

4. Toxicity induced by nitrogen dioxide in experimental animals and isolated cell systems

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There are numerous investigations describing pulmonary effects in experimental animals exposed to nitrogen dioxide (NO_2). Studies on acute, subacute, and chronic NO_2 exposure of animals of different species and ages have been performed. Even at relatively low doses several effects are observed, including impaired lung function, structure, metabolism and biochemistry, as well as decreased host defense against infectious pulmonary diseases. Generally, effects are seen at an exposure level below $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm). Many investigations have also used isolated cell systems which have been exposed to different concentrations of NO_2 . This chapter summarizes the pulmonary effects observed after exposure to NO_2 and also possible molecular mechanisms behind the effects of NO_2 . Representative investigations using low to moderate exposure levels were of primary interest. Effects on the immune system and other systemic effects are also briefly discussed.

The primary targets of NO_2 in the lung are the terminal conducting airways, the alveolar ducts, and the alveoli. The most sensitive cells appear to be the ciliated cells of the bronchial epithelium and the type I and type II cells of the alveolar epithelium. In addition alveolar macrophages are also sensitive to NO_2 exposure, and several studies have shown altered macrophage structure and function following NO_2 exposure.

Effects on pulmonary function

Several investigations using different animal species have shown that NO_2 exposure causes decreased pulmonary function. For example, 52 weeks of exposure of mice at a dose of $380 \mu\text{g}\cdot\text{m}^{-3}$ (0.2 ppm) with regular daily spikes of $1500 \mu\text{g}\cdot\text{m}^{-3}$ (0.8 ppm) caused a decrease in lung dynamic compliance and respiratory capacity (1).

In other studies changes in lung function have been shown to coincide with the morphological alterations in the proximal alveolar region. For instance, the thickening of the alveolar interstitium was associated with decreased compliance in adult rats exposed to NO_2 concentrations of 1880 – $2760 \mu\text{g}\cdot\text{m}^{-3}$ (1–2 ppm) for six weeks (2).

Increased airway hyperresponsiveness after acute NO_2 exposure, which has been shown to occur in

humans, has also been demonstrated in guinea pigs. In one study exposure for 7 d to an NO_2 concentration of $7500 \mu\text{g}\cdot\text{m}^{-3}$ (4 ppm) significantly increased airway hyperresponsiveness to histamine inhaled directly after NO_2 exposure (3). In another study exposure of guinea pigs to $13\ 110 \mu\text{g}\cdot\text{m}^{-3}$ (7 ppm) and higher levels of NO_2 for 10 min caused a concentration-related increase in the response to histamine inhaled directly after the termination of NO_2 exposure. Two or 19 hours after NO_2 exposure no increased sensitivity could be observed (4).

Effects on lung morphology

Many investigations deal with the effect of NO_2 exposure on lung morphology. Acute (hours and up to a few days), subchronic (weeks), and chronic (months) treatments have been studied. Acute exposure of rats to NO_2 at concentrations less than $18\ 800 \mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) does not cause any significant morphological changes. Exposure of rats to $20\ 000 \mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) for 36 h has been shown to result in cilia loss and hypertrophy of the bronchiolar epithelium (5). Exposure to 15 ppm for 24 h caused hyperplasia of type II cells (6). Exposure to 25 ppm for 30 min also resulted in hyperplasia of the type II cells (7). After exposure of rats to 20 ppm of NO_2 for 24 h, severe changes were noted, including thickening of type I cells, reduction in secretory granules in Clara cells and increased division of type II cells (8).

Guinea pig appears to be considerably more sensitive to NO_2 than rat, and in one study exposure to $3760 \mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) for 3 d resulted in edema, thickening of the alveolar wall, and loss of cilia (9).

