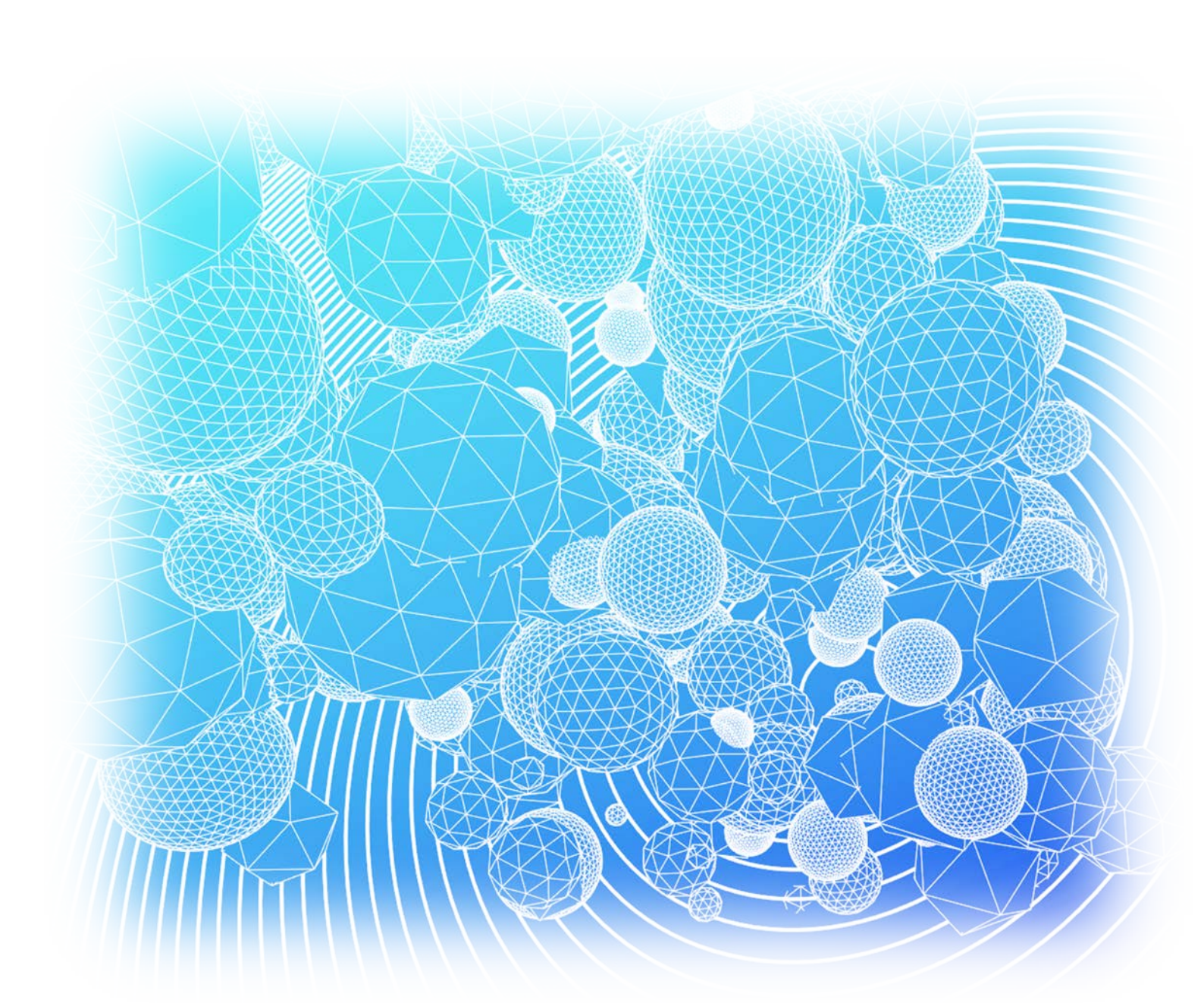

Literature Review and Risk Characterisation of Nitrogen Dioxide

Long and Heavily Trafficked Road Tunnels

Prepared for: NSW Roads and Maritime Services (RMS)

13 April 2018

EnRiskS

Document History and Status

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Limitations

Environmental Risk Sciences Pty Ltd has prepared this report for the use of New South Wales Roads and Maritime Services (RMS) in accordance with the usual care and thoroughness of the consulting profession. It is based on generally accepted practices and standards at the time it was prepared. No other warranty, expressed or implied, is made as to the professional advice included in this report.

It is prepared in accordance with the scope of work and for the purpose outlined in the **Section 1** of this report.

The methodology adopted and sources of information used are outlined in this report. Environmental Risk Sciences Pty Ltd has made no independent verification of this information beyond the agreed scope of works and assumes no responsibility for any inaccuracies or omissions. No indications were found that information provided for use in this assessment was false.

This report was prepared from October to December 2017 and is based on the information provided and reviewed at that time. The report has been revised from January to April 2018 to address review comments. Environmental Risk Sciences Pty Ltd disclaims responsibility for any changes that may have occurred after this time.

This report should be read in full. No responsibility is accepted for use of any part of this report in any other context or for any other purpose or by third parties. This report does not purport to give legal advice. Legal advice can only be given by qualified legal practitioners.

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Appendices:

Glossary of Terms

EPHC	Environment Protection and Heritage Council
NEPC	National Environment Protection Council
NEPM	National Environment Protection Measure
NHMRC	National Health and Medical Research Council
NO	Nitric Oxide
NO2	Nitrogen Dioxide
NOx	Oxides of Nitrogen
US EPA	United States Environmental Protection Agency
WHO	World Health Organization

Executive Summary

Environmental Risk Sciences Pty Ltd (enRiskS) was commissioned by New South Wales Roads and Maritime Services on behalf of the NSW Advisory Committee on Tunnel Air Quality to review nitrogen dioxide health evidence with reference to current interpretations of in-tunnel nitrogen dioxide guidelines in the New South Wales (NSW), Australia and Sweden.

The current NSW guideline is based on an evaluation of health evidence relevant to exposures to nitrogen dioxide up to 30 minutes in duration. This review is being undertaken to address the use of a larger network of tunnels, where in-tunnel exposures may extend up to 60 minutes.

Nitrogen dioxide is a reactive gas that humans are exposed to through inhalation. Exposure to nitrogen dioxide has been associated with respiratory and cardiovascular health effects along with mortality. It is believed that nitrogen dioxide can cause health effects by reacting with the fluid in the lungs. This reaction forms other chemicals which then affect lung tissue through direct contact and other tissues through their absorption into the blood stream. Affecting the lung or other bodily tissues such as the blood vessels have the consequence of initiating inflammatory, allergic and neural responses which can lead to adverse health outcomes.

This review is designed to provide the NSW Advisory Committee on Tunnel Air Quality with evidence to inform their evaluation of the current NSW in-tunnel nitrogen dioxide limit of 0.5 ppm as a rolling 15-minute average by:

- Reviewing the relevant international literature
- Advising on the appropriateness of the Swedish guideline approach for the NSW context
- Identifying any measures worldwide to characterise or address any risks due to nitrogen dioxide exposures for journeys of up to 60 minutes; and
- Advising on appropriate measures to reduce in-tunnel nitrogen dioxide exposure.

International literature review

This review examined experimental studies to determine if exposures of nitrogen dioxide at 0.5 ppm for up to 60 minutes was likely to cause a clinically relevant health effect. Seventy-eight studies were reviewed and although twelve studies examining health effects of nitrogen dioxide exposure up to 0.5 ppm for up to 60 minutes found a statistically significant result, none of these studies were determined to have a clinically relevant health effect.

Appropriateness of the Swedish guideline approach for the NSW context

Evidence from observational epidemiological studies are being used in Sweden for consideration of in-tunnel oxides of nitrogen concentrations. This approach does not set a guideline value, but rather presents potential health costs and benefits of different in-tunnel concentrations. It is assumed that this cost benefit process will then be used within the planning decision making process to set an in-tunnel limit. If a process such as this were to occur in Australia, it would involve the use of different observational studies and potential health endpoints.

Identification of any measures worldwide to characterise or address any risks due to nitrogen dioxide exposures for journeys of up to 60 minutes

Internationally, 1 hour (60 minute) guideline values for nitrogen dioxide are driven by experimental studies with observational studies adding a weight of evidence or providing the basis for adjusting the value derived from the experimental studies. EnRiskS is unaware of any major health or environmental agency that have set a 1 hour nitrogen dioxide exposure guideline based solely on observational epidemiological studies. While some discussion of taking this approach has occurred, the limitations within observational studies and lack of an agreed approach in the way the data from these studies may be translated into a 1 hour guideline, at this time it is not recommended that observational data be used to solely develop an in-tunnel guideline value.

Risk Characterisation

Currently users of Sydney major road tunnel systems may be exposed to nitrogen dioxide concentrations of up to 0.7 ppm, however for car and truck users these concentrations can be reduced to below 0.2 ppm if they wind up their windows and turn their air conditioning onto recirculation. This is lower than the 0.5 ppm which has been considered, based on the experimental studies, to not induce clinically relevant health effects.

While tunnel users may be exposed to nitrogen dioxide concentrations of up to 0.7 ppm, the average nitrogen dioxide concentration in Sydney's major road tunnels was measured at 0.27 ppm, well below the 0.5 ppm criteria.

Actions to assist in managing in-tunnel nitrogen dioxide exposure

Informing users to wind up their windows and turn their air conditioning onto recirculation, as is currently being done at major Sydney road tunnels will reduce exposure to in-tunnel nitrogen dioxide and is therefore an important health message.

Conclusion

The current NSW in-tunnel guideline was informed by the report *Review of experimental studies of exposures to nitrogen dioxide* which considered nitrogen dioxide exposures up to 30 minutes. This review supports and clarifies the conclusions drawn in the *Review of experimental studies of exposures to nitrogen dioxide* report, even with an extended exposure period from 30 to 60 minutes.

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Section 1. Introduction

1.1 Background

Environmental Risk Sciences Pty Ltd (enRiskS) has been commissioned by New South Wales Roads and Maritime Services (RMS) on behalf of the NSW Advisory Committee on Tunnel Air Quality to review in-tunnel nitrogen dioxide (NO_2) health evidence with reference to current interpretations in the New South Wales (NSW), Australia and Sweden.

In NSW the current policy for NO_2 exposure is defined in the *In-Tunnel Air Quality (Nitrogen Dioxide) Policy* which is based on exposure durations of 20 – 30 minutes and short term experimental NO_2 exposure health evidence. The NSW Advisory Committee on Tunnel Air Quality (the Advisory Committee) is reviewing the appropriateness of this policy for tunnel transits lasting up to one hour. The Advisory Committee is seeking:

- Advice on the appropriateness of the Swedish approach to in-tunnel NO_2 limits.
- A characterisation of the risk to vehicle occupants due to NO_2 exposure resulting from tunnel transit with in-tunnel NO_2 levels at the maximum permitted by the *In-Tunnel Air Quality (Nitrogen Dioxide) Policy*.
- Options for any further actions that may assist in managing the risks due to in-tunnel NO_2 exposure in planned Sydney motorway tunnels.

to inform their review.

In Sweden the planned Stockholm Bypass will consist of 18 kilometres (km) of tunnelled roadway with approximately 140 000 vehicular uses per day. This is comparable to the NSW WestConnex scheme which will have 24km of tunnels with approximately 134 000 vehicular uses per day. In anticipation of the Stockholm Bypass, the Swedish Transport Administration have been developing an NO_2 in-tunnel policy. In contrast to the NSW *In-Tunnel Air Quality (Nitrogen Dioxide) Policy*, the Swedish policy is derived from observational epidemiological studies that examine short term exposure to ambient NO_2.

This report has been prepared in line with the objectives outlined in **Section 1.2** and has been modified to address review comments provided by NSW Health and independent reviewer Professor Brian Priestly. Its aim is to review the current NSW and Swedish approaches and provide advice to assist the Advisory Committee in their review.

1.2 Objectives

The objectives of this report are:

- Clarify the methodology used by the Swedish Transport Administration in its development of its in-tunnel NO_2 policy.
- Review and critique the Swedish methodology, with particular reference to the advantages and limitations of the NSW *In-Tunnel Air Quality (Nitrogen Dioxide) Policy.*
- Undertake a literature review of NO_2 and health impacts relevant to the assessment of in-tunnel air quality
- Undertake a risk characterisation based on the findings of the literature review and likely exposures the public may experience within an in-tunnel environment

- Identify options for actions that may assist in managing the health risks due to in-tunnel NO_2 exposure in planned Sydney motorway tunnels for consideration by the Advisory Committee.

This report has only undertaken a literature review of NO_2 and traffic related pollution human experimental studies, in line with the scope highlighted in (Jalaludin 2015), expanded to include studies up to 60 minutes. It is noted that in Australia the National Environment Protection Council have set Australian specific guidance for observational studies to be used when considering concentration response functions from short term exposures to NO_2 (Jalaludin & Cowie 2012).

The report will not specifically consider the health impact of oxides of nitrogen (NOx) other than NO_2, in line with the NSW *In-Tunnel Air Quality (Nitrogen Dioxide) Policy*. It is however understood that some of the studies reviewed will have considered NOx.

1.3 Methodology

The report has been undertaken to specifically evaluate potential health impacts from NO_2 emissions in a tunnelled environment. As such an assessment has been undertaken in accordance with national guidelines on assessing environmental health issues within the community as outlined in the following:

- enHealth, Environmental Health Risk Assessment, Guidelines for Assessing Human Health Risks from Environmental Hazards (enHealth 2012).
- enHealth, Health Impact Assessment Guidelines (enHealth 2001)
- NSW Health, Healthy Urban Development Checklist, A guide for health services when commenting on development polices, plans and proposals (NSW Health 2009)

1.4 Current policy

The NSW *In-Tunnel Air Quality (Nitrogen Dioxide) Policy* specifies an in-tunnel NO_2 limit of **0.5 ppm** as a rolling 15-minute average. This limit is based on evidence from a 2015 review of NO_2 exposure and health effects (Jalaludin 2015), comparative guidelines around the world, and evidence of reduced exposure in vehicles that have their windows up and air vents set to recirculate.

Section 2. Health Impacts of Nitrogen Dioxide

2.1 General

Nitrogen dioxide (NO_2) has been associated with increases in respiratory symptoms, cardiovascular effects, cancer incidence, adverse birth outcomes and mortality, and while epidemiological studies have found these associations, the strength of evidence supporting these associations is varied across health outcome (US EPA 2016; WHO 2013). The effect nitrogen dioxide has on the respiratory system is viewed as the most robust health outcome (WHO 2013). The US EPA has classified respiratory effects from short term (minutes to 1 month) exposure to NO_2 as a causal relationship (US EPA 2016), meaning they believe there is enough evidence to show that short term exposure to NO_2 causes respiratory health effects. With regard to the other health outcomes, the evidence is less certain (WHO 2013), and tends to be suggestive of, but not sufficient to infer, a causal relationship (US EPA 2016).

A complicating factor when examining the independent effects of NO_2 on health is that most of the observational epidemiological studies that consider NO_2 exposure also have a range of other air pollutant exposures that may contribute to the health effect. The strong correlation between NO_2 and other (mostly traffic related) pollutants make it difficult, but not impossible to determine the independent effect of NO_2. This complicating factor is less of a concern in this instance as setting an NO_2 in-tunnel guideline needs to have some consideration of other traffic related pollutants and the potential surrogate nature the NO_2 guideline.

This chapter attempts to address the underlying mechanisms believed to be responsible for the health effects induced by NO_2 exposure. Essentially this involves exploring the potential modes of action linked to causing the health effects. Given the level of knowledge for the potential modes of action, respiratory and cardiovascular health effects will be the primary outcomes considered. The chapter will also explore clinical relevance of the main health outcome, respiratory effects.

2.2 General information on Nitrogen Dioxide

Nitrogen dioxide is a highly reactive gas that is soluble in water and a strong oxidant. Being a gas at room temperature means humans are exposed to NO_2 primarily through breathing it in.

On a global scale, emissions from natural sources outweigh those generated from human activities. However since the natural sources are distributed all over the earth, unlike human contributions which are concentrated in certain areas, the natural contribution to the levels of NO_2 in the atmosphere are small (WHO 2000).

Human generation of NO_2 is primarily through the burning of fossil fuels. This can be through stationary sources, such as heating and power generation, or mobile sources such as motor vehicles.

Exposure to NO_2 can cause health effects, which are further explored in **Section 2.4**.

2.3 Anatomy of the lung

Since breathing nitrogen dioxide is the main way a human can be exposed to NO_2, understanding the anatomy of the lung is important when considering the health effects from exposure to NO_2.

The lung may be divided into a number of discrete parts, consisting of a series of branching tubes which become narrower, shorter and more numerous as they penetrate deeper into the lung (West 2012). When air is breathed in, it first passes down the trachea which divides into the right and left main bronchi. The main bronchi divide into the lobar and segmented bronchi which intern divides into the terminal bronchioles. All these bronchi make up the conducting airways, and their function is to lead air into the gas exchanging regions of the lung. Because they are conducting airways, they take no part in gas exchange and are thus known as anatomical dead space (West 2012).

Gas exchange begins at the respiratory bronchioles which being at the end of the terminal bronchioles. The respiratory bronchioles, which have some alveoli budding from their walls, finally branch into the alveolar ducts. The alveolar ducts are completely lined with alveoli (West 2012). **Figure 2.1** provides a graphical representation of this process.

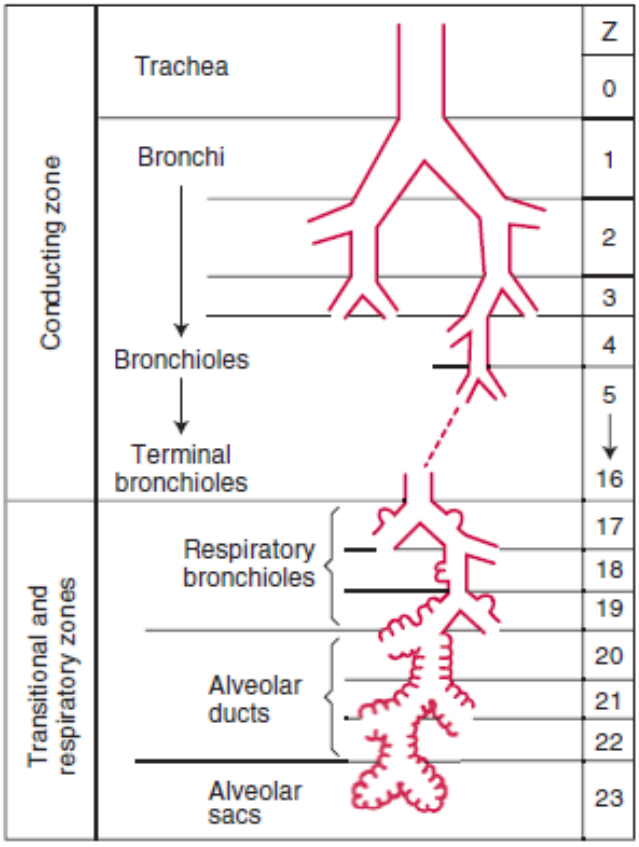


Figure 2.1: Idealisation of the human airways (taken from (West 2012))

2.4 Effects of Nitrogen Dioxide

The US EPA (US EPA 2016) provide a comprehensive review of the dosimetry and mechanistic effects of NO_2 which is summarised below.

Nitrogen dioxide is a highly reactive gas that humans are exposed to either environmentally (through air breathed in) or endogenously (through the body's internal processes producing NO_2). It is believed that the body produces NO_2 via three main pathways. These include:

- the acidification of nitrite (as can transpire in phagolysosomes)
- the decomposition of peroxynitrite and / or the nitrosoperoxylcarbonate anion
- the action of peroxidases when using nitrite and hydrogen peroxide as substrates

Internal production of NO_2 is thought to increase with the consumption of nitrite and nitrate, which are present in substantial concentrations in some leafy vegetables like spinach. Immune responses and inflammation are also known to produce nitrite and nitrate. Due to its reactivity potential, NO_2 is unlikely to become systemically distributed, meaning that the effect of NO_2 is likely to be localised to where it was produced in the body. For environmental exposure to NO_2, this means that tissues other than the lung are unlikely to be affected from inhaled NO_2.

Being highly reactive, NO_2 does not stay as NO_2 for long once breathed into the lungs. When inhaled, NO_2 is met by the epithelial lining fluid. This fluid is a biologically complex aqueous fluid layer that covers all the respiratory tract surfaces. It contains a complex mix of surface active lipids, antioxidants and other biologically active substances that either impede or enhance the movement of NO_2.

While some substances like dipalmitoyl phosphatidylcholine impede the movement of NO_2 through the lungs, others such as the antioxidants ascorbate, glutathione or urate, along with unsaturated lipids and thiol groups react with the NO_2 to form radical biological substances capable of migrating through the epithelial lining fluid and into the lung tissue. The radical biological substance will take the form of either a reactive oxygen species, organic radical, or reactive nitrogen species. The underlying base reaction of the NO_2 with the antioxidants and lipids is believed to convert the NO_2 into nitrous acid or nitrite, producing an organic radical form of the initial antioxidant or lipid (radical biological substance). As these radical biological substances can traverse the epithelial lining fluid and into the lung tissue, it is these radical biological substances that are believed responsible for most of health effects seen from NO_2 exposure.

The conversion of NO_2 in the epithelial lining fluid is governed by a process called "reactive absorption" that involves the NO_2 dissolving followed by chemical reaction. The properties of NO_2 mean it chemically reacts more quickly with the antioxidants and lipids, than with water. Therefore, it is the antioxidants and lipids which are the main chemicals responsible for the NO_2 mass transfer into the epithelial lining fluid. In some studies, the main chemicals have been identified as ascorbate and glutathione, along with albumin, cysteine, urate, unsaturated fatty acids and vitamins A and E.

The thickness of epithelial lining fluid will impede the transfer of NO_2 and its chemical products (radical biological substances) across the epithelial lining fluid and into the underlying lung tissue. The epithelial lining fluid in the tracheobronchial region of the lung is thicker and has more impeding chemicals than the epithelial lining fluid in the alveolar region of the lung. Therefore, from a physiological perspective, it is believed that the alveolar region of the lung is the major site of NO_2 interaction with lung.

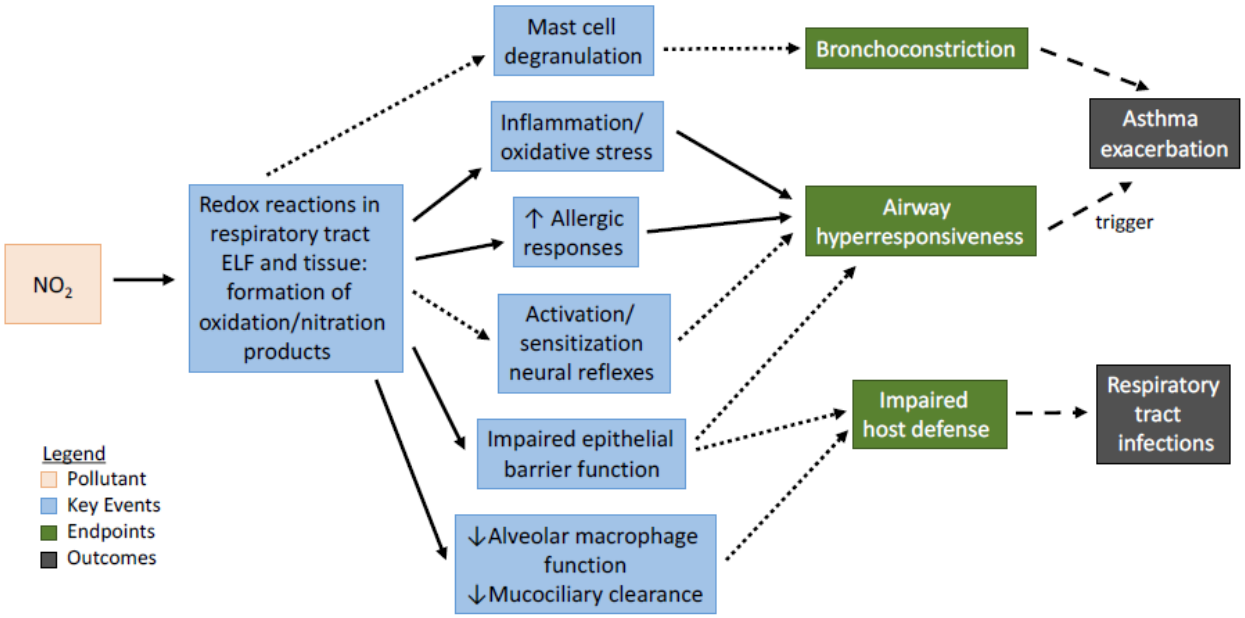
However, modelling of NO_2 dose uptake in the lungs, which does not account for the biochemical and thickness variation of the epithelial lining fluid, tells a slightly different story. The modelling has shown that NO_2 absorbed dose is low in the trachea, increasing to a maximum in the terminal bronchioles, before rapidly decreasing in alveolar sacs. This in part may be due to the total surface area of the alveolar sacs compared to other parts of the lung, as dose is measured as the amount of NO_2 per lung surface area.

The impact NO_2 has on the human body is dependent on which chemical (lipid, antioxidant or thiol) the NO_2 reacts with. NO_2 oxidation of membrane fatty acids can alter cell membrane fluidity and permeability, while oxidation of protein thiols may result in enzyme dysfunction. Consumption of antioxidants may lead to decreased antioxidant defences. The effect of these actions may then cause a cascade resulting in the release of further reactive oxygen species or reactive nitrogen species by leucocytes responding to cell damage. The cell damage may also result in the upregulating of enzymes, and an influx of inflammatory cells or proliferation of resident epithelial or mesenchymal cells.

Short term exposure to NO_2 and respiratory effects

From experimental evidence it is believed that asthma exacerbation and respiratory tract infections are key health outcomes from short term exposure to NO_2, which may be explained through a mode of action approach. **Figure 2** diagrammatically explains this approach. In this diagram key events are defined as subclinical effects (in blue boxes), endpoints are in green boxes as are clinical effects potentially associated with an outcome, while those in the black boxes are human health outcomes.

So far, this chapter has described the key event of NO_2 reacting with antioxidants, lipids and thiol groups to develop radical biological substances (the first blue box in **Figure 2**). Once these radical biological substances are formed, they can have numerous effects.



Note: Pathways indicated by a dotted line are those for which evidence is limited to findings from experimental animal studies, while evidence from controlled human exposure studies is available for pathways indicated by a solid line. Dashed lines indicate proposed links to the outcomes of asthma exacerbation and respiratory tract infections. Key events are subclinical effects, endpoints are effects that are generally measured in the clinic, and outcomes are health effects at the organism level. NO_2 = nitrogen dioxide; ELF = epithelial lining fluid.

Figure 2.2: Summary of evidence for the mode of action linking short term exposure to nitrogen dioxide and respiratory effects (taken from (US EPA 2016))

While mechanisms underlying the effects of NO_2 on airway responsiveness and bronchoconstriction are not well understood, a lack of antioxidants (due to their reaction with NO_2) has been found to increase airway responsiveness. Several different inflammatory pathways may also underlie the increased airway responsiveness following NO_2 exposure. Firstly, mast cells can release histamine and cause smooth muscle contraction. Secondly, neutrophils can release mediators which can alter calcium sensitivity in smooth muscle causing contraction. Third, eosinophils can release mediators that cause epithelial shedding (increasing epithelial permeability) and mucociliary dysfunction which allow allergens greater access to airway epithelium and submucosa. This can lead to exposure of sensory nerve endings and smooth muscle contraction.

The mechanism for increased epithelial permeability from NO_2 exposure is believed to be from the NO_2 reacting with cell membrane lipids. It is thought that lipid peroxidation and altered phospholipid composition following NO_2 exposure affects cell membrane fluidity and airway epithelial barrier function. Inflammatory responses (such as the eosinophil process described above) further impairs the barrier function which can lead to an increase in vascular permeability and influx of plasma proteins such as albumin into the airway lumen. When this happens biomarkers of cellular injury such as lactate dehydrogenase and shed epithelial cells can be detected.

Stimulation of sensory nerve endings may occur as a result of the increased epithelial permeability (described above). The increased epithelial permeability allows greater access to the afferent nerve endings in the lung resulting in the stimulation of smooth muscle receptors causing

bronchoconstriction. It is through this sensory nerve stimulation mechanism that NO_2 is classified as a pulmonary irritant.

Inflammation through cell membrane changes (NO_2 reacting with cell membrane lipids) is brought about by the formation of eicosanoid products, which play an important role in the recruitment of neutrophils. Exposure to NO_2 has been shown to activate nuclear factor kappa – light -chain enhancer of activated B cells (NF-κB) resulting in the production of proinflammatory cytokines including interleukin 6 and interleukin 8. Other studies have shown exposure to NO_2 increases other inflammatory cell types such as macrophages, lymphocytes, eosinophils and mast cells.

Inflammation resulting from NO_2 exposure is considered to create an asthmatic response with two potential phases. Within the early phase the key players are mast cells and basophils which release mediators such as histamine that binds to airway smooth muscle receptors to induce contraction. The mediators also activate other cells which release cytokines. These cytokines recruit more mast cells which cause more smooth muscle contraction, eosinophils and neutrophils which release their own mediators, along with B lymphocytes which results in the production of immunoglobulin E (the late phase).

In contrast to inducing airway constriction, exercise during NO_2 exposure has been shown to minimise airway responsiveness (constriction) in subjects with asthma when compared to airway changes at rest. The reasons for it are not understood but two hypothesises have been speculated. The first is that exercise induced stimulation of the airways makes them insensitive to further stimulation by NO_2. The second is that nitrite forms by reaction of NO_2 in the epithelial lining fluid and mediates relaxation of the smooth muscle. It has been shown that some reactive nitrogen species may act as bronchodilators, and have a direct effect on relaxing smooth muscle.

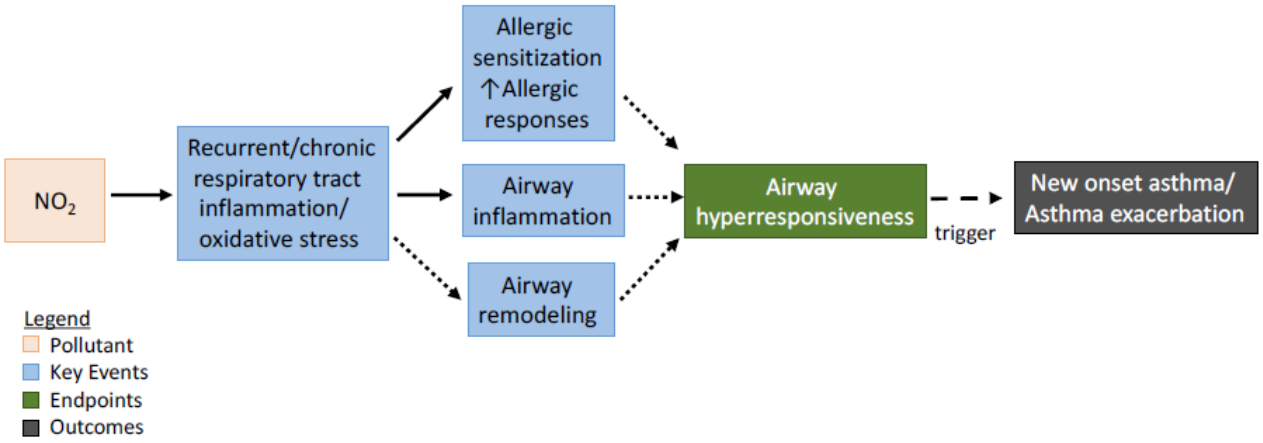
Finally, for the outcome of respiratory tract infections potential mechanisms by which NO_2 exposure may impair host defences include ciliary dyskinesis, damage to ciliated epithelial cells and altered alveolar macrophage function. Altered alveolar macrophage function along with pro-inflammatory mediators and cytokines, increased immunoglobulin E concentrations and altered lymphocyte subsets that have been found post NO_2 exposure, demonstrating a modification and / or adaption in immunity.

