

TOXICOLOGY OF NITRIC OXIDE

Since nitric oxide (NO) is the only oxide of nitrogen present in the smoke from cigarettes made from flue-cured tobacco, this review is concerned primarily with the toxicology of NO. However, the smoke from cigarettes made from air-cured tobaccos may contain very small amounts of nitrogen dioxide (NO₂), and furthermore NO can react with oxygen to form NO₂ with therefore the possibility that some NO₂ may be formed in the lung. It would therefore be unsound to produce a review on nitric oxide without also assessing the possible effects of nitrogen dioxide, or at least demonstrating that nitrogen dioxide is unlikely to be formed.

Toxicological studies on nitric oxide are difficult due to the fact that nitrogen dioxide may be produced in the exposure system by oxidation. Were this to happen, any effects found could be due to NO₂ and not NO.

NITRIC OXIDE

Acute Toxicity

Humans inhaling 15 PPM for 15 minutes are reported to show no effect on lung diffusion capacity, but at levels >20 PPM airway resistance decreased (7,11).

When humans breathed more than 15 PPM for 15 minutes there was a decrease in arterial oxygen partial pressure but alveolar pO₂ was unchanged(11). Airway resistance only increased after the inhalation of >20 PPM.

When 8 healthy male volunteers were exposed to 1 PPM for 2 hours while carrying out intermittent light exercise no symptoms were observed during the exposure period. A small but significant decrease in specific air-way conductance was observed in 4 of the subjects (34).

Dogs exposed to 0.5% and 2% for between 7 and 50 minutes died due to a critical reduction in arterial oxygen content despite inhaling 98% oxygen (14). This was due to either the formation of methemoglobin, low arterial pO₂ or acidemia. These effects are probably due to nitrogen dioxide since it was reported that considerable oxidation of nitric oxide occurred. It has been reported that nitric oxide converts haemoglobin to methemoglobin and induces non-specific bronchopneumonia in dogs (4).

Nitrosile haemoglobin was found in the blood of rats, mice and rabbits which had been exposed to 10 PPM for 1 hour (10). In mice the maximum level was not quite 1.3% of the total haemoglobin and in air this decreased with a half life of 10 minutes. A dose response was found between NO concentration and maximum level of nitrosile haemoglobin.

Rats were injected ip with 5 ml of NO or NO₂ and blood was collected 30 minutes later. Three times as much methemoglobin was formed by NO as by NO₂ (38).

In a study of the transformation of nitrogen oxides when inhaled by animals it was found that in guinea pigs and rabbits who inhaled 20 ml NO₂/M³ for 6 hours nitrate was excreted in the plasma. It was suggested that this was due to rapid oxidation of NO₂ to nitrate by the haemoglobin. In the presence of No the rate of clearance of nitrate was slower due to the fact that NO had first to be oxidised to NO₂ (31).

When primary lung cells from rats were exposed to 8-27 PPM for 3 hours, only the highest concentration (27 PPM) significantly increased mutation (39).

103546458

Sub-Acute Toxicity

When nitric oxide was added to cigarette smoke gas phase the mucous flow in rat trachea was reduced as compared with the gas phase alone (5).
IMPERIAL NEED TO STATE THE CONCENTRATION OF NITRIC OXIDE AND OF THE CIGARETTE SMOKE GAS PHASE USED, FOR DURATION OF EXPOSURE.

No correlation was found between the level of nitric oxide in cigarette smoke and ciliastasis in cats (6).

No ultra-structural changes were found in the hearts of rabbits which had been exposed to 5 PPM NO + 0.5 PPM HCN + 200 PPM CO (24,28,29). IMPERIAL NEED TO INDICATE THE PERIOD OF EXPOSURE.

Experiments have shown that NO and also cigarette smoke increases the activity of guanylate cyclase (17,25,27,33,37). It has been suggested that a close relationship exists between coronary arterial relaxation and guanylate cyclase activation (25). The effect of this activation has been shown to give rise to increased levels of cyclic guanosine 3',5'-mono-phosphate. (17,25,27,32). It has been suggested that NO inhibited platelet aggregation in humans as a result of guanylate cyclase activation which increased the level of cyclic guanosine 3',5'-mono-phosphate in platelets.

Emphysematous changes and thinning of the aveolar wall were found in rats exposed to .2 PPM NO for 6 weeks (15,16).

An exposure of 10 PPM (containing 1-1.5 PPM NO₂) for up to 6 months increased the lung weight of mice. Degenerative and necrotic changes with hyperplasia of the broncheolar epithelium and dilatation of aveolar walls was noted (12).

In rabbits exposed to 5 PPM for 14 days there were changes in the arterioles and a thickening of the aveolar capillary membrane (19). However, no morphological changes were found in rabbits which had been exposed to 43 PPM for 6 days or to 5 PPM + 0.5 PPM HCN + 200 PPM CO for 2 weeks (20,21).