At subchronic exposures, changes occur at lower NO_2 concentrations. In mice, exposure to $640 \mu\text{g}\cdot\text{m}^{-3}$ (0.34 ppm) (6 h·d⁻¹, 5 d a week for 6 weeks) caused type II cell hypertrophy, hyperplasia and the like (10). In rats, effects have been observed after a month of exposure to $940 \mu\text{g}\cdot\text{m}^{-3}$ (0.5 ppm). Hypertrophy of type II cells, an increased number of macrophages, and an alteration of the air-blood barrier are some of the effects observed (2, 11–13). Exposure to $20\ 000 \mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) for up to 28 d caused more severe changes, such as hypertrophy of ciliated cells and Clara cells and hyperplasia of the bronchioli (5).

Chronic exposure to NO_2 also causes changes at low exposure levels. In one study, morphological changes were observed after rats were exposed to $800 \mu\text{g}\cdot\text{m}^{-3}$ (0.4 ppm) for 9-21 months (14). Exposure to $940 \mu\text{g}\cdot\text{m}^{-3}$ (0.5 ppm) for seven months caused swelling of the terminal bronchiolar cilia and hyperplasia of type II cells (15). In another study Hayashi et al (16) observed interstitial edema after four months of exposure to $940 \mu\text{g}\cdot\text{m}^{-3}$ (0.5 ppm). At higher doses more severe changes were observed. Only a few studies with species other than rat have been performed with chronic NO_2 exposure. In one, mice exposed to $940 \mu\text{g}\cdot\text{m}^{-3}$ (0.5 ppm) for 6-12 months exhibited cilia loss in respiratory bronchioles and bronchiolar inflammation (17).

A point of discussion has been whether or not the NO_2 -induced morphological changes develop into emphysema. The results presented are conflicting. Studies on rats have shown that subchronic exposures of high NO_2 levels ($56\ 500 \mu\text{g}\cdot\text{m}^{-3}$, 30 ppm) do not produce irreversible emphysematous lesions (18), neither was this exposure shown to potentiate protease-induced emphysema or bring about a progression in preexisting emphysema. Another recent study using hamsters showed, however, that chronic exposure to an NO_2 level of $3760 \mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) for 8 h a day, 5 d a week for eight weeks caused mild emphysema and significantly potentiated elastase-induced emphysema (19).

Biochemical mechanisms for lung injury

The biochemical mechanisms behind the effects induced by NO_2 are poorly understood. Nitrogen dioxide is a free radical and readily reacts with polyunsaturated fatty acids, as well as with certain amino acids in proteins. For instance, nitrotyrosine formation has been demonstrated to occur during reactions between NO_2 and various proteins. The oxidation of cysteine thiolate ions in proteins by NO_2 has also been observed. Interaction with DNA (deoxyribonucleic acid) can also occur. Still, most studies have focused on the NO_2 induction of lipid peroxidation and lipid peroxide formation *in vivo*, as well as in different *in vitro* systems.

Lipid peroxidation

Lipid peroxidation induced by NO_2 exposure has been detected at exposure levels as low as $75 \mu\text{g}\cdot\text{m}^{-3}$ (0.04 ppm). Lipid peroxidation, measured as ethane exhalation, was detected after nine months of exposure of rats to $75\text{--}750 \mu\text{g}\cdot\text{m}^{-3}$ (0.04-0.4 ppm) (20). Lipid peroxidation has also been evaluated by measuring the content of lipid peroxides or substances reactive to thiobarbituric acid in alveolar lavage fluid and lung tissue after exposure to similar NO_2 con-

centrations (21, 22). Acute or subacute exposure to higher concentrations of NO_2 has also been shown to cause a rapid increase in lung peroxide levels in several species.

Lipid peroxidation results in an alteration in phospholipid composition. Exposure of either mice or guinea pigs to an NO_2 level of $750 \mu\text{g}\cdot\text{m}^{-3}$ (0.4 ppm) for a week resulted in a decreased concentration of phosphatidyl ethanolamine and a relative increase in the phosphatidyl choline concentration (23).