Long term exposure to NO_2 and respiratory effects

Long term exposure to NO_2 is hypothesised to be linked to an onset of asthma or asthma exacerbation. As with the short term exposure model (**Figure 2.2**) the key event of NO_2 reacting with antioxidants, lipids and thiol groups to develop radical biological substances (the first blue box in **Figure 2.3**) is the same. However recurrent or chronic respiratory tract inflammation and oxidative stress is thought lead to allergic sensitisation, airway inflammation and airway remodelling that in turn leads to new asthma (**Figure 2.3**).

While the evidence is minimal, there is some evidence to suggest that repeat exposures to NO_2 may have a pro allergic influence. This is drawn from one human study which showed higher allergic markers after repeat exposure to NO_2, along with some animal studies that have also shown some effect. Regarding airway remodelling, long term exposure to higher than ambient levels of NO_2 has been shown to induce morphological changes in the centriacinar region of the lungs, including the terminal conducting airways, the alveolar ducts and the alveolar. The cells most injured were the

ciliated cells of the bronchiolar epithelium and Type I cells of the alveolar epithelium. These were replaced with nonciliated bronchiolar cells and Type II cells.

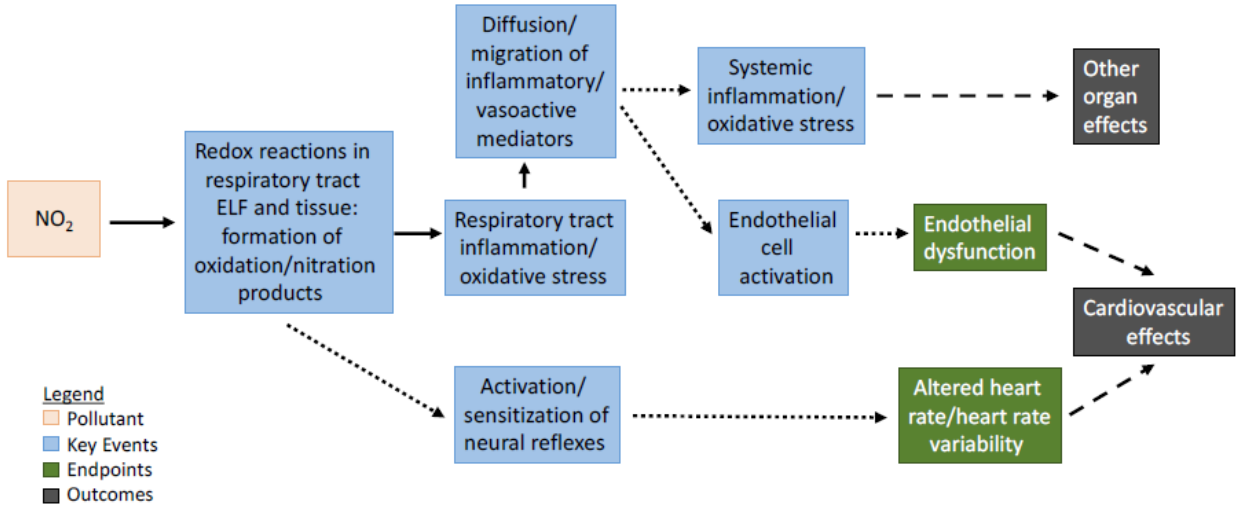


Note: Pathways indicated by a dotted line are those for which evidence is limited to findings from experimental animal studies, while evidence from controlled human exposure studies is available for pathways indicated by a solid line. The dashed line indicates a proposed link to the outcome of new onset asthma/asthma exacerbation. Key events are subclinical effects, endpoints are effects that are generally measured in the clinic, and outcomes are health effects at the organism level. NO_2 = nitrogen dioxide.

Figure 2.3: Summary of evidence for the mode of action linking long term exposure to nitrogen dioxide and respiratory effects (taken from (US EPA 2016))

Exposure to NO_2 and extrapulmonary effects

There is greater uncertainty regarding the mode of action for extrapulmonary effects of inhaled NO_2. As with **Figures 2.2 and 2.3** the key event of NO_2 reacting with antioxidants, lipids and thiol groups to develop radical biological substances (the first blue box in **Figure 2.4**) is the same. From this point three possible pathways have been hypothesised. First, the radical biological substances could diffuse and migrate into circulation causing systemic inflammation and affecting other organs. Second, inflammatory or vasoactive mediators triggered in the lung may migrate from the lung and into the circulatory system resulting in systemic inflammation, affecting other organs or alternatively affecting endothelial cells and causing cardiovascular effects. Finally, the radical biological substances could activate pulmonary irritant receptors resulting in cardiovascular reflex responses (**Figure 2.4**).



Note: Pathways indicated by a dotted line are those for which evidence is limited to findings from experimental animal studies, while evidence from controlled human exposure studies is available for pathways indicated by a solid line. Dashed lines indicate potential links to outcomes related to cardiovascular or other organ effects. Key events are subclinical effects, endpoints are effects that are generally measured in the clinic, and outcomes are health effects at the organism level. NO_2 = nitrogen dioxide; ELF = epithelial lining fluid.

Figure 2.4: Summary of evidence for the mode of action linking exposure to nitrogen dioxide with extrapulmonary effects (taken from (US EPA 2016))

2.5 Clinically relevant outcomes from NO_2 exposure

Respiratory effects such as asthma exacerbation are the health outcomes most robustly associated with NO_2 exposure. But for this exacerbation to occur subclinical effects, such as inflammatory changes, need to trigger a clinical outcome, such as airway responsiveness, and that clinical outcome needs to be of sufficient magnitude to enact the health outcome (asthma exacerbation). Experimental studies examining NO_2 exposure have measured both changes in subclinical effects and clinical outcomes, with clinical outcomes being the main focus for the protection from adverse health outcomes (Brown 2015; Folinsbee, L. J. 1992; Goodman et al. 2009; WHO 2000), while NO_2 values that enact subclinical effects provide a potential reality check for the NO_2 values that enact a clinical outcome. The subclinical studies are also used as evidence to support the mode of action.

Lung function and airway responsiveness are the most robust clinical outcomes on the path to a potential health outcome. Both lung function and airway responsiveness are measured in terms of changes in lung function and airway resistance. While there are numerous measures of lung function, forced expiratory volume over 1 second **(FEV_1)** is a key measure. Airway resistance is measured in terms of the resistance of the respiratory tract to airflow during inhalation and exhalation, that accounts for the changing nature of airway resistance within the lung **(sRaw)**. Airway responsiveness may also be induced in a measurement termed provocative dose. A provocative dose is the dose of chemical or allergen required to reduce a lung function in question by X%. For example, the amount of dose required to reduce FEV_1 by 20%, or increase airways resistance (sRaw) by 100%.

The following agencies refer to “adverse effect” or “adverse outcome”. For this report an “adverse effect” or “adverse outcome” is considered a clinically relevant outcome[1]. A clinically relevant outcome identified by meeting one of the criterion in **Section 2.6**.

United States Environmental Protection Agency (US EPA)

In 2014 to define what magnitude of airway responsiveness is clinically relevant, the US EPA declared a decrease of 15% in FEV_1 as potentially adverse in active healthy adults, while for people with asthma and lung disease a decrease of 10% may be more appropriate (US EPA 2014). This conclusion was drawn in part from statements from the American Thoracic Society and a previous US EPA publication. In this previous publication (US EPA 2006) the US EPA graded lung functional loss. For a person with impaired respiratory system, an FEV_1 decremental change of < 3% was considered as no functional change while a decremental change of 3 to ≤ 10% was considered small. Changes above these values were considered either moderate or large. For airway resistance (sRaw), the comparative no change was a value ≤ 20% while a small change was < 100%. Finally, with respect to provocative dose, a 50% decrease in the provocative dose was defined as a small change (US EPA 2006). Drawing on these documents, it is concluded that the US EPA define a clinically relevant effect as either:

- an FEV1 change of greater than 10%,
- a sRaw change of greater than 100%, or
- a 50% or greater decrease in provocative dose.

World Health Organisation (WHO)

The WHO have a more pragmatic approach when it comes to determining adverse (clinically relevant) health effects from air pollution. They acknowledge that a significant degree of subjectivity and uncertainty remains in determining what might be considered adverse (**Figure 2.5**). It notes

> *The distinction between adverse and non-adverse effects poses considerable difficulties. Of, course more serious effects are generally considered adverse. As one considers effects that are either temporary or reversible, or involve biochemical or functional changes whose clinical significance is uncertain, judgements must be made as to which of the less serious effects should be considered adverse.* (WHO 2000)

[1] In this report a clinically relevant outcome is based on the mean response of the epidemiological studies reviewed. This may result in some sensitive individuals in the population experiencing a clinically relevant outcome. This is explored further in **Table 3.5** and **Section 3.3**

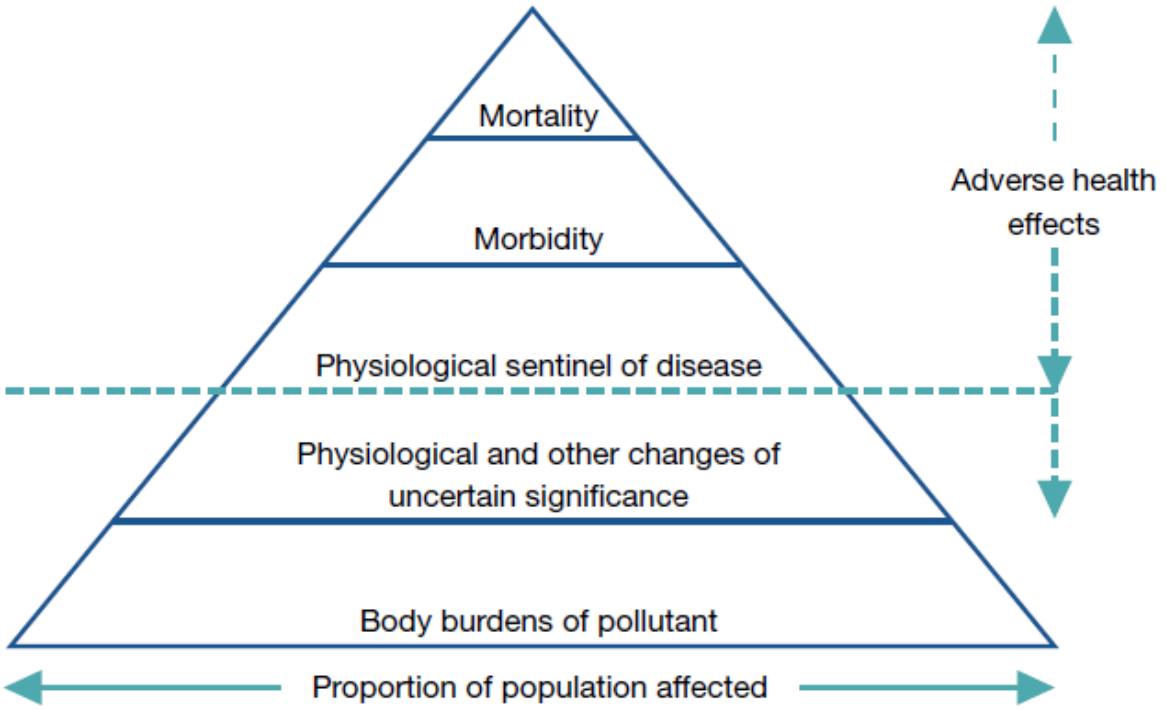


Figure 2.5: Schematic spectrum of biological response to pollutant exposure (taken from (WHO 2017))

To help determine what health effect might be considered adverse, the WHO provides three categories on which to evaluate the evidence. These categories revolve around the strength of evidence and seriousness of health outcome (WHO 2000).

Australian National Health and Medical Research Council (NHMRC)

Like the WHO, the NHMRC identify a zone of uncertainty around what could be considered no adverse effect versus an adverse (clinically relevant) effect (**Figure 2.6**).

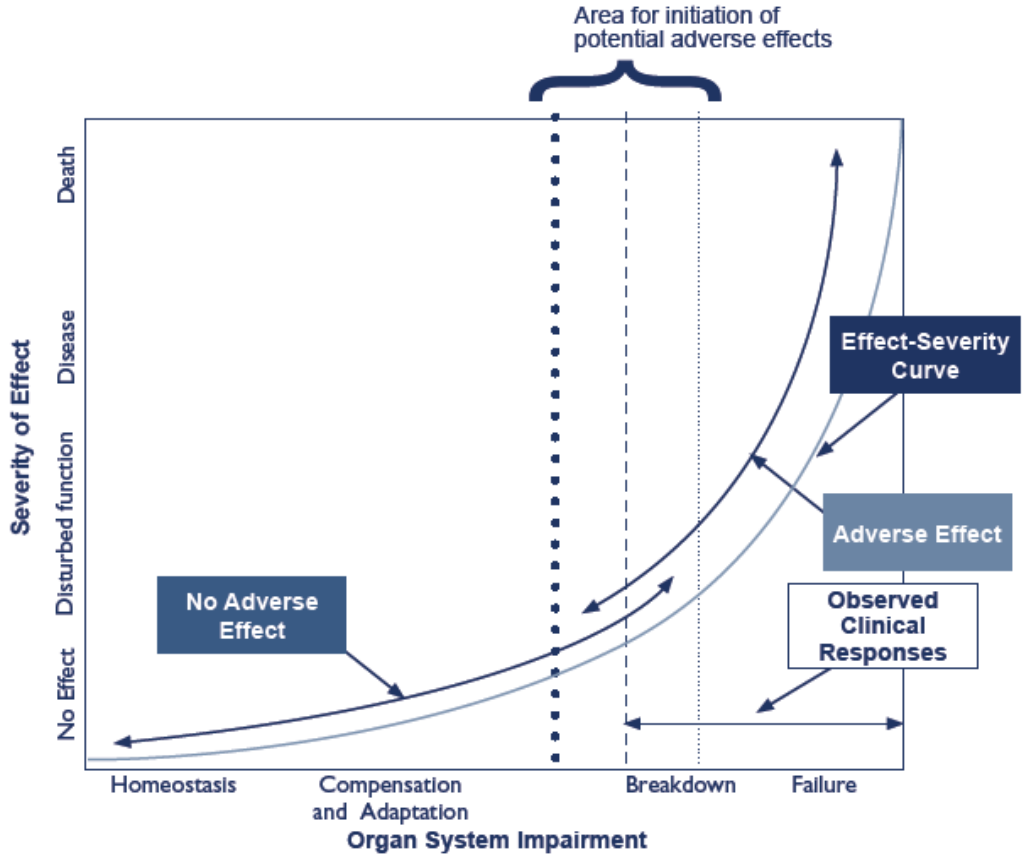


Figure 2.6: Schematic representation of the continuum of adverse and no-adverse health effects and the potential overlap (taken from (NHMRC 2006))

The NHMRC acknowledge the use of subclinical data in the determination of an adverse effect, but note that the science is not robust enough at this time for this subclinical data to be relied upon in determining an adverse effect. Instead the NHMRC drew on guidance released by the American Thoracic Society in 1985 and 2000. This guidance revolved around eight points, those being:

- physiological impact;
- clinical symptoms;
- clinical outcomes;
- mortality;
- population health versus individual risk;
- other potential adverse respiratory effects;
- non-respiratory effects; and
- quality of life.

Many of these points consider the severity of the outcome or breath of the effects, however of interest for this report is the physiological impact and population versus individual risks.

In defining physiological impact the NHRMC noted that “a small transient loss of lung function by itself should not be considered adverse” (NHMRC 2006). Specifically, the report states that

changes in FEV_1 of >12% and >200ml are likely to be clinically important and it is likely that this definition will be adopted in the future in Australia (although the TSANZ (Thoracic Society of Australia and New Zealand) *has favoured a cut off 15% to indicate significant short-term changes). Smaller reductions of FEV_1 could still be of public health importance, but reductions of < 8% would fall within the error of measurement and so are unlikely to be detected or considered significant.*

Smaller changes in FEV_1 with symptoms was also considered clinically relevant (NHMRC 2006).

Population health versus individual risk draws of the concept of population burden. In this concept it is argued that a small change in individual health status applied over a large population may result in a population shift of health status creating an overall large health burden. Given the distribution of severity of effects within a population, a mean application of a clinical effect will result in some members of the public experiencing clinical symptoms. This argument is far more significant for permanent reductions rather than transient reductions in lung function.

The NHMRC view of clinical relevance was reiterated in 2011 by the Australian National Environment Protection Council in their report on setting air quality standards (NEPC 2011).

Drawing on these documents, it is concluded that Australian government agencies define a clinically relevant effect from exposure to air pollution as

- an FEV_1 change of greater than 12%.

Australian societies with an interest in respiratory health - Thoracic Society of Australia and New Zealand (TSANZ) and National Asthma Council of Australia (NACA)

In 2015 the NACA released the Australian Asthma Handbook, which was endorsed by the TSANZ, Royal College of General Practitioners and the Australian Primary Health Care Nurses Association. This handbook provides the following definitions for clinical relevance with regard to lung function testing (NACA 2015).

- *a clinical important increase in FEV_1 (change in FEV_1 of at least 200mL and 12% from baseline for adults, or at least 12% from baseline for children) 10 – 15 minutes after administration of bronchodilator*
- *clinically important variation in lung function (at least 20% change in FEV_1) when measured repeatedly over time*
- *a clinically important reduction in lung function (decrease in FEV_1 of at least 200mL and 12% from baseline on spirometry, or decrease in peak expiratory flow* (PEF) *by at least 20%) after exercise*
- *a clinically important increase in lung function (at least 200mL and 12% from baseline) after a trial of 4 or more weeks of treatment with an inhaled corticosteroid*
- *a clinically important variation in peak expiratory flow (diurnal variability of more than 10%)*
- ***a clinically important reduction in lung function (15 -20%, depending on the test) during a test for airway hyperresponsiveness (exercise challenge test or bronchial provocation test) measured by a respiratory function laboratory.***

Those definitions highlighted in red are of particular importance to this report, the last definition (bolded) being of greatest importance. The Asthma handbook also clarified that the lung function

measurement forced volume capacity (FVC), while a measure of clinical importance, is a less reliable measure than FEV_1.

Drawing on this document, it is concluded that Australian societies with an interest in respiratory health define a clinically relevant effect as

- an FEV_1 change of greater than (12% - 20%) for lung function, or
- a PEF change of greater than (10% - 20%) for lung function, or
- a lung function change of greater than 12% from baseline, or
- an FEV_1 change of greater than (15% - 20%) for airway responsiveness, or
- a lung function change of greater than (15% - 20%) for airway responsiveness.

International societies with an interest in respiratory health - European Respiratory Society and American Thoracic Society

In 2016 the European Respiratory Society and American Thoracic Society released a joint policy statement on what constitutes an adverse health effect of air pollution (Thurston et al. 2017). The authors' intensions were for this statement to offer a set of ideas to be considered when determining the boundary between adverse and non-adverse health effects rather than a strict set of rules or criteria. These ideas were

- **Fatality** – Did the air pollution exposure lead to an increase in short-term or long-term mortality?
- **Persistence of effect** – How persistent over time is the effect?
- **Population risk** – Is there a shift in population risk distribution of an adverse event?
- **Medical / functional significance** - Is there evidence of one or more of the following? 1) severe interference with a normal activity of the affected person or persons; 2) incapacitating illness; 3) permanent injury; 4) progressive dysfunction; 5) reduced quality of life.

In considering lung function and airway responsiveness, the statement reiterated that for healthy individuals that small, transient loss of lung function or change in airway responsiveness, by itself, should not automatically be designated as adverse", however, for individuals with extant compromised function, such as results from asthma, small lung function changes or clinically relevant increases in airway responsiveness should be considered adverse. The statement also reiterates the population versus individual effects argument, in that "a small but statistically significant mean reduction in FEV_1 in a population means that some people (have) larger reductions, with the likelihood that reductions in a subset of susceptible subjects can have passed a threshold for clinical importance". The same argument applies for airway responsiveness. This argument is far more significant for permanent reductions rather than transient reductions in lung function.

Interestingly, with regard to provocative dose, the statement does provide an example of adversity evidenced with regard to a health person and airway responsiveness. A methacholine provocative dose of < 8mg/ml which produces a decrease in FEV_1 of 20% or greater is considered adverse. However, when searching the referenced document in this statement, this criterion could not be found.

It should be noted that this statement has been developed with an emphasis on evaluating all evidence (observational and experimental epidemiological studies, toxicological studies and molecular and cellular biology studies) regarding adverse effects from air pollution, therefore some ideas suggested in the statement, such as fatality, will be more relevant to certain study types, such as observational studies.

2.6 Clinical criteria used for this report

Epidemiological studies attempt to compare two sets of data and, with some established statistical criteria in mind, aim at determining if one set of data is statistically difference from the other. In the case of NO_2 experimental exposure studies, this might involve gathering data on the lung function of people not exposed to NO_2, and comparing that to their lung function once they are exposed to NO_2. If an increase or decrease in lung function between the two exposures (data sets) sit outside the established statistical criteria, the results are known as statistically significant. In this case, little consideration is given as to whether this statistical difference has a biological effect[2], and all that can generally be said is that there appears to be a genuine change of X in lung function resulting from the NO_2 exposure.

Section 2.5 provides an overview of national and international statements and guidelines regarding clinically relevant effects from air pollution exposure. These statements provide measured changes in lung function considered clinically relevant. Continuing with the example in the previous paragraph, the statistical change determined by the NO_2 experimental exposure study may now be compared to the clinically relevant value, to determine if the statistically significant lung function change is clinically relevant.

To assess if the statistical significance outcomes found in NO_2 experimental exposure studies are clinically relevant this report draws on the guideline values outlined in **Section 2.5.** The following criteria has been proposed for clinical relevance:

- an FEV_1 change of greater than 12% for lung function, or
- a PEF change of greater than 10% for lung function, or
- a lung function change of greater than 12%, or
- an FEV_1 change of greater than 15% for airway responsiveness, or
- a lung function change of greater than 15% for airway responsiveness, or
- a sRaw change of greater than 100%, or
- a 50% or greater decrease in provocative dose.

2.7 Limitations of clinical relevance

Clinical relevance within this report will be applied as the mean change of study participants. Applying the mean change does not account for population variability. Therefore, while the average participant may not experience changes of clinical relevance, sensitive individuals within the population may. This limitation has been documented by the NHMRC and European Respiratory Society and American Thoracic Society regarding individual versus population effects (see above).

[2] Some consideration of biological effect may occur when determining sample size

Graphically this limitation is described in **Figure 2.7**, where the bell curve represents the range of responses (e.g. change in FEV_1) within the population from an exposure (e.g. 0.5ppm NO_2).

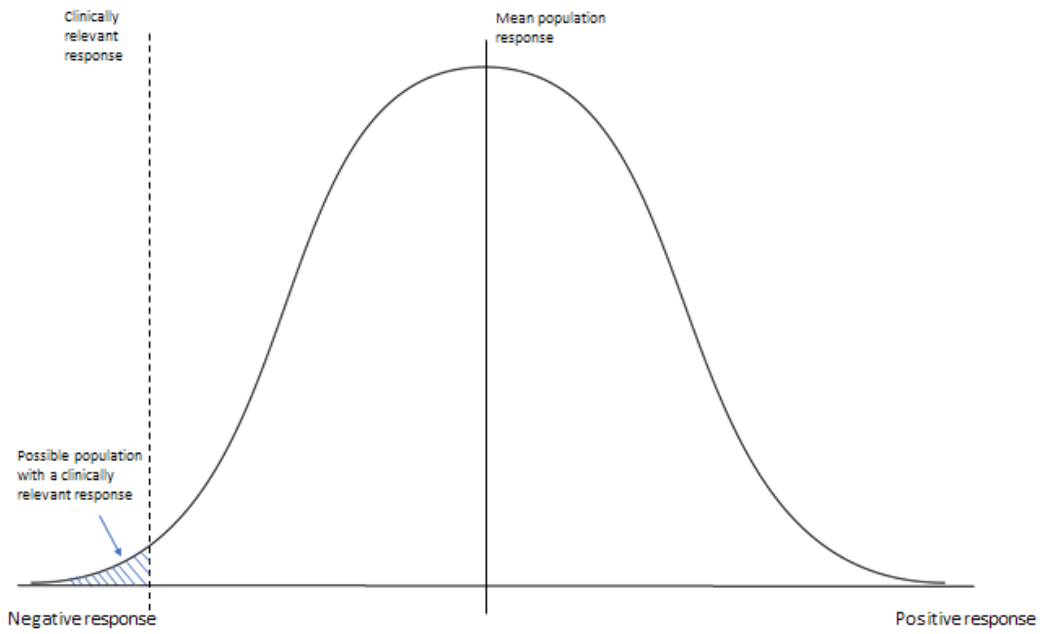


Figure 2.7: Graphical representation of population response from chemical exposure

If a sensitive sub population (such as mild asthmatics) are the participants of the study (as in this report), then this represents a subpopulation of the population bell curve. The mean response from this subpopulation will be more negative than the mean for the population as a whole (**Figure 2.8)** and therefore more likely to account for responses from sensitive populations and provide greater certainty to decision making.

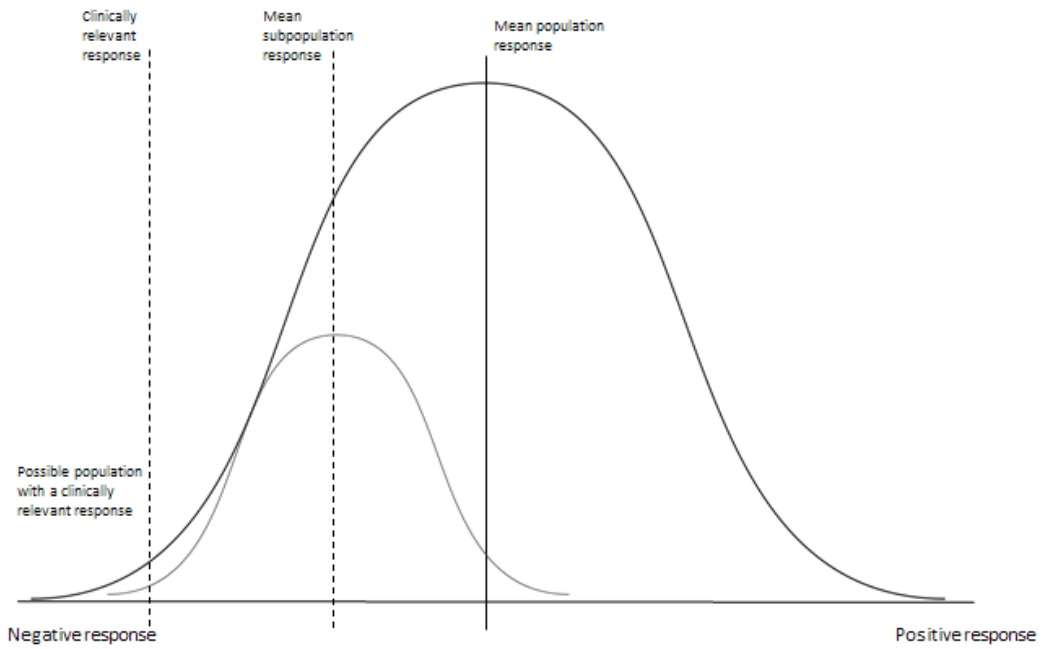


Figure 2.8: Graphical representation of sensitive subpopulation response within the overall population response from chemical exposure

Further, assessing clinical relevance against the 95% lower confidence interval of the sub population sample mean provides a potential added allowance for sensitive subpopulations (**Figure 2.9**). Along with an examination of the mean subpopulation response, an examination of clinical relevance against the 95% lower confidence interval of the sub population sample has been undertaken in **Section 3.2.3 – Meta analysis and reviews**. Specifically, **Table 3.5** provides the results of this examination.

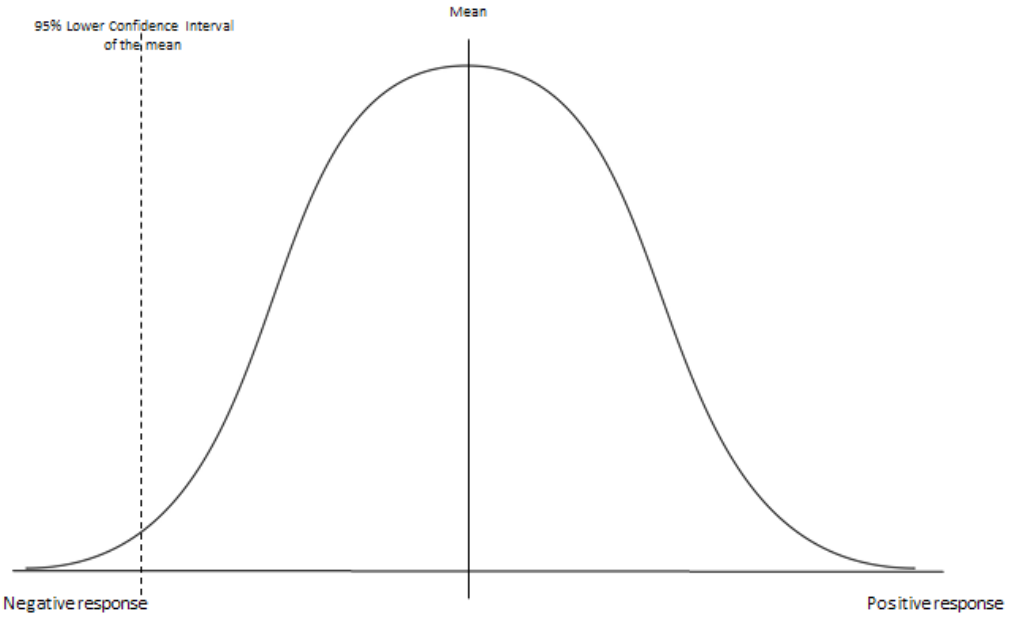


Figure 2.9: Graphical representation of 95% lower confidence interval of the mean response from chemical exposure, compared to the estimated mean.

