Review of experimental studies of exposures to nitrogen dioxide

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1. Introduction

Nitrogen dioxide (NO₂) has been associated with adverse effects on hospital admissions, decrements in measures of lung function and lung function growth, increases in respiratory symptoms, asthma prevalence and incidence, cancer incidence, adverse birth outcomes and mortality (US EPA, 2015; WHO Europe, 2013).

In recent years, it has not been clear whether the effects are caused by NO₂ itself, or by some other air pollutant(s) with which it is correlated in the ambient air. However, as the associations between NO₂ and short-term health effects in many studies remain after adjustment for other air pollutants and that there is consistent short-term epidemiological evidence and some mechanistic support for causality, particularly for respiratory outcomes, the REVIHAPP project concluded that NO₂ also has direct effects (WHO Europe, 2013).

It is more difficult to assess the independent long-term effects of NO₂ as the correlations between concentrations of NO₂ and other pollutants are often high, so that NO₂ might represent the mixture of traffic-related air pollutants. However, some epidemiological studies do suggest that long-term exposures to NO₂ have independent effects on respiratory and cardiovascular mortality and on children’s respiratory symptoms and lung function. The mechanistic evidence, particularly on respiratory effects, and the weight of evidence on short-term associations are suggestive of a causal relationship (Committee on the Medical Effects of Air Pollutants, 2015; WHO Europe, 2013).

The US EPA’s current draft conclusion also is that there is a causal relationship between NO₂ exposure and respiratory effects (US EPA, 2015). The evidence of associations of ambient concentrations of NO₂ with a range of effects on health has strengthened in recent years and is robust to adjustment for other pollutants including some particle metrics. The combined evidence from epidemiologic and experimental studies is suggestive of, but not sufficient to infer, a causal relationship between short-term NO₂ exposure and cardiovascular effects (US EPA, 2015).

A recent review of the effects of traffic related air pollution (Health Effects Institute, 2010) reported that there was

- Sufficient evidence for increased asthma prevalence and increased asthma symptoms and exacerbations in children;
- Suggestive but not sufficient evidence for asthma causation, asthma symptom exacerbations in adults and reduced lung function;
• Suggestive evidence for mortality, especially cardiovascular mortality; and,
• Insufficient evidence for allergic sensitization, adult onset of asthma, increased health-care use both in children and adults, development of chronic obstructive pulmonary disease, birth outcomes, childhood cancers and lung cancer.

The current consensus is that there is no evidence that there is a threshold for NO$_2$ exposure (WHO Europe, 2013). However, the evidence base for assessing the existence of a threshold or the shape of the concentration–response curve is weaker than for fine particles (WHO Europe, 2013).

The investigation of the effects of inhaled air pollutants on human health has followed a multidisciplinary approach using animal toxicology, molecular and cellular biology, controlled human exposure studies, and epidemiology. Each approach has specific strengths and weaknesses in evaluating the effects of air pollution on human health.

Epidemiological studies examine the relationship between exposure and health effects in the community setting. Accurate estimation of exposure and effects of a particular air pollutant is difficult, as air pollution is a complex mixture. The extent of potential confounding, time considerations in air pollution effects (lags and latent periods), individual variation in air pollution exposure and exposure misclassification leads to uncertainty about the causal nature of any observed association with the health outcome under investigation.

Human experimental studies (or chamber studies) are directed at understanding mechanisms of injury and permit strict control of the exposures and the characteristics of the exposed persons. These studies typically involve small homogeneous groups of subjects and outcome measures can be carefully characterised. However, ethical and practical considerations often limit the use of such studies. Exposures must be at concentrations below the levels that are likely to produce unacceptable short-term or long-term effects. Feasibility usually limits exposure to a few hours, and chronic effects cannot be readily addressed. Despite these constraints, controlled human exposure studies have been used to investigation the acute effects of exposure to pollutants including NO$_2$, SO$_2$ and O$_3$ in both healthy people and in people with asthma. Although experimental studies generally only address a single pollutant, this can be helpful when it is unclear whether the health effects reported in epidemiological studies are related to the pollutant itself, or a mixture of pollutants for which the pollutant serves as a surrogate. The results from the experimental studies are therefore useful in determining whether NO$_2$ should be considered toxic to the
population in its own right or should be considered a marker for the complex mixture of air pollution.

2. Scope of work
The scope of work is to undertake a literature review and develop a summary report to NSW Health summarising the health evidence health effects from in-tunnel and short term exposure to NO₂. The literature review should include, but not be limited to, the following:

- evidence of health effects from 'short-term' exposure to NO₂ at 15 and 30 minutes exposure;
- whether the proposed NO₂ 'in-tunnel' 15 minute rolling average of 0.5ppm is appropriately protective of health;
- health effects of traffic pollution with NO₂ as a marker;
- any evidence of independent effects of NO₂ when other traffic pollutants are controlled for; and
- whether NO₂ is considered a 'non-threshold' pollutant

This report will review:

- Experimental studies where the NO₂ exposure is ≤ 30 minutes;
- Experimental studies where the NO₂ exposure is ≥ 60 minutes; and
- Ambient studies in road tunnels, subways and busy roads.

3. Experimental studies where the NO₂ exposure is ≤ 30 minutes
The papers (N=18) reviewed in this section are presented in Table 1 and were published between 1985 and 2014 (Barck et al., 2005a; Barck et al., 2005b; Barck et al., 2002; Bauer et al., 1986; Bylin et al., 1988; Bylin et al., 1985; Ezratty et al., 2014; Helleday et al., 1994; Jorres and Magnussen, 1990; Jorres and Magnussen, 1991; Kim et al., 1991; Koenig et al., 1987; Rubinstein et al., 1990; Sandstrom et al., 1990; Sandstrom et al., 1991; Strand et al., 1997; Strand et al., 1996; Strand et al., 1998).

In three studies (Helleday et al., 1994; Sandstrom et al., 1990; Sandstrom et al., 1991), NO₂ concentrations were between 2.25ppm and 5.5ppm to mimic occupational exposures. These three studies are discussed separately at the end of this section.

In the remaining 15 studies, NO₂ exposure was less than 0.5ppm except in the study by Ezratty et al. (Ezratty et al., 2014) where in addition to 0.2ppm, an exposure of 0.6ppm was also used. These studies have only a small number of subjects (generally less than 20) who
are either healthy or have mild asthma. The duration of exposure ranged from 15 to 30 minutes. Exposure to NO$_2$ in the experimental chamber was either at rest or whilst engaging in mild to moderate intermittent exercise. Main outcome measures were lung function, airway responsiveness, and cellular and inflammatory markers in blood, bronchoalveolar lavage fluid (BALF) and nasal washings.

Thirteen of the 15 studies measured changes in lung function before and after NO$_2$ exposures and filtered air. The lung function indices that were commonly measured included forced vital capacity (FVC), forced expiratory volume in one second (FEV$_1$), peak expiratory flow (PEF) and specific airway resistance (sRaw). In 12 of these studies, there were no differences in any of the lung function indicators after exposure to NO$_2$ compared to filtered air. In these 12 studies, the concentration of NO$_2$ ranged from 0.12ppm to 0.5ppm and the duration of exposure was from 15 to 30 minutes. In the remaining study (Bylin et al., 1985), healthy subjects demonstrated an increase in sRaw when exposed to 0.22ppm NO$_2$ for 20 minutes but a decrease in sRaw when exposed to 0.44ppm NO$_2$ for 20 minutes. Changes in sRaw were not seen in subjects with asthma. In the study by Koenig et al. (Koenig et al., 1987), there were no differences in lung function between healthy and asthmatic adolescents.

Eight of the fifteen studies measured airway responsiveness to non-specific bronchoconstricting agents (histamine, methacholine, sulphur dioxide, cold air) (Bauer et al., 1986; Bylin et al., 1988; Bylin et al., 1985; Jorres and Magnussen, 1990; Jorres and Magnussen, 1991; Rubinstein et al., 1990; Strand et al., 1997; Strand et al., 1996) or allergen challenge (Strand et al., 1997). Five studies (Bauer et al., 1986; Bylin et al., 1988; Bylin et al., 1985; Jorres and Magnussen, 1990; Strand et al., 1996) showed increased airway responsiveness after exposure to NO$_2$ compared to exposure to air whereas three studies (Jorres and Magnussen, 1991; Rubinstein et al., 1990; Strand et al., 1998) did not show an effect after exposure to NO$_2$. Seven of these studies recruited only subjects with asthma. Bylin et al. (Bylin et al., 1985) also studied healthy adults. In contrast to subjects with asthma, there were no changes in airway responsiveness after exposure to NO$_2$ in healthy subjects.