Several investigators have also demonstrated NO_2 -induced lipid peroxidation in *in vitro* systems. The cell type most commonly used is the endothelial cell from either pig arteries or aorta. Studies using these cell types have recently attempted to relate the effect on lipid metabolism to functional parameters such as membrane fluidity and enzyme activation or inactivation.

Thus membrane fluidity changes are related to lipid peroxidation. NO_2 -induced changes in membrane fluidity have been demonstrated in alveolar macrophages and endothelial cells in culture. Endothelial cells exposed to an NO_2 level of $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm), for instance, exhibit a decreased membrane fluidity already after 3 h. Thus NO_2 changes the physical state of the membrane lipids, perhaps through initiating lipid peroxidation and hence impair membrane functions (24).

Lipid peroxidation can also activate phospholipase activities. Activation of phospholipase A_1 in cultured endothelial cells by NO_2 has been demonstrated. This activation, which is specific for phospholipase A_1 , occurs at an NO_2 concentration of $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm) after 40 h of exposure and is speculated to depend on a specific NO_2 -induced increase in phosphatidyl serine in the plasma membranes (25).

One function of phospholipases is the release of arachidonic acid. The effect of NO_2 on the release and metabolism of arachidonic acid has been studied both *in vivo* and *in vitro*. Both an increase and a depression in the metabolism of arachidonic acid has been observed in several species. *In vivo* exposure of rats to $18\ 800 \mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) for 2 h resulted in decreased levels of prostaglandins E_2 and $\text{F}_2\alpha$, as well as thromboxane B_2 , in lavage fluid. On the other hand, at an exposure level of $1880 \mu\text{g}\cdot\text{m}^{-3}$ (1 ppm), the concentrations of thromboxane B_2 were increased (26).

Effect on proteins

Several investigators have studied the effect of NO_2 exposure on enzymes and receptors of the lung. A significant increase in the activity of glycolytic enzymes was observed after the exposure of guinea pigs to NO_2 levels of $2760\text{--}18\ 800 \mu\text{g}\cdot\text{m}^{-3}$ (2-10 ppm) for one to two weeks (27). Alkaline phosphatase and lactate dehydrogenase activities also increased after

exposure to 5 ppm of NO_2 for a week. Decreased 5-hydroxytryptamine uptake and increased angiotensin receptor binding were observed at $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm).

An important protective system against proteolysis in the lung is the α -1-proteinase inhibitor (1-PI). This enzyme has also been shown to be sensitive to oxidants. However, the effect of NO_2 on this enzyme is difficult to evaluate. One study showed small inhibition of 1-PI activity, whereas another showed significantly increased 1-PI activity after acute NO_2 exposure of $5640 \mu\text{g}\cdot\text{m}^{-3}$ (3 ppm) for 3 h (28).

An important target for NO_2 -induced toxicity is probably the structural proteins of the lung. Accordingly, NO_2 exposure has been shown to increase collagen synthesis (important for the development of pulmonary fibrosis). It is estimated that this increase would occur at doses as low as $1880 \mu\text{g}\cdot\text{m}^{-3}$ (1 ppm) (29).

Antioxidant defense systems

Since NO_2 is an oxidant and lipid peroxidation is believed to be a major molecular event responsible for the toxic effects of NO_2 , much attention has been focused on the effect on the antioxidant defense system in the epithelial lining fluid and in pulmonary cells. Investigations with subacute and chronic NO_2 exposure at levels of 75 – $62\ 040 \mu\text{g}\cdot\text{m}^{-3}$ (0.04–33 ppm) have been performed both in vivo and in vitro and focused on effects on low-molecular antioxidants such as glutathione, vitamin E, and vitamin C, as well as on some enzymes involved in the synthesis and catabolism of glutathione. Experiments made in vitro using human plasma have shown a rapid depletion of vitamin C and glutathione and a loss of vitamin E. This result was achieved with a concentration of $26\ 320 \mu\text{g}\cdot\text{m}^{-3}$ (14 ppm) (30).