Ultimately however, without sampling the whole population or the most sensitive subpopulation (such as severe asthmatics), it is impossible to understand the clinical effect of every individual. This situation is not uncommon for the development of health guidelines, where limitations in data, feasibility of the guideline implementation and societal acceptance of risk may all be contributing factors to its development. Given this, it is acknowledged that setting a guideline value does not rely solely on population studies, but rather is a socio-political matter (enHealth 2012).

Section 3. Experimental Studies

3.1 General

3.1.1 What are experimental studies?

An experimental study is the strongest epidemiological study design because it can control and modify exposures to a subject, so that an exposure of interest can be directly related to the outcome of interest (causation). By their design an experimental study is always prospective, that is, will always examine a future outcome. Randomising an experimental study limits any chance of bias that may occur within selection of the study groups, or in the case of the literature reviewed in this report, within the timing of exposure measures.

A key strength of an experimental design is the ability to assign causation to an outcome, e.g. to prove that an exposure to NO_2 caused a decrease in lung function. In the context of NO_2 experimental studies, key weaknesses include a small and potentially bias sample size that may not represent the community of interest, along with the limited outcome measures that can be explored. For example, while testing for changes in lung function is acceptable, testing for premature mortality is not.

3.1.2 Experimental study types used in nitrogen dioxide studies

Experimental studies that have explored human exposure to NO_2 are one of three main types. The most common is the randomised cross over study. In this type of study, the participant enters an air tight chamber for a set period of time and is randomly exposed to either air free of NO_2, or air containing a set concentration of NO_2. Outcome measurements, for example lung function, are taken at set times during and / or after this exposure. After a washout period, usually between 1 to 4 weeks, the exposure that the participant initially missed out on is given, be that air free of NO_2 or air containing NO_2. The cross over design works because the participant act as their own control, that is the results from their outcome measures when the chamber was free of NO_2 is compared to the results from the outcome measures when the chamber contained NO_2.

A variation of the randomised cross over study design is the non-randomised cross over design. Here all procedures are the same as the randomised cross over design with the exception that the order of exposure is not randomised. All participants are either exposed to air free of NO2, then air containing NO_2, or vice versa. The disadvantage of this approach is that bias may be induced into the study which could have otherwise been minimised by randomising the exposure.

The third main type of study is the experimental exposure. Here outcome measures are taken from participants before they enter the exposure chamber. They then enter the exposure chamber where they are exposed to a set concentration of NO_2. The same outcome measures are taken during and / or after this NO_2 exposure and compared to the outcome measures taken before the participant was exposed. The disadvantage of this approach from the randomised and non-randomised cross over design is that the baseline or control expose is not quantified, i.e. the concentration of NO_2 and other exposure conditions that the participant has been exposed to prior to entering the chamber is not known.

While not prominent, randomised control trials have also been undertaken when exploring human exposure to NO_2. In this study design a group of participants are randomly assigned to being

exposed or not exposed to NO_2, and the results from the outcome measures of the two exposure groups are compared.

Lastly, real world applications of the non-randomised cross over design have been used where participants are exposed to NO_2 by sitting in a road tunnel or walking along a busy street, and the outcome measures of this exposure is compared to the outcome measures from sitting in an office or walking in a park.

3.1.3 Outcome measures in nitrogen dioxide experimental studies

The outcome measures used in experimental NO_2 studies can be divided into four broad categories – lung function; airway responsiveness; inflammatory, cellular and biochemical markers; and cardiovascular effects.

Lung function

Lung function, as the name suggests, measures the function of the lungs. This is generally done by measuring physiological lung parameters such as how much air a person can exhale in 1 second (FEV_1), how much air in total a person can exhale after taking in a full breath (FVC), and calculating the airway resistance.

Airway responsiveness

A variation of the lung function test is airway responsiveness. This is because lung function is still measured, but only after the lungs have been exposed to a non-specific agent (such as methacholine or cold air) or specific agent (such as birch or dust mite) that may cause the lungs to reduce their function. The main aim of this outcome is to determine if an exposure to NO_2 makes the lungs more vulnerable when put under stress. To determine this, the experiment will examine whether it takes a significantly smaller dose of the agent, when exposed to NO_2 compared to air, to achieve a predetermined outcome, such as a 100% increase in airway resistance. Alternatively, a person may be exposed to an agent, post exposure to NO_2 or air, and lung function will be measured and compared.

Inflammatory, cellular and biochemical markers

Inflammatory, cellular and biochemical markers are outcome measures that are taken from either a participant's sputum, bronchioalveolar lavage fluid, nasal fluid or blood. These tests are design to determine if exposure to NO_2 may lead to preclinical changes in the body, primarily focusing on inflammation and allergic reaction. The inflammatory, cellular and biochemical markers used in the NO_2 experimental studies are defined in **Appendix A**.

Cardiovascular effects

Very few NO_2 experimental studies have considered cardiovascular effects. For those that have, they generally measured physiological parameters such as heart rate and cardiac output.

Appendix A contains a list and explanation of the outcome measures used in NO_2 experimental studies.

3.2 Literature review

3.2.1 Search strategy

The literature review to discover experimental human studies of NO_2 exposure was conducted examining key national and international documents along with a search in the Medline database. References were obtained from the Australian *Review of experimental studies of exposures to nitrogen dioxide* (Jalaludin 2015), United States Environmental Protection Agency *Integrated Science Assessment for Oxides of Nitrogen – Health Criteria* (US EPA 2016) and the World Health Organisation *Review of evidence on health aspects of air pollution* (WHO 2013). A search in Medline of literature from 1946 till September 2017 using the search terms “nitrogen dioxide” and “chamber” was undertaken. A total of 82 papers which included 4 meta-analyses or reviews were found suitable for inclusion in this review.

Thirty seven of the seventy eight studies identified included asthmatic participants, forty six involved ‘healthy’ participants, while five studies involved participants with chronic obstructive pulmonary disease (COPD). Exposure times of NO_2 varied from five minutes to six hours. While most studies considered exposure to NO_2 in isolation, sixteen studies considered NO_2 exposure as part of traffic pollution. Eight studies performed repeat exposures to the same NO_2 concentration over a one-week period, while fifty three included intermitted exercise to be undertaken while exposures were given. Further information can be found in **Appendices B - D**.

3.2.2 Key outcome results

Appendices B - D present the key findings of the literature review, which are summarised below.

Lung function

Sixty two studies, containing 92 NO_2 exposure concentrations, examined the relationship between NO_2 exposure and lung function. The concentrations of NO_2 exposure ranged from 0.01 ppm to 5.5 ppm.

When considering studies that examined NO_2 exposures 60 minutes or less (**Tables B.1 & B.2**), there was 30 exposure concentrations that were equal or less than 0.5 ppm of which 5 found a statistically significant result. The study outcomes of these 5 studies were assessed to determine if they were clinically relevant (**Figure 3.1** & **Table 3.1**).

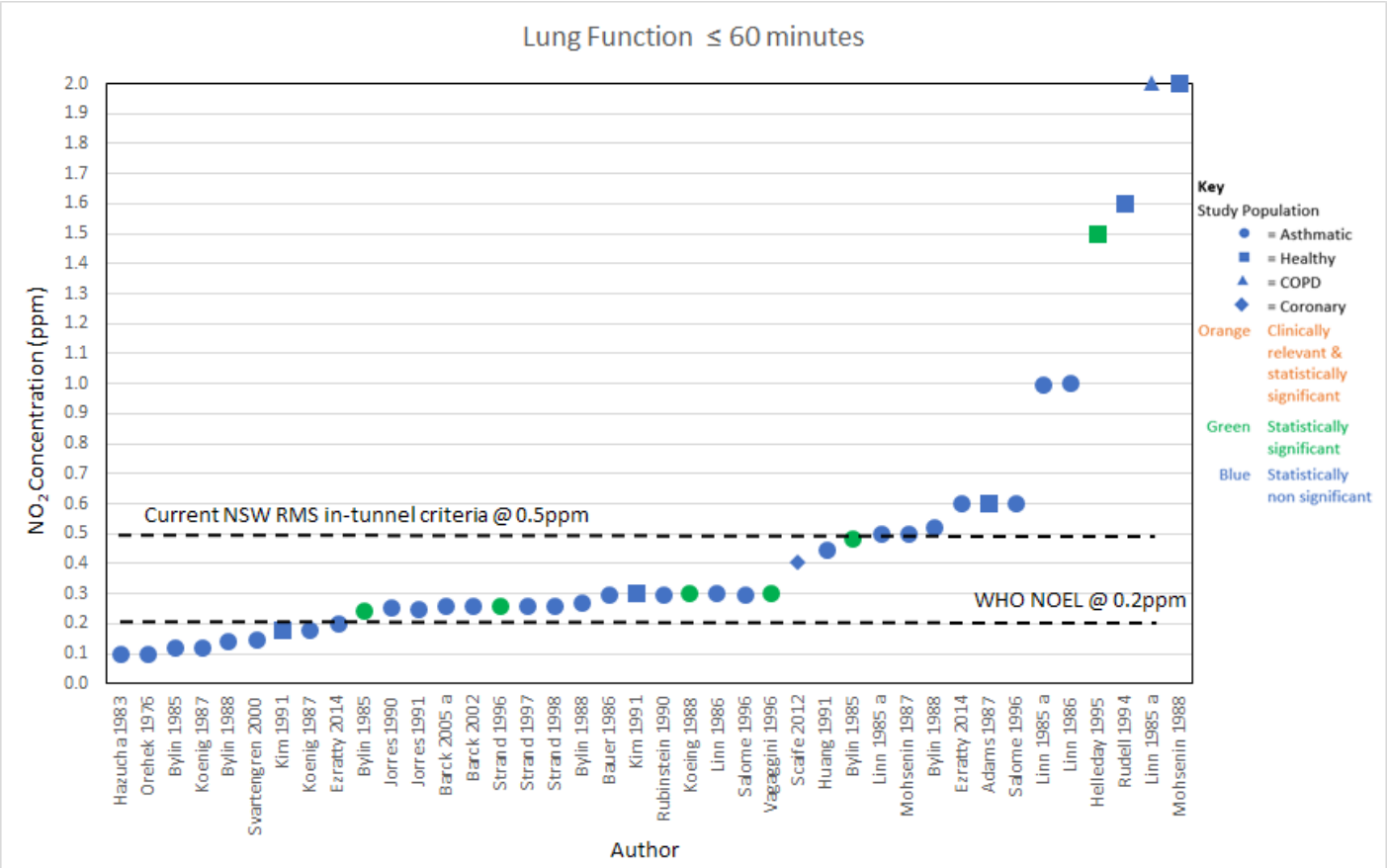


Figure 3.1: Graph of studies examining exposures to NO_2 of ≤ 60 minutes and lung function up to 2ppm, ranked by NO_2 concentration

Table 3.1: Determination of clinical relevance of statistically significant lung function NO_2 exposure studies ≤ 0.5ppm, ≤ 60 minutes exposure

Study	NO_2 (ppm)	Exposure time (mins)	Study population affected	Statistically significant outcome#	Clinically relevant	Basis*
Studies ≤ 30 minutes						
Bylin 1985	0.24	20	Healthy	17% ↑ sRaw	x	6
Strand 1996	0.26	30	Asthmatics	9% ↓ TGV ^	x	3
Bylin 1985	0.48	20	Healthy	32% ↓ sRaw	x	6
Studies >30 to ≤ 60 minutes						
Koeing 1988	0.3	60	Asthmatics	4% ↓ FVC	x	3
Vagaggini 1996	0.3	60	COPD	8% ↓ FEV_1	x	1

***Criterion 1** - an FEV_1 change of greater than 12% for lung function; **Criterion 2** - a PEF change of greater than 10% for lung function; **Criterion 3** - a lung function change of greater than 12%; **Criterion 4** - an FEV_1 change of greater than 15% for airway responsiveness; **Criterion 5** - a lung function change of greater than 15% for airway responsiveness; **Criterion 6** - a sRaw change of greater than 100%; **Criterion 7** - a 50% or greater decrease in provocative dose
#Papers were selected based on reported statistically significant outcome, however not all lung function measurements adjusted for the percentage change in control exposure. Where the study permitted, percentage change in lung function measurements are based on mean percentage change in lung function between time 0 and time X from NO_2 exposure minus the mean percentage change in lung function between time 0 and time X from the control exposure. Those studies where this could not be done are marked with a ^.

Of the 5 statistically significant studies, none were found to meet the criteria of clinical relevance.

Airway responsiveness

Thirty three studies, containing 51 NO_2 exposure concentrations, examined the relationship between NO_2 exposure and airway responsiveness. The concentrations of NO_2 exposure ranged from 0.075 ppm to 3 ppm.

When considering studies that examined NO_2 exposures 60 minutes or less (**Tables B4 & B5**), there was 24 exposure concentrations that were equal or less than 0.5 ppm of which 10 found a statistically significant result. The study outcomes of these 10 studies were assessed to determine if they were clinically relevant (**Figure 3.2** & **Table 3.2**).

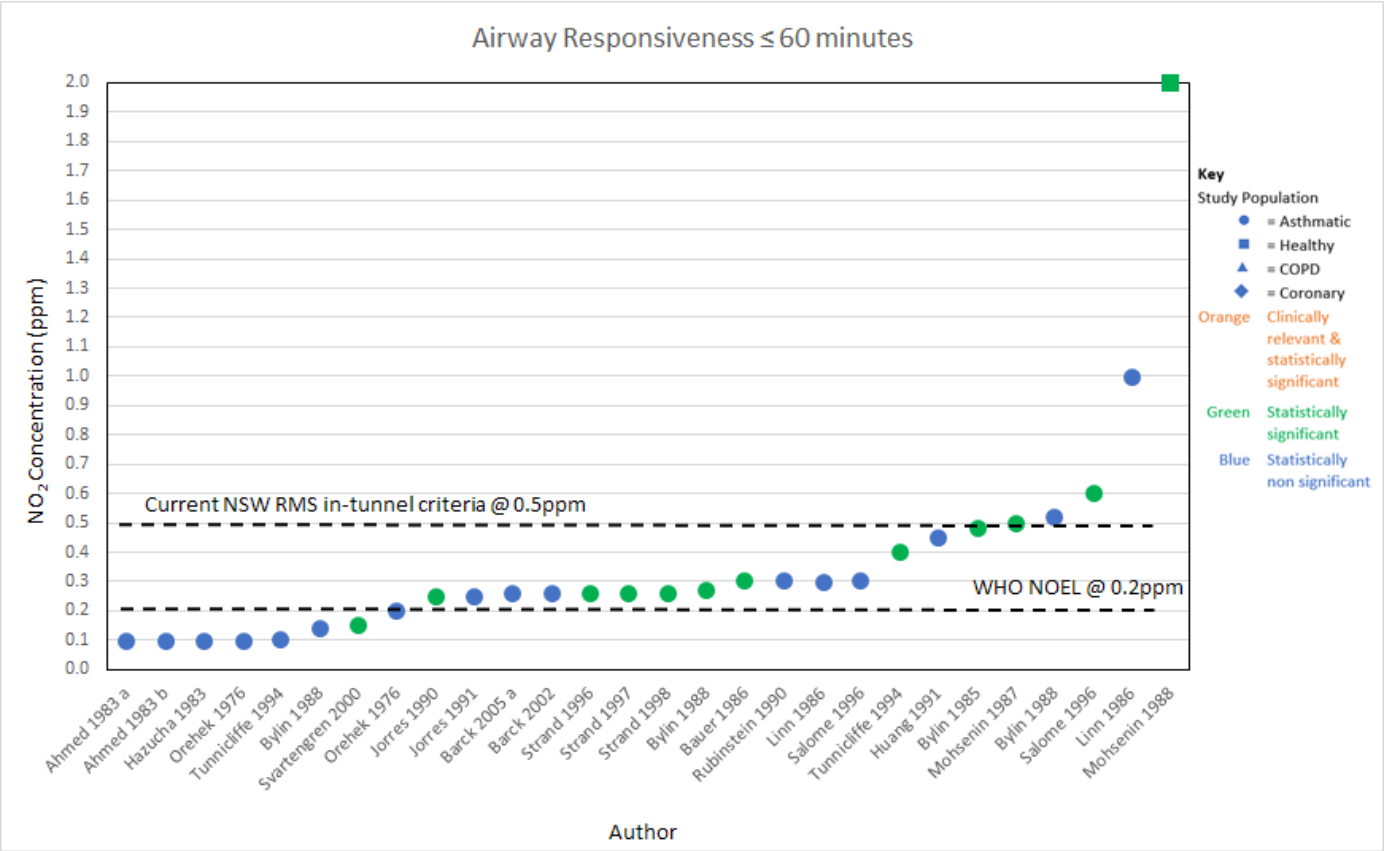


Figure 3.2: Graph of studies examining exposures to NO_2 of ≤ 60 minutes and airway responsiveness up to 2ppm, ranked by NO_2 concentration

Table 3.2: Determination of clinical relevance of statistically significant airway responsiveness NO_2 exposure studies ≤ 0.5ppm, ≤ 60 minutes exposure

Study	NO_2 (ppm)	Exposure time (mins)	Study population affected	Statistically significant outcome#	Clinically relevant	Basis*
Studies ≤ 30 minutes						
Svartengren 2000	0.15	30	Asthmatics	13% ↑ sRaw; 12% ↑ TGV	X	5,6
Jorres 1990	0.25	30	Asthmatics	19% ↓ PD	X	7
Strand 1996	0.26	30	Asthmatics	46% ↓ PD	X	7
Strand 1997	0.26	30	Asthmatics	4% ↓ FEV_1; 7% ↓ PEF	X	4,5
Strand 1998	0.26	30	Asthmatics	Up to 5% ↓ FEV_1	X	4
Bylin 1988	0.27	30	Asthmatics	38% ↓ PD	X	7

Study	NO_2 (ppm)	Exposure time (mins)	Study population affected	Statistically significant outcome#	Clinically relevant	Basis*
Bauer 1986	0.3	30	Asthmatics	9% ↓ FEV_1; 20% ↓ sGaw; 35%^ ↓ PD	X	4,6,7
Bylin 1985	0.48	20	Asthmatics	Mean PD not calculated		
Studies >30 to ≤ 60 minutes						
Tunnicliffe 1994	0.4	60	Asthmatics	Up to 5% ↓ FEV_1	X	4
Mohsenin 1987	0.5	60	Asthmatics	Mean PD not reported		

***Criterion 1** - an FEV_1 change of greater than 12% for lung function; **Criterion 2** - a PEF change of greater than 10% for lung function; **Criterion 3** - a lung function change of greater than 12%; **Criterion 4** - an FEV_1 change of greater than 15% for airway responsiveness; **Criterion 5** - a lung function change of greater than 15% for airway responsiveness; **Criterion 6** - a sRaw change of greater than 100%; **Criterion 7** - a 50% or greater decrease in provocative dose

#Papers were selected based on reported statistically significant outcome, however not all lung function measurements adjusted for the percentage change in control exposure. Where the study permitted, percentage change in lung function measurements are based on mean percentage change in lung function between time 0 and time X from NO_2 exposure minus the mean percentage change in lung function between time 0 and time X from the control exposure. Those studies where this could not be done are marked with a ^.

Of the 10 statistically significant studies, 8 did not meet the criteria of clinical relevance, while 2 did not report enough data to determine clinical relevance.

Inflammatory, cellular and biochemical markers

Forty studies, containing 52 NO_2 exposure concentrations, examined the relationship between NO_2 exposure and inflammatory, cellular and biochemical markers. The concentrations of NO_2 exposure ranged from 0.01 ppm to 5.5 ppm.

Seven studies examined NO_2 exposures 60 minutes or less (**Tables B7 & B8**) of which 3 had a statistical significant result. All 3 of these studies were at a NO_2 concentration of 0.26 ppm, while a further 2 studies at this concentration did not find statistical significance. The 3 statistically significant studies examined asthmatic participants and found differing significant markers, except for eosinophilic cationic protein (ECP) that was found increased in two of the studies.

It is important to consider the studies using inflammatory, cellular and biochemical markers with caution. Samples taken from the epithelial lining fluid too long after the NO_2 exposure may not reflect the impact of the NO_2 exposure, as the chemical processes in the epithelial lining fluid are dynamic and short lived. Further, incorrect sample handling can skew the results (US EPA 2016). Also, many of the studies undertook testing of multiple markers (and their percentages) with no consideration of or adjustment for multiple comparisons. What this means is that the studies are likely to find a statistically significance result purely by chance. While many studies found statistical significance, very few have the same marker driving the statistical significance, which provides further evidence of issues with multiple comparisons. A further analysis of individual markers across studies may reveal the lack of consistency between the NO_2 exposure and the marker in question.

Cardiovascular effects

Four studies were identified that considered NO_2 exposure and cardiovascular effects (**Table B10**). There were no significant endpoints identified at exposure concentrations less than or equal to 0.5 ppm NO_2.

Traffic related experimental studies

Sixteen studies examined exposure traffic pollution, while identifying the NO_2 concentration within this pollution mix (**Appendix C**). With regard to lung function there were no statistically significant studies that examined exposure of 60 minutes or less, however there were two statistically significant studies below a NO_2 concentration of 0.5 ppm that examined exposure over 2 hours (**Table 3.3**). For airway responsiveness there was one statistically significant study (**Table 3.3**).

Table 3.3: Determination of clinical relevance of statistically significant traffic related exposure studies ≤ 0.5ppm

Study	NO_2 (ppm)	Exposure time (mins)	Study population affected	Statistically significant outcome[#]	Clinically relevant	Basis*
Lung Function						
McCreanor 2007	0.075	120	Asthmatics	4% ↓ FEV_1; 4% ↓ FVC	**x**	1,3
Larsson 2010	0.14	120	Asthmatics	P value only		
Airway Responsiveness						
Svartengren 2000	0.15	30	Asthmatics	13% ↑ sRaw; 12% ↑ TGV	**x**	5,6

***Criterion 1** - an FEV_1 change of greater than 12% for lung function; **Criterion 2** - a PEF change of greater than 10% for lung function; **Criterion 3** - a lung function change of greater than 12%; **Criterion 4** - an FEV_1 change of greater than 15% for airway responsiveness; **Criterion 5** - a lung function change of greater than 15% for airway responsiveness; **Criterion 6** - a sRaw change of greater than 100%; **Criterion 7** - a 50% or greater decrease in provocative dose

#Papers were selected based on reported statistically significant outcome, however not all lung function measurements adjusted for the percentage change in control exposure. Where the study permitted, percentage change in lung function measurements are based on mean percentage change in lung function between time 0 and time X from NO_2 exposure minus the mean percentage change in lung function between time 0 and time X from the control exposure. Those studies where this could not be done are marked with a ^.

Of the 3 statistically significant studies, 2 did not meet the criteria of clinical relevance, while 1 did not report enough data to determine clinical relevance.

Repeat exposure to the same concentration

Eight studies examined repeat exposures to the same concentration of NO_2, 4 with NO_2 exposures below 0.5 ppm (**Appendix D**). Of these 4 studies only one had a statistically significant result for either lung function or airway responsiveness, which was not clinically relevant (**Table 3.4**).

Table 3.4: Determination of clinical relevance of statistically significant airway responsiveness NO_2 exposure studies ≤ 0.5ppm, ≤ 60 minutes exposure

Study	NO_2 (ppm)	Exposure time (mins)	Study population affected	Statistically significant outcome#	Clinically relevant	Basis*
Airway Responsiveness						
Strand 1998	0.26	30	Asthmatics	Up to 5% ↓ FEV_1	**X**	4

***Criterion 1** - an FEV_1 change of greater than 12% for lung function; **Criterion 2** - a PEF change of greater than 10% for lung function; **Criterion 3** - a lung function change of greater than 12%; **Criterion 4** - an FEV_1 change of greater than 15% for airway responsiveness; **Criterion 5** - a lung function change of greater than 15% for airway responsiveness; **Criterion 6** - a sRaw change of greater than 100%; **Criterion 7** - a 50% or greater decrease in provocative dose

#Papers were selected based on reported statistically significant outcome, however not all lung function measurements adjusted for the percentage change in control exposure. Where the study permitted, percentage change in lung function measurements are based on mean percentage change in lung function between time 0 and time X from NO_2 exposure minus the mean percentage change in lung function between time 0 and time X from the control exposure. Those studies where this could not be done are marked with a ^.

3.2.3 Reviews and meta-analyses

A review provides a summary of available evidence on an issue, as has been conducted in **Section 3.2.2**. It attempts to provide a description of this evidence. A comprehensive review such as a systematic review, can analyse the evidence in terms of consistency, a criterion used in assessing epidemiological evidence. Consistency refers to the ability of different studies to show the same effect, and can be used when judging evidence.

Another way of assessing the evidence is through a meta-analysis. A meta-analysis involves statistically combining the results of several individual studies. This is undertaken to increase the number of participants in the analysis, thereby providing greater power to detect an effect, if any. In this way a well-constructed meta-analysis of individual studies provides stronger evidence regarding the true relationship between the exposure and outcome than an individual study.

There has been one review (Hesterberg et al. 2009) and three meta-analyses (Brown 2015; Folinsbee, L. J. 1992; Goodman et al. 2009) that have attempted to summarise and synthesise the experimental evidence regarding short term NO_2 exposure. These papers were reviewed, with the following summaries provided in (Jalaludin 2015).

> *Folinsbee (Folinsbee, 1992) reviewed experimental studies of exposures to NO_2 (20 studies of people with asthma and five studies of healthy people) to test the hypothesis that exposure to NO_2 increased airway responsiveness. In the 20 studies of people with asthma, the NO_2 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 NO_2 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 NO_2 compared to exposure to filtered air. In a subgroup analysis, increased airway responsiveness was only seen in subjects resting when exposed to NO_2 and not when*

exercising during exposure. There was no dose response relationship for subjects with asthma (0.05<NO_2<0.2ppm; 0.2<NO_2<0.3ppm; 0.3<NO_2). In healthy subjects, increased airway responsiveness was only demonstrated in those exposed to more than 1ppm of NO_2.

Hesterberg et al. (Hesterberg et al., 2009) reviewed more than 50 experimental studies that focussed on inhaled NO_2 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 NO_2 up to about 0.6ppm, although some sensitive asthmatics may be affected by NO_2 levels as low as 0.2ppm;

• Healthy subjects exposed to NO_2 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 NO_2 concentrations below 2ppm increase susceptibility to viral infection;

• For asthmatics and individuals with chronic obstructive pulmonary disease, NO_2 induced lung inflammation is not expected below 0.6ppm;

• Changes in blood chemistry generally required NO_2 concentrations above 1–2ppm.

Goodman et al. (Goodman et al., 2009) conducted a meta-analysis and meta-regression of controlled human NO_2 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 FEV_1 after an airway challenge; and, the proportion of subjects with increased airway responsiveness. The authors examined 41 exposure scenarios from 38 (28) *studies published between 1976 and 2002. Concentrations of NO_2 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 NO_2 effects on airway responsiveness, the effects were too small to be considered significant at NO_2 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 NO_2 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.

All four papers attempt to provide some estimation of effect for NO_2 exposure. Folinsbee used a sign test method to show a statistically significant fraction of individuals having a response to NO_2 exposure. However, his work did not include the magnitude of the response, and did not provide a recommended NO_2 concentration for susceptible populations. Hesterberg et al provided a summary of individual papers (including those that considered inflammatory, cellular and biochemical markers), to draw their conclusions that a "*health-protective, short-term NO_2 guideline level for susceptible (and healthy) populations would reflect a policy choice between 0.2 and 0.6*". Goodman et al provided a comprehensive search of the literature and reanalysis of the data. Concentrating on lung function and airway responsiveness, they examined the magnitude of effect through the analysis of change in forced expiratory volume over 1 second (FEV_1) and change in provocative dose. They used a US EPA classification to measure adverse outcome from change in FEV_1, along with a halving of the provocative dose as a definition of adverse. Using these classifications Goodman et al concluded that "*the extent of the effects observed ….*(with) *NO_2 exposure, ..are sufficiently small* (as to not have) *a significant adverse effect on airway hyper-responsiveness at concentrations up to 0.6 ppm".* Brown built on the definition of adverse effect used in Goodman et al, reanalysing the data on provocative dose to conclude a "*significantly significant fraction* (of individuals with asthma exposed to NO_2 (at rest)) *experience increases in airway responsiveness following 30 minute exposures to NO_2 in the range of 0.2 ppm and 0.3 ppm and following at 60 minute exposures to 0.1ppm*.

The above studies show airway responsiveness as the key outcome for setting NO_2 guidelines, and the two key studies in this regard are Goodman et al and Brown. The difference between these metanalyses is driven by their statistical technique. Both metanalyses examined all populations presented in the literature review, but the subpopulation in question are asthmatics, who have been exposed to a non-specific agent (such as histamine, methacholine or cold air), at rest.

Goodman et al defined the change in provocative dose in this population as the difference between the provocative dose from NO_2 exposure and provocative dose from air exposure, normalised to the provocative dose from air exposure. They calculated change in provocative dose based on individual subjects (where available) and on group data (where it was not). Mean change in provocative dose and standard deviations were calculated. Examining the means using meta-analysis, Goodman et al concluded that NO_2 does not cause clinically relevant effects on airway hyper-responsiveness in asthmatics at concentrations up to 0.6 ppm.

Brown argued that using the arithmetic mean, as was done in Goodman et al, may affect the validity of some of the analysis, as airway responsiveness data is recognised as being log-normally distributed. Brown used individual subject data only and defined the change in provocative dose as the ratio of provocative dose from NO_2 exposure to the provocative dose from air exposure. A value of 0.5 indicating a halving of the provocative dose while a value of 2 indicating a doubling of the provocative dose. The median and geometric standard deviation was calculated. A sign test was used to determine whether there were a statistically greater number of individuals experiencing increase in airway responsiveness compared to those experiencing a reduction in airway responsiveness. Brown found a statistically significant number of asthmatics at rest had an increase in airway responsiveness at 0.1ppm (33 ↑ versus 17 ↓), 0.1 - 0.2 ppm (47 ↑ versus 23 ↓), 0.2 - 0.3

ppm (28 ↑ versus 8 ↓), and greater than 0.3 ppm (24 ↑ versus 9 ↓). Within this sample, of the 37 individuals who had a double dose change in provocative dose, 76% had a halving of the provocative dose, while 24% had a doubling of the dose. There is little information provided as to the NO_2 exposure concentration for these 37 individuals that caused this significant dose change. Of the 37 individuals a large proportion of them (25) come from one study that examined NO_2 exposures of 0.14 ppm, 0.27 ppm and 0.53 ppm for 30 minutes (Bylin et al. 1988), while other studies identified examined NO_2 exposures of 0.2 ppm at 60 minutes (Orehek et al. 1976), NO_2 exposures of 0.48 ppm at 20 minutes (Bylin et al. 1985), and NO_2 exposures of 0.5 ppm at 60 minutes (Mohsenin 1987). Although Brown did find some individuals with a halving of provocative dose, the median change in provocative dose calculated by Brown for all the asthmatics at rest was 0.75 (three quarters of the provocative dose).