Six studies (Barck et al., 2005a; Barck et al., 2005b; Barck et al., 2002; Ezratty et al., 2014; Strand et al., 1997; Strand et al., 1996), also measured inflammatory markers in blood, sputum, nasal fluid and/or BALF. In these six studies, NO$_2$ exposure ranged from 0.2ppm to 0.6ppm and the duration of exposure ranged from 15 to 30 minutes. A range of inflammatory markers were measured and include eosinophil cationic protein (ECP; six studies),
myeloperoxidase (MPO; four studies), tryptase (one study) and interleukins (one study) as well as different types of white blood cells (for example, eosinophils and neutrophils; five studies). The results are mixed. There were increases in ECP after NO₂ exposure in three of six studies (Barck et al., 2005a; Barck et al., 2002; Ezratty et al., 2014) but no increases in MPO, tryptase or interleukins. Eosinophils and/or neutrophils were increased in two of the five studies.

Strand et al. (Strand et al., 1996) investigated whether any adverse effects from exposure to NO₂ persisted over seven days. Exposure to 0.26ppm NO₂ or purified air for 30 minutes was followed by histamine challenge at 30 minutes, five hours, 27 hours and seven days after the end of exposure. There was a significant increase in airway responsiveness to histamine only at five hours after exposure. There were no changes in specific airway resistance, inflammatory markers (ECP, MPO, tryptase) or in self-reported symptoms in the 24 hours after exposure. This suggests that any change in airway responsiveness is transient.

Three studies have also exposed subjects to different concentrations of NO₂ in an attempt to establish a dose-response relationship (Bylin et al., 1988; Ezratty et al., 2014; Koenig et al., 1987). Koenig et al. (Koenig et al., 1987) did not demonstrate differences in lung function between exposures to 0.12ppm NO₂, 0.18ppm NO₂ or filtered air for 30 minutes. Bylin et al. (Bylin et al., 1988) exposed adults with mild asthma to 0.13ppm, 0.25ppm, 0.49ppm NO₂ or filtered air for 30 minutes and were only able to demonstrate increased airway responsiveness to histamine challenge in subjects exposed only to NO₂ concentration of 0.25ppm. These two studies did not demonstrate a dose-response relationship. Ezratty et al. (Ezratty et al., 2014) exposed adults with seasonal allergic rhinitis and mild asthma to 0.2ppm NO₂, 0.6ppm of NO₂ or filtered air and measured lung function as well as inflammatory markers in sputum. Exposure to NO₂ or filtered air occurred on two consecutive days - one exposure for 30 minutes on day one and twice for 30 minutes each on day two. There were no differences in lung function (FEV₁, PEF) after exposure to NO₂ (0.2ppm and 0.6ppm) or filtered air. However, the percentage of eosinophils and ECP in sputum increased after exposure to 0.6ppm NO₂ but not after 0.2ppm NO₂ or filtered air.

A small number of studies have also studied the impact of repeated exposures to NO₂ (Barck et al., 2005a; Ezratty et al., 2014; Strand et al., 1998). Strand et al. (Strand et al., 1998) exposed subjects with mild asthma to 0.26ppm of NO₂ for 30 minutes for four consecutive days and measured lung function (FEV₁, sRaw) after inhaling an allergen, as well as self-reported symptoms and medication use. There were small but significant changes in FEV₁ with both single and repeated exposures but no evidence of a cumulative
There were no changes in airway responsiveness to histamine challenge, reported symptoms and beta-agonist use. Barck et al. (Barck et al., 2005a) exposed subjects with mild asthma to 0.26ppm NO₂ for 15 minutes on day one followed by two exposures of 0.26ppm NO₂ for 15 minutes each on day two. Lung function tests and sputum were collected after an allergen challenge. There were no changes in lung function and sRaw. However, ECP in sputum increased significantly after the second day of NO₂ exposure compared to the first NO₂ exposure suggesting that brief repeated exposures are necessary to promote an airway inflammatory response to allergen. Ezratty et al. (Ezratty et al., 2014) exposed adults with seasonal allergic rhinitis and mild asthma to 0.2ppm NO₂, 0.6ppm NO₂ or filtered air and measured lung function as well as inflammatory markers in sputum. Exposure to NO₂ or filtered air occurred on two consecutive days - one exposure for 30 minutes on day one and twice for 30 minutes each on day two. There were no changes in eosinophil inflammation after the first day of NO₂ exposure but there were significant increases in eosinophil inflammation after the second day of NO₂ exposure suggesting either a delayed response or a cumulative effect of NO₂.

Lastly, the three studies where NO₂ exposures were greater than 2.25ppm are presented here. Sandstrom et al. (Sandstrom et al., 1990) studied 32 healthy males and showed increased mast cells and lymphocytes in BALF at four to 24 hours and increased macrophages at 24 and 48 hours following exposure to 4ppm NO₂ and light exercise for 20 minutes. In a further study, Sandstrom et al. (Sandstrom et al., 1991) exposed healthy adults to 2.25, 4.0 and 5.5ppm NO₂ and light exercise for 20 minutes. There were no changes in FVC and FEV₁ or non-cellular markers after any of the NO₂ exposures. There was an increase in lymphocytes (at 4.0ppm and 5.5ppm NO₂) and mast cells at all three NO₂ exposures. Finally, Helleday et al. (Helleday et al., 1994) exposed smokers and non-smokers to 3.5ppm NO₂ and light exercise for 20 minutes. Smokers had increased alveolar macrophages and neutrophils in BALF at 24 hours after exposure while non-smokers had increased lymphocytes in BALF and increased neutrophils in bronchial fluid. There were no changes in white blood cell counts in blood in either of the groups.

In summary:
- There were no differences in lung function after exposure to NO₂ compared to filtered air. NO₂ concentrations ranged from 0.12ppm to 0.5ppm for 15 to 30 minutes.
- There is an effect of exposure to NO₂ on airway responsiveness. Five of eight studies showed increased airway responsiveness after exposure to NO₂.
compared to exposure to filtered air. All five studies recruited subjects with asthma.

- The results for inflammatory markers are mixed. The most consistent results were for increases in ECP after NO$_2$ exposure (increases in three of six studies).
- The one study that measured airway responsiveness over a longer period (at 30 minutes, five hours, 27 hours and seven days after exposure) suggests that any change in airway responsiveness is transient.
- There is no clear evidence of a dose-response relationship for effects on lung function and airway responsiveness at NO$_2$ levels below 0.5ppm.
- There is no clear evidence that repeated exposure to NO$_2$ lead to cumulative effects. The two studies that demonstrated probable cumulative effects exposed subjects to a higher dose on the second day. Therefore it is not clear whether it was the cumulative doses or the increased dose that lead to greater effects after the second day of exposure.

4. **Experimental studies where the NO$_2$ exposure is $\geq 60$ minutes**

Fourteen studies were reviewed in this section (Table 2). These studies were conducted between 1989 and 2012, with NO$_2$ exposures between 0.3 and 4ppm and duration of exposure between one to six hours (1 hour: 4 studies; 2 hours: 2 studies; 3 hours: 4 studies; $\geq 4$ hours: 4 studies).

Of the nine studies that investigated changes in lung function (NO$_2$ exposures ranged from 0.3 to 4ppm and the duration of exposure ranged from one to four hours) (Blomberg et al., 1999; Frampton et al., 2002; Frampton et al., 1991; Frampton et al., 1989; Langrish et al., 2010; Rubinstein et al., 1991; Salome et al., 1996; Vagaggini et al., 1996; Witten et al., 2005), only one study demonstrated decreases in lung function after NO$_2$ exposure compared to exposure to air. In this study (Blomberg et al., 1999), 12 healthy subjects were exposed to 2ppm NO$_2$ or air for fours a day on four consecutive days. There were significant reductions in FEV$_1$ and FVC after the first NO$_2$ exposure. Lung function changes attenuated with repeated exposures.