In vivo protective effects of vitamin C and vitamin E against the pulmonary toxicity of NO_2 have also been demonstrated. Thus in one study animals fed a diet deficient in vitamin C showed increased amounts of protein in lung lavage fluid after an NO_2 exposure of $9000 \mu\text{g}\cdot\text{m}^{-3}$ (4.8 ppm) for 3 h. There was no effect on animals fed with a normal diet (31). Similar results using exposure of $1880 \mu\text{g}\cdot\text{m}^{-3}$ for 72 h were obtained in an earlier study (32). Rats fed with a diet deficient in vitamin E similarly showed a significant increase in lipid peroxidation after 7-d exposure to $5640 \mu\text{g}\cdot\text{m}^{-3}$ (3 ppm) when compared with controls (33). In addition vitamin E supplementation decreased the toxicity of NO_2 (34, 35). The only enzymes which appear to be significantly affected after NO_2 exposure are glutathione reductase, glucose-6-phosphatase dehydrogenase and glutathione peroxidase. The activities of these enzymes have been shown to be increased after NO_2 exposure of 1880 – $28\ 000 \mu\text{g}\cdot\text{m}^{-3}$ (1–15 ppm) for up to 7 d both in vivo and in vitro (36–38).

Effects on host defense

The host defense of the lung includes both physical defense mechanisms, including the mucociliary system, the mucous layer and the epithelial lining fluid, and cellular defense mechanisms, of which the first and probably most important are the alveolar macrophages. The role of alveolar macrophages in host defense is diverse, including the detoxification and removal of inhaled particles, the killing of inhaled microorganisms, interacting with lymphoid cells in a variety of immunological reactions, and the removal of damaged or dying cells from the alveoli through phagocytosis.

Mucociliary clearance

One of the main functions of ciliated bronchial epithelial cells is to participate in mucociliary clearance. These cells are sensitive to NO_2 , and studies have shown loss of cilia, increased ciliary fragility, inhibited ciliary growth, and loss of ciliary motility. There is also evidence of swelling, hypertrophy, and focal hyperplasia of the epithelial cells. Acute effects on the ciliated epithelial cells are seen only at concentrations of NO_2 exceeding those relevant to human exposure.

In rats exposed to $940 \mu\text{g}\cdot\text{m}^{-3}$ (0.5 ppm) loss of cilia was observed after seven months of exposure (15). A more pronounced effect was observed with increasing dose. Exposure to $3760 \mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) for 3 d showed a loss of cilia and indications of cellular swelling, hypertrophy, and hyperplasia (39). Similar effects were observed when rats were exposed for six weeks (40). In hamsters a two week exposure to $3760 \mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) resulted in decreased ciliary beating activity and morphological changes (41).

The effect of NO_2 on ciliated epithelial cells in vitro has also been investigated, and Kita & Omichi (42) showed that an NO_2 concentration of $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm) caused a decrease in ciliary beating.

In conclusion, it is evident that NO_2 affects the ciliated epithelial cells and that this effect is dependent on both the concentration and duration of the NO_2 exposure. Interestingly, however, no significant impairment of tracheobronchial clearance rates could be observed in sheep even after an NO_2 exposure of $14\ 100 \mu\text{g}\cdot\text{m}^{-3}$ (7.5 ppm). Some effect was seen at $28\ 200 \mu\text{g}\cdot\text{m}^{-3}$ (15 ppm) (43). Neither did exposure of rabbits to $18\ 000 \mu\text{g}\cdot\text{m}^{-3}$ (10 ppm) for 2 h cause any decreased mucociliary transport (44). Thus it appears that the airway epithelium has a general overcapacity in mucus transport.