Comparing the analysis undertaken by Goodman et al and Brown several issues are apparent. These include:

- **Outcome change:** Goodman et al is attempting to examine a definitive change of outcome while Brown is primarily focused on the proportionality of positive to negative outcome change.
- **Study focus:** Goodman et al focus is on the clinical relevance of the outcome, while Brown's main focus is on statistical significance of the outcome.
- **Estimation of clinical importance:** Goodman et al argument focuses on mean clinical outcome while Brown's clinical argument is focused on the most extreme individual outcomes within the study.
- **Dose response:** Neither analysis could show a dose response effect with NO_2 exposure
- **Overall clinical relevance:** Both studies show a non-clinically relevant mean / median provocative dose change.

The mean / median provocative dose change in both studies was greater than 0.5, meaning a non-clinically relevant change in airway responsiveness. This provides further evidence of a lack of clinical relevance in NO_2 exposures under 0.5 ppm as presented in **Section 3.2.2**.

However, as highlighted by Brown (and covered further in **Sections 2.5 & 3.3**) there is some concern over the appropriateness of using a mean provocative dose change to define a clinically relevant impact of NO_2 at a population level. This concern centres on the issue that if the average response within a population is not clinically relevant, given the individual variability within a population, there will still be some members of this population which may have a clinically relevant effect. Some way of addressing this issue is to consider the 95th percentile of the mean. The 95% confidence interval of the mean represents the likely range of where the true mean lies. Thus, for a 95% lower confidence interval, 97.5% of the time the true mean will be above this number. Therefore the 95% lower confidence interval could be considered a potential conservative estimate of the true mean.

Goodman et al have provided 95 percent confidence interval estimates of the mean for a number of scenarios. **Figure 3.3** summarises the approach Goodman et al undertook in their meta-analysis. Essentially, Goodman et al combined similar studies for meta-analysis based on whether the studies reported individual participant results or group summary results. For the meta-analysis of group data studies Goodman et al analysed the effect of NO_2 exposure on airway responsiveness,

while for the meta-analysis of individual data studies Goodman et al analysed the effect of NO_2 exposure on airway responsiveness and FEV_1 (**Table 3.5**).

Figure 3.3: Meta-analysis approach undertaken by Goodman et al

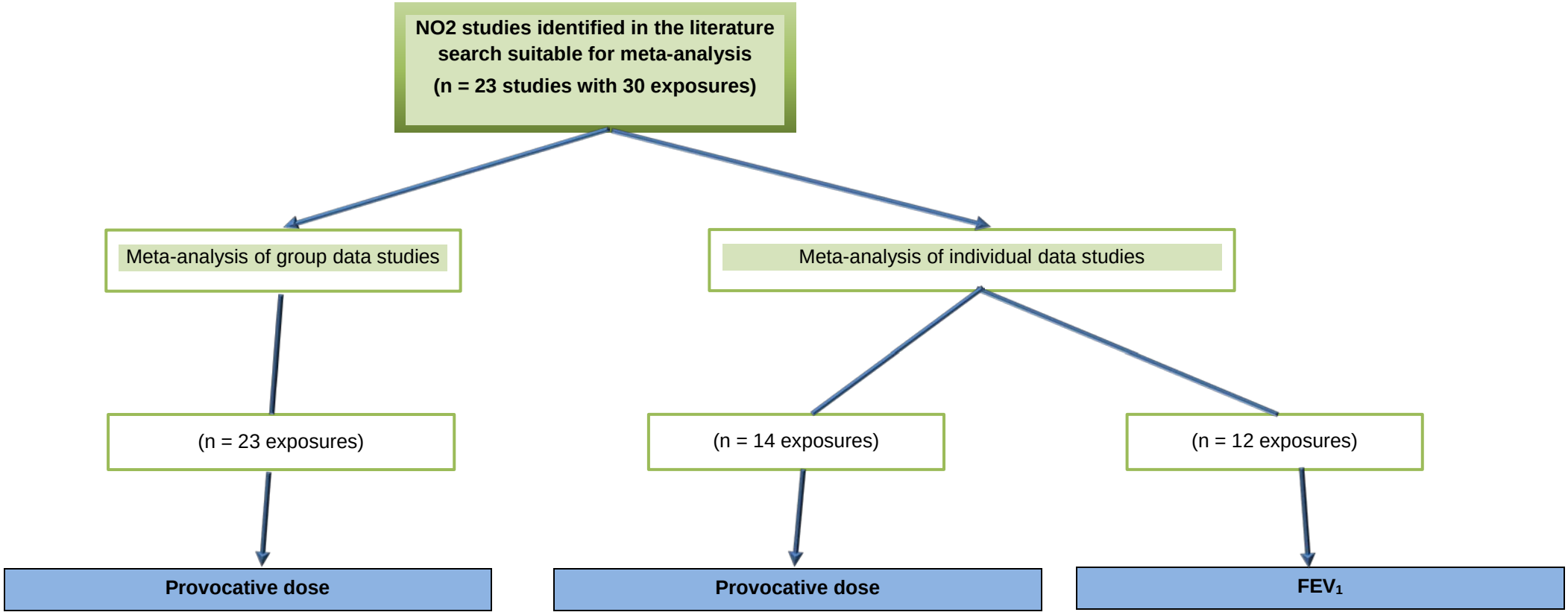


Table 3.5: Measure of airway responsiveness as percentage change in provocative dose (group data)

Effect Size (%)	Clinically relevant	Basis*	95% lower Confidence Interval (%)	Clinically relevant	Basis*
Measure of airway responsiveness as percentage change in provocative dose (group data)					
-27.04	**x**	7	**-35.59**	**x**	7
Measure of airway responsiveness as percentage change in provocative dose (individual data)					
-11.49	**x**	7	**-21.63**	**x**	7
Measure of lung function change (FEV_1) (individual data)					
-1.75	**x**	1	**-3.29**	**x**	1

***Criterion 1** - an FEV_1 change of greater than 12% for lung function; **Criterion 2** - a PEF change of greater than 10% for lung function; **Criterion 3** - a lung function change of greater than 12%; **Criterion 4** - an FEV_1 change of greater than 15% for airway responsiveness; **Criterion 5** - a lung function change of greater than 15% for airway responsiveness; **Criterion 6** - a sRaw change of greater than 100%; **Criterion 7** - a 50% or greater decrease in provocative dose

As seen in column 1 of **Table 3.5**, no mean estimates of effect size were found to be clinically relevant. Considering the lower 95 percent confidence interval estimates of the mean, no clinically relevant effect was found for the meta-analysis of airway responsiveness change when analysed with group data (-35.59%, (95% LCI)) or individual data (-21.63%, (95% LCI)). Further, no clinically relevant effect was found for the meta-analysis of change in lung function (FEV_1) (-3.27%, (95% LCI)). Goodman et al also stratified the meta-analysis by dose, airway challenge, activity level and delivery. No mean estimates of effect size for any of these stratified analyses were found to be clinically relevant, as were most of the lower 95 percent confidence interval estimates of the mean.

Considering the merits of the individual variability argument raised by Brown (covered further in **Sections 2.7 & 3.3**), this argument must be taken in the context of the analysis undertaken by Brown. Firstly, the studies analysed were undertaken in asthmatic (but not severe asthmatic) participants, thereby already considering a susceptible subpopulation. Further, the statistical significance was only found in airway responsiveness and not lung function, only when the asthmatics were at rest, not when exercising, and only when challenged to a non-specific agent (such as histamine, methacholine or cold air) and not when challenged to a specific agent (such as birch or dust mite). Therefore, a specific subset of the population and exposure scenarios is already drawn to determine the statistical significance, and this should be considered when wanting to analyse further into individual variation within the study population.

3.3 Key limitations of NO_2 experimental studies

There are a number of key limitations with the use of NO_2 experimental studies to set an in tunnel 1-hour guideline value. These include:

- **NO_2 surrogacy for vehicular pollutants** – Developing an in-tunnel NO_2 guideline will limit, but not eliminate, commuter exposure to other vehicular pollutants. The NO_2 guideline could be considered as a surrogate measure of vehicular pollutants. Therefore, studies that consider NO_2 exposure in isolation of other vehicular pollutants will not account for the

exposure to these other pollutants, and therefore may underestimate the true impact of the in-tunnel pollutant exposure. This report considered experimental studies that used NO_2 as a proxy for vehicular pollutants and did not find any clinically relevant outcomes from these short term (60 minutes or less) exposures at NO_2 concentrations less than or equal to 0.5ppm **Section 3.2.2 – Traffic related experimental studies & Appendix C**.

- **Cost** – undertaking a human experimental study is costly in terms of time and resources. As such many studies can only recruit a limited number of participants (limiting their statistical power to detect an effect), or can only undertake the experiment over a limited time period (limiting the ability to study long term health outcomes).
- **Ethical aspects** – These will limit the impact of effects that can be examined from NO_2 exposure and may be divided into:
    - **Health endpoints** – Only mild and reversible health endpoints, such as change in lung function, will generally receive ethics approval for a human experimental study. Studies examining development of cancer, birth defects or mortality will not (although it is expected that mild and reversible health endpoint should be more sensitive endpoints than those that are not reversible); and
    - **Study population** – In most cases only a study population where non-life threatening impacts are expected to occur will receive ethics approval. For example, undertaking an NO_2 study on severe asthmatics, where the impact may result in hospitalisation will generally not be approved. Therefore, the most vulnerable population may not be studied.
- **Study Population** – Not examining the most vulnerable population, along with individual variability within a population, will mean a guideline set based on results from experimental studies may not be protective of every individual within our society. However, this situation is not uncommon for the development of health guidelines, where limitations in data, feasibility of the guideline implementation and societal acceptance of risk may all be contributing factors to its development.

3.4 Comparison of current review with 2015 Review

This review identified 38 more studies than that undertaken by Jalaludin in 2015 (Jalaludin 2015), including 8 more traffic related exposure studies. A key difference in this review was the need to examine studies with NO_2 exposures between 30 – 60 minutes. Despite the inclusion of the extra studies, this review did not find any differing evidence from the review by Jalaludin, that would alter the summary bore out in his report.

3.5 Summary of Australian 1-hour NO_2 ambient guideline

The Australian National Environment Protection Council have set a 1-hour NO_2 guideline of 0.12 ppm. This is based on experimental evidence indicating a lowest observed effect level in the range of 0.2 – 0.3 ppm, plus chronic exposure concentrations of 0.04 – 0.08 ppm, and respiratory symptoms in observational data (NEPC 1997), along with consideration of achievability in urban centres (NEPC 1998).

3.6 Summary of World Health Organization (WHO) 1-hour NO_2 ambient guideline

The WHO have set a 1-hour NO_2 guideline of 0.1 ppm. This is based on evidence of changes in lung function and changes in airway responsiveness identified in several studies with exposures to NO_2 between 0.2 to 0.3 ppm. Thus the WHO have defined a Lowest Observed Effect Level (LOEL) of 0.2 ppm (WHO 2000). To develop the guideline, the WHO then divided the LOEL by 2 to account for possible airway responsiveness effects below 0.2 ppm.

This WHO value has been adopted by the European Environment Agency (EEA 2016).

3.7 Summary of United States Environmental Protection Agency (US EPA) 1-hour NO_2 ambient guideline

The US EPA have set a 1-hour NO_2 guideline of 0.1 ppm. The choice of this level was based on a policy decision after considering the available experimental and observational epidemiological evidence, along with the likely exposure impacts of setting a guideline at this level.

> *In setting the level of the new 1-hour standard at 100 ppb* (0.1 ppm)*, the Administrator noted that there is no bright line clearly directing the choice of level. Rather, the choice of what is appropriate is largely a public health policy judgment entrusted to the Administrator. This judgment must include consideration of the strengths and limitations of the evidence and the appropriate inferences to be drawn from the evidence and the exposure and risk assessments* (US EPA 2017)

The US EPA noted the evidence provided by Brown (Brown 2015) regarding statistically significant outcomes at an NO_2 exposure concentration of 0.1 ppm, along with an expectation that setting a level of 0.1 ppm would be expected to limit area-wide NO_2 concentrations to below 0.085 ppm, which *was the lowest 98th percentile 1-hour daily maximum NO_2 concentration in the cluster of five key epidemiologic studies which reported associations with respiratory-related hospital admissions or emergency department visits and which the Administrator gave substantial weight* (US EPA 2017).

3.8 Conclusion

This chapter has reviewed the experimental epidemiological evidence regarding NO_2 exposure and clinical health impacts. When considering the reported statistically significant mean changes in lung function and airway responsiveness, for studies of 60 minutes or less exposure time and at exposure concentrations of NO_2 less than or equal to 0.5 ppm, the review found these changes to be minor and not clinically relevant.

The evidence examined, and limitations highlighted are consistent with a previous review undertaken by Jalaludin in 2015 (Jalaludin 2015), and conclusions drawn in the meta-analysis by Goodman et al (Goodman et al. 2009).

However, all the studies reviewed are subject to limitations as outlined in **Section 3.3**. Specifically, the studies do not specifically address the most sensitive members of the population, namely severe asthmatics. For these individuals the current approach of advising motorists to wind up windows and put air conditioning on recirculation significantly reduced NO_2 exposures in the cabin (refer to **Section 6.5.1**), providing an additional margin of safety in relation to in-tunnel exposures for these individuals.

Section 4. Observational studies - Concentration response functions

4.1 General

Unlike experimental studies, observational studies do not control an exposure, rather they "observe" exposures in a population and health outcomes in that population. This observation can occur over a time period (either prospectively (i.e. into the future) or retrospectively (ie observing past exposure)), or at a single time point. Depending on how well the exposures are observed (along with other exposures that might effect or "bias" the health outcomes in the study), this will impact on the ability of the study to draw conclusions regarding the exposure and health outcome. Therefore, while experimental studies are generally able to show that an exposure caused a health outcome, this is less certain for an observational study, which may only be able to suggest an exposure might have caused a health outcome. There is however a set of rules (Bradford Hill (Hill 1965)) that can be applied to observational studies to overcome this limitation.

The main advantages of an observational study over an experimental study is the ability to study a large number of people and over long time periods, along with the ability to study health endpoints not considered acceptable for experimental studies (such as mortality). Studying a large number of people means you are more likely to detect a health impact at a lower exposure concentration because your study has more statistical "power". For many observational studies (as is the case for some short term NO_2 observational studies) much of the exposure and health data is routinely collected by government environmental and health agencies, meaning the costs of undertaking these studies is dramatically reduced.

The downside to observational studies is that the exposure data is inevitably never as good as for an experimental study. This is especially the case for NO_2 observational studies where a person's NO_2 exposure may be estimated from a community air monitoring station some distance from where they live, along with a lack of or weak data on other exposures which may also cause the health outcome being studied. These other exposures can distort the 'real' effect the NO_2 exposure is having on the health outcome, if they are not properly accounted for. Assuming a person's individual NO_2 exposure is related to an estimated community NO_2 exposure may also distort the 'real' effect the NO_2 exposure is having on the individual health outcome.

NO_2 observational studies are designed to examine various NO_2 exposures and comparing those exposures to the rate of the health outcome. Ideally, as the concentration of NO_2 increases, so should the rate of the health outcome. Graphically this looks like **Figure 4.1** and is known as a concentration response function (also known as a dose response or exposure response function).

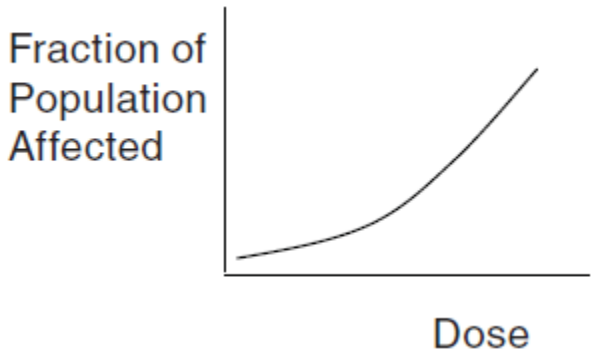
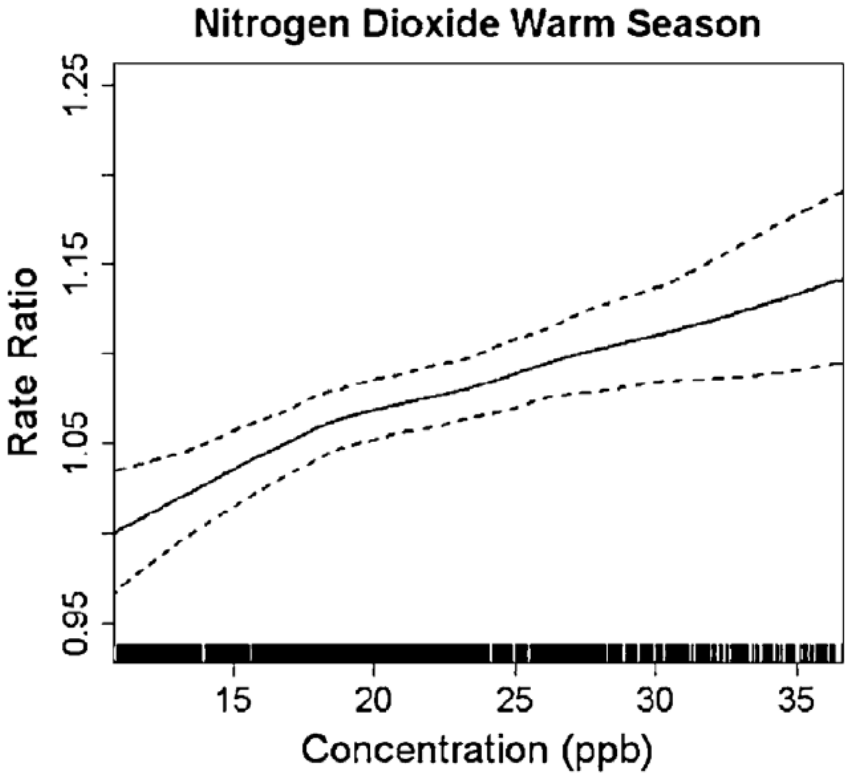


Figure 4.1: Example of a conceptual model of a population dose response relationship (taken from (NRC 2008))

Generally, for NO_2 observational studies these concentration response functions are presented as the ratio of rates of the health outcome for the different NO_2 exposures (also known as the rate ratio, risk ratio or relative risk) as shown in **Figure 4.2**.



Note: ppb = parts per billion. Solid line = locally weighted scatterplot smoothing concentration-response estimates. Dashed lines = twice-standard error estimates. Results are from generalized additive models. Results are presented for the 5th to 95th percentiles of nitrogen dioxide concentrations.

Figure 4.2: Concentration- response function for the association between 3-day average (lag0-2) nitrogen dioxide concentrations and emergency department visits for paediatric asthma in Atlanta (taken from (US EPA 2016))

Analysis of existing observational studies to develop concentration response functions has been unable to determine if there is a concentration of NO_2 below which no health effects are observed. Therefore, based on this observational data, it is concluded that for short term NO_2 exposure there no evidence of a threshold (where no health effects will occur). It is noted however, that the evidence base for assessing the existence of a threshold or the shape of the concentration–response curve for short term NO_2 is potentially confounded and weaker than for fine particles (WHO 2013).

4.2 National Environment Protection Council guidance

In Australia, the National Environment Protection Council has provided a number of concentration response functions for health outcomes from exposure to NO_2 (**Table 4.1**). The values obtained from these concentration response functions can be applied to a population to estimate excess morbidity or mortality in that population as a result of increase in NO_2 exposure. It is important to note that the evidence supporting these concentration response functions is varied, with the strongest evidence for respiratory hospital admissions, and some support around mortality when considering the short term effects from NO_2 exposure (WHO 2013). Therefore, if assessing the short term impacts of NO_2 using concentration response functions it is recommended that the assessment of respiratory hospital admissions and possibly mortality be the core analysis (WHO 2013). Other health endpoints can be assessed, but as the evidence around these health endpoints is less certain the results from this analysis should be used with caution and only to provide supplementary evidence (WHO 2013). **Table 4.1** provides a list of the concentration response functions recommended for use in Australia, with the studies deriving these concentration response functions explored below.

Table 4.1: Concentration response functions recommended for use in Australia (taken from (Jalaludin & Cowie 2012))

Study	Health Outcome	Concentration Response Function Value (95%CI)
Short term		
Mortality		
Environment Protection and Heritage Council (EPHC 2010)	Non trauma	1.7 (0.3-3.2) % per 8.98 ppb NO2
Environment Protection and Heritage Council (EPHC 2010)	Cardiovascular	1.6 (0.4-2.8) % per 8.98 ppb NO2
Environment Protection and Heritage Council (EPHC 2010)	Respiratory	3.9 (0.6-7.4) % per 8.98 ppb NO2
Morbidity		
Environment Protection and Heritage Council (EPHC 2010)	Cardiovascular (hospitalisations)	1.3 (0.3-2.3) % per 8.98 ppb NO_2 in 15 -64 year olds; 2.6 (1.8-3.3) % per 8.98 ppb NO_2 in 65+ year olds
Environment Protection and Heritage Council (EPHC 2010)	Cardiac (hospitalisations)	1.2 (0.0-2.4) % per 8.98 ppb NO_2 in 15 -64 year olds; 3.3 (2.4-4.3) % per 8.98 ppb NO_2 in 65+ year olds

Study	Health Outcome	Concentration Response Function Value (95%CI)
Environment Protection and Heritage Council (EPHC 2010)	Cardiac failure (hospitalisations)	7.5 (5.3-9.7) % per 8.98 ppb NO_2 in 65+ year olds
Environment Protection and Heritage Council (EPHC 2010)	Arrhythmia	3.4 (0.7-6.3) % per 8.98 ppb NO_2 in 15 -64 year olds
Environment Protection and Heritage Council (EPHC 2010)	Myocardial infarction	4.8 (2.3-7.4) % per 8.98 ppb NO_2 in 65+ year olds
Environment Protection and Heritage Council (EPHC 2010)	Respiratory	3.6 (1.5-5.7) % per 9.0 ppb NO_2 in 1 -4 year olds; 4.0 (1.1-7.1) % per 9.0 ppb NO_2 in 5-14 year olds; 1.6 (0.5-2.8) % per 9.0 ppb NO_2 in 15 -64 year olds
(Simpson et al. 2005)	Ischemic heart disease	1.0017 (1.0007-1.0027) per 1 ppb NO2
(Simpson et al. 2005)	Respiratory	1.0016 (1.0006-1.0026) per 1 ppb NO_2 in 65+ year olds
(Jalaludin et al. 2008)	Asthma	1.1 (0.6-1.6) % per 9.5 ppb NO2
(Williams et al. 2012)	Lung Function	-0.0025 (-0.0047- -0.0002) per 1 ppb NO2
(Williams et al. 2012)	Change in PEF	-0.4042 (-0.7318- -0.0767) per 1 ppb NO2
Long term		
Morbidity		
(Williams et al. 2012)	Incidence of asthma	1.27 (1.04-1.56) per 4.31 ppb NO2
(Williams et al. 2012)	Change in FEV1	-45.4 (-74.3-16.5) ml per 4.31 ppb NO2
(Williams et al. 2012)	change in FVC	-43.1 (-72.2-14.1) ml per 4.31 ppb NO2
(Williams et al. 2012)	Airways inflammation	1.03 (1.01-1.05) per 1 ppb NO2

Environment Protection and Heritage Council (EPHC) - Multi-City Mortality & Morbidity Study

In 2003 the EPHC began a time series study with the aim of examining any association between air pollution and daily mortality and morbidity in Australian cities. A time series study being where exposures and health outcomes in population are measured over several different time periods, thereby allowing trends to be detected. For example, one technique used in a time series study is a case crossover analysis. This is where the levels of air pollution and health outcome in a population at one particular time is compared to air pollution levels and health outcomes at another particular time or times within the same population. If a relationship is found between the higher levels of air pollution and health outcomes that is statistically significant, then the air pollution and health outcome is said to be associated. The term association is used because the design of this observational study alone is unable to prove the air pollution caused the morbidity or mortality (causation), but rather that the two are associated (for example an increase in air pollution is associated with an increase in morbidity).

The outcome of the EPHC analysis was individual Australian city results for air pollution and morbidity and mortality. The EPHC then 'pooled' or combined the results of all the cities together through a statistical technique known as random effects meta-analysis to development estimates of how much morbidity or mortality would be expected to increase with increased levels of NO_2 (**Table 4.1**).

Australian Child Health and Air Pollution Study (ACHAPS) (Williams et al 2012)

In 2007 and 2008 the ACHAPS was undertaken on school children around Australia. This observational study involved a cross sectional study of 2860 school children, with a nested panel study of 270 children with a history of asthma. Essentially, the cross-sectional study involved estimating a child's average NO_2 exposure over a substantial time period and measuring health outcomes for that child over a single time point (one day). The child's NO_2 exposures and health outcomes were compared to the other children to determine if those children exposed to higher concentrations of NO_2 were more likely to have health outcomes.

The nested panel study involves taking a subset of those 2860 school children (270 children with a history of asthma) to follow them more closely. In this study the 270 children were followed for up to 36 days, with daily estimates of NO_2 exposure and measures of health outcomes. The relationship between the NO_2 exposure measures and health outcomes were analysed using a generalised linear model, accounting for confounders along with child and school specific data. Essentially, this means all the NO_2 exposure data and health outcomes data were compared to see if an increase in NO_2 was associated with an increase in health outcome, adjusting for the fact that the results provided by a child or school may affect this relationship.

As with the EPHC study these studies in themselves do not result in causation rather association (**Table 4.1**).

(Simpson et al 2005) The short-term effects of air pollution on hospital admissions in four Australian cities

This observational time series study examined pollution and health outcome data in four Australian cities over 4 years from 1996 to 1999. Like the EPHC study it examined trends in pollution and hospital admissions over time for each of the four Australian cities. To do this it developed a statistical model that accounted for things such as seasonal patterns, temperature and humidity while examining the relationship between NO_2 levels and hospital admissions (this form of statistical analysis was also undertaken in the EPHC study). The results of each city were then combined using random effects meta-analysis to development estimates of how hospital admissions would be expected to increase with increased levels of NO_2 (**Table 4.1**).

(Jalaludin et al 2008) Air pollution and ED visits for asthma in Australian children: a case-crossover analysis

This observational study examined NO_2 exposure and asthma hospital admissions (health outcome) data for children aged 1- 14 years old. The study was restricted to the Sydney metropolitan region and data from a 5-year period from 1997 – 2001 was analysed. The study used a case cross over analysis (described above) to determine the value of the concentration response function listed in **Table 4.1**.

4.3 Swedish approach to NOx standard setting

4.3.1 General

The Swedish Transport Administration have focused on concentrations of NOx and not NO_2 when considering health impacts of transport infrastructure. They have funded a number of publications which provide approaches, but not guideline values, for consideration in setting in-tunnel NOx standards. These publications, described below, are based on observational epidemiological data and the concentration response functions derived from this data.

4.3.2 Potential health impacts of changes in air pollution exposure associated with moving traffic into a road tunnel

In this paper Orru et al (Orru et al. 2015) examined the impact of a proposed 18 km road tunnel in the Swedish city of Stockholm. The tunnel is designed to divert traffic off the streets of Stockholm thereby reducing overall exposure of NOx to the city's residents. To examine the impact of the proposed tunnel Orru et al undertook a cost effectiveness approach. This is where they examined the health gains from reduced NOx exposure on the surrounding population and the health costs of increased pollution exposures to the people using the tunnel.

Health gains or health costs are calculated by an equation that uses the concentration response function (see **section 4.3.3**), the concentration of NOx, the size of the population affected and the underlying rate of the health outcome (e.g. mortality). In the Orru et al paper the calculation of health gains to the population was relatively straight forward. This involved comparing the annual concentration of NOx exposure to the Stockholm population with and without the tunnel to estimate the number of fewer premature deaths, asthma incidence and asthma prevalence. They found developing the tunnel would equate annually to 23.2 fewer premature deaths and 565 and 21 fewer asthma cases in children and adults respectively.

For tunnel users, calculating the health costs required a few extra steps. The annual NOx exposure for a tunnel user was based on a calculation of one tunnel exposure to NOx per day of approximately 20 minutes. This 20-minute exposure per day was then time weighted average to an annual average exposure by multiplying the tunnel concentration by the minutes exposed in a year (from 365 20-minute trips) and dividing this value by the total minutes in a year. The annual average of the 20-minute exposures were calculated either during peak traffic periods or non-peak periods. The annual increase was estimated in the paper to be 8.4 $\mu g/m^3$ NOx (peak traffic exposure) and 4.3 $\mu g/m^3$ NOx (non-peak traffic exposure).

To account for the fact that if a person was not travelling through the tunnel they would still be exposed to NOx from driving on an open road motorway (i.e. they still need to get to work), the annual NOx exposure from this pathway was calculated in the same way as the tunnel scenario. This value was then substracted from the tunnel scenario to obtain a net increase in annual NOx exposure. The paper does not disclose the non-peak NOx concentration, nor the travel time used to estimate this open road motorway scenario. It did find that the open road scenario would lead to an annual increase of 1.2 $\mu g/m^3$ NOx (peak traffic exposure) and 0.5 $\mu g/m^3$ NOx (non-peak traffic exposure). Thus, the health costs to tunnel users was based on an annual NOx exposure of 7.2 $\mu g/m^3$ NOx (peak traffic exposure) and 3.8 $\mu g/m^3$ NOx (non-peak traffic exposure). They found for a tunnel using population between 30 -74 years, these NOx exposures would equate annually to 20.6 premature deaths (peak) or 15.1 premature deaths (non-peak). Further, an NOx annual exposure of 7.6 $\mu g/m^3$ would result in a decrease in life expectancy of 0.23 years.

Both tunnel and open motorway NOx concentrations were modelled estimates of future traffic conditions, with the average speed on the motorway estimated at 30-40km during peak traffic and 65-70 km during non-peak traffic periods. Interestingly, the largest impact of NOx was modelled at tunnel portal exits. In NSW routine portal emissions are not permitted in long road tunnel, so this impact would not be relevant for NSW.

The health costs to tunnel users was based on in-tunnel and on-road NOx concentrations, rather than in-cabin NOx concentrations. In-cabin concentrations can be significantly lower than in-tunnel

and on-road concentrations (see **Section 6.5.1**) meaning the estimation of health impacts using in-tunnel concentrations will be conservative for most tunnel users.

4.3.3 Assessment of long-term health impacts of air quality with different guideline values for NO_x in the planned by-pass tunnel Förbifart Stockholm

Further assessment has been undertaken in a report (Orru & Forsberg 2016). The assessment in this report is the same as in (Orru et al. 2015), with the exception of tunnel exposure scenarios. While (Orru et al. 2015) used future traffic predictions and one tunnel ventilation scenario to estimate NOx impact on tunnel users, (Orru & Forsberg 2016) used 4 ventilation scenarios. Essentially, they assumed a maximum 1-hour concentration of NOx in the tunnel of 1000, 2000, 3000 and 4000 µg/m^3. As this is a maximum level that cannot be passed at any point in the day, the overall concentration in the tunnel over a 24-hour period will be less than the specified 1000, 2000, 3000 or 4000 µg/m^3 (**Figure 4.3**).