When mice were exposed to 10 PPM NO or NO₂ for 2 hours daily up to 30 weeks it was found that NO produced more severe lung pathology than NO₂ with clear evidence of paraseptal emphysema (22).

Rats were exposed to continuous inhalation of .2 to 10 mg NO/M³ and it was found that the changes depended on the duration of exposure and concentration (36). At 10 mg NO/M³ the lungs showed necrobiotic changes, microabscess and emphysema. IMPERIAL NEED TO GIVE THE TIME OF EXPOSURE.

Rats exposed to 0.05 mg/litre NO for 7 weeks, at 6 hours/day, 5 times/week were found to have increased levels of methemoglobin (9).

Rats exposed to 2 PPM NO for 6 weeks showed no alteration in blood parameters but methemoglobin was not found (15,16).

Exposure of humans to 15 PPM is reported to alter the affinity of the blood for oxygen (16). IMPERIAL NEED TO STATE THE PERIOD OF EXPOSURE.

Exposure of mice to 10 PPM (containing 1-1.5 PPM NO₂) for up to 6 months showed an increase in the leukocyte count and a decrease in the blood cholinesterase activity. Heinz bodies were found in red blood cells (12).

103546459

An exposure system for exposure to NO which avoided oxidation to NO₂ has been described (35). It was used for the continuous exposure of rats to 0.2 or 10 mg/NO/M³. Cholinesterase decreased substantially at 10 mg NO/M³ but was unchanged at 0.2 mg NO/M³. Serum alkyl phosphates decreased in a dose dependent manner. IMPERIAL NEED TO GIVE TIME OF EXPOSURE.

Continuous exposure to 2 PPM for 4 weeks did not reduce the resistance of male mice to infection by *Pasteurella multocida*. However, the female mice had a slightly enhanced mortality compared with the controls (30).

Chronic Toxicity

Mice were exposed to 10 PPM NO or NO₂ for 2 hours daily over 30 weeks and at intervals groups were subjected to immunological examination. Both gases were found to suppress the immune function (22).

Golden hamsters which had been exposed to 2 PPM continuously for 16 months showed no evidence of malignant tumours (3). In this experiment 40 PPM of NO₂ was also present.

Exposure of hamster lung cultures to fresh smoke from a variety of cigarettes showed that there was a positive correlation between the NO content of the smoke and malignant transformation (13,14).

However when mice were exposed to 2.4 PPM for a life time no differences from control were detected in the lung (23).

It has been reported that nitric oxide converts haemoglobin to methemoglobin and induces non-specific broncho pneumonia in dogs (4). No effect on the blood viscosity of dogs was found after they had been exposed to air polluted with nitric oxide daily for 4 years (8).

A new method for the measurement of NO (presumably bound to haemoglobin) using isotopic dilution with N¹⁵O and mass spectrometry has been developed (18). This showed that the levels of NO in the blood of non smokers and smokers was not significantly different. It was suggested that a mechanism existed for its rapid detoxification should NO be exogenously derived.

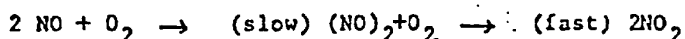
After a life time exposure to 2.4 PPM (NO₂ < 0.04 PPM) the level of nitrosyl haemoglobin in mice was 0.01% and no methemoglobin was formed (26). However, there was a change in the osmotic resistance of the red blood cells and a decrease in serum heptaglobin which suggested haemolysis but at a low level.

Summary

(As in the IMPERIAL Paper)

Comments

I think it is likely that the DHSS will be aware of the possibility of oxidation of NO to NO₂ in the lung of the smoker. I feel we could be thought to be ducking the issue if we do not address the probability of NO₂ formation, and assess the toxicity of NO₂. If we feel it is unlikely that NO₂ will be formed, this should be brought out with the relevant evidence. In this regard the Imperial report is rather lacking. We know the oxidation to be termolecular and the rate of NO₂ formation to be controlled by the rate of formation of the nitric oxide dimer.



103546460

The rate of oxidation is dependent on the square of the nitric oxide concentration and increases with falling temperature.

The Chemists might be able to predict from the kinetics that the amount of NO_2 formed will be small but finite. Where there is inefficient clearance of NO from the lungs larger amounts of NO_2 may be expected.

Another issue which is absent from the Imperial report is whether or not NO is capable of upsetting the protease-antiprotease balance in the lung. Any evidence that NO does not upset this balance should be brought out. This is likely to be a point of concern with the DHSS in view of the implications for emphysema, in the light of the modern theory on emphysema pathogenesis.

There is a possibility of dinitrogen tetroxide formation. This should at least be considered, and if deemed likely to be formed in any amount the toxicology at these amounts should be assessed.

Mike Burke
30 November 1984

c.c. Dr. P. W. Darby
Mr. C. D. Briggs

105546461