Alveolar macrophages

In vivo. Numerous investigations dealing with the effect of NO_2 exposure on the structure and function of alveolar macrophages have been performed. These cells are sensitive to NO_2 , and NO_2 , for instance, has been shown to disrupt membrane integrity and the

viability of the macrophages and also depress their number and phagocytic activity and thereby their bactericidal activity. The phagocytic capacity and mobility of alveolar macrophages are particularly sensitive parameters.

Effects on the function of alveolar macrophages can be observed at concentrations as low as $560 \mu\text{g}\cdot\text{m}^{-3}$ (0.3 ppm). Exposure of rabbits to this dose for 13 d resulted in decreased phagocytic activity and reduced mobility (45). In mice, morphological changes were observed after 21 weeks of exposure to $940 \mu\text{g}\cdot\text{m}^{-3}$ (0.5 ppm) with a peak exposure of $2760 \mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) for 1 h each day (46). In rats exposed to $7520 \mu\text{g}\cdot\text{m}^{-3}$ (4 ppm) for 10 d an increased number of alveolar macrophages was observed in addition to decreased viability and phagocytic activity (47, 48). An interesting finding is that acute exposure (up to 24 h) to $1880 \mu\text{g}\cdot\text{m}^{-3}$ (1 ppm) apparently causes a stimulation of the clearance of particles from the lung, whereas at higher concentrations this clearance decreases. This occurrence has been shown in both rat (49) and rabbit (50).

In vitro. Only a few studies have used *in vitro* exposure of alveolar macrophages to NO_2 . The effects seen are comparable to those found after *in vivo* exposure, and both phagocytic activity and morphological changes in alveolar macrophages have been observed in guinea pigs at concentrations as low as $180 \mu\text{g}\cdot\text{m}^{-3}$ (0.1 ppm) (51). In a recent study, however, exposure of up to 15 ppm for 3 h did not affect the release of either the neutrophil chemotactic factor or interleukin-1 from human alveolar macrophages (52).

Interaction with infectious agents

Several experimental approaches have been used to determine the functional efficiency of the pulmonary defenses of animals after NO_2 exposure. Generally, sensitivity towards infections provoked by bacteria or virus has been investigated. After exposure to NO_2 the animals are exposed by aerosol to different bacteria or virus. Most often mortality enhancement following NO_2 exposure is used as a measure of the NO_2 effect. The sensitivity to NO_2 exposure differs depending on the type of bacteria or viruses used and also on the NO_2 dosing regime.

The species most commonly used for these types of studies is the mouse. Exposure of mice to $370 \mu\text{g}\cdot\text{m}^{-3}$ (0.2 ppm) with two 1-h spikes of $1500 \mu\text{g}\cdot\text{m}^{-3}$ (0.8 ppm) per day for one year, followed by an infection with *Streptococcus*, resulted in greater mortality than among controls. It also appears that spiked exposure was needed to initiate response (1). In another study, intermittent exposure to $940 \mu\text{g}\cdot\text{m}^{-3}$ (0.5 ppm) for 6 or 18 h per day, followed by infection with *Klebsiella pneumoniae*, resulted in increased mortality after six months. Continuous exposure caused effects already at three months (53).

Higher NO_2 concentrations are needed during acute exposure. For instance, exposure of mice to $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm) for 3 d was needed before a significant increase was seen in mortality after infection with *Klebsiella pneumoniae* (54). Exposure of mice to $4700 \mu\text{g}\cdot\text{m}^{-3}$ (2.5 ppm) for 4 h caused impaired antibacterial activity (55). An NO_2 exposure of 5 ppm for 6 h per day for 6 d caused increased susceptibility to cytomegalo virus in mice, and a continued NO_2 exposure of 5 ppm for two months among squirrel monkeys resulted in increased viral-induced mortality. *In vitro* effects occurred already at 2 ppm (56).