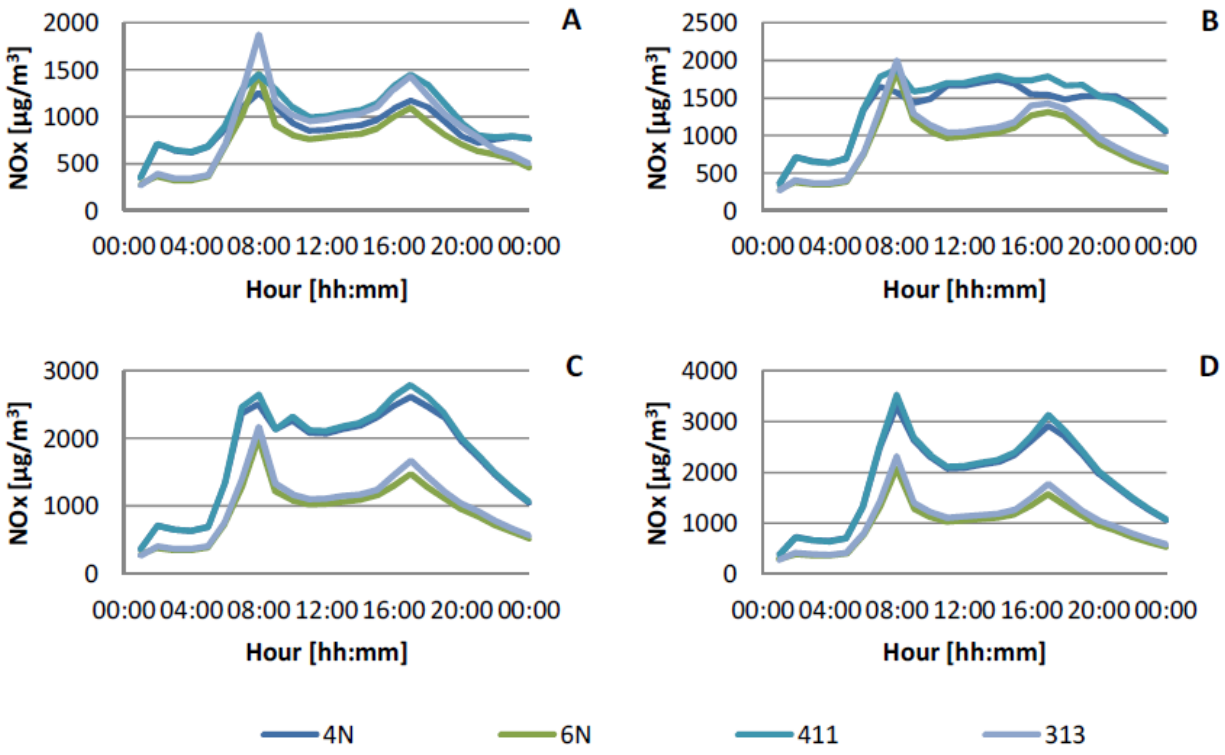


Figure 4.3: Modelled 1-hour NOx concentrations (for different road links, 4N, 6N, 411 and 313) - A = 1000 µg/m^3, B = 2000 µg/m^3, C = 3000 µg/m^3, D = 4000 µg/m^3 (taken from (Orru & Forsberg 2016))

The report highlighted the impossibility of the tunnel achieving the 1000 µg/m^3 scenario, and therefore this scenario is based on the lowest achievable maximum 1-hour NOx concentration of 1789 µg/m^3. Annual averages of the concentrations modelled (**Figure 4.3**) per exposure scenario were calculated and used to estimate an annual NOx dose for a tunnel user. As with (Orru et al. 2015) this dose was adjusted to account for an exposure on the open motorway.

The report estimates an annual increase in premature mortality of 22.2 (1789 µg/m^3 scenario), 35.2 (2000 µg/m^3 scenario), 45.0 (3000 µg/m^3 scenario) and 47.9 (4000 µg/m^3 scenario). It compared these values to an estimated annual decrease of 23.7 premature deaths in the surrounding Stockholm population as a result of the tunnel being built and subsequent traffic diversion, concluding that only under the most conservative exposure scenarios would there be an expected reduction in air pollution effects on the population. Most importantly, the report does not recommend a guideline value, nor set an acceptable level of risk that could be used for a guideline. Instead, it undertakes a 'as low as reasonably practicable' approach, highlighting the health impacts of reducing or increasing an in-tunnel NOx guideline value.

4.3.4 NOx concentration response function

The NOx concentration response function used in (Orru et al. 2015) and (Orru & Forsberg 2016) is based on the study by (Nafstad et al. 2004). This study involved following a cohort of 40 to 49 year old Norwegian men (n = 16 209) living in Oslo from 1972 till 1998. Annual NOx exposures to the men were estimated based on participant's main place of residence in the year, with these yearly averages then averaged over the years of exposure. The relationship between the NOx exposures and national death registry data were analysed using a Cox proportional hazards regression model. This model used average 5-year levels of exposure in most analyses, and NOx levels were analysed as both a continuous and categorical variable. The categorical variable ranges of NOx were 0 -9.99, 10.00-19.99, 20.00-29.99, and ≥ 30.00 µg/m^3. The national death registry data consisted of total deaths from diseases, deaths from respiratory diseases, deaths from lung cancer, deaths from ischemic heart diseases and deaths from cerebrovascular diseases. The outcome of this analysis was a concentration response function (risk ratio) for mortality (total deaths) of 1.08 for a 10 µg/m^3 increase in NOx, which was used in the two above studies by Orru et al.

The annual NOx exposures were based on extrapolation (modelling) from NOx community monitoring station measurements where possible. However, as NOx measurements were not always available, a large proportion of the NOx estimates were based on the distribution of sulphur dioxide concentrations. This distribution was adjusted to reflect NOx concentrations, with consideration of background and local traffic influences. Importantly NO_2 concentrations were not calculated because ground level ozone concentrations were not measured. Therefore, the driver for using NOx and not NO_2 in this study is not the health impact of NOx versus NO_2, but rather an inability to estimate NO_2 exposure.

4.4 Comparison between Swedish and Australian use of observational NO_2 / NOx data to determine health risk

4.4.1 General

There are two important differences in the Swedish approach and approaches that have been undertaken in Australia when assessing the health impacts from NO_2 / NOx air pollution. These are, the selection of the concentration response function and the chemical of impact (NO_2 or NOx).

4.4.2 Selection of concentration response function

The Swedish approach uses a concentration response function based on long term health risk, whereas Australian approaches undertaken for the National Environmental Protection Measure (NEPM) used short term concentration response functions only (Golder 2013).

The use of short versus long term concentration response function may be considered in terms of weight of evidence and biological acceptability.

Weight of evidence

Short term respiratory health effects are considered the most robust health endpoint for NO_2 exposure, with the US EPA classifying respiratory effects from short term (minutes to 1 month) exposure to NO_2 as a causal relationship (US EPA 2016), and the WHO ranking it as the NO_2 concentration response function of greatest certainty of causing health effects (WHO 2013). While other health endpoints, both long and short term, are considered with less certainty, the WHO rank long term all-cause mortality as one of the most uncertain health endpoints from NO_2 exposure (WHO 2013). It is noted that no assessment of the weight of evidence concerning NOx has been made, however NO_2 is an integral part of NOx (see **section 4.4.2**).

Biological acceptability

A limitation to the use of observational studies to assess intermittent (up to 1 hour) but higher exposures to NO_2 is the question of biological acceptability. What is meant by this is can an intermittent exposure (up to 1-hour exposure) be equated to a 24-hour or annual exposure?

Haber's rule provides some evidence to answer this question. It essentially articulates that concentration is directly proportional to time. So, an exposure of 2ppm for 1 hour would be equivalent to an exposure of 1ppm for 2 hours. This rule is used in the basic human health risk assessment calculation and is the approach Orru et al undertook. It, or some variant of it, would also be a likely Australian approach, the difference being that for Australia a 1-hour exposure would be estimated into a 24-hour exposure and that would be imputed into the short term concentration response function, while Orru would estimate the 24-hour exposure, and then average all the 24-hour exposures over a year and apply that figure to the long term concentration response function.

But there are limitations to Haber's rule, and modifications to it approach has been suggested over time (Connell et al. 2016). Importantly, the data on which Haber's rule was founded was lethal dose concentrations to animals. This is a very finite health endpoint and therefore an equally important consideration is how does a lethal health endpoint relate to a non-clinically relevant one? In other words, does the body recover from a non-significant clinical insult from a 1-hour exposure to nitrogen dioxide within a 24-hour period, therefore making it unlikely to cause the observational short term (24 hour) or long term effects. In an attempt to answer this question, those statistically significant experimental studies identified in **Tables 3.1, 3.2, B.7 & B.8** were reviewed (**Table 4.2**). The review focused on whether study outcomes were measured at multiple time periods, and if so which period, or periods were statistically significant. Those time points in red in **Table 4.2** are the time periods of statistical significance.

Table 4.2: Statistically significant experimental studies (≤ 0.5 ppm NO_2 & ≤ 60 minutes exposure) and time period of statistical significance.

Study	NO_2 (ppm)	Exposure time (mins)	Statistically significant outcome	Time of measured health outcome*#
Lung Function				
Bylin 1985	0.24	20	↑ sRaw	Pre; During exposure (10, 20 mins); Post
Strand 1996	0.26	30	↓ TGV	Pre; During exposure (4, 15, 30 mins); Post 10, 20 mins
Koeing 1988	0.3	60	↓ FVC	Pre; Post 2, 7, 20 mins
Vagaggini 1996	0.3	60	↓ FEV_1	Pre; Post 1, 2 hours
Bylin 1985	0.48	20	↓ sRaw	Pre; During exposure (10, 20 mins); Post
Airway Responsiveness				
Svartengren 2000	0.15	30	↑ sRaw; ↑ TGV	Post 4 hours, allergen challenge given, early-phase (4-5) hours, late-phase (7-14) hours post. Statistically significant ↓ FEV_1 for a subset of exposed in late phase
Jorres 1990	0.25	30	↓ PD	Post 15 mins
Strand 1996	0.26	30	↓ PD	Post 30mins, 5 hours, 27 hours, 7 days
Strand 1997	0.26	30	↓FEV_1; ↓ PEF	Post 4 hours, allergen challenge given - early-phase (4) hours, late-phase (7-13) hours post
Strand 1998	0.26	30	↓ FEV_1	Multiple day exposures Post 4 hours, allergen challenge given - early-phase (4.25) hours, late-phase (7-14) hours post. Day 1 - early-phase (4.25) hours, late-phase (7-14) hours post. Day 4 - early-phase (4.25) hours, late-phase (7-14) hours post. Importantly, no difference in baseline FEV_1 or sRaw values to those from the following morning
Bylin 1988	0.27	30	↓ PD	Post 25 mins, not found at higher dose
Bauer 1986	0.3	30	↓ FEV_1; ↓ sGaw; ↓ PD	Post 60 mins (provocation at resting ventilation); 90 mins (provocation at 30L/min); 100 mins (provocation at 60L/min)
Tunnicliffe 1994	0.4	60	↓ FEV_1	Post, allergen challenge immediately given, early-phase (0-

Study	NO_2 (ppm)	Exposure time (mins)	Statistically significant outcome	Time of measured health outcome*#
				2) hours, late-phase (2-6) hours post
Bylin 1985	0.48	20	↓ PD	Post 20 mins
Mohsenin 1987	0.5	60	↓ PD	Post 8-10 mins
Inflammatory, Cellular and Biochemical Markers				
Barck 2005 a	0.26	15	After allergen challenge, ↑ ECP; ↓ MPO	Multiple day exposures, samples Pre Day 1, 2 & 3 Day 1 – Day 3 difference
Barck 2002	0.26	30	After allergen challenge, ↑ % N & ECP levels; ↓ M	Post 23 hours
Strand 1996	0.26	30	↑ MCG	Pre; Post 30 mins, 27 hours

*Statistically significant time period in red; Pre = Pre exposure to NO_2 or Air, Post = Post exposure to NO_2 or Air

Table 4.2 provides some evidence regarding the potential transient nature of health outcomes experienced from exposures of less than or equal to 0.5 ppm NO_2, for the time period of 60 minutes or less. No real patterns of prolonged health outcome from exposure have emerged. Two of the five positive lung function studies had recovery periods within minutes post exposure. Many of the airway responsiveness studies showed only early responses with one commenting that "*no effect of NO_2 or allergen remained from Day 1* (the previous day)" (Strand, V. et al. 1998). There are however, some studies that have statistically significant results on their last time of outcome measure. The inflammatory, cellular and biochemical markers are a little more complex, although one study had markers recovered by 27 hours. The argument regarding the transient nature of the health outcomes is strengthen when the non-statistically significant studies are also considered. Twenty-five studies with exposures less than or equal to 0.5 ppm NO_2, for the time period of 60 were non statistically significant for lung function, 14 studies were non statistically significant for airway responsiveness and 4 studies were non statistically significant for inflammatory, cellular and biochemical markers.

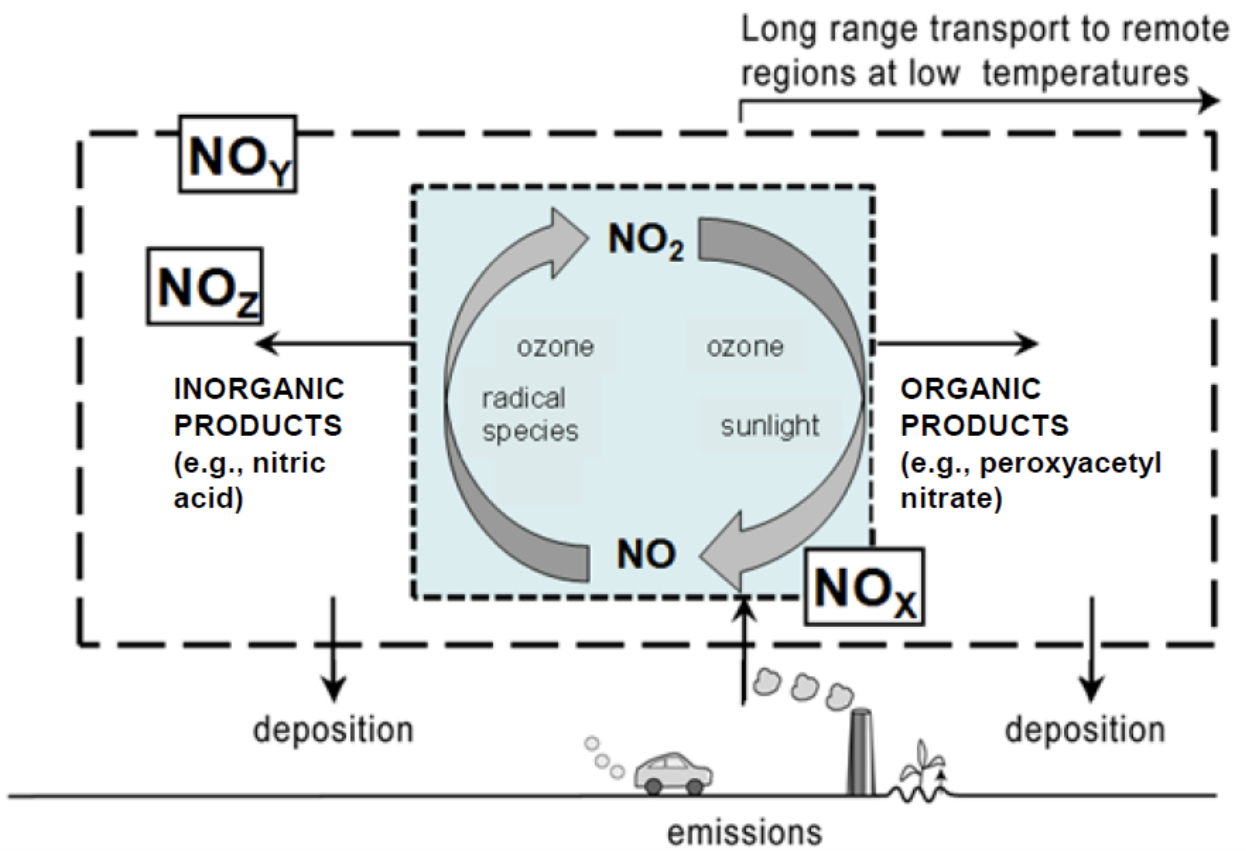
It is important to consider the limitations of the information in **Table 4.2**, which is addressed in **Section 3.3**. Briefly, these studies may not consider all the health endpoints that may drive the observational study outcomes. The experimental studies do not consider the most susceptible populations and are likely to be underpowered.

In summary, the current experimental evidence while limited is weak regarding a prolonged health outcome (≥ 1 day) from a short (≤ 1 hour exposure) to NO_2 at concentrations 0.5ppm or less.

4.4.3 Selection of chemical of impact (NO_2 / NOx)

The Swedish approach by Orru et al of using NOx as a measure of health impact is based on an assessment of geographical and cultural similarities of the Swedish population to the Oslo cohort. Orru et al argue that NOx is a good indicator of the gradients in levels of motor vehicle exhaust and that due to its long atmospheric lifetime (days) it may be considered as inert and without considering photochemical processes, as in the Oslo cohort. Finally, on a yearly basis there is good spatial correlation between NOx and NO_2 (Orru & Forsberg 2016).

NOx is the sum of NO_2 and nitric oxide (NO), which may go onto form other biproducts (**Figure 4.4**). Human emissions of NOx are primarily as NO. The NO rapidly reacts with radicals and ozone to form NO_2 in the air (US EPA 2016). While the information on the health effects of NO is unclear, it is recognised that when considering health impacts of NOx, NO_2 is the main component of interest, and the component whose impact has been assess in a weight of evidence approach (US EPA 2016; WHO 2000).



Note: The inner shaded box depicts NO_X [sum of nitric oxide (NO) and nitrogen dioxide (NO_2)]. The outer box contains oxides of nitrogen formed from reactions of NO_X (NO_Z). Oxides of nitrogen in the outer and inner boxes (NO_X + NO_Z) are collectively referred to as NO_Y by the atmospheric sciences community.

Figure 4.4: Reactions of oxides of nitrogen species in the ambient air (taken from (US EPA 2016))

The use of the Oslo cohort study by Orru et al is understandable. For an environmental epidemiological investigation, it is a well-constructed study that has been determined to reflect the Swedish population. Its limiting factor however is its long-term health estimations from exposure to NOx concentrations, and the applicability of those to an in-tunnel situation. There is an unknown ratio of NO_2 to NO in the Oslo environment, and if this ratio is not similar to an in-tunnel ratio, it can affect the estimated outcome. This is because if NO_2 is assumed to be the main driver of health impact and the ratio of NO_2 to NO is high in the Oslo environment, but low in the tunnel environment, the use of the Oslo concentration response function will overestimate health impacts from in tunnel exposure. Undertaking an assessment using the NO_2 concentrations can help minimise this affect. Further, using short term NO_2 concentration response functions rather than long term ones draws on a more robust evidence base, noting that no formal assessment of NOx has been undertaken in a weight of evidence approach (see **section 4.4.1 weight of evidence**).

It is important to note that all risks assessments and the studies they are based on have strengths and weaknesses, including those used in Australia. Nonetheless in Australia, the impact of short term exposure to NO_2 is the focus health outcomes assessments as has been outlined in **Section 4.2** and the NEPM process (Golder 2013).

4.5 Used of observational studies to set a 1-hour NO_2 concentration

Currently neither the WHO, US EPA or Australian authorities have solely used observational studies to develop their NO_2 1-hour guideline. The WHO reply primarily on experimental data (WHO 2000) , while the US EPA and Australian authorities consider the information provided in NO_2 observational studies in a semi quantitative context with experimental studies providing the strongest influence (NEPC 1997, 1998; US EPA 2017) (see **sections 3.5 – 3.7**). Even the Swedish guidance developed by Orru et al (Orru & Forsberg 2016) does not set a guideline level for in-tunnel NOx concentrations, but rather highlights the potential impacts different in-tunnel concentrations may have on the population (see **section 5**).

The question of using observational studies to set a short term concentration for NO_2 has been considered by the WHO with no firm conclusion drawn (WHO 2013). Rather the report highlighted that “there is an argument for having both a guideline set on the basis of chamber (experimental) studies, where the toxic agent is known to be NO_2, and a further guideline set on the basis of the large body of time-series (observational) studies that show the effects at lower concentrations, but with more uncertainty as to the responsible indicator pollutant for health effects” (WHO 2013). The report further discussed the possibility of adjusting the 24-hour concentrations used in the observational studies to a 1-hour concentration and comparing that adjusted value against the 1-hour concentration values found in experimental studies. Alternatively, the subset of observational studies that used 1-hour averages could be used to set an observational study 1-hour value, noting the limited studies to choose from to develop this guide.

Owing to the limitations of observational studies and lack of agreed approach in the way the data from these studies may be translated into a 1-hour guideline, at this time it is not recommended that observational data be used to solely develop an in-tunnel guideline value.

Section 5. Comparison of the Jalaludin and Orru approaches

5.1 General

The comparison and use of the Australian Jalaludin and Swedish Orru approaches for assessing risk from NO_2 / NOx exposure can be considered in terms of the policy setting in which they will be used, the evidence base from which they have drawn, and the consideration of the interacting effects of other pollutants.

5.2 Policy setting

Developing a guideline value of this type by considering evidence only is rare. There are many other factors that may come into consideration. EnHealth the peak environmental health body in Australia state

> *While acknowledging that setting a level of 'acceptable risk' is often necessary for decision-making purposes, setting the numerical value for such a risk level is a socio-political matter, requiring extensive consultation with stakeholders, including the community likely to be affected by the environmental hazard and those responsible for managing or ameliorating the risks. A socioeconomic or cost–benefit analysis of the risk management options should also be part of the process.* (enHealth 2012)

This process is reflected in both the Jalaludin (Jalaludin 2015) and Orru (Orru & Forsberg 2016; Orru et al. 2015) approaches. Both approaches provide evidence but neither recommend an in-tunnel guideline value. Instead they attempt to provide scientific evidence to inform the risk decision process. In the case of the Jalaludin report, it focused on experimental studies, while the Orru approach involved the use of an observational study.

The report *Review of experimental studies of exposures to nitrogen dioxide* by Jalaludin was considered by the New South Wales (NSW) Advisory Committee of Tunnel Air Quality. This committee is chaired by the NSW Chief Scientist and consists of representatives from NSW Government as well as independent experts in air pollution. The experimental evidence was assessed by the committee in the context of Australian tunnel design and ventilation, vehicular emissions and in-cabin vehicular exposures, who decided on an in-tunnel value of 0.5 ppm rolling average.

The Swedish report *Assessment of long-term health impacts of air quality with different guideline values for NOX in the planned by-pass tunnel Förbifart Stockholm* (Orru & Forsberg 2016) has been developed so the Swedish Transport Administration can consider the potential health impacts from setting in-tunnel NOx at different levels. The report acknowledges the limitations of the Swedish tunnel design in achieving the lowest NOx level of 1000 µg/m^3 (*with the planned ventilation solution, it is not possible to keep the maximum NOx value below 1000 µg/m^3* (Orru & Forsberg 2016)), and makes some recommendations for the tunnel design. However, its primary purpose is to provide an estimate of excess mortality in tunnel users that can be considered in the broader context of policy development. To aid in this broader context, it compares the excess in mortality for tunnel uses against the lower mortality in the wider community from the tunnel development. This cost effectiveness approach can be helpful, but should only be used in a qualitative context. Because the

cost-effective comparison is driven by the numbers of in tunnel users and population affected, a relatively small number of one group compared to a large number could lead to an excessively small or large guideline value. Also, a large shift in the numbers of one group could have the effect of changing the already set guideline value.

5.3 Evidence base

Sections 3 and 4 explored the differing evidence base used by Jalaludin and Orru. In summary Jalaludin has focused on experimental studies while Orru have used results from an observational study. Both approaches have their strengths and weaknesses with experimental studies primarily influencing the setting of NO_2 guidelines at this point in time (see **Section 4.5**)

5.4 Surrogacy for other pollutants

Setting an in-tunnel NO_2 or NOx guideline value will limit exposure to other vehicular pollutants such as particulate matter, which may not have in-tunnel guideline values. Therefore, developing a NO_2 / NOx in-tunnel guideline value that does not consider the other vehicular pollutants it is limiting may underestimate the risk to tunnel users. Most experimental studies for NO_2 do not take this issue into consideration, with only 16 of the 78 studies identified in our literature review considering other vehicular pollutants. Observational studies do better by their design. NO_2 observational studies are based in urban populations where vehicular exhaust is a main contributor to NO_2 concentrations. While some observational studies will attempt to “adjust” for these other vehicular pollutants, the study used by Orru did not. A complicating factor to this is the differing proportions of the pollutants in-tunnel as opposed to in the urban environment. Nonetheless the observational studies (unintentionally) are more likely to take into consideration the impact of other air pollutants (plus other issues which may affect the outcome) than experimental studies.

Section 6. Exposure Assessment

6.1 General

The exposure to NO_2 and other related vehicular pollutants on the road will be dependent on travel route, travel time, travel frequency and vehicular type.

6.2 Travel route

For a person to reach their destination they will have the option of either going through the tunnel system or travel via the above ground street network. Both routes will contain some level of vehicular pollutant exposure.

Tunnel network (in-tunnel NO_2 concentrations)

The assessment of in-tunnel exposure to NO_2 can be considered in terms of what level of NO_2 is permissible in the tunnel and / or what levels have been measured by vehicles travelling through the tunnel.

The maximum concentration of NO_2 currently permissible in the road tunnels being developed in Sydney is 0.5 ppm on a 15-minute rolling average. However, for some existing tunnels this criterion does not apply.

Monitoring of NO_2 levels inside Sydney's major road tunnels has shown NO_2 concentrations measured from outside the transiting vehicle ranging from less than 0.05 ppm in the Sydney Harbour Tunnel to 0.718 ppm in the M5 East tunnel (**Figure 6.1**).

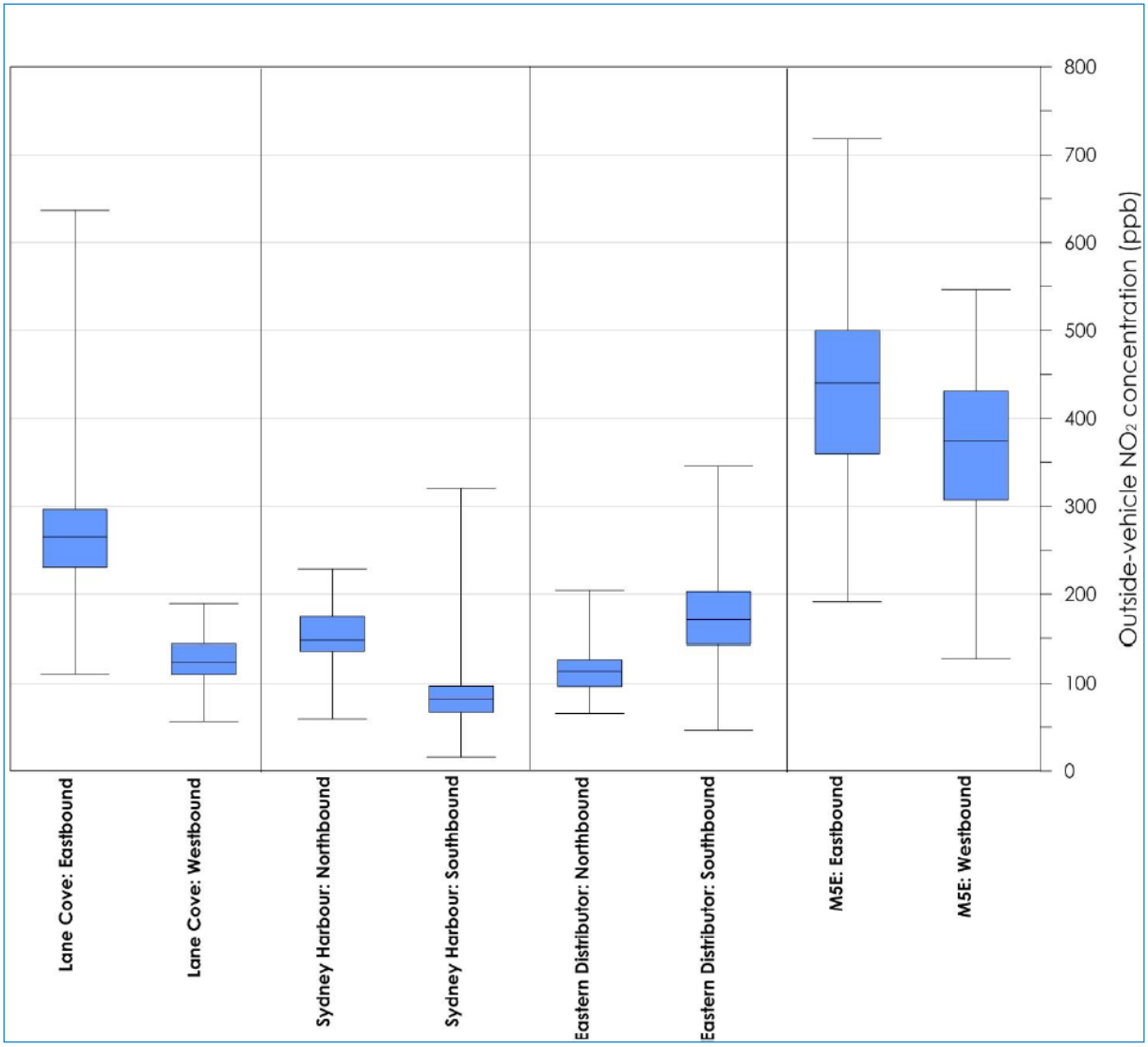


Figure 6.1: Outside vehicle NO_2 concentrations averaged by tunnel transit time (taken from (PEL 2016))

The average outside vehicle NO_2 concentration of all the tunnels was 0.266 ppm, with the average of the southbound Sydney Harbour Tunnel being the lowest NO_2 average at 0.097 ppm and the eastbound M5 East tunnel being the highest at 0.453 ppm (PEL 2016) .

Surface roads (NO_2 concentrations)

Undertaking a trip via surface roads will involve exposures to NO_2. Measured outside vehicle concentrations of NO_2 on the surface roads surrounding Sydney tunnels systems are generally less than 0.15 ppm (PEL 2016). While lower than the in-tunnel concentrations, these measurements have been taken on the surface roads leading into the tunnel systems and not the alternate surface roads which may be more congested. Other studies have examined surface road NO_2 measurements from inside the vehicle cabin (see **Section 6.5**).