Five studies investigated airway responsiveness to a number of agents (carbachol, histamine, house dust mite allergen, hypertonic saline). Airway responsiveness was increased in two studies (Frampton et al., 1991; Salome et al., 1996). In these two studies, NO$_2$ exposures were 0.6ppm for one hour and 1.5ppm for three hours. In the studies where airway responsiveness was not altered, the NO$_2$ exposures were 0.6ppm and 0.05ppm with
three 2ppm peaks for three hours (Frampton et al., 1989); 0.3ppm for one hour (Vagaggini et al., 1996) and 0.4ppm for three hours (Witten et al., 2005).

Seven of these experimental studies also measured various inflammatory markers in BAL, nasal lavage or blood (Blomberg et al., 1997; Blomberg et al., 1999; Frampton et al., 2002; Pathmanathan et al., 2003; Rubinstein et al., 1991; Wang et al., 1995; Witten et al., 2005). Three studies did not show changes to inflammatory markers (Rubinstein et al., 1991; Wang et al., 1995; Witten et al., 2005). Rubinstein et al. (Rubinstein et al., 1991) did not show any changes in lymphocytes in blood or BALF in healthy adults after 0.6ppm NO₂ exposure for two hours per day on four consecutive days; Wang et al. (Wang et al., 1995) did not show changes in nasal lavage after 0.4ppm NO₂ exposure for six hours except that in the subjects who were exposed to a nasal allergen challenge prior to NO₂ exposure, there was increased ECP in nasal fluid compared to those subjects who did not have the nasal allergen challenge; and, Witten et al. (Witten et al., 2005) showed no difference in inflammatory markers in sputum after exposure to 0.4ppm NO₂ for three hours and house dust mite allergen. The remaining four studies showed increases in various inflammatory markers in bronchial washings, bronchial biopsy and BALF. All four studies recruited healthy adults and NO₂ exposures ranged from 2ppm for four hours (Blomberg et al., 1997), 2ppm for four hours on four consecutive days (Blomberg et al., 1999; Pathmanathan et al., 2003) and 0.6 or 1.5ppm for three hours (Frampton et al., 2002).

Three studies also examined cardiovascular disease markers following NO₂ exposures (Channell et al., 2012; Langrish et al., 2010; Scaife et al., 2012). Langrish et al. (Langrish et al., 2010) did not show any differences in blood flow after vasodilators or in fibrinolytic markers in 10 healthy adults exposed to 4ppm NO₂ compared to filtered air for one hour. There were no differences in heart rate, blood pressure and heart rate variability in 18 older adults with a history of heart disease exposed to 4ppm NO₂ compared to filtered air for one hour (Scaife et al., 2012). In an interesting study, Channell et al. (Channell et al., 2012) exposed 14 healthy adults to diesel exhaust (PM=100ug/m³, NO₂=0.8ppm), 0.5ppm or filtered air for two hours and then probed human coronary artery cells for intracellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1). VCAM-1 was increased after both NO₂ and diesel exhaust exposure and ICAM-1 was increased only after NO₂ exposure.
In summary:

- There were no differences in lung function after exposure to NO\textsubscript{2} compared to filtered air. NO\textsubscript{2} concentrations ranged from 0.3 to 4ppm and the duration of exposure ranged from one to four hours.
- Two of five studies showed increased airway responsiveness after exposure to NO\textsubscript{2} compared to exposure to filtered air.
- The results for inflammatory markers are mixed. Overall, inflammatory markers increased after exposure to NO\textsubscript{2}.
- There are only a few experimental studies investigating cardiovascular endpoints after exposure to NO\textsubscript{2}. Currently, it is not possible to make a judgment on the cardiovascular effects of NO\textsubscript{2}.
- In the one study where subjects were exposed to NO\textsubscript{2} or air for four consecutive days, there were significant reductions in FEV\textsubscript{1} and FVC after the first NO\textsubscript{2} exposure which then attenuated with repeated exposures.

5. Ambient air studies in road tunnels, subways and busy roads

Eight studies were reviewed which investigated health effects after exposure to ambient air in road tunnels (n=4), along busy roads (n=1) or in subways (n=3) (Table 3). All eight studies also studied subjects in a control environment.

Seven of the eight studies measured lung function in a number of exposure scenarios where NO\textsubscript{2} levels ranged from 0.01 to 0.5ppm and the duration of exposure from five minutes to two hours (Huang et al., 1991; Klepczynska Nystrom et al., 2012; Klepczynska Nystrom et al., 2010; Larsson et al., 2010; Larsson et al., 2007; McCreanor et al., 2007; Svartengren et al., 2000). Only one study showed a decrease in lung function (McCreanor et al., 2007) where there were significant decreases in FVC and FEV\textsubscript{1} after walking along a busy road for two hours (NO\textsubscript{2}=0.07ppm, PM\textsubscript{2.5}=28ug/m\textsuperscript{3}) compared to spending two hours in a park (NO\textsubscript{2}=0.01ppm, PM\textsubscript{2.5}=12ug/m\textsuperscript{3}). McCreanor et al. (McCreanor et al., 2007) however did not show changes in FEF\textsubscript{25-75} or in exhaled nitric oxide. Six of the studies did not show any changes in lung function although Larsson et al. (Larsson et al., 2010) showed a decrease in PEF but not in VC, FVC and FEV\textsubscript{1}.

Six studies (Klepczynska Nystrom et al., 2012; Klepczynska Nystrom et al., 2010; Larsson et al., 2010; Larsson et al., 2007; McCreanor et al., 2007; Svartengren et al., 2000) also collected information about respiratory symptoms and in only one study was there no difference in symptoms (McCreanor et al., 2007). In the five remaining studies, NO\textsubscript{2}
exposures ranged from 0.01 to 0.15ppm and PM$_{2.5}$ from 28 to 95ug/m$^3$. Upper respiratory symptoms were reported in two studies (Larsson et al., 2010; Larsson et al., 2007) and lower respiratory symptoms were reported in four studies (Klepaczynska Nystrom et al., 2012; Klepaczynska Nystrom et al., 2010; Larsson et al., 2010; Larsson et al., 2007). Svartengren et al. (Svartengren et al., 2000) reported that exposure to road tunnel air resulted in significantly more asthma symptoms in the late phase of the allergen challenge.

Three studies measured airway responsiveness (Huang et al., 1991; Larsson et al., 2010; Svartengren et al., 2000). Twelve children with moderate asthma inhaled concentrated road tunnel air for 5, 15, 35, 65 and 105 breaths where NO$_2$ levels were 0.45 to 0.5ppm (Huang et al., 1991). Methacholine and dust mite allergen challenges after 105 breath exposure did not show increased airway responsiveness. Larsson et al. (Larsson et al., 2010) also did not demonstrate increased airway responsiveness in adults with mild asthma exposed to road tunnel air (NO$_2$=0.17ppm, PM$_{10}$=170ug/m$^3$, PM$_{2.5}$=95ug/m$^3$) compared to exposure to a hospital laboratory. On the other hand, Svartengren et al. (Svartengren et al., 2000) demonstrated increased airway responsiveness to allergen challenge after exposure a 30 minute to road tunnel air (NO$_2$=0.13ppm, PM$_{2.5}$=80ug/m$^3$) compared to exposure to a low air pollution suburban environment (median NO$_2$=0.005ppm).