Effect of nitrogen dioxide on the immune system

When the mucociliary system and the phagocytic activities of alveolar macrophages and polymorphonuclear lymphocytes have failed to remove particles effectively from the respiratory tract, immunologic (humoral and cell-mediated immunity) responses are provided by lymphocytes. The humoral part of this system primarily involves the B cells that function in the synthesis and secretion of antibodies [immunoglobulin (Ig) G, A, M] into the blood and body fluids. The cell-mediated component primarily involves T-lymphocytes, which are the effectors of cellular defense. These cells are responsible for delayed hypersensitivity and defense against viral, fungal, bacterial, and neoplastic diseases. Alveolar macrophages may be responsible for the initiation of humoral and cellular immune responses through their ingestion of particles or soluble antigens and their "processing" and "presenting" these antigens for specific antigen-reactive B and T cells. This process stimulates the B and T cells to proliferate and differentiate into effectors of humoral (antibody production) and cell-mediated (cytotoxic lymphocytes) immunity.

The immune system is important for defense against respiratory infections. So far, relatively few investigations have dealt with the effect of NO_2 near ambient concentrations, and in the studies performed only few of the various immunologic parameters have been tested. There are, for instance, no adequate studies of the effect of NO_2 on pulmonary immune systems. Such studies are needed since these systems form the primary defense against respiratory infections. However, several studies on systemic immune systems in various animals have been performed. Most studies show that long-term exposure to NO_2 causes the suppression of several humoral and cell-mediated immunologic functions.

Antibody-forming cells. NO_2 has been shown to affect the antibody-forming cells in the spleen. Sheep exposed to an NO_2 concentration of $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm) intermittently 1.5 h per day for 10 d, and im-

munized with sheep red blood cells 2 d after the exposure had a lower production of antibody-forming cells as revealed by a decreased output of plaque-forming cells from the spleen (57). Lefkowitz et al (58) exposed mice to an NO_2 level of $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm) for 24 h per day for 6 d and injected the mice with sheep red blood cells after the first day of exposure. NO_2 did not affect hemagglutination antibody titers or cell-mediated immunity (blastogenesis of splenic T cells), but did significantly reduce the number of splenic plaque-forming cells against the sheep red blood cells.

Antibody response. The effect of NO_2 on the humoral antibody response to sheep red blood cells in mice was studied by Fujimaki and his co-workers (59-61). Significant suppression in primary antibody response, apparently due to a reduction in the functioning of B cells, was observed, along with a depression of the secondary response, probably due to effects on T-cell function. Effects on the primary response could be observed already after four weeks' exposure of mice to an NO_2 level of $752 \mu\text{g}\cdot\text{m}^{-3}$ (0.4 ppm). Higher concentrations ($9400 \mu\text{g}\cdot\text{m}^{-3}$) were needed to suppress the secondary response.

It is also evident that antibody titers against influenza virus are decreased after NO_2 exposure. This occurrence has been shown in both mice and monkeys. Three months of NO_2 exposure of $3760 \mu\text{g}\cdot\text{m}^{-3}$ (2 ppm) among mice caused fourfold lower antibody serum neutralizing titers than in controls (62). This finding agrees with those in monkeys exposed to $1880 \mu\text{g}\cdot\text{m}^{-3}$ (1 ppm) for over a year (63).

Kosmider et al (64) exposed guinea pigs to an NO_2 level of $1880 \mu\text{g}\cdot\text{m}^{-3}$ (1 ppm) for six months and reported a significant reduction in all serum immuno-globulin fractions and complement. Decreased levels of these substances may lead to an increase in the frequency, duration, and severity of an infectious disease. Mice exposed to NO_2 had decreased serum levels of IgA, and they exhibited non-specific increases in serum IgM, IgG, and IgG₂ (62).

A noticeable increase in the titer of serum antibodies against lung tissue was found by Balchum et al (65) when they exposed guinea pigs to an NO_2 level of $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm).

