DEVELOPMENTAL AND HEMOTOLOGICAL RESPONSES TO LOW LEVEL CONTINUOUS EXPOSURE OF NITROGEN DIOXIDE IN MICE

Jarnail Singh, Stillman College, Tuscaloosa, Alabama

ABSTRACT

Young healthy mice were continuously exposed to 0ppm, 0.5ppm, 1.0ppm and 5ppm nitrogen dioxide gas for eight weeks. Nitrogen dioxide exposure for eight weeks decreased the average weight of mice, increased the average weight of lungs, heart, and brain and decrease the average weight of liver. Nitrogen dioxide exposure did not have any effects on the WBC and RBC in mice blood but it increased the HCT and HGB in mice blood. Nitrogen dioxide exposure increased the MCV and decreased the MCH and MCHC in mice blood.

Nitrogen dioxide (herein after referred to as NO₂) is a non-explosive, non-flammable colorless gas that most people can smell at concentrations from 1 to 3ppm. Eye and nasal irritation becomes apparent at concentrations of about 13ppm and accidental exposure to concentrations of 150 to 200ppm can be fatal (1). Due to some unknown reasons intermittent exposure, such as for a worker in a factory, is much less harmful than continuous exposure to similar concentrations, such as breathing the city air, for the same total number of hours. Rats given continuous exposure to 5ppm of NO₂ had 18 percent mortality, where as those exposed intermittently to 5ppm and 25ppm for an equivalent number of hours had no mortality (1). The maximum allowed concentration set by the Environmental Protection Agency (EPA) is not more than 0.05ppm of NO₂ in air on an annual basis.

Air pollution by nitrogen dioxide is a potentially important cause of respiratory infection (2-4). Mice continuously exposed to NO₂ covertly developed pulmonary emphysema, bronchial epithelial hypertrophy and persistent lesions (5-6). Investigations have shown that the pathogenesis of the oxidant induced enhancement in susceptibility to infection involves an inhibition in pulmonary antibacterial defense systems due to alveolar macrophage dysfunction (7-9). Most of these studies, however, confine to the adult laboratory animals. Furthermore, almost nothing is known about the effects of low level continuous exposure of nitrogen dioxide on the newly born to adult laboratory animals.
This study was an effort to determine the response of low continuous exposure of nitrogen dioxide on the development and hematology in mice.

Materials and Methods

Environmental chambers and gas mixtures: Four environmental chambers (one each for 0ppm, 0.5ppm, 1.0ppm and 5.0ppm NO₂) were placed in an air conditioned room in which the average temperature and humidity were 74± 2°F and 54± 2 respectively. The animals were placed in these four chambers for 7 days a week, 24 hours a day for 8 weeks. Each environmental chamber was connected to the gas mixture cylinder on one side and exhaust fan on the other side by means of rubber tubing. Gas mixtures of 0ppm, 0.5ppm, 1.0ppm and 5.0ppm NO₂ were obtained from Matheson Gas Products. The cylinders were fastened with NO₂ regulators and the regulators were fastened with nupro needle valves (micro meter). The valves were connected to the environmental chambers by rubber tubing. The gas flow was set at 200 ml per minute.

Animals: One litter of 3 day old mice along with the mother was placed in plastic cages with wire covers. Three litters in 3 cages with a total of 25-30 mice were placed in each environmental chambers with gases on all the time except for 5 hours a week. This time was used to clean, change, and sterilize the cages. This was carried out once a week for the first three weeks and twice a week for the second five weeks. The mice were given five minutes of fresh air everyday. The food was a special sterilized commercial product (lab blox) for mice. Tap water was provided in sterilized bottles and like the food was available to the mice at all times. The litters were weaned after the first two weeks of exposure and were separated by sexes. At the end of eight weeks, 20 mice from each chamber were weighed, sacrificed and organs removed. These were weighed and blood samples for hemotological studies were collected. Forty four point seven (44.7) lambda blood was transferred to a vial with 10 ml of isoton in it. The vials were capped and the contents were mixed immediately to avoid any coagulation of blood. These samples were read for White Blood Corpuscles, Red Blood Corpuscles, Hemoglobin, Hematocrit, Mean Corpuscle Hemoglobin, Mean corpuscle volume and Mean Corpuscle Hemoglobin Concentration (hersin after referred to as WBC, RBC, HGB, HCT, MCH, MCV and MCHC respectively) on a coulter counter in the local pathology laboratory.