Six studies which investigated inflammatory markers. Inflammatory markers were measured in BALF, nasal fluid, sputum and blood. Three studies (Klepaczynska Nystrom et al., 2012; Klepaczynska Nystrom et al., 2010; Larsson et al., 2010) did not demonstrate increases in BALF inflammatory markers (interleukins, tumour necrosis factor) or in blood counts in mild asthmatics after exposure to road tunnel air for two hours (median NO$_2$=0.13ppm; median PM$_{2.5}$=80ug/m$^3$) (Larsson et al., 2010) or to a subway for two hours (mean NO$_2$=0.01ppm, PM$_{2.5}$=71-77ug/m$^3$) (Klepaczynska Nystrom et al., 2012; Klepaczynska Nystrom et al., 2010). On the other hand, McCreanor et al. (McCreanor et al., 2007) demonstrated increased neutrophils and MPO in subjects exposed to a busy road but not in IL-8 and ECP; Larsson et al. (Larsson et al., 2007) showed that subjects exposed to road tunnel air had increased lymphocytes in BALF but no changes to peripheral blood (blood count, plasminogen activator inhibitor-1, fibrinogen), fibronection and total metallo-proteinease-9; Klepaczynska Nystrom et al. (Klepaczynska Nystrom et al., 2010) showed increases in blood fibrinogen and regulatory T-cells in blood after subway exposure but not in plasminogen activator inhibitor-1; and, Steenhof et al. (Steenhof et al., 2014) showed that exposure to NO$_2$ (geometric mean 0.02ppm) in a number of environments was associated with decreased lymphocytes and eosinophils and that exposure to PM was associated with increased neutrophils and monocytes in blood.
In summary:

- In these eight studies of exposure to road tunnel air and subway air, NO2 concentrations were relatively low (less than 0.2ppm in seven studies and 0.5ppm in one study). NO2 concentrations were similar to published mean concentration in other road tunnels (0.015-0.15ppm) (National Health and Medical Research Council, 2008). PM2.5 concentrations in these eight studies were at the lower end of reported concentrations from other road tunnels (100-300ug/m3) (National Health and Medical Research Council, 2008).

- There were generally no differences in lung function after exposure to road tunnels or subways compared to control environments.

- Both upper and lower respiratory symptoms were more commonly reported after exposure to road tunnel and subway environments.

- The effect of exposure to road tunnels on airway responsiveness is unclear. Two of three studies did not show increased airway responsiveness. All three studies recruited subjects with asthma.

- There are mixed results for inflammatory markers following exposure to road tunnels or subways.

6. Discussion

Folinsbee (Folinsbee, 1992) reviewed experimental studies of exposures to NO2 (20 studies of people with asthma and five studies of healthy people) to test the hypothesis that exposure to NO2 increased airway responsiveness. In the 20 studies of people with asthma, the NO2 exposures ranged from 0.1ppm to 0.6ppm (17 studies ≤0.5ppm) and the duration of exposure ranged from 20 to 120 minutes (14 studies ≤60 minutes and nine studies ≤30 minutes). In the 5 studies of healthy people, the NO2 exposures ranged from 0.1ppm to 2ppm (three studies ≤0.5ppm) and the duration of exposure ranged from 20 to 180 minutes (four studies ≤60 minutes and one study ≤30 minutes). Overall, a significant proportion (59%) of subjects demonstrated increased airway responsiveness on exposure to NO2 compared to exposure to filtered air. In a subgroup analysis, increased airway responsiveness was only seen in subjects resting when exposed to NO2 and not when exercising during exposure. There was no dose response relationship for subjects with asthma (0.05<NO2<0.2ppm; 0.2<NO2<0.3ppm; 0.3<NO2). In healthy subjects, increased airway responsiveness was only demonstrated in those exposed to more than 1ppm of NO2.
Hesterberg et al. (Hesterberg et al., 2009) reviewed more than 50 experimental studies that focussed on inhaled NO₂ concentrations between 0.1 to 3ppm during short-term exposures (30 minutes to six hours) both at rest or combined with exercise. Their findings were:

- Airway responsiveness in asthmatic individuals is not affected by NO₂ up to about 0.6ppm, although some sensitive asthmatics may be affected by NO₂ levels as low as 0.2ppm;
- Healthy subjects exposed to NO₂ below 1ppm do not show pulmonary inflammation;
- At 2ppm for four hours, neutrophils and cytokines in lung-lavage fluid can increase, but these changes do not necessarily correlate with significant or sustained changes in lung function;
- There is no consistent evidence that NO₂ concentrations below 2ppm increase susceptibility to viral infection;
- For asthmatics and individuals with chronic obstructive pulmonary disease, NO₂ induced lung inflammation is not expected below 0.6ppm;
- Changes in blood chemistry generally required NO₂ concentrations above 1–2ppm.

Goodman et al. (Goodman et al., 2009) conducted a meta-analysis and meta-regression of controlled human NO₂ exposure and airway responsiveness studies in asthmatic subjects. The three endpoints (compared to filtered air) were: change in provocative dose of a challenge agent necessary to cause a specified change in lung function; the change in FEV₁ after an airway challenge; and, the proportion of subjects with increased airway responsiveness. The authors examined 41 exposure scenarios from 38 studies published between 1976 and 2002. Concentrations of NO₂ were between 0.2ppm and 0.6ppm and the duration of exposure between 30 minutes and six hours. The overall meta-analysis results were statistically significant for all three endpoints. Dose-response relationships assessed by meta-regression for all the three endpoints were not significant. The conclusion was that although there were NO₂ effects on airway responsiveness, the effects were too small to be considered significant at NO₂ levels below 0.6ppm. Similar conclusions were expressed by Hesterberg et al. (Hesterberg et al., 2009).

Brown (Brown, 2015), in a recent analysis, examined the effects of exposure to NO₂ on airway responsiveness in asthmatics using data from human experimental studies. Analysis was stratified by whether exposure occurred while subjects were resting (16 exposure scenarios, 12 studies, 0.1-0.53ppm, duration 20-60 minutes) or by whether exposure was combined with exercise (17 exposure scenarios, 12 studies, 0.15-0.60ppm, duration 30-360 minutes). There were significant increases in airway responsiveness in individuals with
asthma exposed to NO₂ (at rest) to between 0.2ppm and 0.3ppm for 30 minutes and at 0.1ppm for 60 minutes. There was also a median decrease of 25% in the provocative dose with a clinically relevant halving of the provocative dose occurring in 25% of the asthmatic subjects (three studies with exposures ≤30 minutes and two studies with exposures of 60 minutes). This is in contrast to the conclusions drawn by Hesterberg et al. and Goodman et al. (Goodman et al., 2009; Hesterberg et al., 2009) who suggested that the effects on airway responsiveness are sufficiently small so as not to be clinically significant. The meta-analyses showed no effect for exposures during exercise. Linear regression models did not show an association between provocative dose and NO₂ exposure.

7. Summary
From the review of the experimental studies and meta-analytical studies of short-term (≤30 minutes) exposures to low levels of NO₂ (≤0.5ppm), the strongest evidence is for effects on airway responsiveness. These effects are generally seen in asthmatics, and the effects are small and transient. However, clinically relevant halving of the provocative dose occurred in 25% of asthmatic subjects.

In New South Wales, the proposal is to set the in-tunnel 15 minute rolling average NO₂ guideline at 0.5ppm. The NO₂ concentration will be a marker for air pollution concentration within the road tunnels. However a number of salient points should be borne in mind when setting in-tunnel NO₂ guidelines:

- The above studies examined small numbers of healthy or mildly asthmatic subjects, whereas the general population will include people who are more sensitive to NO₂ and may therefore experience more pronounced effects at low concentrations.
- Clinically relevant halving of the provocative dose occurred in 25% of asthmatic subjects in experimental studies where NO₂ concentrations were generally <0.5ppm and the duration of exposure between 20-60 minutes.
- Subjects exposed to road tunnel, subway or a busy road were more likely to report upper and lower respiratory symptoms. The NO₂ concentration ranged from 0.01 to 0.15ppm and the exposure duration from 30 minutes to two hours.
- In-tunnel air pollution is a complex mixture and NO₂ is a marker for this complex mixture. Therefore, even if there are no health effects at a certain level of NO₂ in experimental studies (for example, 0.5ppm in this instance), this does not mean that there will not be any health effects if in-tunnel NO₂ levels are <0.5ppm. Other components of in-tunnel air pollution, such as fine and ultra fine particles and air toxics, may yet be at high enough levels to lead to adverse health effects.
• Although the respiratory effects of NO₂ may be small in any given individual, the fact that large numbers of people will be unavoidably exposed means that the overall burden of illness could be large.

Whether the proposed NO₂ in-tunnel 15 minute rolling average of 0.5ppm is appropriately protective of health is an important question. At the moment, experimental studies suggest that brief exposures to NO₂ (≤30 minutes) at concentrations ≤0.5ppm increases airway responsiveness and that in some people with asthma such adverse effects may be clinically relevant.

Therefore, it is recommended that further consultations take place prior to finalising the NO₂ in-tunnel guideline and that consideration be given to setting a lower NO₂ in-tunnel guideline.

