Paper No. 17

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 NO_2) 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 NO_2 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 NO_2 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 NO_2 covertly developed pulmonary emphysema, bronchial epithelial hypertrophy and persistent lesions (5-6). Investigations have shown that the pathogenesis of the oxidant induced enhancement in suceptibility to infection involves an inhibition in pulmonary antibacteria defense systems due to alveolar macrophage dysfuction (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 hemotology in mice.

Materials and Methods

Environmental chambers and gas mixtures: Four environmental chambers (one each for 0ppm, 0.5ppm, 1.0ppm and 5.0ppm NO_2) 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 NO_2 were obtained from Matheson Gas Products. The cylinders were fastened with NO_2 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, sacrified 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 Carpuscles, Red Blood Corpuscles, Hemoglobin, Hematocrit , Mean Corpuscle Hemoglobin, Mean corpuscle volume and Mean Corpuscle Hemoglobin Concentration (herein after referred to as WBC, RBC, HGB, HCT, MCH, MCV and MCHC respectively) on a coulter counter in the local pathalogy 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.

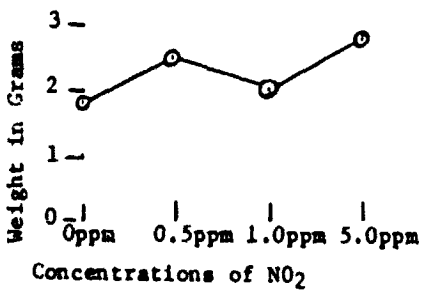


Figure 2 Effects of different concentrations of NO_2 on the average weight change percent of control in mice.

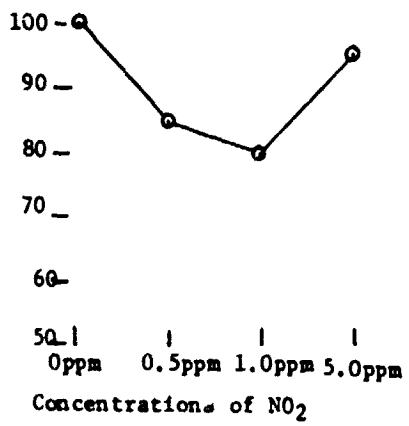


Figure 3 Effects of different concentrations of NO_2 on the average weight (± standard error) of liver in mice.

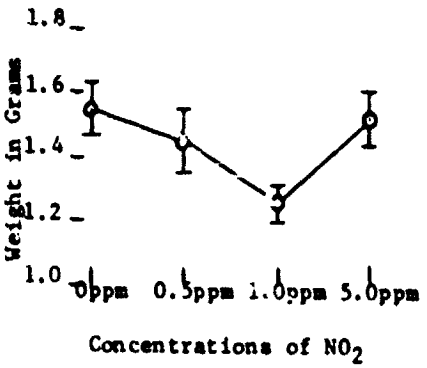
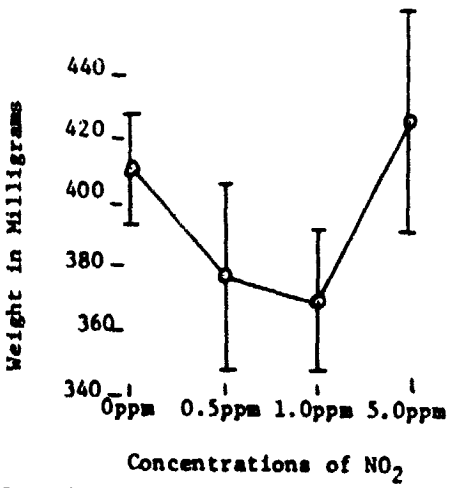


Figure 4 Effects of different concentrations of NO_2 on the average weight (± standard error) of kidney in mice.



start of the experiment for 0, 0.5, 1.0 and 5.0 ppm NO_2 concentrations were 1.8, 2.5, 2.0 and 2.8 gms respectively (Figure 1). At the end of the experiment mice expcsed to 0.5, 1.0 and 5.0ppm

concentrations produced an average weight of 85, 80 and 95
ent of control exposed mice respectively (Figure 2).

e 5 Effects of different
ntrations of NO_2 on the
ge weight (± standard
) of lung in mice.

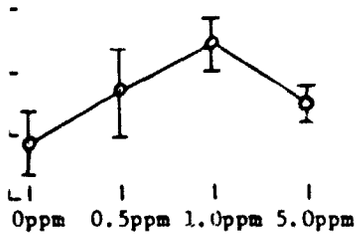
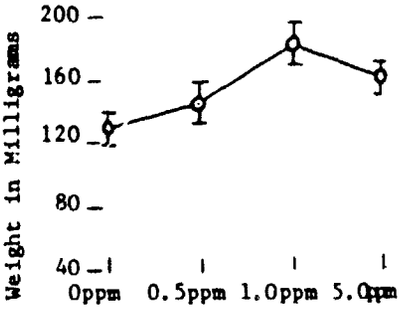


Figure 6 Effects of different
concentrations of NO_2 on the
average weight (± standard
error)of heart in mice.