6.3 Travel time

One of the primary aims of road tunnels is to keep traffic moving and reduce travel time. Increased travel (exposure) time will increase the overall exposure to NO_2. Mathematically this can be explained through Haber's rule where a 1-hour exposure to 0.5 ppm of NO_2 would be equivalent to 2-hour exposure at 0.25 ppm of NO_2. So, an exposure to a lower NO_2 concentration on a surface road but for a longer time period could be considered as detrimental as a higher concentration for a shorter time period.

Haber's rule does have limitations (see **Section 4.4. 1 – biological acceptability**). Biological acceptability, such as whether the lower concentration can produce a health effect that may be seen at the higher concentration being an important limitation.

Increased travel time can have other health outcomes not associated with the NO_2 exposure. There is moderate evidence to suggest that driving for long hours elicits a stress response over an extended period of time (Antoun et al. 2017). Travel time has also been associated with weight gain, cardiometabolic risk, insufficient physical activity, insufficient sleep and worse physical and mental health (Ding et al. 2014; McCormack & Virk 2014; Sugiyama et al. 2016). This evidence is based on observational studies which have limitations (see **Section 4.1**) and can only be considered in a qualitative manner, but provides some comparative information when assessing the subclinical effects of NO_2 exposure.

6.4 Travel frequency

Driving occupations such as taxi drivers and couriers are likely to make multiple trips per day within the tunnel network. As such their exposure to NO_2 will be more frequent.

6.5 Vehicular type

6.5.1 Cars and trucks

Cars and trucks have the ability to wind up their windows and turn on the air flowing through the vehicle cabin space to recirculation. Undertaking these actions has be ability to significantly reduce in-cabin NO_2 concentrations (**Figures 6.1 & 6.2**), with the maximum value recorded being less than 0.2 ppm versus 0.718 ppm for outside vehicle NO_2 concentrations (PEL 2016). In-cabin measurements of cars travelling through Sydney tunnels found that NO_2 concentrations were on average a third to less than one tenth inside the car cabin compared to outside when winding up their windows and turning on air recirculation (PEL 2016). Even without air recirculating through the cabin, NO_2 concentrations were less, reduced by between an average of a quarter to three quarters from the outside in-tunnel concentrations.

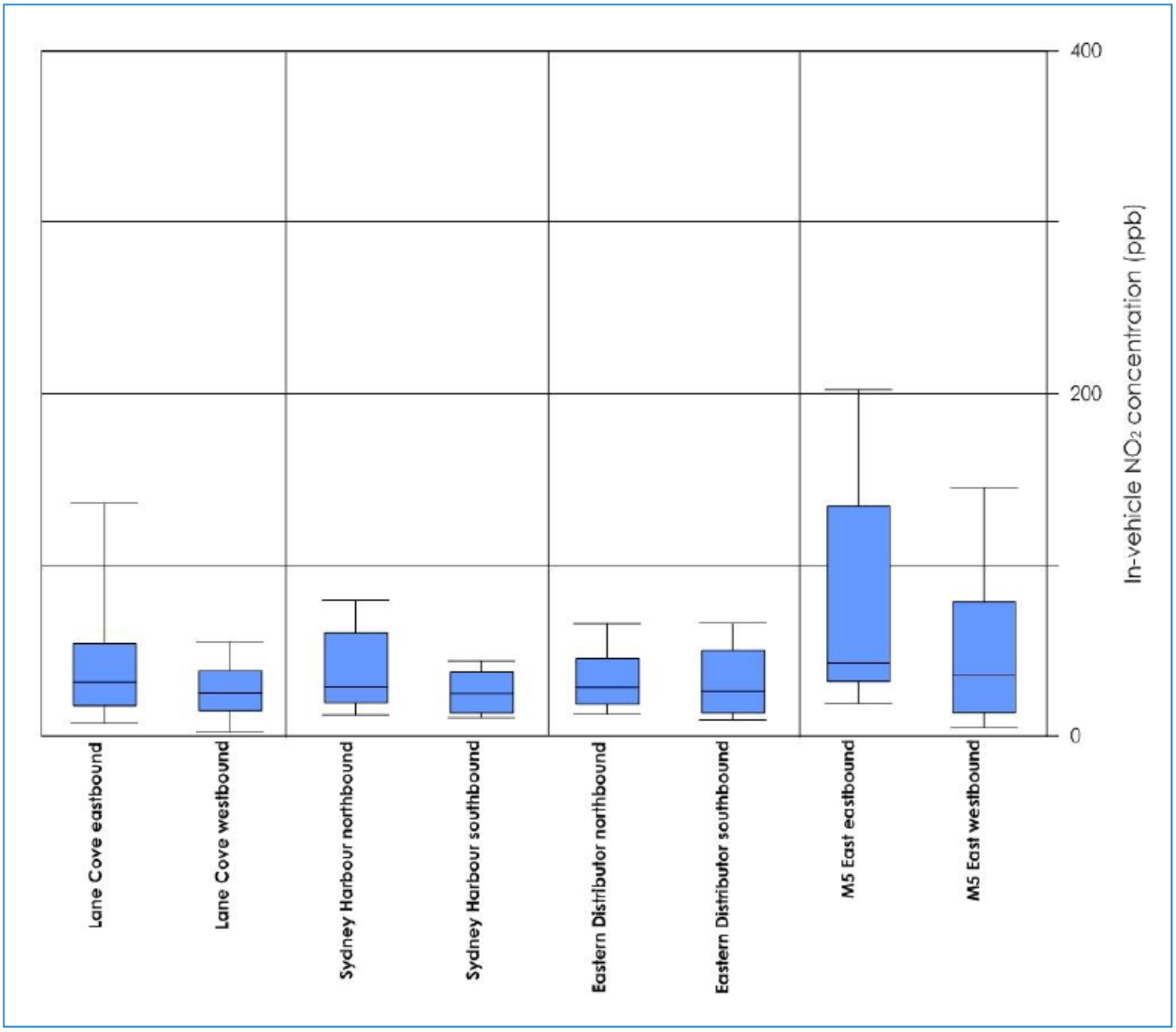


Figure 6.2: In-cabin NO_2 concentrations with recirculation on, averaged by tunnel transit time (taken from (PEL 2016)) (Note: this graph shows a maximum concentration for M5Eastbound at greater than 0.2ppm, however the report documents the maximum exposure as less than 0.2 ppm).

In-cabin surface road NO_2 measurements, where windows were wound up and recirculation was on, were lower than those measured in-tunnel, averaging 0.05 ppm. This concentration increased up to 0.1 ppm when recirculation was off (PEL 2016). Other studies have found average in-cabin NO_2 concentrations between 0.03 to 0.07 ppm, with a maximum range up to 0.55ppm (HEI 2013).

6.5.2 Motorcycles

Motorcyclists do not have the ability to wind up their windows and turn on air recirculation and it is assumed that the outside vehicular NO_2 levels measured (see **Section 6.1**) are the same as found inside their helmet.

6.6 Summary

Current NO_2 in-tunnel exposures are greater than surface road exposures, however the in-tunnel exposure to NO_2 may be significantly reduced to below 0.2 ppm for car and truck drivers if they wind up their windows and place their ventilation on recirculation. This is not the case for motorcycles who do not have this ability.

Driving (exposure) time is important, and reduced driving time can have its own health benefits. These benefits should not be used to justify discounting established adverse health effects from NO_2 exposure, but may be taken into consideration when evaluating the subclinical effects of NO_2 exposure.

Frequent tunnel users such as couriers and taxi drivers, along with motorcyclists, are a particularly vulnerable subpopulation with extended or higher periods of exposure to NO_2.

Section 7. Conclusions

Environmental Risk Sciences Pty Ltd (EnRiskS) has undertaken a review of the epidemiological evidence regarding health impact nitrogen dioxide exposure with respect to informing an in-tunnel nitrogen dioxide guideline for New South Wales. At present the NSW Government requires an in-tunnel nitrogen dioxide guideline value for all new road tunnels of 0.5 ppm as a rolling 15-minute average. The review of experimental epidemiological evidence did not identify any studies that contained clinically relevant health results at nitrogen dioxide concentrations less than or equal to 0.5 ppm. There are however limitations with this evidence, including the ability of the 0.5 ppm value to consider health effects that severe asthmatics and those with significant cardiopulmonary issues may experience.

Currently users of Sydney major road tunnel systems may be exposed to nitrogen dioxide concentrations of up to 0.7 ppm, however for car and truck users these concentrations can be reduced to below 0.2 ppm if they wind up their windows and turn their air conditioning onto recirculation. Therefore, winding up windows and turning air conditioning onto recirculation can reduce the potential 0.5 ppm in-tunnel concentration to below a 0.2 ppm exposure for car or truck users. This reduction to below 0.2 ppm effectively acts as a margin of safety for severe asthmatics and those with significant cardiopulmonary issues, provided they follow this advice. It is noted however that motorcyclists do not have this ability to wind up their windows and turn their air conditioning onto recirculation. Further frequent tunnel users such as taxi drivers and couriers may be exposed multiple times during the day. While tunnel users may be exposed to nitrogen dioxide concentrations of up to 0.7 ppm, the average nitrogen dioxide concentration in Sydney's major road tunnels was measured at 0.27 ppm.

This review examined an in-tunnel approach undertaken in Sweden. This approach involved drawing on an epidemiological observational study to determine excess mortality risk from exposure to in-tunnel oxides of nitrogen. The approach did not set a guideline value, rather provided the estimated impact of differing in-tunnel levels so these impacts can be considered within the broader decision-making process. It also compared the mortality risk of tunnel users against the mortality benefit to the surrounding community.

EnRiskS is unaware of any major health or environmental agency that have set a 1- hour nitrogen dioxide (or oxides of nitrogen) exposure guideline based solely on observational epidemiological studies. At present, evidence from these studies are considered in a semi quantitative manner, and included in the policy mix with experimental epidemiological data setting the guideline starting point.

The merit of the Swedish approach lies in its ability to consider evidence beyond experimental studies and for this evidence to be considered semi quantitatively within the broader decision-making process. If a similar approach was undertaken in Australia it is recommended that Australian relevant observational studies and more robust health endpoints be used. However, owing to the limitations of observational studies and lack of agreed approach in the way the data from these studies may be translated into a 1-hour guideline, at this time it is not recommended that observational data be used to solely develop an in-tunnel guideline value.

It is acknowledged that in setting the current NSW in-tunnel nitrogen dioxide guideline value health evidence was considered in the broader policy framework. The health evidence was drawn from the report *Review of experimental studies of exposures to nitrogen dioxide* which considered nitrogen

dioxide exposures up to 30 minutes. This review supports and clarifies the conclusions drawn in the *Review of experimental studies of exposures to nitrogen dioxide* report, even with an extended exposure period from 30 to 60 minutes.

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Appendix A – Key terms used in NO_2 experimental studies

Section 3.1.2 highlights the four broad outcome measures used in in NO_2 experimental studies. The following provides an explanation of the specific measures within these four categories that can be used in the explanation of **Appendix B**.

2,3-diphosphoglycerate **(2,3-BPG)**	Present in human red blood cells- increased levels forces oxygen to be released by the blood haemoglobin (ie. Increased levels increase oxygen supply to tissues)
Acetylcholinesterase **(AChE)**	Enzyme that destroys acetylcholine. Acetylcholine is a neurotransmitter (a chemical that is released by nerve cells to send signals to other cells)
Activator protein – 1 **(AP-1)**	Factor that regulates gene expression. Involved in cell growth, proliferation and planned cell death. It is involved in skin regeneration and also breast cancer cell growth
Aerosol bolus test **(Abt)**	The inhalation of aerosol particles with the specific aim of monitoring inhaled and exhaled aerosol patterns. The shapes of these patterns are used to assess lung function
Airways resistance **(Raw)**	A measure of the resistance of the respiratory tract to airflow during inhalation and exhalation
Albumin **(Alb)**	A protein found in plasma that stops fluid from leaking out of blood vessels. It also transports hormones, vitamins and medications throughout the body.
Alpha-1 antitrypsin **(A1AT)**	Protein that breaks down enzymes released by neutrophils at sites of inflammation. Deficiency of this can lead to chronic obstructive pulmonary disease (emphysema)
Alpha – Tocopherol **(VitE)**	The most active form of vitamin E and is an antioxidant. (Disables the production of oxygen free radicals in tissues)
Alveolar Macrophages **(AM)**	Macrophages found in alveolar fluid
Alveolar permeability **(Ap)**	Ability for gas exchange across the alveoli
Angiotension-converting enzyme **(ACE)** activity	It is part of the renin-angiotensin system that controls blood pressure. It is an enzyme (mainly found in the lung) that converts angiotensin I to angiotensin II. Angiotensin II constricts blood vessels, increasing blood pressure.
Ascorbic acid **(AsA)**	Vitamin C. Acts as an antioxidant.
Basophils **(B)**	A type of white blood cell (the least common type). They are involved in many different inflammatory reactions, particularly found in tissues where an allergic reaction is occurring.
Blood count **(Blc)**	This includes levels of red blood cells, white blood cells (total count and breakdown of neutrophils, basophils, eosinophils, monocytes and lymphocytes) and platelets.
Blood pressure **(BP)**	Blood pressure- normal 120/80
Cardiac output **(CO)**	Amount of blood pumped out by the heart per minute
Chemokine ligand 1 **(CXCL1)**	Protein/chemical that attracts neutrophils
Chemokine ligand 5 **(CCL5)**	Protein that guides migration of white blood cells (especially basophils and eosinophils) and mast cells into a site of inflammation
Coagulation factors **(CoF)**	Enzymes that are an integral part of the clotting cascade to form a blood clot
Eosinophils **(E)**	A type of white blood cell. They are attracted to tissues (by basophils and mast cells) where allergic reactions occur.
Eosinophilic cationic protein **(ECP)**	A protein found in eosinophils and is secreted during inflammation when eosinophils are activated. Measuring levels of ECP in the serum can be a marker of asthma severity.
Eotaxin **(Eot)**	Chemical that specifically attracts eosinophils- produced in asthmatic lungs
Epithelial cells **(Ep)**	Line the cavities of organs and blood vessels in the body and are involved in secretion of chemicals, transport, protection and absorption.
Exhaled nitric oxide **(eNO)**	A measure of airway inflammation. Nitric oxide is produced by certain cell types in an inflammatory response
Factor VII **(F-VII)**	Coagulation factor that initiates clotting when there is an injury to a blood vessel
Fibrinogen **(Fi)**	Protein essential for blood clot formation
Fibronectin **(Fib)**	Involved in wound healing

Forced expiratory flow **(FEF)**	The flow (or speed) of air coming out of the lung during the middle portion of forced expiration, generally defined by what fraction remains of the FVC. It can be at one time interval, say 50% (FEF_{50}) or over a period, say from 25% to 75 % (FEF_{25-75})
Forced expiratory volume in 1 second **(FEV_1)**	The volume of air that can be exhaled in one second after full inhalation
Functional residual capacity **(FRC)**	The volume of air in the lungs at the end of passive expiration
Forced vital capacity **(FVC)**	The volume of air that can be exhaled after full inhalation
Glucose-6- phosphate dehydrogenase **(G6PD)**	Enzyme in a metabolic pathway in red blood cells that helps to protect them from being damaged during any states of stress, like infection or chemical exposure.
Glutathione **(GSH)**	Important antioxidant that mops up free radicals that can cause damage to red blood cells
Glutathione disulphide **(GSSG)**	Derived from two glutathione molecules. Involved in modulation of the receptors in nerves. Ratio of GSH:GSSG is an important indicator of health of cells.
Glutathione peroxidase **(GPx)**	Enzyme that is involved in protecting an organism from damage from free radicals
Glutathione reductase **(GR)**	Enzyme that has a role in protecting red blood cells from damage when exposed to certain chemicals or drugs. Deficiency can result in destruction of red blood cells
Granuloctye macrophage colony stimulating factor **(GM-CSF)**	White blood cell growth factor. Stimulates stem cells to produce granulocytes (types of white blood cells including neutrophils, basophils, eosinophils) and monocytes.
Haeomatocrit **(Hct)**	The volume percentage of red blood cells in blood- normal 45% in men, 40% in women
Haemoglobin **(Hb)**	Found in red blood cells. Carries oxygen from the lung to the tissues.
Heart rate **(HR)**	The number of contractions of the heart in one minute
Heart rate variability **(HRv)**	Normal variation in the time interval between heartbeats
High density lipoprotein cholesterol **(HDL-chol)**	Lipoproteins transport fat molecules around the body. They are high density because they have the highest proportion of protein to lipids. Thought to be protective against atherosclerosis (build up of fat in arteries)
Hyaluronic acid **(HLA)**	Major component of joint cartilage and skin. Also contributes to movement and activation of certain cells, including lymphocytes, involved in the immune response.
Immunoglobulin-A **(IgA)**	Antibody (structure that recognises a foreign particle) found in mucous secretions such as in saliva, trachea, lung and genitourinary tract.
Immunoglobulin-E **(IgE)**	Antibody found on basophils and mast cells. Associated with allergic diseases and parasitic infections.
Immunoglobulin-G **(IgG)**	Main antibody found in human serum. It is the major antibody involved in controlling infection through the secondary immune response.
Immunoglobulin-G4 **(IgG4)**	Subclass of immunoglobulin G, elevated in IgG4 related disease (rare chronic inflammatory condition).
Immunoglobulin-M **(IgM)**	Largest antibody and is the first to appear in response to a foreign particle or infection
Interferon gamma **(IFNɣ)**	Cytokine (substance secreted by certain cells of the immune system that have an effect on other cells) critical for immunity against mainly viruses and protozoa.
Interleukin 1 **(IL-1)**	Cytokine that generates a fever and inflammation during infection
Interleukin 1β **(IL-1β)**	Part of the interleukin 1 family- may contribute to inflammatory pain
Interleukin 4 **(IL-4)**	Cytokine that regulates the immune response. Higher levels are seen in allergies.
Interleukin 5 **(IL-5)**	Cytokine that attracts eosinophils.
Interleukin 6 **(IL-6)**	Cytokine important in generating a fever during infection/inflammation. Also involved in mediating inflammation.
Interleukin 8 **(IL-8)**	Chemical that causes neutrophils to migrate to a site of infection and also activates the neutrophils to kill the bacteria.
Interleukin 10 **(IL-10)**	Anti-inflammatory cytokine
Interleukin 12 **(IL-12)**	Cytokine that stimulates T cells. (T cells are important in defence against intracellular organisms)
Interleukin 13 **(IL-13)**	Cytokine secreted by T cells that are involved in allergic responses

Interleukin 12p70 **(IL-12p70)**	The combination of IL-12 subunits p35 and p40. Possibly related to immunity to intracellular bacteria like mycobacteria.
Jun N-terminal kinase **(JNK)**	
Lactate Dehydrogenase **(LDH)**	Enzyme found in body tissues and is released during tissue damage.
Leucocyte coping capacity **(Lcc)**	A new/novel method for testing stress response in vertebrates
Leucocytes **(Le)**	White blood cells- cells of the immune system involved in protecting the body against infection and foreign particles
Lymphocytes **(L)**	Type of white blood cell found in lymph- includes B, T and Natural Killer (NK) cells
Lymphocyte subpopulations **(LSP)**	Subsets of B, T and NK cells, including CD3, CD4, CD8, CD19, CD25, CD16+56+, CD44, CD45RO, CD 69, CD 62.
Lysozyme positive macrophages **(LPM)**	Lysozyme is an enzyme found in the granules of macrophages. It causes the integrity of bacterial cell walls to be compromised, resulting in the rupture/death of the bacteria.
Mac-1 expression on granulocytes **(MCG)**	Mac-1 is a receptor found on the membrane of granulocytes. It allows attachment of the cell to the wall of vessels. It can also enable granulocytes to bind microbes.
Mast cells **(MC)**	A type of white blood cell that play a role in allergy and anaphylaxis
Mast cell tryptase **(MCT)**	Chemical found in granules of mast cells. Serum concentration is increased in anaphylaxis and allergic conditions.
Maximal (mid) expiratory flow **(MMEF or MEF or MEFR or MMFR)**	The peak of expiratory flow as taken from the flow volume curve. It should in theory be identical to the PEF.
Mean transit time **(MTT)**	Is the area under the flow-volume curve divided by the forced vital capacity
Macrophages **(M)**	Type of white blood cell that digest foreign substances, dead cells and microbes. Also important for the body's immune response
Macrophage phagocytosis **(Mp)**	Ingests and destroys foreign particles, including dead cells and cellular debris. Important role in chronic inflammation
Malondialdehyde **(Mal)**	Is a marker of oxidative stress (see Thiobarbituric acid reactive substance (TBARS). Oxidative stress results in an excess of reactive oxygen species/free radicals. This can damage all components of cells.
Methyl—histamine **(M-his)**	Major metabolite of histamine, produced by mast cells. Urinary levels can be measured to monitor conditions with increased mast cell activity such as mastocytosis.
Monoclonal endothelial antibody **(Mea)**	Antibody to endothelial cells
Monocytes **(Mo)**	Type of white blood cell that differentiates into macrophages and dendritic cells
Monocyte Chemoattractant Protein-1 **(MCP-1)**	Chemokine that regulates the movement of monocytes/macrophages
Neuroendocrine cell **(NE)**	Cells that receive input from nerves (via neurotransmitters) and release a hormone (signalling molecule) in response to this input. These cells link the nervous and endocrine systems in the body.
Neutrophils **(N)**	The most abundant type of white blood cell (granulocyte). First inflammatory cells to migrate towards sites of inflammation/infection, particularly bacterial infection.
Myeloperoxidase **(MPO)**	Enzyme found in neutrophil granules, which assists in killing bacteria and other foreign molecules.
Nuclear factor – κB **(NF-κB)**	Protein found in almost all cells. Plays a role in regulating the immune systems response to infection.
Oxidised glutathione **(OGSH)**	Same as glutathione disulphide (GSSG)
Oxygen uptake **(O2)**	Oxygen consumption per kilogram of body weight
p38 Kinase **(p38)**	Involved in the cell differentiation, cell death and regulation/recycling of unnecessary cell components. It is a group of kinases who function in response to stress stimuli, such as cytokines.
Provocative dose **(PD XXX)**	The dose of chemical or allergen required to reduce a lung function in question by X%. For example, $PD_{20}FEV_1$ is the dose required to drop FEV_1 by 20%.

Peak expiratory flow **(PEF or PEFR)**	The maximal flow (or speed) achieved during the maximally forced expiration initiated at full inhalation
Prostaglandin E2 **(PGE2)**	Lipid compounds found in most tissues and cells. It directly dilates vessels and relaxes smooth muscle. Often used in obstetrics.
Phagocytosis potential **(Pp)**	The ability of the neutrophils or macrophages to phagocytose foreign particles or microbes
Plasminogen activator inhibitor-1 **(PAI-1)**	Protein that inhibits the breakdown of blood clots.
Platelet **(Pl)**	A component of blood that stops bleeding when there is injury to a blood vessel
Polymorphonuclear cells **(PMN)**	Neutrophil
Red blood cell count **(Rbc)**	Number of red blood cells in the blood.
Respiratory frequency **(Rf)**	Respiratory rate- number of breaths per minute
Respiratory resistance **(Rt)**	A measure of the resistance of the respiratory tract.
Residual volume **(RV)**	The volume of air remaining in the lungs after a maximal exhalation
Selenium **(Se)**	Trace element nutrient that functions as a cofactor for the reduction of enzymes such as glutathione peroxidases.
Skin conductance **(SkC)**	Continuous variation in the electrical characeristics of the skin. Measure of emotional and sympathetic responses- eg. Increased sweating increases skin conductance
Soluble intercellular adhesion molecule **(sICAM)**	Found on the surface of cells involved in inflammation and immune response.
Soluble intercellular adhesion molecule 1 **(sICAM-1)**	Present on the surface of leucocytes and lining on vessels. When stimulated by cytokines (IL-1 and TNF), the concentrations increase. When activated, the leucocyte attaches to the vessel wall and moves into tissues.
Specific airway conductance **(sGaw)**	This is the inverse to sRaw and measures the conductance of the respiratory tract to airflow during inhalation and exhalation.
Specific airway resistance **(sRaw)**	A measure of the resistance of the respiratory tract to airflow during inhalation and exhalation, that accounts for the changing nature of airway resistance within the lung
Superoxide release **(SpR)**	Superoxide is found in neutrophils and released when they come in contact with bacteria
Thiobarbituric acid reactive substance **(TBARS)**	By product of degradation of fats. Can measure TBARS to measure the damage caused by oxidative stress. Malondialdehyde is one of the substances measured.
Tissue plasminogen activator **(tPA)**	Protein involved in the breakdown of blood clots.
Total cell **(TC)**	Total white cell count in the blood
Total cholesterol **(Tchol)**	Total amount of cholesterol in the blood including LDL, HDL and triglycerides
Total lung capacity **(TLC)**	Is the maximum volume of air present in the lungs
Total protein **(Tp)**	Total amount of protein in the serum. Made up of albumin and globulin.
T-lymphocytes **(TL)**	Type of white cell that plays a key role in the immune response in the body. There are a number of different subtypes with different functions.
Tidal volume **(TV)**	The volume or amount of air inhaled or exhaled normally at rest
Tryptase **(Try)**	Secreted by granules in mast cells. Elevated levels are seen in anaphylaxis.
Tumour necrosis factor - α **(TNF-α)**	Cytokine involved in systemic inflammation. Produced mainly by macrophages. Can also be produced by other cell types including mast cells.
Tyrosine (nuclear phosphorylated) **(Tys)**	Tyrosine is an amino acid, which is a building block for cells to produce proteins. Phosphorylated tyrosine is important for regulation of enzyme activity.
Uric acid **(UA)**	Produced from the natural breakdown of cells and from foods. Passes out of the body via the kidney, and is normal component of urine. Elevated levels in the blood can lead to gout.
Vascular cell adhesion molecule 1 **(VCAM-1)**	Enables the adhesion of lymphocytes, eosinophils, basophils and monocytes to the vessel wall

Ventilation rate **(VR)**	Number of breaths per minute
Virus inactivation **(VirA)**	Viruses are unable to infect their host
Vital Capacity **(VC)**	The volume of air breathed out after the deepest inhalation
Volume of Thoracic gas **(TGV or Vtg)**	Is the volume of air in the lungs after expiration, typically equal to FRC, but measured by another technique.
Von Willebrand factor **(vWF)**	Blood protein involved in clotting
White cell **(Wc)**	Leucocytes- cells of the immune system involved in protecting the body against infection and foreign particles

Appendix B - Graphed and tabulated experimental studies of NO_2 exposure

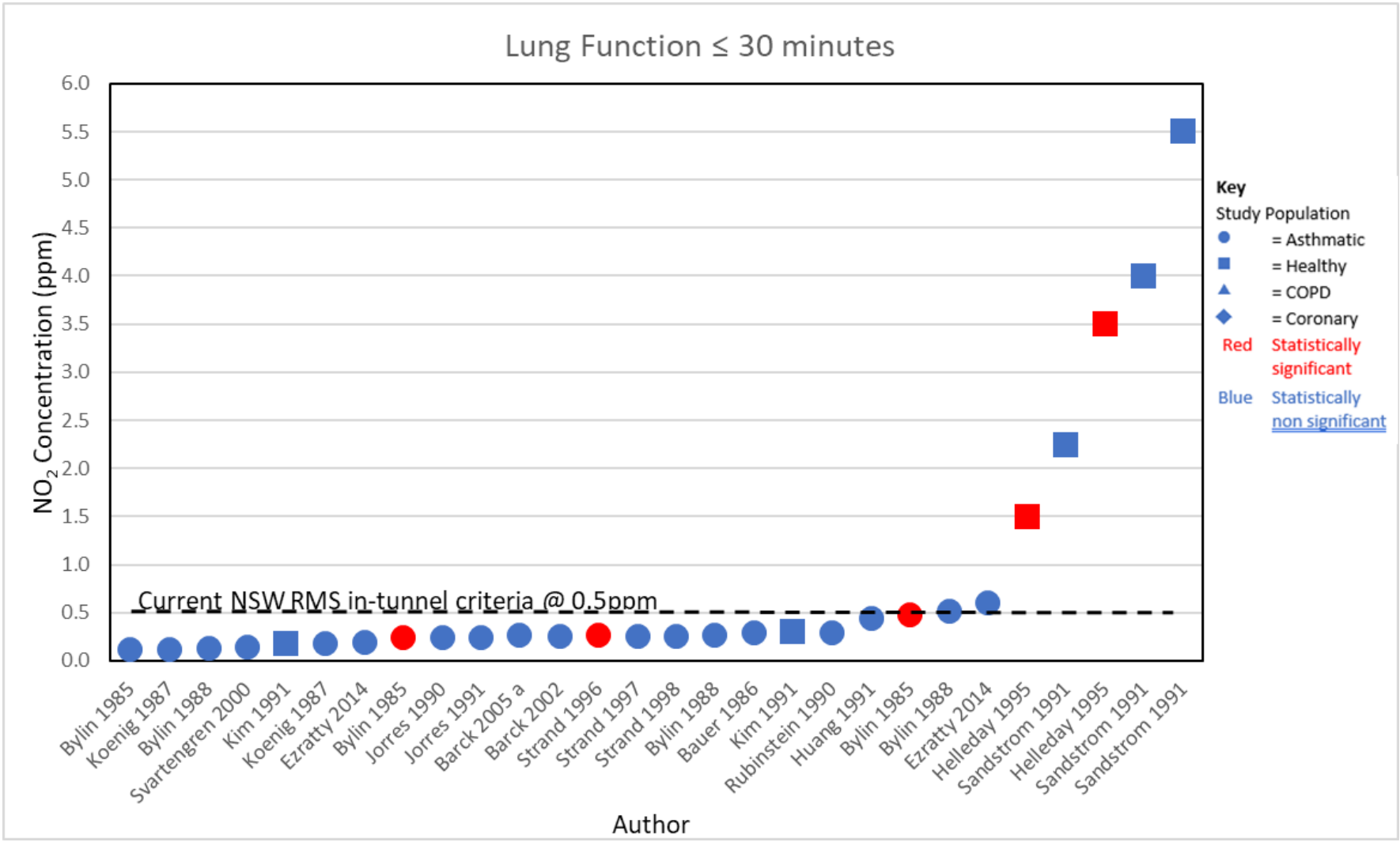

Figure B.1 Graph of studies examining exposures to NO_2 of ≤ 30 minutes and lung function, ranked by NO_2 concentration