Cellular immunity. Studies performed by Richters & Damji (66, 67) showed a decrease in the total T-lymphocyte population following the NO_2 exposure of mice. Significant effects were observed already after seven weeks of exposure to $470 \mu\text{g}\cdot\text{m}^{-3}$ (0.25 ppm). After 8 h of exposure to 4 ppm, the percentages of total T-lymphocytes, T-helper/inducer lymphocytes and T-cytotoxic/suppressor lymphocytes were significantly lower in the NO_2 -exposed mice. Large T-cytotoxic/suppressor cells were found

to be the most susceptible subpopulation. Exposed mice also had significantly lower spleen weights (68).

In 1978 Maigetter et al (69) showed that exposure of mice to an NO_2 concentration of $940 \mu\text{g}\cdot\text{m}^{-3}$ (0.5 ppm) for three months resulted in suppression of the response of splenic T and B cells to phytochemagglutinin and bacterial lipopolysaccharide.

Hilliam et al (70) studied effects of acute NO_2 exposure on cellular immunity after lung immunization in rats. Twenty-four hours of exposure to $9400 \mu\text{g}\cdot\text{m}^{-3}$ (5 ppm) and higher concentrations of NO_2 caused an elevated cellular immunity against intratracheally administered sheep red blood cells. The authors suggested that NO_2 can be responsible for the production of abnormally elevated immune responses to antigens deposited in the damaged lung.

Taken together, these results, although still incomplete, indicate that the immune system is sensitive to NO_2 exposure. More extensive studies using lower exposure levels for longer times are needed, as are studies of the effects on the pulmonary immune system. This should be an important area for future research.

Interaction between nitrogen dioxide and allergens in animal studies

The interaction between air pollutants and allergens on the allergic sensitization process has been investigated in several animal studies (43, 71-81). From these studies it can be concluded that pollutants can facilitate sensitization at least at relatively high exposure concentrations of pollutants. However, the majority of these studies has dealt with the effects of ozone, and only a few with the effects of nitrogen dioxide.

Matsumura (71) found that a great proportion of guinea pigs exposed to $131\ 600 \mu\text{g}\cdot\text{m}^{-3}$ (70 ppm) developed anaphylactic shock to inhaled egg albumin, while in a control group none or only one developed anaphylaxis. In another study, Matsumura (72) showed that the exposure of guinea pigs to $75\ 200 \mu\text{g}\cdot\text{m}^{-3}$ (40 ppm) increased the sensitization to egg albumin via the intraperitoneal route. Exposure to concentrations as high as $1212\ 000 \mu\text{g}\cdot\text{m}^{-3}$ (400 ppm) did not affect sensitization in the latter study. This finding suggests that there are differences among pollutants in their mechanisms of action.

Even if nitrogen dioxide increases sensitization to allergens in guinea pigs at concentrations about 100-fold those in ambient air, it remains to be shown that this effect exists even at lower concentrations, as has been shown for ozone in animal models. The question of whether nitrogen dioxide at ambient concentrations exerts such an enhancing effect on sensitization is important to study in animal models, since there are reports from epidemiologic studies showing a higher prevalence of allergy in cities and in areas with increased exposure to car exhausts.

Systemic effects of nitrogen dioxide

As described in chapter 3, absorbed NO_2 is transformed into nitrite and nitrate, the main metabolites found in blood and urine. Thus systemic effects of NO_2 are expected to be either related to metabolites or to secondary effects resulting from pulmonary injury.

Systemic immunotoxic effects were described in the preceding section. Genotoxic effects, including effects of nitrite, nitrate, and endogenous formation of carcinogenic nitrosamines, are discussed in chapter 8.

There is relatively little substantial evidence of the systemic toxicity of NO_2 . No effect was seen on body weight in several species after chronic exposure to 2000–47 000 $\mu\text{g}\cdot\text{m}^{-3}$ (82). In the same study, no changes in hemoglobin, hematocrit, or leucocyte count were found in dogs. However, other studies have shown a decrease in the number of erythrocytes and an increase in the number of leucocytes in rabbits (63) and polycythemia in rats (83). Changes in erythrocyte membranes have been observed in several subacute studies. (See, eg, reference 84.)