:cts on Vital Organs: Nitrogen dioxide exposure at 1.0ppm
:entration significantly decrease the average weight of liver
ice as compared with 0,0.5 and 5.0ppm NO_2 exposure in mice
ure 3). Nitrogen dioxide exposure did not produce any sign-

re 7 Effects of different
ntrations of NO_2 on the
ge weight (± standard
r) of Brain in mice.

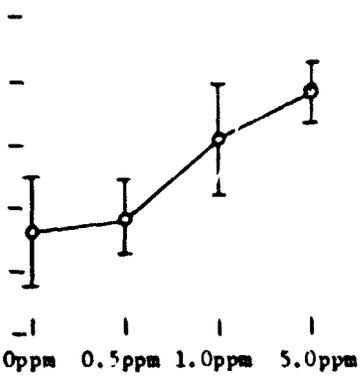
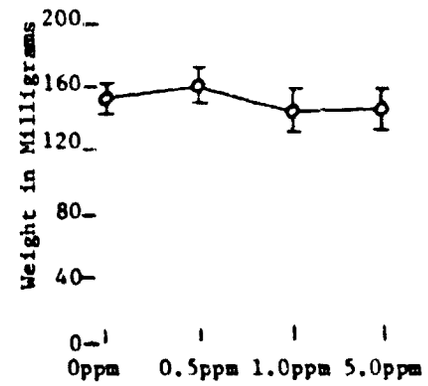


Figure 8 Effects of different
concentrations of NO_2 on the
average weight (± standard
error) of spleen in mice.



cant changes in the average weight of kidneys (Figure 4) and
een (Figure 8) in mice. One ppm NO_2 exposure significantly

increased the average weight of lungs (Figure 5) in mice as compared with the control exposed mice and 1 and 5ppm NO_2 exposure

Figure 9 Effects of different concentrations of NO_2 on the average RBC count (± standard error) in mice.

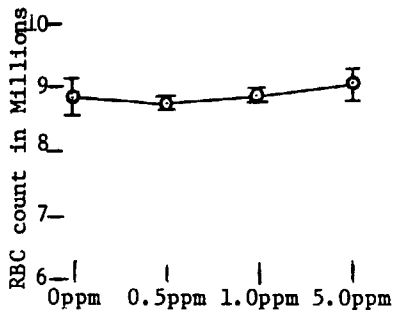
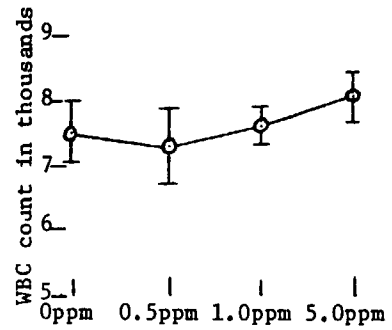


Figure 10 Effects of different concentrations of NO_2 on the average WBC count (± standard error) in mice.



significantly increased the average weight of heart (Figure 6) in mice as compared with control exposed mice. Five ppm NO2 exposure significantly increased the average weight of brain in mice (Figure 7) as compared with the control and 0.5ppm NO_2 exposed mice.

Figure 11 Effects of different concentrations of NO_2 on the average HCT percent (± standard error) in mice.

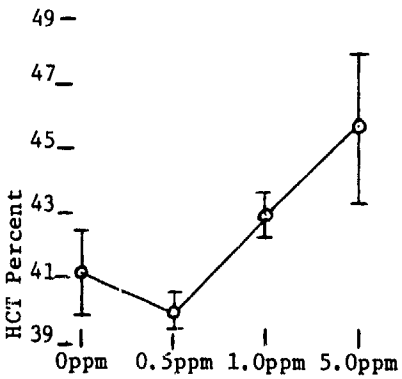
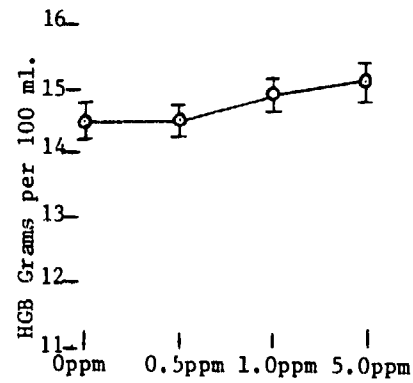


Figure 12 Effects of different concentrations of NO_2 on the average HGB (± standard error) in mice.



Hemotological Changes: Nitrogen dioxide exposure at different concentrations did not produce any significant changes on the

Figure 13 Effects of different concentrations of NO_2 on the average MCV (± standard error) in mice.