Table B.1 Studies examining exposures to NO_2 of ≤ 30 minutes and lung function, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Bylin 1985	Non-randomised cross over	A, H	17-45(A), 20-36(H)	8(A), 8(H)	0.12$	N	20	N	N	sRaw, TGV	
Koenig 1987	Randomised Cross over	A, H	11 to 19	10(A), 10(H)	0.12	N	30	Y	N/Y	FEF50, FEF75, FEV1, FVC	
Bylin 1988	Randomised Cross over	A, N, ES	17-56	20	0.14$	N	30	N	N	sRaw, TGV	
Svartengren 2000	Non-randomised cross over	A, N, ES	19-55	20	0.15$	Y	30	N	N	sRaw, TGV, FEV1	
Kim 1991	Randomised Cross over	H	18-23	9	0.18	N	30	N	Y	FEV1, PEF, RT, FEF50	
Koenig 1987	Randomised Cross over	A, H	11 to 19	10(A), 11(H)	0.18	N	30	Y	Y	FEF50, FEF75, FEV1, FVC	
Ezratty 2014	Randomised Cross over	A,N	20-69	19	0.2	N	30	Y	N	FEV1, PEF	
Bylin 1985	Non-randomised cross over	A, H	17-45(A), 20-36(H)	8(A), 8(H)	0.24$	N	20	N	N	sRaw, TGV	Healthy only - ↑sRaw at 20 mins compared to 10 mins
Jorres 1990	Randomised Cross over	A,N	34(mean)	14	0.25	N	30	N	N	sRaw	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Jorres 1991	Randomised Cross over	A, N, S	17 -44	11	0.25	N	30	N	Y	sRaw	
Barck 2005 a	Randomised Cross over	A, NS, ES	23-48	18	0.26$	N	15	Y	N	FEV1, sRaw, TGV	
Barck 2002	Randomised Cross over	A,NS	23-39	13	0.26$	N	30	N	N	sRaw, TGV, FEV1	
Strand 1996	Randomised Cross over	A, N, ES	20-48	19	0.26	N	30	N	Y	sRaw, TGV	TGV significantly lower 20 minutes after exposure
Strand 1997	Randomised Cross over	A, N, ES	18-50	18	0.26$	N	30	N	N	sRaw, Vtg, FEV1, PEF, FVC, FEF.	
Strand 1998	Randomised Cross over	A, N, ES	21-52	16	0.26$	N	30	Y	N	sRaw, TGV, FEV1	
Bylin 1988	Randomised Cross over	A, N, ES	17-56	20	0.27$	N	30	N	N	sRaw, TGV	
Bauer 1986	Randomised Cross over	A,N	20-45	15	0.3	N	30	N	Y	FEV1, sGaw	
Kim 1991	Randomised Cross over	H	18-23	9	0.3	N	30	N	Y	FEV1, PEF, RT, FEF50	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Rubinstein 1990	Randomised Cross over	A, N	23-34	9	0.3	N	30	N	Y	sRaw, FEV1, FVC	
Huang 1991	Experimental exposure	A,N	10 to 14	6	0.45-0.5 NOx	Y	5	N	N	FVC, FEV1, Raw, MMF, PEFR, FEF	
Bylin 1985	Non-randomised cross over	A, H	17-45(A), 20-36(H)	8(A), 8(H)	0.48[$]	N	20	N	N	sRaw, TGV	Healthy only - ↓sRaw during and post exposure compared to pre exposure
Bylin 1988	Randomised Cross over	A, N, ES	17-56	20	0.52[$]	N	30	N	N	sRaw, TGV	
Ezratty 2014	Randomised Cross over	A,N	20-69	19	0.6	N	30	Y	N	FEV1, PEF	
Helleday 1995	Experimental exposure	H,N	23-30	8	1.5	N	20	N	N	Mucociliary activity	↓ mucociliary activity 45mins after exposure
Sandstrom 1991	Experimental exposure	H, N	22-32	18	2.25	N	20	N	Y	FEV1, FVC	
Helleday 1995	Experimental exposure	H,N	23-30	8	3.5	N	20	N	N	Mucociliary activity	↓ mucociliary activity 45mins after exposure

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Sandstrom 1991	Experimental exposure	H, N	22-32	18	4	N	20	N	Y	FEV1, FVC	
Sandstrom 1991	Experimental exposure	H, N	22-32	18	5.5	N	20	N	Y	FEV1, FVC	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease
$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

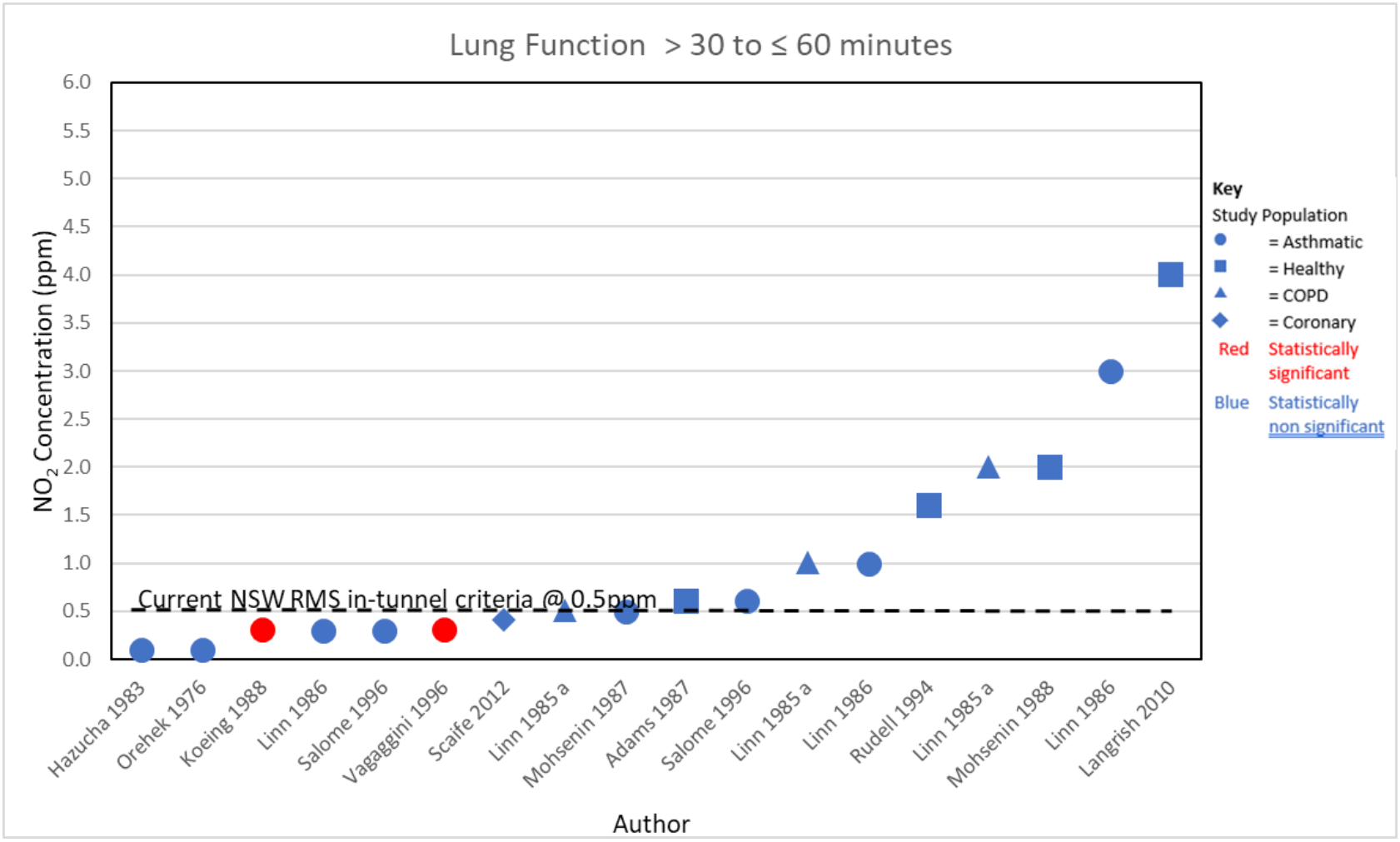

Figure B.2 Graph of studies examining exposures to NO_2 of >30 to ≤ 60 minutes and lung function, ranked by NO_2 concentration

Table B.2 Studies examining exposures to NO_2 of >30 to ≤ 60 minutes and lung function, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Hazucha 1983	Randomised Cross over	A, H, N	18-35	15(A), 15 (N)	0.1	N	60	N	N	sRaw	
Orehek 1976	Randomised Cross over	A	15-44	20	0.1	N	60	N	N	sRaw	
Koeing 1988	Randomised Cross over	A, H	12 to 17	12(A), 12(H)	0.3	N	60	N	Y	FEF50, FEF75, FEV1, FVC	Asthmatics only - ↓ FVC at 3mins, gone by 8 mins
Linn 1986	Randomised Cross over	A	20-34	21	0.3	N	60	N	Y	sRaw, FVC, FEV1, MMFR	
Salome 1996	Randomised Cross over	A	7 to 65	20	0.3	N	60	N	N	FEV1, FVC, PEF	
Vagaggini 1996	Randomised Cross over	A, H, COPD	29 (A), 34 (H), 58 (COPD)	7 (A), 8 (H), 7 (COPD)	0.3	N	60	N	Y	FEV1	COPD only - ↓ FEV1
Scaife 2012	Randomised Cross over	Coronary heart disease with reduced LV systolic function, N	56-76	18	0.4	N	60	N	N	FEV1, FVC, O2sat	
Linn 1985 a	Randomised Cross over	COPD, N, ES, S	50-69	22	0.5	N	60	N	Y	FVC, FEV1, FEV2, PEFR,	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
										MMFR, Vtg, sRaw	
Mohsenin 1987	Randomised Cross over	A	22-40	10	0.5	N	60	N	N	FVC, FEV1, FEF40, FRC, sGaw	
Adams 1987	Randomised Cross over	H, N	18-30	40	0.6	N	60	N	Y	FVC, FEV1, FEF25-75, sRaw	
Salome 1996	Randomised Cross over	A	7 to 65	20	0.6	N	60	N	N	FEV1, FVC, PEF	
Linn 1985 a	Randomised Cross over	COPD, N, ES, S	50-69	22	1	N	60	N	Y	FVC, FEV1, FEV2, PEFR, MMFR, Vtg, sRaw	
Linn 1986	Randomised Cross over	A	20-34	21	1	N	60	N	Y	sRaw, FVC, FEV1, MMFR	
Rudell 1994	Experimental exposure	H,N	19-27	8	1.6	Y	60	N	Y	FVC, FEV1, FEF25-75, MTT	
Linn 1985 a	Randomised Cross over	COPD, N, ES, S	50-69	22	2	N	60	N	Y	FVC, FEV1, FEV2,	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
										PEFR, MMFR, Vtg, sRaw	
Mohsenin 1988	Randomised Cross over	H, N	18-33	18	2	N	60	N	N	FVC, FEV1, FEF40, FRC, sGaw	
Linn 1986	Randomised Cross over	A	20-34	21	3	N	60	N	Y	sRaw, FVC, FEV1, MMFR	
Langrish 2010	Randomised Cross over	H,N	22-28	10	4	N	60	N	Y	FVC, FEV1, VC, eNO	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

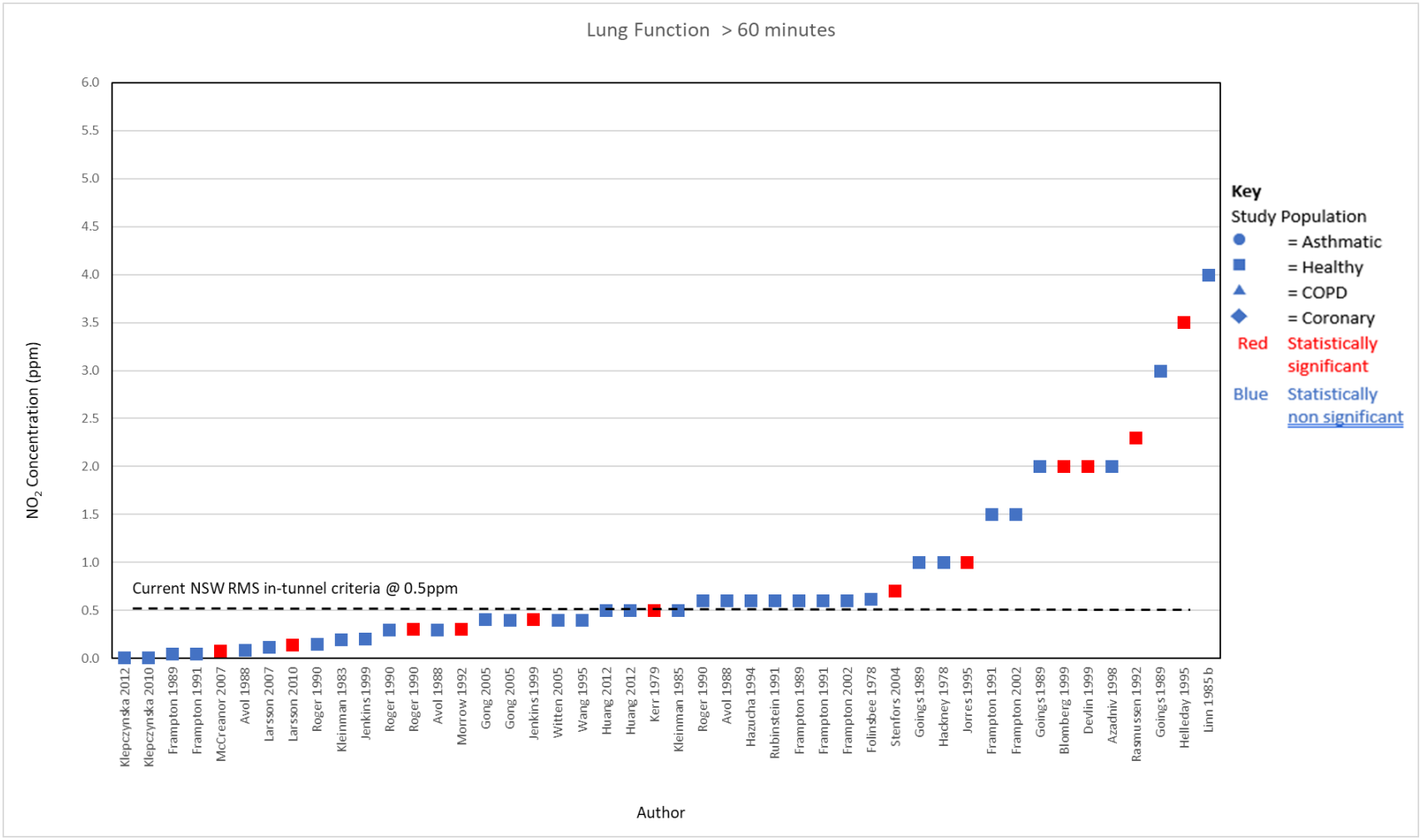

Figure B.3 Graph of studies examining exposures to NO_2 of > 60 minutes and lung function, ranked by NO_2 concentration

Table B.3 Studies examining exposures to NO_2 of > 60 minutes and lung function, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Klepczynska 2012	Randomised Cross over	A	18-52	16	0.01[$]	Y	120	N	Y	VC, FEV1, FVC, PEF, exhaled NO	
Klepczynska 2010	Randomised Cross over	H	18-46	20	0.012[$]	Y	120	N	Y	VC, FEV1, FVC, PEF, eNO	
Frampton 1989	Randomised Cross over	H, N	19-37	15	0.05 with three 15min peaks of 2.0	N	180	N	Y	FVC, FEV1, sGaw	
Frampton 1991	Randomised Cross over	H, N	19-37	15	0.05 with three 15min peaks of 2.0	N	180	N	Y	FVC, FEV1, sGaw, PEFR, MEFR	
McCreanor 2007	Randomised Cross over	A,N	19-55	60	0.075[$]	Y	120	N	Y	FEV1, FVC, FEF, PEF, eNO	↓FEV1, FVC
Avol 1988		A		36	0.086	Y	120	N			
Larsson 2007	Randomised Cross over	H,N	19-59	16	0.12[$]	Y	120	N	Y	FEV1, FVC	
Larsson 2010	Randomised Cross over	A, N	18-55	14	0.14[$]	Y	120	N	Y	VC, FEV1, PEF, FVC, exhaled NO	↓ PEF
Roger 1990	Randomised Cross over	A, N	19-35	21	0.15	N	75	N	Y	sRaw, FVC, FEV1	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Kleinman 1983	Randomised Cross over	A	31±11	31	0.2	N	120	N	Y	Rt, FEV1, FVC	
Jenkins 1999	Randomised Cross over	A,NS	22-41	11	0.2	N	360	N	Y	FEV1, FEC ,FVC	
Roger 1990	Randomised Cross over	A, N	19-35	21	0.3	N	75	N	Y	sRaw, FVC, FEV1	
Roger 1990	Randomised Cross over	A, N	19-35	13	0.3	N	110	N	Y	sRaw, FVC, FEV1	↓ sRaw, FVC , FEV1
Avol 1988		A		59	0.3	N	120	N			
Morrow 1992	Randomised Cross over	H, N, COPD, S	49-69(H), 47-70 (COPD)	20(H), 20(COPD)	0.3	N	240	N	Y	FEV1, FVC	COPD only - ↓ FVC, FEV1
Gong 2005	Semi randomised cross over	H, COPD	mean 68(H), 72(COPD)	6(H), 18(COPD)	0.4	N	120	N	Y	MMEF, FEV1, FVC	
Gong 2005	Semi randomised cross over	H, COPD	mean 68(H), 72(COPD)	6(H), 18(COPD)	0.4	Y	120	N	Y	MMEF, FEV1, FVC	
Jenkins 1999	Randomised Cross over	A,NS	22-41	10	0.4	N	180	N	Y	FEV1, FEC ,FVC	↓ FEV1 with 0.2ppm ozone co-exposure
Witten 2005	Randomised Cross over	A, N, allergy	21-48	15	0.4	N	180	N	Y	FEV1	
Wang 1995	Randomised Cross over	N, allergic rhinitis	18-55	16 (8 had allergen challenge)	0.4	N	360	N	N	Nasal airway resistance	
Huang 2012	Semi randomised cross over	H,N	20-36	23	0.5	N	120	N	Y	FVC, FEV1, FEF25-75	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Huang 2012	Semi randomised cross over	H,N	20-36	23	0.5	Y	120	N	Y	FVC, FEV1, FEF25-75	
Kerr 1979	Experimental exposure	A, H, chronic bronchitis (CB), N, S	22-63 (A,CB) 19-50 (H)	20(A,CB), 10 (H)	0.5	N	120	N	Y	FVC, FEV1, Vtg, ERV, sGaw, TLC, FRC, RV.	Asthmatics and chronic bronchitis only - ↑ TLC, FRC, RV
Kleinman 1985	Randomised Cross over	H	18-55	20	0.5	N	135	N	Y	FVC, FEV1, FEV2, PEFR, plus others	
Roger 1990	Randomised Cross over	A, N	19-35	21	0.6	N	75	N	Y	sRaw, FVC, FEV1	
Avol 1988		A		59	0.6	N	120	N			
Hazucha 1994	Randomised Cross over	H,N	18-35	21	0.6	N	120	N	Y	FEV1, sRaw	
Rubinstein 1991	Randomised Cross over	H, N	18-45	5	0.6	N	120	Y	Y	sRaw, FEV1, FVC	
Frampton 1989	Randomised Cross over	H, N	24-37	9	0.6	N	180	N	Y	FVC, FEV1, sGaw	
Frampton 1991	Randomised Cross over	H, N	24-37	9	0.6	N	180	N	Y	FVC, FEV1, sGaw, PEFR, MEFR	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Frampton 2002	Randomised Cross over	H,N	18-40	21	0.6	N	180	N	Y	FVC, FEV1, sGaw	
Folinsbee 1978		H, N	20-25	15	0.62	N	120	N	Y	FEV1, FVC, FEF, TGV, Raw	
Stenfors 2004	Randomised Cross over	A, H, N	22-52(A), 19-42(H)	15(A), 25(N)	0.7	Y	120	N	Y	FEV1, FVC, sRaw	↑sRaw
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	1	N	120	N	N	FEV1	
Hackney 1978	Non-randomised cross over	H, N, ES, S	23-48	16	1	N	140	N	Y	FVC, PEF, FEV1, sGaw	
Jorres 1995	Randomised Cross over	A, H	21-37 (A)	12(A), 8 (H)	1	N	180	N	Y	FEV1	Asthmatics only - ↓ FEV1
Frampton 1991	Randomised Cross over	H, N	19-37	15	1.5	N	180	N	Y	FVC, FEV1, sGaw, PEFR, MEFR	
Frampton 2002	Randomised Cross over	H,N	18-40	21	1.5	N	180	N	Y	FVC, FEV1, sGaw	
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	2	N	120	N	N	FEV1	
Blomberg 1999	Non-randomised cross over	H, N	21-32	12	2	N	240	Y	Y	FEV1, FVC	↓ FEV1 ,FVC
Devlin 1999	Randomised Cross over	H, N	18-35	8	2	N	240	N	Y	FEV1, sRaw, Abt	↓Abt

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Azadniv 1998	Randomised Cross over	H, N	22-35	12	2	N	360	N	Y	sRaw, FEV1	
Rasmussen 1992	Randomised Cross over	H,N	22-66	14	2.3	N	300	N	N	FEV1, FVC, TLC	↑ FEV1, FVC
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	3	N	120	N	N	FEV1	
Helleday 1995	Experimental exposure	H,N	23-30	8	3.5	N	240	N	N	Mucociliary activity	↑ mucociliary activity 24 hours after exposure
Linn 1985 b	Randomised Cross over	A, H, NS	18-34(A), 20-36(H)	23(A), 25(H)	4	N	75	N	Y	sRaw	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

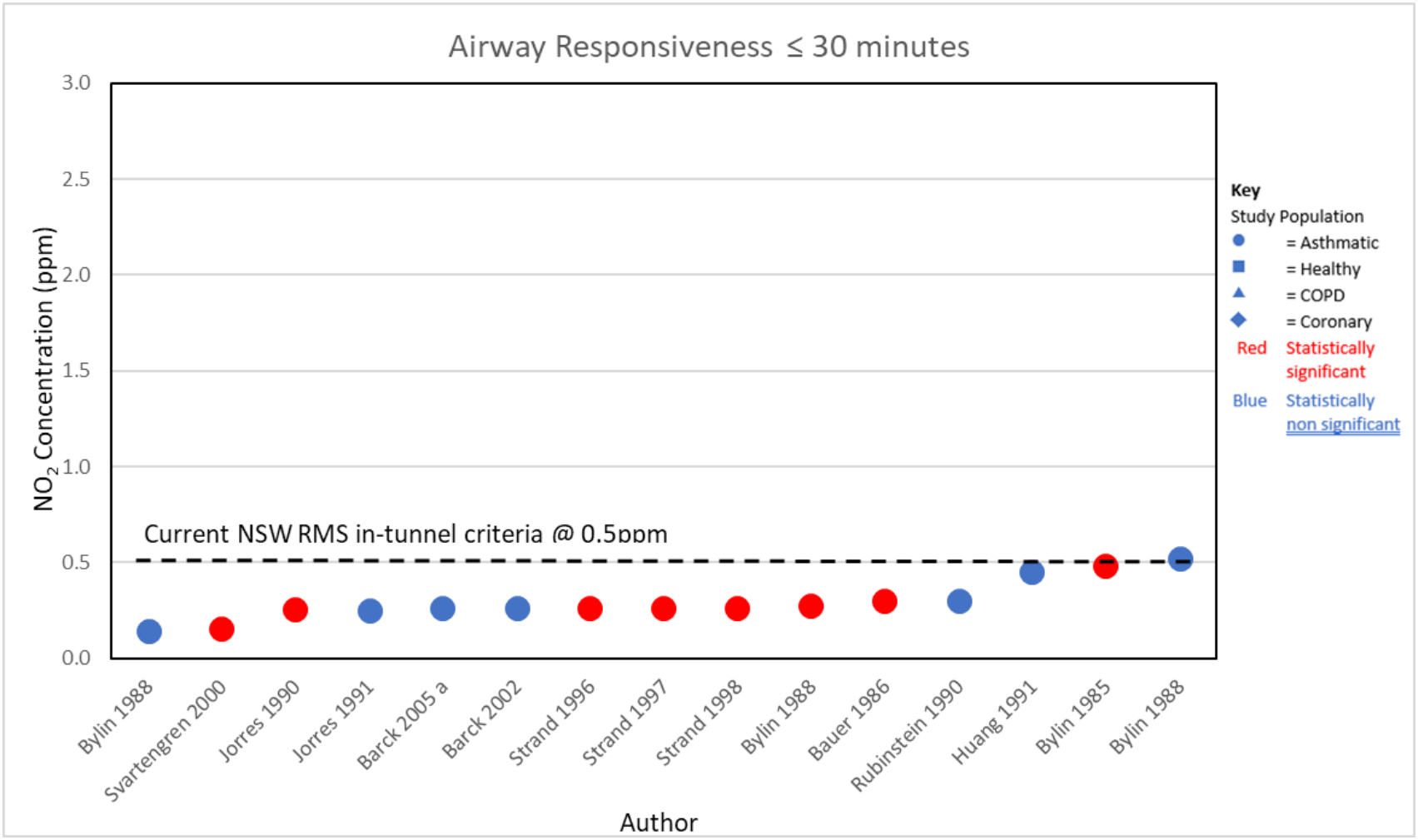

Figure B.4 Graph of studies examining exposures to NO_2 of ≤ 30 minutes and airway responsiveness, ranked by NO_2 concentration

Table B.4 Studies examining exposures to NO_2 of ≤ 30 minutes and airway responsiveness, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Bylin 1988	Randomised Cross over	A, N, ES	17-56	20	0.14[$]	N	30	N	N	sRaw, TGV	
Svartengren 2000	Non-randomised cross over	A, N, ES	19-55	20	0.15[$]	Y	30	N	N	sRaw, TGV, FEV1	After allergen - ↑ sRaw, TGV
Jorres 1990	Randomised Cross over	A,N	34(mean)	14	0.25	N	30	N	N	sRaw, PD	↓PD
Jorres 1991	Randomised Cross over	A, N, S	17 -44	11	0.25	N	30	N	Y	sRaw	
Barck 2005 a	Randomised Cross over	A, NS, ES	23-48	18	0.26[$]	N	15	Y	N	FEV1, sRaw, TGV	
Barck 2002	Randomised Cross over	A,NS	23-39	13	0.26[$]	N	30	N	N	sRaw, TGV, FEV1	
Strand 1996	Randomised Cross over	A, N, ES	20-48	19	0.26	N	30	N	Y	sRaw, TGV	Significant difference noted for histamine challenge for sRaw at 5 hours only, but not at 30 minutes, 27 h or 7 days.

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Strand 1997	Randomised Cross over	A, N, ES	18-50	18	0.26$	N	30	N	N	sRaw, Vtg, FEV1, PEF, FVC, FEF.	After allergen - ↓PEF, FEV1
Strand 1998	Randomised Cross over	A, N, ES	21-52	16	0.26$	N	30	Y	N	sRaw, TGV, FEV1	↓FEV1
Bylin 1988	Randomised Cross over	A, N, ES	17-56	20	0.27$	N	30	N	N	sRaw, TGV, PD	↓PD - Not repeated at higher dose in this experiment
Bauer 1986	Randomised Cross over	A,N	20-45	15	0.3	N	30	N	Y	FEV1, sGaw, PD	↓ PD, FEV1, sGaw
Rubinstein 1990	Randomised Cross over	A, N	23-34	9	0.3	N	30	N	Y	sRaw, FEV1, FVC	
Huang 1991	Experimental exposure	A,N	10 to 14	6	0.45-0.5 NOx	Y	5	N	N	FVC, FEV1	
Bylin 1985	Non-randomised cross over	A, H	17-45(A), 20-36(H)	8(A), 8(H)	0.48$	N	20	N	N	sRaw, TGV ,PD	Asthmatics only - ↓ PD
Bylin 1988	Randomised Cross over	A, N, ES	17-56	20	0.52$	N	30	N	N	sRaw, TGV	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

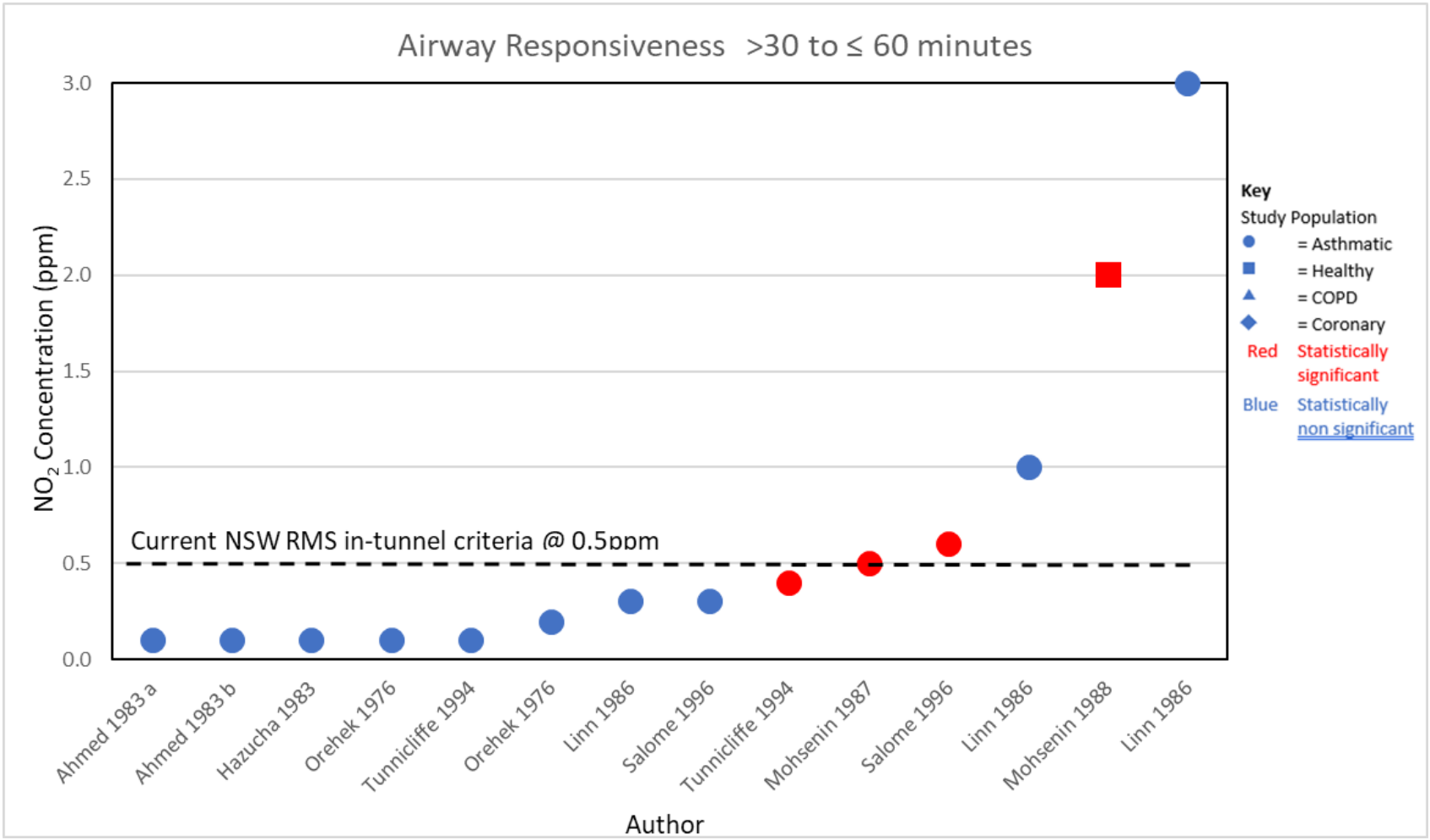

Figure B.5 Graph of studies examining exposures to NO_2 of >30 to ≤ 60 minutes and airway responsiveness, ranked by NO_2 concentration