An increase in spleen weight and an increased number of erythrocytes in the spleen occurred after six weeks of exposure of mice to as a low concentration as 660 $\mu\text{g}\cdot\text{m}^{-3}$ (85). A somewhat longer exposure to relatively low concentrations enhanced endogenous retrovirus expression in the spleen of mice (86).

Some studies indicate hepatic effects, such as an increased phenobarbital-induced sleeping time, reduced cytochrome P-450 levels in the liver, and a decrease in serum proteins, lipoproteins and plasma cholinesterase (87–90).

NO_2 exposure has induced decreased litter size but no teratogenic effects (91).

The host defense systems are also affected by NO_2 exposure. There is a loss of cilia from the ciliated epithelial cells after chronic exposure to 940 $\mu\text{g}\cdot\text{m}^{-3}$ NO_2 . Higher concentrations are needed, however, to induce any significant inhibition of the mucociliary transport of particles. The function of alveolar macrophages is also impaired after NO_2 exposure, and a decreased function of alveolar macrophages can be seen after subchronic exposure to 560 $\mu\text{g}\cdot\text{m}^{-3}$. In *in vitro* experiments effects have been seen at NO_2 concentrations as low as 180 $\mu\text{g}\cdot\text{m}^{-3}$. However, these results have not been confirmed.

The decreased host defense can be demonstrated as an increased susceptibility to infections provoked by bacteria or viruses. Such occurrences have been shown after chronic NO_2 exposure of 370 $\mu\text{g}\cdot\text{m}^{-3}$ with regular peaks of 1500 $\mu\text{g}\cdot\text{m}^{-3}$.

NO_2 causes several biochemical alterations. Effects on antioxidant defense mechanisms, lipid peroxidation, and protein function have been demonstrated. Lipid peroxidation appears to be the most sensitive, and one study reported increased lipid peroxidation after chronic NO_2 exposure of 75 $\mu\text{g}\cdot\text{m}^{-3}$. The significance of these findings can be questioned, however, primarily because of the methodology used. Generally, NO_2 -induced biochemical alterations have been studied at considerably higher exposure levels. The relevance of the biochemical alterations for the observed toxicity of NO_2 *in vivo* is not known.

Data on the effect of NO_2 on the immune system is still incomplete, but they indicate that this system is rather sensitive to NO_2 exposure. For instance, effects on T-lymphocyte populations were observed already after NO_2 exposure of 470 $\mu\text{g}\cdot\text{m}^{-3}$ for seven weeks, and suppression of the humoral antibody response occurred in mice after a four-week exposure to 750 $\mu\text{g}\cdot\text{m}^{-3}$. Other systemic effects of NO_2 only occur at high exposure levels.

Summary and concluding remarks

In experimental animals NO_2 exposure induces several types of pulmonary toxicity. Generally, effects in experimental animals are seen at higher exposure levels than those causing effects in humans. The absolute lung exposure levels at a given NO_2 concentration may differ, however, between humans and experimental animals since their breathing patterns vary. There are clear signs of decreased pulmonary function after chronic NO_2 exposure to 380 $\mu\text{g}\cdot\text{m}^{-3}$ with regular peaks of 1500 $\mu\text{g}\cdot\text{m}^{-3}$. Effects on lung morphology can be observed following subchronic exposure to 640 $\mu\text{g}\cdot\text{m}^{-3}$, whereas concentrations of at least 3760 $\mu\text{g}\cdot\text{m}^{-3}$ are needed to cause any changes in acute exposure. Acute exposure to 7500 $\mu\text{g}\cdot\text{m}^{-3}$ also causes increased airway hyperresponsiveness towards histamine.

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