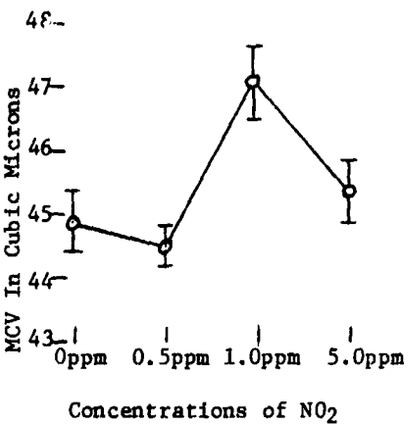


Figure 14 Effects of different concentrations of NO2 on the average MCH (± standard error) in mice.

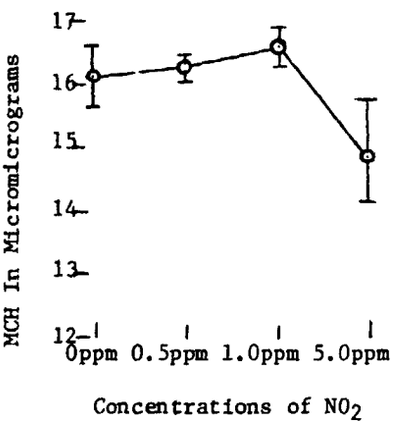
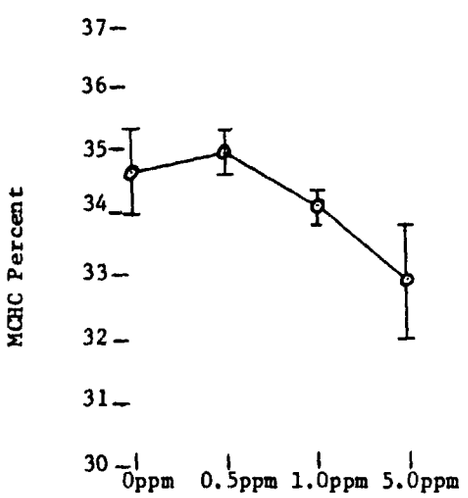


Figure 15 Effects of different concentrations of NO_2 on the average MCHC (± standard error) in mice.



average count of RBC and WBC in the mice blood (Figure 9 and 10). Five ppm NO_2 exposure significantly increased the HCT and HGB in the mice blood (Figure 11 and 12) as compared with the control exposed mice. Mice exposed to 1ppm NO_2 concentration had a

significantly higher amount of MCV as compared with the remaining of the NO_2 concentration exposure (Figure 13) and 5ppm NO_2 exposure significantly reduced the MCH in mice blood as compared with the rest of the NO_2 concentrations (Figure 14). Nitrogen dioxide exposure at 1 and 5ppm concentrations significantly decreased the amount of MCHC in mice as compared with the 0 and 0.5ppm NO_2 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 suceptible to NO_2 stress. The increase in the average weight of liver, lungs, heart and brain as a result of NO_2 exposure may be due to an increase of blood volume in these organs (6). Five ppm NO_2 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 organisms's abilities to extract oxygen from the NO_2 environments. The increase in hematocrit (as a response to stress) is further substantiated by the data, that every increase in the NO_2 concentration exposure increased the total serum proteins in mice (11).

References

1. _____ Chemical Villains: A biology of pollution pp. 120 (1974).
2. Bates, D. V.: Air Pollutants and the human lung. Am. Rev. Respir. Dis. 105: 1-13 (1972).
3. Goldsmith, J. R.: Effects of Air Pollution on Human Health. Air Pollution. New York, Academic Press Inc. 547-615 (1968).
4. Goldstein, E.: Evolution of the role of nitrogen dioxide in the development of respiratory diseases in man. Calif. Med. 115: 21-27 (1971).
5. Freeman, G. and Haydon, G. B.: Emphysema after low level exposure to NO_2. Arch. Environ. Health 8:125-28 (1964).
6. Haydon, G. B., Freeman, G. and Furiosi, N. J.: Covert Pathogenesis of NO_2 Induced Emphysema in Rat. Arch. Environ. Health. 11:776-783 (1965).
7. Purvis, M. R., Miller, S. and Ehrlich, R. J.. Effects of atmospheric pollutants on suceptibility to respiratory infection. Jour. Infec. Dis. 109:238-42 (1961).
8. Ehrlich, R.: Effect of nitrogen dioxide on resistance to respiratory infection. Bacterial. Rev. 30:604-614 (1966).

9. Goldstein, E., Eagle, M.C. and Hoeprich, P.D.: Effect of nitrogen dioxide on Pulmonary bacterial defense mechanism. Arch. Environ. Health 26:202-204 (1973).
10. Freeman, G., Furiosi, N.J. and Haydon, G. B.: Effects of continuous exposure of 0.8ppm NO_2 on respiration of rats. Arch. Environ. Health. 13: 454-456 (1966).
11. Singh, J.: Unpublished data. (1976).

Supported by NIH-MBS
Grant Number 08021