Results

Gross Weight Changes: The initial average weight of mice at the
Figure 1 Average weight of mice at the start of the experiment.

Figure 2 Effects of different concentrations of NO₂ on the average weight change percent of control in mice.

Figure 3 Effects of different concentrations of NO₂ on the average weight (± standard error) of liver in mice.

Figure 4 Effects of different concentrations of NO₂ on the average weight (± standard error) of kidney in mice.

Concentrations of NO₂ start of the experiment for 0, 0.5, 1.0 and 5.0 ppm NO₂ concentrations were 1.8, 2.5, 2.0 and 2.8 gms respectively (Figure 1). At the end of the experiment mice exposed to 0.5, 1.0 and 5.0ppm
concentrations produced an average weight of 85, 80 and 95 gmt of control exposed mice respectively (Figure 2).

Figure 6 Effects of different concentrations of NO₂ on the average weight (± standard error) of heart in mice.

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0 ppm 0.5 ppm 1.0 ppm 5.0 ppm

Concentrations of NO₂

Effects on Vital Organs: Nitrogen dioxide exposure at 1.0 ppm concentration significantly decreased the average weight of liver mice as compared with 0, 0.5 and 5.0 ppm NO₂ exposure in mice (Figure 3). Nitrogen dioxide exposure did not produce any significant changes in the average weight of kidneys (Figure 4) and spleen (Figure 8) in mice. One ppm NO₂ exposure significantly
increased the average weight of lungs (Figure 5) in mice as compared with the control exposed mice and 1 and 5 ppm NO\textsubscript{2} exposure.

Figure 9 Effects of different concentrations of NO\textsubscript{2} on the average RBC count (± standard error) in mice.

Figure 10 Effects of different concentrations of NO\textsubscript{2} on the average WBC count (± standard error) in mice.

Concentrations of NO\textsubscript{2} significantly increased the average weight of heart (Figure 6) in mice as compared with control exposed mice. Five ppm NO\textsubscript{2} exposure significantly increased the average weight of brain in mice (Figure 7) as compared with the control and 0.5 ppm NO\textsubscript{2} exposed mice.

Figure 11 Effects of different concentrations of NO\textsubscript{2} on the average HCT percent (± standard error) in mice.

Figure 12 Effects of different concentrations of NO\textsubscript{2} on the average HGB (± standard error) in mice.

Concentrations of NO\textsubscript{2} Hematological Changes: Nitrogen dioxide exposure at different concentrations did not produce any significant changes on the
Figure 13 Effects of different concentrations of NO₂ on the average MCV (± standard error) in mice.

Figure 14 Effects of different concentrations of NO₂ on the average MCH (± standard error) in mice.

Figure 15 Effects of different concentrations of NO₂ on the average MCHC (± standard error) in mice.

Concentrations of NO₂

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average count of RBC and WBC in the mice blood (Figure 9 and 10). Five ppm NO₂ exposure significantly increased the HCT and MCH in the mice blood (Figure 11 and 12) as compared with the control exposed mice. Mice exposed to 1ppm NO₂ concentration had a
significantly higher amount of MCV as compared with the remaining of the NO2 concentration exposure (Figure 13) and 5ppm NO2 exposure significantly reduced the MCH in mice blood as compared with the rest of the NO2 concentrations (Figure 14). Nitrogen dioxide exposure at 1 and 5ppm concentrations significantly decreased the amount of MCH in mice as compared with the 0 and 0.5ppm NO2 exposure (Figure 15).

Comments

It is apparent from the experimental data that nitrogen dioxide exposure decreased the average weight in mice. These findings are in contrast to the earlier investigations (10). Young developing animals have a large metabolism rate than the large ones and as a result are probably more susceptible to NO2 stress. The increase in the average weight of liver, lungs, heart and brain as a result of NO2 exposure may be due to an increase of blood volume in these organs (6). Five ppm NO2 exposure significantly increased the HCT (hematocrit) and HGB (hemoglobin) in mice blood as compared with the control exposed mice. It largely may be due to an adoptive compensatory mechanism in which the organism compensate for the reduced availability of oxygen by increasing the organism's abilities to extract oxygen from the NO2 environments. The increase in hematocrit (as a response to stress) is further substantiated by the data, that every increase in the NO2 concentration exposure increased the total serum proteins in mice (11).

References


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