8. References


Larsson, B.-M. et al., 2010. Limited airway effects in mild asthmatics after exposure to air pollution in a road tunnel. Respiratory Medicine, 104: 1912-1918.


National Health and Medical Research Council, 2008. Air quality in and around traffic tunnels, Canberra, Australia.


WHO Europe, 2013. Review of evidence on health aspects of air pollution - REVIHAPP Project, WHO Regional Office for Europe, Copenhagen, Denmark.
<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental group</th>
<th>Level and duration of exposure to NO$_2$</th>
<th>Health outcomes measured</th>
<th>Results and comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bylin 1985 (Bylin et al., 1985)</td>
<td>N=8, 20-36 years, healthy. N=8, 17-45 years, asthma.</td>
<td>230ug/m$^3$ (0.11ppm), 460ug/m$^3$ (0.22ppm) or 910ug/m$^3$ (0.44ppm) NO$_2$ or filtered air at rest for 20 minutes.</td>
<td>Airway responsiveness to histamine (only for NO$_2$ at 0.44ppm and filtered air), sRaw.</td>
<td>In healthy adults, sRaw increased with 0.22ppm NO$_2$ and decreased with 0.44ppm NO$_2$. No changes in airway responsiveness. In the asthmatic group, increase in airway responsiveness with exposure to 0.44ppm NO$_2$. No differences sRaw following NO$_2$ exposure compared to filtered air between the two groups.</td>
</tr>
<tr>
<td>Bauer 1986 (Bauer et al., 1986)</td>
<td>N=15, 20-45 years, asthma, non-smokers.</td>
<td>0.3ppm NO$_2$ or air for 30 minutes. Exercise for last 10 minutes of the exposure.</td>
<td>Lung function (FEV$_1$, PEF) and airway reactivity to isocapnic cold air hyperventilation.</td>
<td>No difference in FEV$_1$ and PEF between NO$_2$ and air exposures during resting exposures. No fall in FEV$_1$ and PEF between NO$_2$ and air exposures during exercise. Exposure to NO$_2$ potentiated airway reactivity to isocapnic cold air hyperventilation.</td>
</tr>
<tr>
<td>Koenig 1987 (Koenig et al., 1987)</td>
<td>N=10, 14-19 years, healthy adolescents. N=10, 11-19 years, adolescents with asthma.</td>
<td>0.12ppm, 0.18ppm NO$_2$ or filtered air for 30 minutes. Moderate exercise for 10 minutes following exposures.</td>
<td>Lung function (PEF, FEV$_1$, maximal flow at 50% and 75% of VC).</td>
<td>No differences in any of the endpoints following NO$_2$ exposure compared to filtered air both within and between the two groups of subjects.</td>
</tr>
<tr>
<td>Bylin 1988 (Bylin et al., 1988)</td>
<td>N=20, 17-56 years, mild asthma, non-smokers.</td>
<td>260ug/m$^3$ (0.13ppm), 510ug/m$^3$ (0.25ppm) or 1000ug/m$^3$ (0.49ppm) NO$_2$ or purified air at rest for 30 minutes.</td>
<td>Airway responsiveness to histamine (about 25 minutes after exposure), sRaw, sGaw.</td>
<td>Increased airway responsiveness to 0.25ppm NO$_2$ only. No differences in sRaw and sGaw with the different NO$_2$ exposures.</td>
</tr>
<tr>
<td>Rubinstein 1990</td>
<td>N=9, 23-34 years, stable asthma, non-</td>
<td>0.3ppm NO$_2$ or filtered</td>
<td>Airway responsiveness to sulphur dioxide (0.25-0.4ppm following</td>
<td>No differences in any of the endpoints following NO$_2$ exposure compared to filtered air.</td>
</tr>
<tr>
<td>Study (Year)</td>
<td>Participants</td>
<td>Exposure Conditions</td>
<td>Lung Function and Airways</td>
<td>Results</td>
</tr>
<tr>
<td>-------------</td>
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</tr>
<tr>
<td>Rubinstein et al., 1990</td>
<td>smokers.</td>
<td>air for 30 minutes. Light to moderate exercise.</td>
<td>NO$_2$ exposure, lung function (FVC, FEV$_1$), sRaw, and respiratory symptoms.</td>
<td>air.</td>
</tr>
<tr>
<td>Sandstrom 1990 (Sandstrom et al., 1990)</td>
<td>N=32, 21-37 years, healthy, non-smokers, males.</td>
<td>4ppm NO$_2$ for 20 minutes. Light exercise.</td>
<td>BAL prior to and 4, 8, 24 and 48 hours after exposure for cell count and differential, mast cells, macrophages and albumin.</td>
<td>Increased mast cells and lymphocytes in BALF 4-24 hours after exposure and increased lysozyme positive macrophages at 24 and 48 hours after exposure.</td>
</tr>
<tr>
<td>Jorres 1990 (Jorres and Magnussen, 1990)</td>
<td>N=14, 21-55 years, mild asthma non-smokers.</td>
<td>0.25ppm NO$_2$, 0.5ppm SO$_2$ or filtered air for 30 minutes at rest.</td>
<td>Lung function (sRaw) and airway responsiveness to isocapnic hyperventilation with 0.75ppm SO$_2$.</td>
<td>Increased airway responsiveness after NO$_2$ exposure compared to SO$_2$ or air exposures.</td>
</tr>
<tr>
<td>Jorres 1991 (Jorres and Magnussen, 1991)</td>
<td>N=11, 17-55 years, mild stable asthma.</td>
<td>0.25ppm NO$_2$ or filtered air for 30 minutes. Exercising for the last 10 minutes of exposure.</td>
<td>Airway responsiveness to methacholine one hour after exercise.</td>
<td>No differences in airway responsiveness after NO$_2$ exposure compared to filtered air.</td>
</tr>
<tr>
<td>Kim 1991 (Kim et al., 1991)</td>
<td>N=9, 18-23 years, healthy competitive athletes.</td>
<td>0.18ppm, 0.3ppm NO$_2$ or filtered air for 30 minutes. Exercise on treadmill during exposure.</td>
<td>Lung function (FEV$_1$, PEF, R$_T$, Vmax50%) just prior to and immediately after exposure.</td>
<td>No differences in FEV$_1$, PEF, R$_T$ and Vmax50% after NO$_2$ exposures compared to filtered air.</td>
</tr>
<tr>
<td>Sandstrom 1991 (Sandstrom et al., 1991)</td>
<td>N=18, 22-32 years, healthy, non-smokers, males.</td>
<td>2.25ppm, 4.0ppm or 5.5ppm NO$_2$ for 20 minutes. Eight subjects in each exposure category. Light exercise.</td>
<td>BAL prior to and 24 hours after exposure for cell count and differential, mast cells, macrophages and albumin and for non-cellular components (albumin, fibronectin, angiotensin-converting enzyme, hyaluronan, β$_2$-microglobulin).</td>
<td>No changes in FVC and FEV$_1$ or non-cellular markers after NO$_2$ exposures. Increase in lymphocytes (at 4.0ppm and 5.5ppm NO$_2$) and mast cells at all three NO$_2$ exposures.</td>
</tr>
</tbody>
</table>
| Helleday 1994 (Helleday et al., 1994) | N=8, 24-35 years, non-smokers.  
