was stabilized in 72 µL respectively 30 µL 0.17 mol/L K₂ EDTA and kept on ice until centrifugation (2200 RCF at 4°C for 5 min) within 60 min of collection. The number of uterine implantation sites was determined in the dams, and the thymus, lungs, liver, and spleen were dissected, weighed, and snap frozen in cryotubes (NUNC) in liquid N₂. All samples were stored at - 80°C until analysis.

Post weaning growth testing and behavioral testing (Learning and memory)

All investigations were performed during the animals' light cycle, i.e. between 8.00 a.m. and 4.30 p.m. From PND 3, the experimenter was kept unaware as to which group an individual mouse belonged. The same observer was used in any one test. Exposed and control animals were tested alternately. Female and male animals were tested during different weeks. After weaning, the pups were weighed once every month until termination of behavioral tests (19 weeks).

One female and one male per litter were tested. Females were tested at 12 and 16 weeks of age, males at age 13 and 17 weeks as described earlier (Hougaard KS et al., 2005) with minor modifications. The maze consisted of a circular white plastic pool, with a diameter of 100 cm and a height of 45 cm. The pool was surrounded by many external cues (wall lights, wall decorations of geometric figures in black plastic foil etc.), which were visible from within the pool and could be used by the mouse for spatial orientation. The pool was filled to a depth of 27 cm with water at room temperature. Four points on the rim of the pool, N, E, S and W (not true magnetic directions), were used as starting points and divided the pool into four arbitrary quadrants. A circular transparent platform (diameter 10 cm) was situated on a solid support and submerged 1 cm below the water surface, and thus invisible from water level.

The animals were tested in four daily trials using the four starting points assigned in a pseudorandom sequence. One standard-cage with 5–6 animals was placed near the pool. In each trial, the mouse was gently placed in the water facing the wall at the designated starting position. When the mouse swam to and climbed onto the platform, the trial was completed. If the animal failed to locate the platform within 60 sec, it was led to the platform. All animals were left to sit on the platform for 15 sec, before it was returned to the cage. The latency to reach the platform was measured by stopwatch.

Results

Evaluation of Exposure

The inhalation exposure was performed on a daily basis on 13 successive days for both polluted air and control air, each organized in two groups. The polluted air concentration was controlled by filter sampling. The control group was exposed to lab air diluted by HEPA filtered compressed air.

Maternal measures and Litter parameters

No clinical signs of toxicity were observed in the dams during the exposure period and the number of females presenting offspring was similar in the two groups. Air polluted females tended to gain more weight during gestation than did controls (Air polluted weight gain 13.97 ± 0.59 g (SEM) vs. weight gain in controls: 11.87 ± 0.66 g (SEM), $p = 0.05$), but lactational body weights and absolute and relative maternal organ weights at weaning were similar in control and Air polluted dams.

Litter size, gender ratio, and implantation loss did not differ between groups, but statistically lactational body weights were significantly lower in Air polluted than in control offspring. Post hoc analyses showed that shortly after birth, body weights of Air polluted offspring were numerically, but not statistically significantly lower than in controls (controls 1.78 ± 0.03 g (SEM) vs. Air polluted 1.57 ± 0.04 g (SEM)). However, by weaning Air polluted offspring weighed approximately 10% less than control progeny.

When weight gain from postnatal day (PND) 2 to 9 and from PND 9 to 22 was analyzed, the overall analysis showed weight gain to be statistically significantly lower in Air polluted litters compared to control litters. Post hoc analysis indicated that the decreased weight gain was only statistically different in the latter part of gestation. At weaning, one male and one female from each litter were selected for behavioral testing. Body weights of these randomly selected animals did not differ at the day of weaning, and when the weights of these animals were analyzed until termination of behavioral tests, no differences pertaining to prenatal exposure were revealed.

Behavioral tests

At the age of two months, male and female offspring were tested for learning and memory ability in the Morris water maze. The mean escape latency for both groups decreased to approximately 10 sec over the course of the 20 learning trials. When memory was tested three weeks later, all animals recalled the location in the south west quadrant, i.e. only a single male animal used the maximally allowed period of 60 sec without climbing onto the platform. When the platform was moved to a new location in the north east quadrant, time to locate the platform increased momentarily and then quickly decreased again. With the platform in the central position the animals quickly learned the position. Both learning and memory performance was similar in the two prenatal exposure groups of both males and females, but female Air polluted offspring used less time than controls to locate the platform

on the first trial of the spatial reversal learning task ($p < 0.05$). A similar, although statistically insignificant, pattern was observed for male Air polluted offspring. In the Open field, there was a tendency towards increased activity in female Air polluted offspring (Control 8.97 ± 1.0 m (SEM) vs. Air polluted 12.0 ± 2.1 m (SEM), ($p < 0.1$)), whereas male offspring from the two groups behaved similarly (Control 13.0 ± 2.4 m (SEM) vs. Air polluted 14.9 ± 2.5 m (SEM)).

Discussion and Conclusion

The present study assessed the effects of prenatal exposure to air pollution on gestation, postnatal development, behavior, and biomarkers of prenatal exposure. Cognitive function and selected biomarkers only differed slightly between prenatal exposure groups, whereas body weight gain differed between offspring from control and exposed dams, particularly during the latter half of the nursing period. Thus, statistically body weight of pups differed significantly from controls on the day of weaning and rate of weight gain was significantly reduced during the latter half of lactation, i.e. from PND 9 to PND 22. Growth restriction is a common effect of chemical exposure, also when exposure occurs during fetal life. Thus, chemical exposure of the mother during gestation may be associated with prenatal growth restriction and thus reduced birth weight. In the present study, body weights of Air polluted offspring were slightly, but insignificantly, lower than weights of controls a few days after birth. This difference increased during lactation and body weight was especially affected during the latter part of the period, even if Air polluted exposure was terminated just prior to birth. At the lowest dose levels, the body weights were increased in male diesel pups at PND 11 compared to controls, but no differences were observed later in life for this group. In the highest dose-group body weights were decreased from postnatal week 3 to 10, whereas the middle dose was associated with decreased body weights during postnatal week 6 to 9 (Tsukue N et al., 2002). Male offspring from pregnant ICR mice exposed to air pollution particulates with 1.0 or 3.0 mg/m³ Air polluted 12 h/day during GD 2–16 exhibited increased body weights on PND 28, the only time point measured, but no effect was observed at 0.3 mg/m³.

This is possibly owing to gradual release of chemicals attached to emission of Air pollution deposited in maternal lungs, extending the period of internal exposure and thus of induction of effects beyond the period of dosing. Further-more, substances may be secreted into milk. Pregnant rats were exposed to Air polluted on GD 7–20, or during the two last

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weeks of prenatal and two first weeks of postnatal life. Polycyclic aromatic hydrocarbons (PAHs) were measured in fetuses and maternal blood at GD 20, and in breast milk at PND 14. Exposure increased PAH levels in fetal tissues and maternal milk, indicating that PAHs from Air pollution inhaled by the mother transfer to the offspring via the placenta and breast milk (Tozuka Y et al., 2004). If the effects of Air pollution are due to soluble compounds leaching from these particles, the blood levels of these compounds may even increase very slowly, and thus the biological effect may be delayed compared to the period of exposure (Pepelko WE et al., 1983). Finally, chemical substances transferred from mother to fetus may act as toxicants later in life, even if diesel particles were inhaled only during gestation (Newbold RR et al., 2004, Barker DJ et al., 1998).

In utero exposure affected weight gain during lactation. The chemical composition of pollutants and differences in exposure method may play a significant role on the biological effects observed.

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