N=8, 28-32 years, smokers, median 20 cigarettes/day. | 3.5ppm NO₂ for 20 minutes.  
Light exercise. | BAL and peripheral blood sampling for cell count and differential prior to and 24 hours after exposure. | After NO₂ exposure, smokers had increased alveolar macrophages and neutrophils in BALF.  
After NO₂ exposure, non-smokers had increased lymphocytes in BALF and increased neutrophils in bronchial fluid.  
No differences in blood cell count and differential. |
|---|---|---|---|---|
| Strand 1996 (Strand et al., 1996) | N=19, 20-48 years, mild asthma, atopic, life-time non-smokers and ex-smokers. | 488μg/m³ (0.26 ppm) NO₂ or purified air for 30 minutes.  
Intermittent exercise. | Airway responsiveness to histamine and sRaw measured 30 minutes, 5 hours, 27 hours and 7 days after exposure.  
Peripheral blood inflammatory markers (ECP, MPO or tryptase) and expression of an adhesion molecule (MAC-1) on granulocytes measured at baseline and at 30 minutes and 27 hours after the end of exposure.  
Self-reported respiratory symptoms during exposure and for the week after the end of exposure. | Significant increase in airway responsiveness to histamine (at 5 hours after exposure).  
No change in sRaw.  
Increased expression of MAC-1 30 mins after exposure.  
No change in ECP, MPO or tryptase.  
No differences in self-report symptoms in the 24 hours after exposure. |
| Strand 1997 (Strand et al., 1997) | N=18, 18-50 years, mild asthma, pollen allergy (birch, timothy), life-time non-smokers and ex-smokers. | 500μg/m³ (0.26 ppm) NO₂ or purified air for 30 minutes followed by allergen challenge at 4 hours after exposure.  
. | Airway responsiveness to allergen and sRaw to histamine challenge 24 hours after NO₂ exposure.  
Lung function (FEV₁, PEF, FVC, FEF₅₀%, FEF₇₅%).  
Peripheral blood inflammatory | Pre-exposure to NO₂ made no differences to airway responsiveness to allergen or to histamine challenge, sRaw, inflammatory markers (ECP, eosinophils) and respiratory symptoms.  
No difference in lung function immediately after allergen challenge but a significant decrease in
| Study | Participants | Exposure Details | Measured Outcomes | Summary
<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Strand 1998 (Strand et al., 1998)</td>
<td>N=16, 21-52 years, mild asthma, atopic, life-time non-smokers.</td>
<td>500ug/m³ (0.26 ppm) NO₂ or purified air for 30 minutes on 4 consecutive days. Allergen challenge 4 hours after end of exposure.</td>
<td>PEF and FEV₁ with NO₂ 3-9 hours after allergen challenge.</td>
<td>Lung function (FEV₁), sRaw, respiratory symptoms, medication use. Self-reported respiratory symptoms. Histamine challenge on day 5. No changes in FEV₁ after NO₂ exposure. Small but significant changes in lung function (FEV₁) with single and repeated NO₂ exposure and allergen challenge. No change in histamine responsiveness, respiratory symptoms or medication (inhaled beta-agonist) use.</td>
</tr>
<tr>
<td>Barck 2002 (Barck et al., 2002)</td>
<td>N=13, 23-39 years, atopy, mild-asthma, non-smokers.</td>
<td>500ug/m³ (0.26ppm) NO₂ for 30 minutes. Allergen inhalational challenge 4 hours after exposure.</td>
<td>No effect on lung function, airway resistance or symptoms. Increased neutrophils in bronchial wash and BAL and increased ECP in bronchial wash after NO₂ and allergen challenge. No differences in other inflammatory markers (eosinophils, IL-5, IL-8, sICAM-1, eotaxin and MPO) between the two groups. No change in respiratory symptoms or medication use.</td>
<td>Lung function (FEV₁ 3-10 hours after allergen challenge) and sRaw Bronchial wash and BAL performed 19 hours after allergen challenge for inflammatory mediators. Respiratory symptoms.</td>
</tr>
<tr>
<td>Barck 2005 (Barck et al., 2005a)</td>
<td>N=18, 23-48 years, atopy, mild-asthma, non-smokers.</td>
<td>Day 1: 500 ug/m³ (0.26ppm) NO₂ or filtered air for 15 minutes. Day 2: 500ug/m³ (0.26ppm) NO₂ or purified air for 15 minutes, after 1 hour a further 500ug/m³ NO₂ or purified air for 15 minutes On both days, exposure</td>
<td>No effect of NO₂ exposure on FEV₁ and sRaw. Significant increase in ECP between days 1 and 3 in both sputum and blood after NO₂ exposure plus allergen challenge. No changes in neutrophils. Decreases in the levels of MPO in blood but not in sputum after NO₂ exposure. No change in respiratory symptoms or medication use.</td>
<td>Lung function (FEV₁), sRaw and symptoms following NO₂ exposure and allergen challenges. Inflammatory mediators and cells in sputum and blood at baseline (day 1) and days 2 and 3.</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure</td>
<td>Challenge</td>
<td>Findings</td>
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<tr>
<td>Barck 2005 (Barck et al., 2005)</td>
<td>N=16, 22-48 years, seasonal allergic rhinitis, mild asthma.</td>
<td>500ug/m³ (0.26ppm) NO₂ or filtered air for 30 minutes at rest.</td>
<td>Nasal challenge (cells, ECP, MPO) with birch or timothy pollen 4 hours after exposure. Blood sampling at baseline and 24 hours later (ECP, MPO).</td>
<td>No enhancing effect of NO₂ exposure to nasal challenge compared to purified air. No differences in ECP, MPO, eosinophils and neutrophils in blood and nasal fluid.</td>
</tr>
<tr>
<td>Ezratty 2014 (Ezratty et al., 2014)</td>
<td>N=19, 20-69 years, mild intermittent asthma, non-smokers.</td>
<td>0.2ppm or 0.6ppm NO₂ or filtered air. One exposure for 30 minutes on day 1 and twice for 30 minutes each on day 2.</td>
<td>Inflammatory response (cell count and differential, ECP) in sputum 6, 32 and 48 hours after first exposure. Lung function (FEV₁, PEF) at baseline and daily for 3 days.</td>
<td>Percentage of eosinophils and ECP in sputum increased after 0.6ppm NO₂ but not after 0.2ppm NO₂ or filtered air. Dose related trend for percentage eosinophil increase on days 2 and 3 and for ECP on day 3. No differences in FEV₁ and PEF.</td>
</tr>
</tbody>
</table>

BAL=bronchoalveolar lavage; ECP=eosinophil cationic protein; FEF<sub>50%</sub>=forced expiratory flow at 50% volume of forced vital capacity; FEF<sub>75%</sub>=forced expiratory flow at 75% volume of forced vital capacity; FEV<sub>1</sub>=forced expiratory volume in 1 second; FVC=forced vital capacity; MPO=myeloperoxidase; PEF=peak expiratory flow; R<sub>T</sub>=total respiratory resistance; sGaw=specific airway conductance; sRaw=specific airway resistance; Vmax50%=maximal expiratory flow at 50% volume of forced vital capacity
Table 2 Experimental studies where the NO$_2$ exposure is ≥60 minutes

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental group</th>
<th>Level and duration of exposure to NO$_2$.</th>
<th>Health outcomes measured</th>
<th>Results and comments</th>
</tr>
</thead>
</table>
| Frampton 1989 (Frampton et al., 1989) | N=19, 19-37 years, healthy non-smokers. | Continuous 0.6ppm NO$_2$ for 3 hours (n=9).  
Continuous 0.05ppm NO$_2$ for 3 hours which included 3 2ppm peaks each of 15 minutes (n=15).  
Five subjects participated in both protocols. | Lung function (FVC, FEV$_1$) and carbachol challenge  
Macrophages from BALF (taken 3.5 hours after exposure) examined for inactivation of influenza virus and IL-1 production in vitro. | No changes in lung function (FVC, FEV$_1$) and carbachol challenge when exposed to NO$_2$ compared to air.  
No significant changes in inactivation of influenza virus and IL-1 production in vitro between NO$_2$ and air exposure. |
| Frampton 1991 (Frampton et al., 1991) | N=39, 19-37 years, healthy, never smoked. | 9 subjects exposed to 0.6ppm NO$_2$ or purified air for 3 hours.  
15 subjects exposed to 0.05ppm NO$_2$ with 3 peaks of 2ppm for 15 minutes each.  
15 subjects exposed to 1.5ppm NO$_2$ or purified air for 3 hours.  
Exercise. | Lung function (sGaw, PEFR, MEFR, FVC, FEV$_1$) and airway reactivity to carbachol 30 minutes after exposure. | No effect on lung function for any of the three NO$_2$ exposure protocols.  
Only exposure to 1.5ppm NO$_2$ for 3 hours increased airway reactivity. |
<p>| Rubinstein 1991 (Rubinstein et al., 1991) | N=5, 18-45 years, healthy, non-smokers. | 0.6ppm NO$_2$ for 2 hours/day on 4 separate days within a six day period. | Respiratory symptoms, lung function (FVC, FEV$_1$, sRaw), circulating and BALF lymphocyte subtypes. | No effect on symptoms and lung function, or changes in circulating and BALF lymphocyte subtypes. |</p>
<table>
<thead>
<tr>
<th>Study (Author, Year)</th>
<th>Participants</th>
<th>Exposure Details</th>
<th>Nasal Provocation Test</th>
<th>Airway Changes</th>
<th>Overall Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wang 1995 (Wang et al., 1995)</td>
<td>N=16, 18-55 years, seasonal allergic rhinitis, non-smokers</td>
<td>0.4 ppm NO₂ or air for 6 hours</td>
<td>8 subjects also had a nasal allergen provocation test prior to measurement of nasal airway resistance and nasal lavage (ECP, MPO, IL-8, MCT)</td>
<td>No change in nasal airway resistance, ECP, MPO, IL-8 and MCT after NO₂ or air exposure. After allergen challenge, those exposed to NO₂ compared to air had higher ECP in nasal fluid and there were no differences in increases in MCT. No changes in MPO and IL-8 after allergen challenge.</td>
<td></td>
</tr>
<tr>
<td>Salome 1996 (Salome et al., 1996)</td>
<td>N=9, 19-65 years, current asthma. N=11, 7-15 years, severe asthma</td>
<td>1 hour exposures to 5 different scenarios: Ambient air 0.3 ppm NO₂ in ambient air 0.6 ppm NO₂ in ambient air Ambient air+gas heater combustion by-products+0.3 ppm NO₂ Ambient air+gas heater combustion by-products+0.3 ppm NO₂</td>
<td>Airway responsiveness to histamine 1 hour and 1 week after exposure. Lung function and symptoms during, 1 hour and 1 week after exposures.</td>
<td>No significant effect on symptoms or lung function (FEV₁, PEFR). Small significant increase in airway responsiveness at 0.6 ppm NO₂ in ambient air but not in the mixture with combustion by-products.</td>
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<tr>
<td>Vagaggini 1996 (Vagaggini et al., 1996)</td>
<td>N=7, mean age=34 years healthy, non-</td>
<td>0.3 ppm NO₂ for 1 hour. Moderate</td>
<td>Lung function (FEV₁) and symptoms before and 2 hours after exposure. Nasal lavage and induced sputum 2</td>
<td>No significant changes in lung function (except for a small significant decrease in COPD group), airway responsiveness to hypertonic saline</td>
<td></td>
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<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposures</td>
<td>Methods</td>
<td>Results</td>
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<tr>
<td>Blomberg 1997</td>
<td>N=30, 20-30 years, healthy, non-asthmatics, non-smokers.</td>
<td>2ppm NO₂ or air for 4 hours. Light exercise.</td>
<td>Endobronchial biopsies and bronchial lavage at 1.5 (n=15) or 6 hours (n=15) after exposures.</td>
<td>Significant increases in IL-8, neutrophils in bronchial washings and increases in lymphocytes in BALF after exposure to NO₂. No up-regulation of adhesion molecules or increase in inflammatory cells in bronchial biopsies.</td>
<td></td>
</tr>
<tr>
<td>Blomberg 1999</td>
<td>N=12, 21-32 years, healthy, non-asthmatic non-smokers.</td>
<td>2ppm NO₂ or air for 4 hours/day on 4 consecutive days. Light exercise.</td>
<td>Lung function and inflammatory mediators in the lungs. Bronchial biopsies, BAL and bronchial washes 1.5 hours after last exposures.</td>
<td>Significant reductions in FEV₁ and FVC after first NO₂ exposure which attenuated with repeated NO₂ exposures. Significant increases in neutrophils and MPO in bronchial washings but not in BALF. No change in anti-oxidants.</td>
<td></td>
</tr>
<tr>
<td>Frampton 2002</td>
<td>N=21, 18-40 years, healthy, lifetime non-smokers.</td>
<td>0.6ppm NO₂, 1.5ppm NO₂ or air for 3 hours. Intermittent exercise.</td>
<td>Spirometry (FEV₁, FVC) before and after exposure. Blood sampling and BAL 3.5 hours after exposure.</td>
<td>No differences in FEV₁ and FVC after NO₂ exposure compared to air. Decrease in haematocrit, haemoglobin and lymphocytes in blood with NO₂ exposure. Increase neutrophils and lymphocytes in bronchial lavage and increased CD4 lymphocytes in alveolar lavage with NO₂ exposure. Increased susceptibility of airway</td>
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<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure</td>
<td>Procedures</td>
<td>Outcomes</td>
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<tr>
<td>Pathmanathan 2003 (Pathmanathan et al., 2003)</td>
<td>N=12, 21-32 years, healthy, non-asthmatics, non-smokers.</td>
<td>2ppm NO₂ or air for 4 hours/day on 4 consecutive days. Light exercise.</td>
<td>Inflammatory mediators in the lungs (bronchial biopsies at 1.5 or 6 hours after exposures).</td>
<td>Significant increases in IL-5, IL-10, IL-13, ICAM-1 following NO₂ exposure compared to air.</td>
<td></td>
</tr>
<tr>
<td>Witten (2005) (Witten et al., 2005)</td>
<td>N=15, 21-48 years, mild-moderate atopic asthmatics, non-smokers and past smokers, house dust mite allergy.</td>
<td>0.4ppm NO₂ or filtered air for 3 hours. Intermittent exercise.</td>
<td>Lung function (FEV₁) before and after exposure. House dust mite allergen challenge immediately after exposure. Sputum collected at 6 and 26 hours after allergen challenge.</td>
<td>No differences in FEV₁, allergen provocative dose and respiratory symptoms between NO₂ and filtered air. No differences in inflammatory markers (IL-5, IL-8, ECP, GM-CSF) between NO₂ and filtered air after allergen challenge.</td>
<td></td>
</tr>
<tr>
<td>Langrish (2010) (Langrish et al., 2010)</td>
<td>N=10, median age=24 years, healthy males, non-smokers.</td>
<td>4ppm NO₂ or filtered air for 1 hour. Intermittent exercise.</td>
<td>Lung function (FVC, FEV₁, VC) before and after exposure. Blood flow after vasodilators. Fibrinolytic markers (plasminogen activator, plasminogen-activator inhibitor type 1) measured at 4 hours after exposure. Exhaled nitric oxide measured at baseline, and 1 and 4 hours after exposure.</td>
<td>No differences in lung function, blood flow after vasodilators, fibrinolytic markers and exhaled nitric oxide between the two groups.</td>
<td></td>
</tr>
<tr>
<td>Scaife 2012 (Scaife et al., 2012)</td>
<td>N=18, 56-76 years, undergone CABG or had a previous history of a heart attack.</td>
<td>0.4ppm NO₂ or air for 1 hour.</td>
<td>Holter monitoring during and for 24 hours after exposure.</td>
<td>No significant changes in heart rate, blood pressure and indices of heart rate variability for exposure to NO₂ compared to air.</td>
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</tr>
<tr>
<td>Channell 2012 (Channell et al., 2012)</td>
<td>N=14, healthy adults. Mean age diesel</td>
<td>Diesel exhaust (PM=100µg/m³, NO₂=0.8ppm) or</td>
<td>Blood sampling before, immediately after and 24 hours after exposures. Human coronary artery endothelial</td>
<td>ICAM-1 expression increased at both time points after NO₂ exposure and VCAM-1 expression increased at both time points for NO₂ exposure and at 24 hours.</td>
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<tr>
<td>exhaust exposure=24.9 years. Mean age NO₂ exposure=25.3 years.</td>
<td>filtered air. NO₂ (0.5 ppm) or filtered air. All exposures for 2 hours with intermittent moderate exercise.</td>
<td>cells probed for ICAM-1 and VCAM-1 expression.</td>
<td>hours for diesel exhaust exposure. IL-8 increased in human coronary artery endothelial cells incubated with plasma after NO₂ exposure.</td>
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</table>

BAL = bronchoalveolar lavage; BALF = bronchoalveolar fluid; CABG = coronary artery bypass graft; COPD = chronic obstructive pulmonary disease; ECP = eosinophil cationic protein; FEV₁ = forced expiratory volume in 1 second; FVC = forced vital capacity; GM-CSF = granulocyte/macrophage colony stimulating factor; ICAM = intracellular adhesion molecule; IL = interleukin; MCT = mast cell tryptase; MEFR = mid-expiratory flow rate; PEF = peak expiratory flow; sGAW = specific airway conductance; sRaw = specific airway resistance; VC = vital capacity; VCAM = vascular cell adhesion molecule
<table>
<thead>
<tr>
<th>Study</th>
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<th>Level and duration of exposure to NO₂</th>
<th>Health outcomes measured</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Huang (1991) (Huang et al., 1991)</td>
<td>N=6, mean age=12 years, dust mite sensitive, moderate asthma.</td>
<td>Concentrated road tunnel air inhaled for 5, 15, 35, 65 and 105 breaths. Inhaling 105 breaths took about 5 minutes. SO₂=70-120ppb and NO₂=0.45-0.50ppm was which were 6 and 20 fold above ambient air respectively.</td>
<td>Lung function tests before and after each breath category. Methacholine challenge and mite allergen challenge before and after taking 105 breaths.</td>
<td>Exposure to concentrated road tunnel air did not affect lung function and did not increase airway sensitivity to methacholine challenge and mite allergen challenges.</td>
</tr>
<tr>
<td>Svartengren (2000) (Svartengren et al., 2000)</td>
<td>N=20, 19-55 years, mild atopic (birch, timothy grass) asthma. Exposed to pollutants inside a car within a road tunnel.</td>
<td>30 minute median exposure to: NO₂=313ug/m³ (0.15ppm) (range 203–462ug/m³) PM₁₀=170ug/m³ (range 103–613ug/m³) PM₂.₅=95ug/m³ (range 61–218ug/m³). As a control, subjects also exposed to lower pollution levels in a suburban area – median NO₂=11ug/m³ (0.005ppm) (range 0-51ug/m³).</td>
<td>Following allergen challenge 4 hours after exposure, lung function (FEV₁), airway resistance, symptoms and medication used measured.</td>
<td>Concentrations of NO₂ in the car and in the tunnel were similar. After allergen exposure, a significant increase in airway resistance but no difference in fall in FEV₁ between tunnel air exposure and the control exposure. Exposure to road tunnel air had a significantly more asthma symptoms during the late phase after the allergen challenge.</td>
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<tr>
<td>McCreanor (2007) (McCreanor et al., 2007)</td>
<td>N=60, 19-55 years, mild to moderate asthma.</td>
<td>2 hours walking along a busy road (median NO₂=142ug/m³ (0.07ppm); range 10.7-289ug/m³; median PM₂.₅=28.3ug/m³, range 62-161ug/m³). 2 hours in a park (NO₂=21.7ug/m³ (0.01ppm);</td>
<td>Lung function (FEV₁, FVC, FEF₂₅₋₇₅), PEF, symptoms, exhaled nitric oxide. Sputum for inflammatory markers (IL-8, MPO, ECP).</td>
<td>Significantly greater decrements in FEV₁ and FVC in the street group. Changes were greater in moderate asthma group compared to the mild asthma group. No differences in FEF₂₅₋₇₅, asthma symptoms and medication use, and exhaled nitric oxide between the two exposures.</td>
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<tr>
<td>Study</td>
<td>Participants</td>
<td>Exposure</td>
<td>Measures</td>
<td>Findings</td>
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<td>Larsson (2007) (Larsson et al., 2007)</td>
<td>N=16, 19-59 years, healthy, non-smokers.</td>
<td>2 hour exposure to a road tunnel (median NO$<em>2$=230ug/m$^3$; range 180-269ug/m$^3$; median PM$</em>{2.5}$=64ug/m$^3$, range 46-81ug/m$^3$). 2 hour exposure to an urban area (24-hr mean NO$<em>2$=46ug/m$^3$, PM$</em>{2.5}$=13ug/m$^3$). Intermittent light exercise.</td>
<td>Lung function (VC, FEV$_1$, FVC), symptoms, peripheral blood (fibrinogen) and BALF (lymphocytes, fibronectin, total metallo-proteinease-9).</td>
<td>Higher neutrophils and MPO in the road exposure group. No differences in IL-8 and ECP.</td>
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<td>Klepczynska-Nystrom (2010) (Klepczynska Nystrom et al., 2010)</td>
<td>N=20, 18-46 years, healthy, non-smokers.</td>
<td>2 hour exposure to a subway environment (mean NO$<em>2$=24ug/m$^3$ (0.01ppm), PM$</em>{2.5}$=77ug/m$^3$). 2 hour exposure to an office environment (NO$<em>2$ and PM$</em>{2.5}$ concentrations not provided). Intermittent light exercise.</td>
<td>Lung function (VC, FEV$_1$, FVC, PEF, exhaled nitric oxide), symptoms, peripheral blood (blood count, plasminogen activator inhibitor-1, fibrinogen), BALF (inflammatory markers – IL-1B, IL-6, IL-8, IL-10, IL-12p70, tumour necrosis factor).</td>
<td>Increased reporting of lower airways symptoms with subway exposure. No differences in upper airway, eye and nose symptoms. Increase in blood fibrinogen and regulatory T-cells in blood after subway exposure. No differences in lung function, plasminogen activator inhibitor-1 and BALF inflammatory markers.</td>
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<td>Larsson (2010) (Larsson et al., 2010)</td>
<td>N=14, 18-55 years, mild asthma, non-smokers.</td>
<td>2 hour exposure to a road tunnel (median NO$<em>2$=265ug/m$^3$ (0.13ppm); range 112-579ug/m$^3$; median PM$</em>{2.5}$=80ug/m$^3$, range 41-93ug/m$^3$). 2 hour exposure to hospital laboratory (NO$<em>2$ and PM$</em>{2.5}$ concentrations not provided).</td>
<td>Lung function (VC, FEV$_1$, FVC, PEF, exhaled nitric oxide), blood count, methacholine challenge for airway responsiveness, nasal lavage and induced sputum 7 hours after exposure.</td>
<td>Significantly increased reporting of upper and lower airways symptoms and decreased PEF with road tunnel exposure. No differences in VC, FEV$_1$, FVC, airway responsiveness after methacholine challenge, inflammatory markers (IL-1B, IL-6, IL-8, IL-10, IL-12p70, tumour necrosis factor, interferon-gamma) and blood count.</td>
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<tr>
<td>Study</td>
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<td>Outcome Measures</td>
<td>Results/Findings</td>
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<tr>
<td>Klepczynska-Nystrom (2012) (Klepczynska</td>
<td>N=16, 18-52 years, mild asthma, non-smokers</td>
<td>2 hours to a subway environment (mean NO$<em>2$=20ug/m$^3$ (0.01ppm), PM$</em>{2.5}$=71ug/m$^3$)</td>
<td>Intermittent light exercise.</td>
<td>Lung function (VC, FEV$_1$, FVC, PEF, exhaled nitric oxide), symptoms, peripheral blood (blood count, plasminogen activator inhibitor-1, fibrinogen), BALF (inflammatory markers – IL-1B, IL-6, IL-8, IL-10, IL-12p70, tumour necrosis factor). Increased reporting of eye and nose symptoms with subway exposure. No differences in lower airway symptoms. Increase in CD4 cells expressing activation marker CD25 in BALF after subway exposure. No differences in lung function, plasminogen activator inhibitor-1, fibrinogen, blood count and BALF inflammatory markers.</td>
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<td>Nystrom et al., 2012)</td>
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<td>2 hours to an office environment (NO$<em>2$ and PM$</em>{2.5}$ concentrations not provided). Moderate light exercise.</td>
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<td>Steenhof (2014) (Steenhof et al., 2014)</td>
<td>N=31, 19-26 years, healthy, non-smokers</td>
<td>5 hour exposure to 5 different environments – 2 traffic sites, an underground train station, a farm and an urban background site. All outdoor sites combined: geometric mean NO$<em>2$=0.02ppm, range=0.009-0.034ppm; geometric mean PM$</em>{2.5}$=23ug/m$^3$, range=8-95ug/m$^3$. Underground train station: geometric mean NO$<em>2$=0.02ppm, range=0.014-0.026ppm; geometric mean PM$</em>{2.5}$=140ug/m$^3$, range=123-167ug/m$^3$. Intermittent exercise.</td>
<td>White blood count at baseline at 2 and 18 hours after exposure.</td>
<td>In regression models, decreased lymphocytes and eosinophils counts associated with NO$_2$. In regression models, increased white blood cell count, neutrophils and monocytes counts associated with PM at the underground train station.</td>
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