Table B.5 Studies examining exposures to NO_2 of >30 to ≤ 60 minutes and airway responsiveness, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Ahmed 1983 a		A		20	0.1	N	60			sGaw	
Ahmed 1983 b		A		20	0.1	N	60			sGaw	
Hazucha 1983	Randomised Cross over	A, H, N	18-35	15(A), 15 (N)	0.1	N	60	N	N	sRaw	
Orehek 1976	Randomised Cross over	A	15-44	20	0.1	N	60	N	N	sRaw	
Tunnicliffe 1994	Randomised Cross over	A, N	16 - 60	8	0.1	N	60	N	N	FEV1	
Orehek 1976	Randomised Cross over	A	15-44	4	0.2	N	60	N	N	sRaw	
Linn 1986	Randomised Cross over	A	20-34	21	0.3	N	60	N	Y	sRaw, FVC, FEV1, MMFR	
Salome 1996	Randomised Cross over	A	7 to 65	20	0.3	N	60	N	N	PD20FEV1	
Tunnicliffe 1994	Randomised Cross over	A, N	16 - 60	8	0.4	N	60	N	N	FEV1	↓ FEV1
Mohsenin 1987	Randomised Cross over	A	22-40	10	0.5	N	60	N	N	FRC, sGaw, PD	↓PD

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Salome 1996	Randomised Cross over	A	7 to 65	20	0.6	N	60	N	N	PD20FEV1	↓PD for change in FEV1
Linn 1986	Randomised Cross over	A	20-34	21	1	N	60	N	Y	sRaw, FVC, FEV1, MMFR	
Mohsenin 1988	Randomised Cross over	H, N	18-33	18	2	N	60	N	N	PD	↓PD to reduce sGaw
Linn 1986	Randomised Cross over	A	20-34	21	3	N	60	N	Y	sRaw, FVC, FEV1, MMFR	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

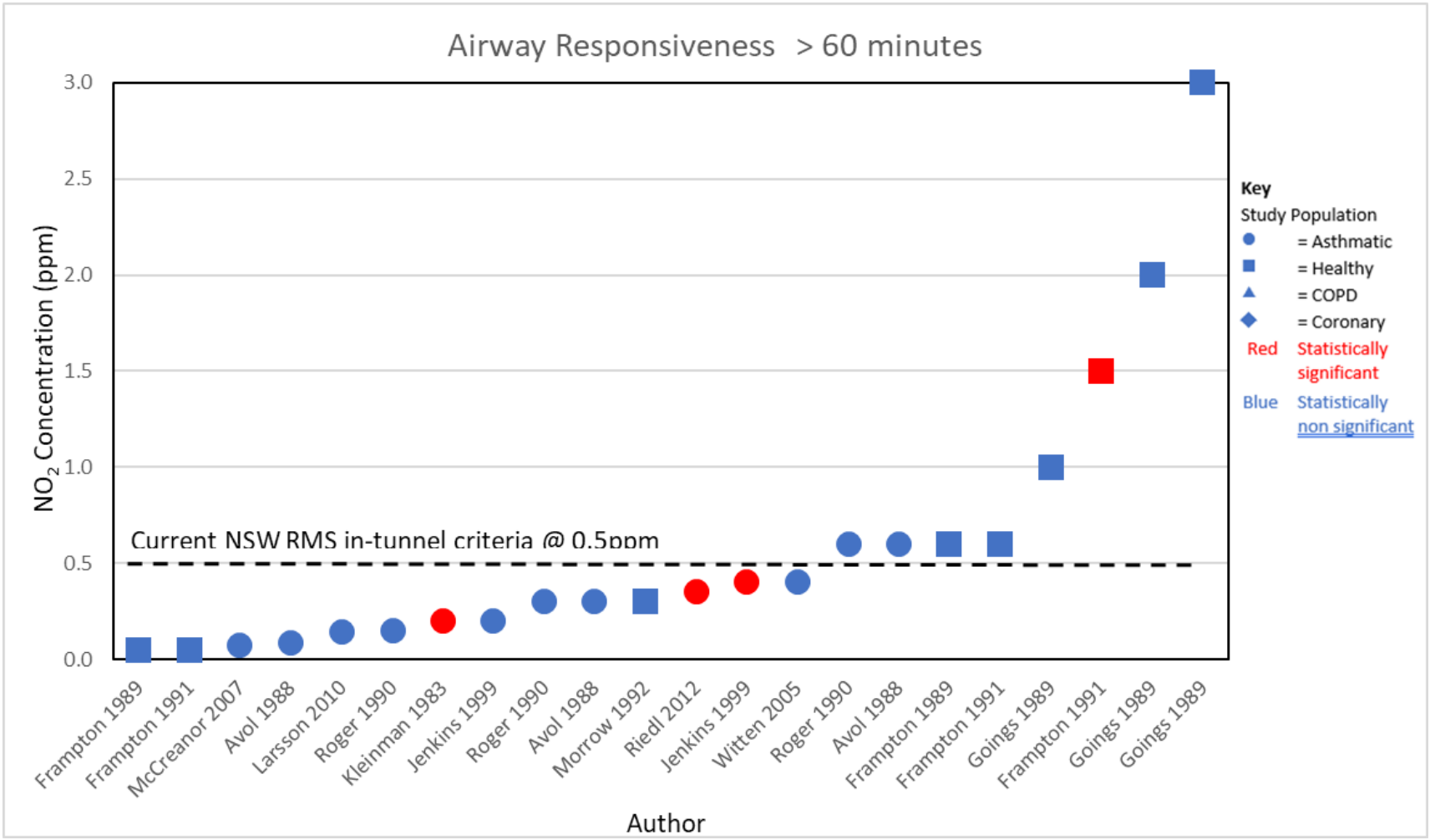

Figure B.6 Graph of studies examining exposures to NO_2 of > 60 minutes and airway responsiveness, ranked by NO_2 concentration

Table B.6 Studies examining exposures to NO_2 of > 60 minutes and airway responsiveness, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Frampton 1989	Randomised Cross over	H, N	19-37	15	0.05 with three 15min peaks of 2.0	N	180	N	Y	FVC, FEV1, sGaw	
Frampton 1991	Randomised Cross over	H, N	19-37	15	0.05 with three 15min peaks of 2.0	N	180	N	Y	FVC, FEV1, sGaw, PEFR, MEFR	
McCreanor 2007	Randomised Cross over	A,N	19-55	60	0.075[$]	Y	120	N	Y	FEF 25-75	
Avol 1988		A		36	0.086	Y	120	N			
Larsson 2010	Randomised Cross over	A, N	18-55	14	0.14[$]	Y	120	N	Y	VC, FEV1, PEF, FVC	
Roger 1990	Randomised Cross over	A, N	19-35	21	0.15	N	75	N	Y	sRaw, FVC, FEV1	
Kleinman 1983	Randomised Cross over	A	31±11	31	0.2	N	120	N	Y	Rt, FEV1, FVC, PD	↓ PD for FEV1 only
Jenkins 1999	Randomised Cross over	A,NS	22-41	11	0.2	N	360	N	Y	FEV1	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Roger 1990	Randomised Cross over	A, N	19-35	21	0.3	N	75	N	Y	sRaw, FVC, FEV1	
Avol 1988		A		59	0.3	N	120	N			
Morrow 1992	Randomised Cross over	H, N, COPD, S	49-69(H), 47-70 (COPD)	20(H), 20(COPD)	0.3	N	240	N	Y	FEV1	
Riedl 2012	Randomised Cross over	A, N	19-55	15	0.35	N	120	N	Y	FEV1	↑ FEV1 post allergen exposure or exercising
Jenkins 1999	Randomised Cross over	A,NS	22-41	10	0.4	N	180	N	Y	FEV1, PD	↓PD
Witten 2005	Randomised Cross over	A, N, allergy	21-48	15	0.4	N	180	N	Y	FEV1	
Roger 1990	Randomised Cross over	A, N	19-35	21	0.6	N	75	N	Y	sRaw, FVC, FEV1	
Avol 1988		A		59	0.6	N	120	N			
Frampton 1989	Randomised Cross over	H, N	24-37	9	0.6	N	180	N	Y	FVC, FEV1, sGaw	
Frampton 1991	Randomised Cross over	H, N	24-37	9	0.6	N	180	N	Y	FVC, FEV1,	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
										sGaw, PEFR, MEFR	
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	1	N	120	N	N	FEV1	
Frampton 1991	Randomised Cross over	H, N	19-37	15	1.5	N	180	N	Y	FVC, FEV1, sGaw, PEFR, MEFR	↓ FEV1, FVC
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	2	N	120	N	N	FEV1	
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	3	N	120	N	N	FEV1	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

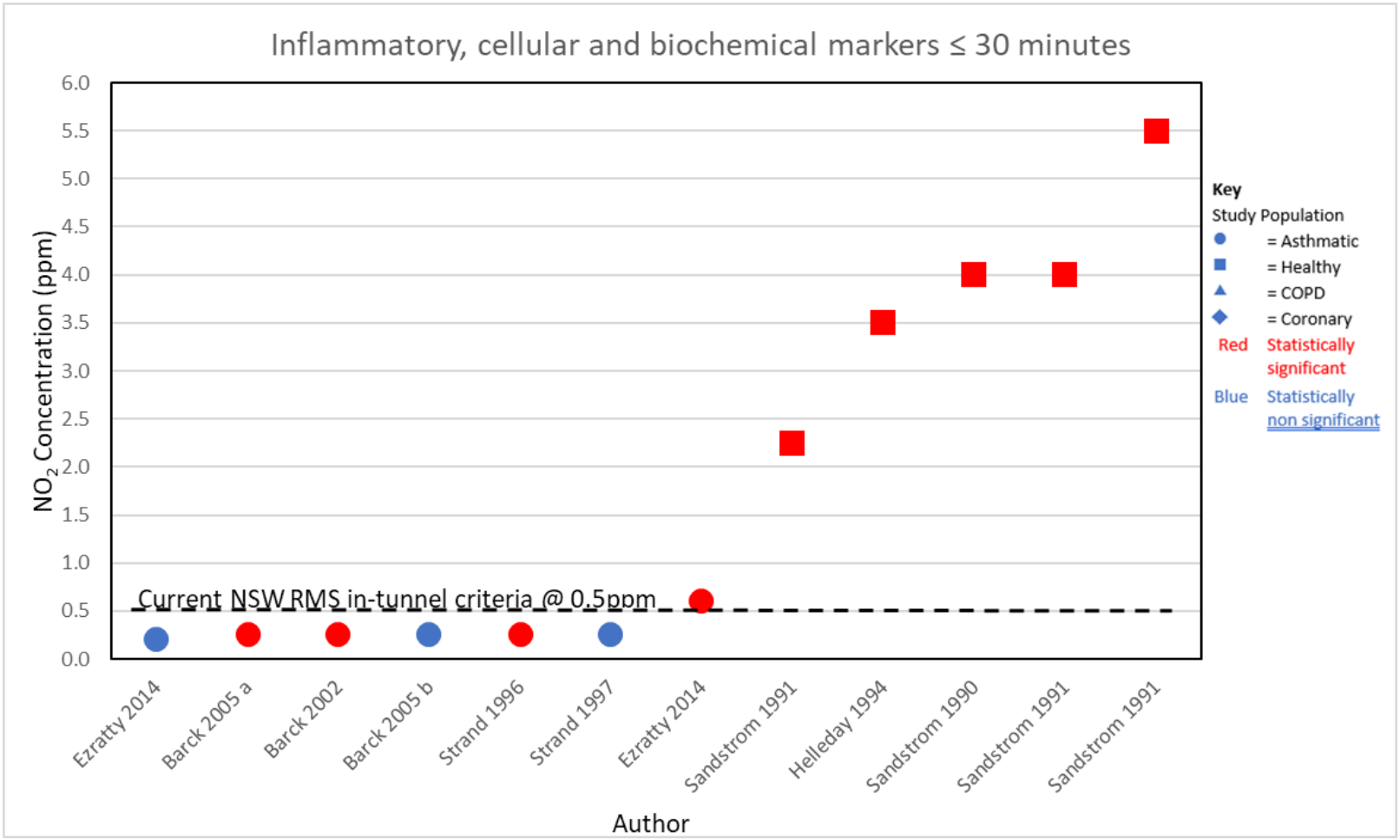

Figure B.7 Graph of studies examining exposures to NO_2 of ≤ 30 minutes and inflammatory, cellular and biochemical markers, ranked by NO_2 concentration

Table B.7 Studies examining exposures to NO_2 of ≤ 30 minutes and inflammatory, cellular and biochemical markers, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Ezratty 2014	Randomised Cross over	A,N	20-69	19	0.2	N	30	Y	N	E, N, M, ECP	
Barck 2005 a	Randomised Cross over	A, NS, ES	23-48	18	0.26[$]	N	15	Y	N	E, N, ECP, MPO	After allergen challenge, ↑ ECP; ↓ MPO
Barck 2002	Randomised Cross over	A,NS	23-39	13	0.26[$]	N	30	N	N	E, M, L, N, MC, Alb, ECP, Eot, IL-8, MPO, sICAM	After allergen challenge, ↑ % N & ECP levels; ↓ M
Barck 2005 b	Randomised Cross over	A, rhinitis	22-48	16	0.26[$]	N	30	N	N	E, N, ECP, MPO	
Strand 1996	Randomised Cross over	A, N, ES	20-48	19	0.26	N	30	N	Y	ECP, MPO, Try, MCG	↑ MCG
Strand 1997	Randomised Cross over	A, N, ES	18-50	18	0.26[$]	N	30	N	N	L, N, E, B, Mo, ECP	
Ezratty 2014	Randomised Cross over	A,N	20-69	19	0.6	N	30	Y	N	E, N, M, ECP	↑ %E; ↑ ECP compared to baseline measure
Sandstrom 1991	Experimental exposure	H, N	22-32	18	2.25	N	20	N	Y	L, AM, MC, Alb, Fib, ACE, Mp	↑ MC

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Helleday 1994	Experimental exposure	H,N,S	24-35(N), 28-32(S)	8(N), 8(S)	3.5	N	20	N	N	AM, LPM, N, L, LSP, MC, Pp	Smokers - ↑AM, N. Non smokers - ↑L, N
Sandstrom 1990	Experimental exposure	H, N	21-37	32	4		20	N	Y	L, M, N, MC, Alb, E, Ep, LPM	↑ L, MC, %LPM
Sandstrom 1991	Experimental exposure	H, N	22-32	18	4	N	20	N	Y	L, AM, MC, Alb, Fib, ACE, Mp	↑ MC, L
Sandstrom 1991	Experimental exposure	H, N	22-32	18	5.5	N	20	N	Y	L, AM, MC, Alb, Fib, ACE, Mp	↑ MC, L

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

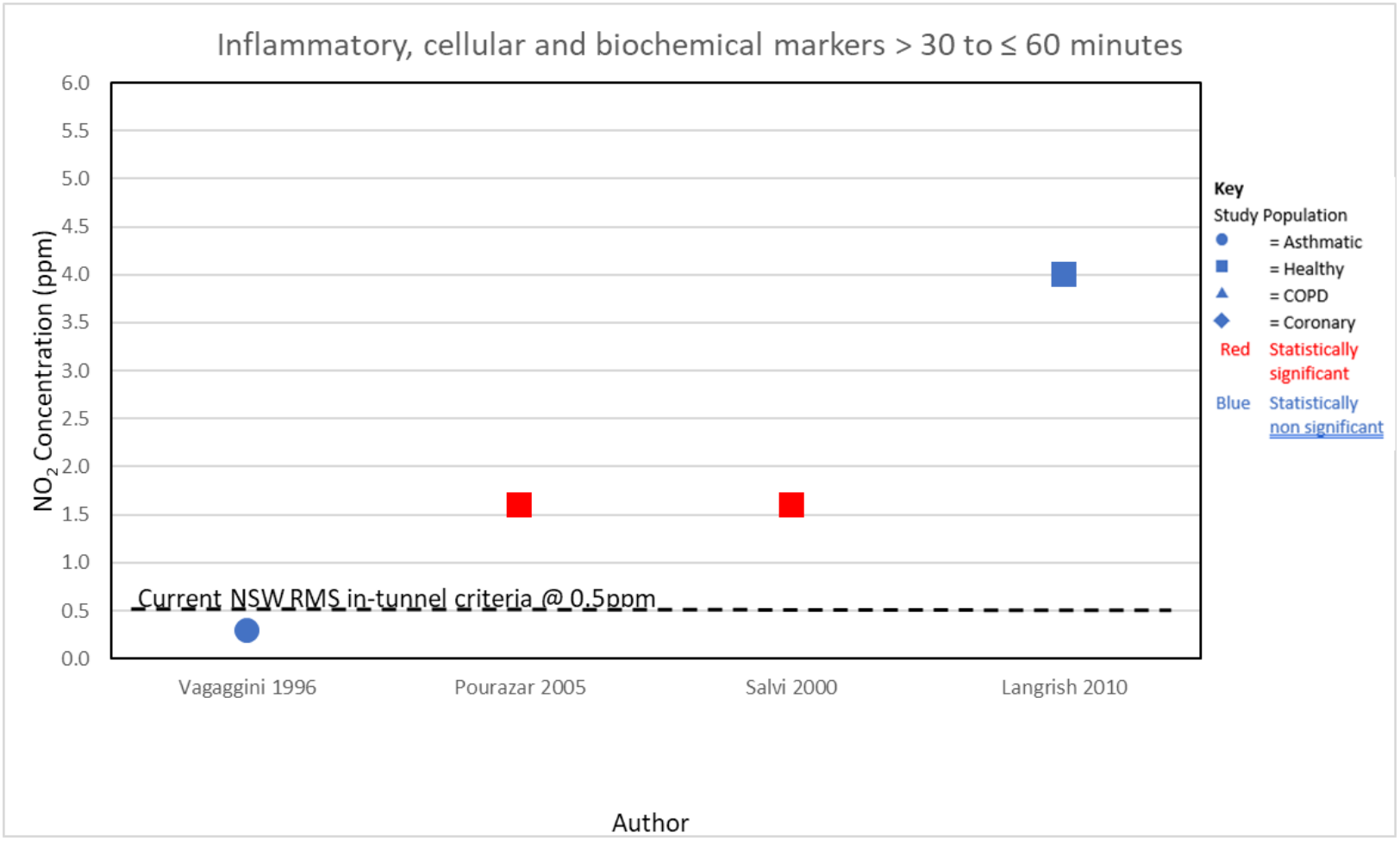

Figure B.8 Graph of studies examining exposures to NO_2 of >30 to ≤ 60 minutes and inflammatory, cellular and biochemical markers, ranked by NO_2 concentration

Table B.8 Studies examining exposures to NO_2 of >30 to ≤ 60 minutes and inflammatory, cellular and biochemical markers, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Vagaggini 1996	Randomised Cross over	A, H, COPD	29 (A), 34 (H), 58 (COPD)	7 (A), 8 (H), 7 (COPD)	0.3	N	60	N	Y	E, N, M, L	
Pourazar 2005	Randomised Cross over	H, N	21-28	15	1.6	Y	60	N	Y	NF-κB,AP-1, p38, JNK, Tys	↑NF-κB,AP-1, p38, JNK, Tys
Salvi 2000	Randomised Cross over	H, N	21-28	15	1.6	Y	60	N	Y	IL-4, IL-5, IL-8, IL-1β, IFNɣ, TNF -α, GM-CSF, CXCL1	↑IL-8, CXCL1
Langrish 2010	Randomised Cross over	H,N	22-28	10	4	N	60	N	Y	Hb, Wc, Pl, N, L, Mo	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

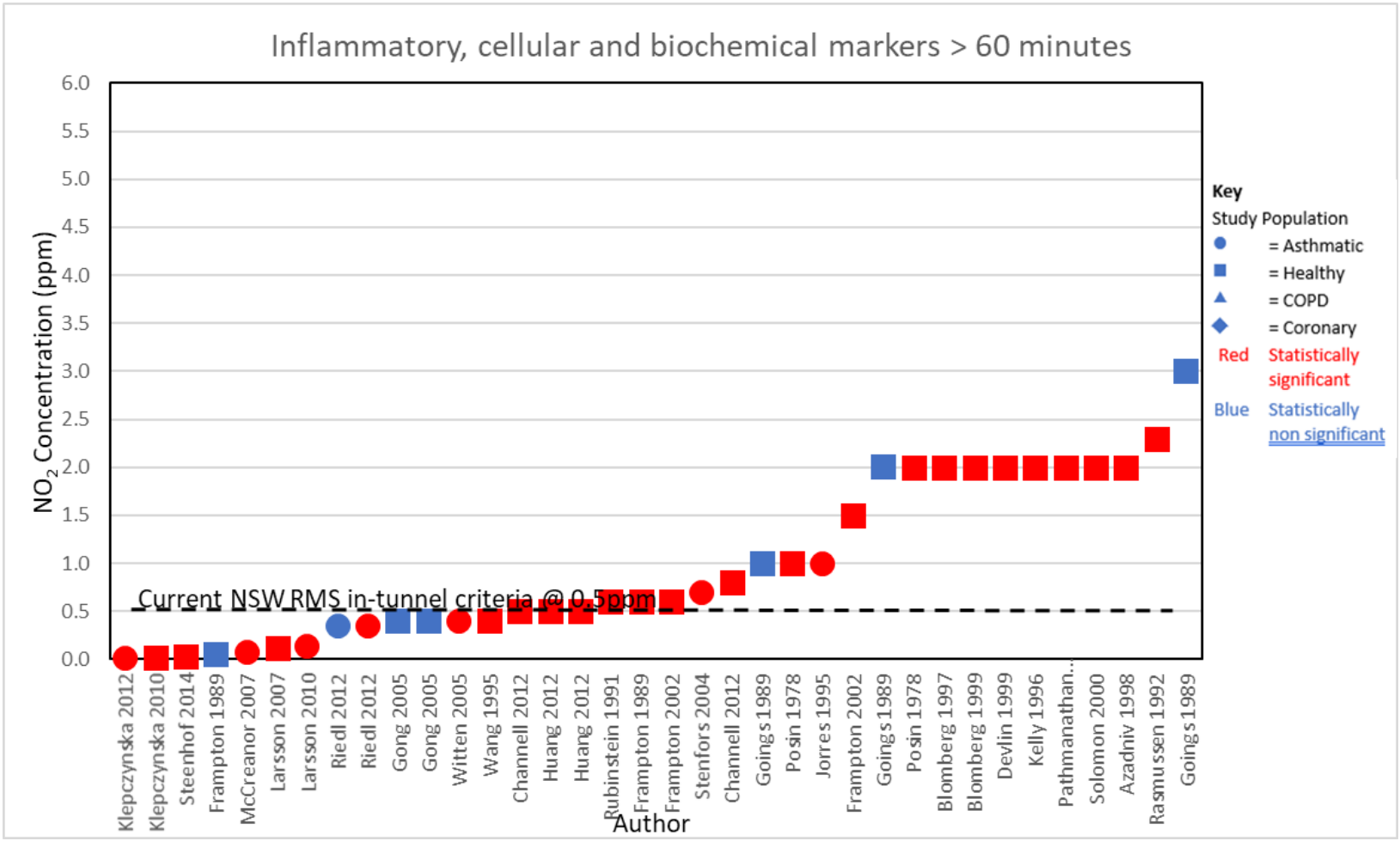

Figure B.9 Graph of studies examining exposures to NO_2 of > 60 minutes and inflammatory, cellular and biochemical markers, ranked by NO_2 concentration

Table B.9 Studies examining exposures to NO_2 of > 60 minutes and inflammatory, cellular and biochemical markers, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Klepczynska 2012	Randomised Cross over	A	18-52	16	0.01[$]	Y	120	N	Y	Blc, Fi, Le, N, E, B, L, LSP, Mo, M, IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, PAI-1	↑ LSP activity
Klepczynska 2010	Randomised Cross over	H	18-46	20	0.012[$]	Y	120	N	Y	Blc, Fi, Le, N, E, B, L, LSP, Mo, M, IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, PAI-1	↑ Fi, some LSP
Steenhof 2014	Experimental exposure	H, N, ES	19-26	31	0.02 (GM) 0.009 - 0.034 (Range)	Y	300	N	Y	Wc, N, Mo, L, E, B	Regression analysis - ↓L,E associated with NO2 exposure
Frampton 1989	Randomised Cross over	H, N	19-37	15	0.05 with three 15min	N	180	N	Y	M, TC, IL-1	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
					peaks of 2.0						
McCreanor 2007	Randomised Cross over	A,N	19-55	60	0.075[$]	Y	120	N	Y	N, E, MPO, IL-8, ECP	↑MPO
Larsson 2007	Randomised Cross over	H,N	19-59	16	0.12[$]	Y	120	N	Y	E, N, B, AM, L, LSP, MC, Fib	↑ L, some LSP, AM
Larsson 2010	Randomised Cross over	A, N	18-55	14	0.14[$]	Y	120	N	Y	E, N, B, L, Mo, , IL-1B, IL-6, IL-8, IL-10, TNF-α, IL-12p70.	↑ IL-10, TNF-α, IL-12p70
Riedl 2012	Randomised Cross over	A, N	19-55	15	0.35	N	120	N	Y	E, M, L, PMN	
Riedl 2012	Randomised Cross over	A, N	19-55	15	0.35	N	120	N	Y	IgA, IgE, IgG, IgG4, IgM, IL-4, IL-5, IL-8, IL-12, IFNɣ, TNF-α, ECP, CCL5, Eot, GM-CSF, Try, F-VII, Fi, vWF	↓ IgG4

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Gong 2005	Semi randomised cross over	H, COPD	mean 68(H), 72(COPD)	6(H), 18(COPD)	0.4	N	120	N	Y	Ep, Mo, N, E	
Gong 2005	Semi randomised cross over	H, COPD	mean 68(H), 72(COPD)	6(H), 18(COPD)	0.4	Y	120	N	Y	Ep, Mo, N, E	
Witten 2005	Randomised Cross over	A, N, allergy	21-48	15	0.4	N	180	N	Y	Le, M, N, E, L, IL-5, ECP, IL-8, GM-CSF, Tp	After allergen - ↓E
Wang 1995	Randomised Cross over	N, allergic rhinitis	18-55	16 (8 had allergen challenge)	0.4	N	360	N	N	ECP, MCT, MPO, IL-8	After allergen - ↑ ECP
Channell 2012	Non-randomised cross over	H	24.9 (mean)	7	0.5[$]	N	120	N	Y	sICAM -1, VCAM-1, IL-8, MCP-1	↑ sICAM-1, VCAM-1, IL-8
Huang 2012	Semi randomised cross over	H,N	20-36	23	0.5	N	120	N	Y	LDH, IL-6, IL-8, A1AT, CoF, Tchol, HDL-chol	↑ LHD, HDL-chol
Huang 2012	Semi randomised cross over	H,N	20-36	23	0.5	Y	120	N	Y	LDH, IL-6, IL-8, A1AT, CoF, Tchol, HDL-chol	↑ LHD

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Rubinstein 1991	Randomised Cross over	H, N	18-45	5	0.6	N	120	Y	Y	L, LSP	
Frampton 1989	Randomised Cross over	H, N	24-37	9	0.6	N	180	N	Y	M, TC, IL-1	
Frampton 2002	Randomised Cross over	H,N	18-40	21	0.6	N	180	N	Y	Hct, Hb, Rbc, L, TL, PMN, M, E, LDH	↑L; Dose related ↑PMN; Dose related ↓Hct, L, TL
Stenfors 2004	Randomised Cross over	A, H, N	22-52(A), 19-42(H)	15(A), 25(N)	0.7	Y	120	N	Y	N, L, MC, E, ECP, IL-1β, IL-6, IL-8, IL-10, IFNɣ, CXCL1, TNF -α, NF-κB, CCL5, LSP, VCAM-1, sICAM-1	For healthy - ↑ N, L, LSP, IL-6, IL-8, VCAM-1; For asthmatics - ↑ IL-10; ↓E
Channell 2012	Non-randomised cross over	H	25.3 (mean)	7	0.8[$]	Y	120	N	Y	ICAM -1, VCAM-1, IL-8, MCP-1	↑ VCAM-1
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	1	N	120	N	N	IgG, IgA	

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Posin 1978	Experimental exposure	H		10	1	N	150	N	Y	AChE, GSH, G6PD, LDH, GR, GPx, VitE, TBARS, 2,3-BPG, Hb, Hct	↑ GR; ↓ AChE, Hb, Hct
Jorres 1995	Randomised Cross over	A, H	21-37 (A)	12(A), 8 (H)	1	N	180	N	Y	E, N, M, L, Ep, MC, TxB, 6PG, PDG2, plus others	↑TxB; Asthmatics only - ↑ 6PG, PGD2
Frampton 2002	Randomised Cross over	H,N	18-40	21	1.5	N	180	N	Y	Hct, Hb, Rbc, L, TL, PMN, M, E, LDH	Dose related ↑PMN; Dose related ↓Hct, L, TL; 40% increase in LDH
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	2	N	120	N	N	IgG, IgA	
Posin 1978	Experimental exposure	H		10	2	N	150	N	Y	AChE, GSH, G6PD, LDH, GR, GPx, VitE, TBARS, 2,3-BPG, Hb, Hct	↑ G6PD, TBARS; ↓ AChE, Hb, Hct

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Blomberg 1997	Randomised Cross over	H, N	20-30	15	2	N	240	N	Y	M, L, N, MC, LSP, sICAM-1, VCAM-1, NE, MCT, Mea, Tp, Alb, IgA, IL-8.	↑ N, IL-8 ; ↓ %M, Tp, IgA, Alb, sICAM-1; Δ LSP
Blomberg 1999	Non-randomised cross over	H, N	21-32	12	2	N	240	Y	Y	M, L, N, MC, LSP, ICAM-1, NE, MCT, Tp, Alb, M-his, IL-8, MPO, HLA, GSH, GSSG, AsA, UA	↑N, MPO; ↓N, Alb
Devlin 1999	Randomised Cross over	H, N	18-35	8	2	N	240	N	Y	M, L, PMN, Ep, Tp, LDH, IL-6, IL-8, PGE2, Fib, A1AT, tPA, Mp, SpR, AsA, UA, GSH, VitE	↑PMN, IL-6, IL-8, A1AT, tPA,; ↓ %Mp, SpR, Ep

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Kelly 1996	Randomised Cross over	H, N	19-45	44	2	N	240	N	Y	UA, AsA, GSH, OGSH, Mal	↑GSH; ↓AsA; Δ UA
Pathmanathan 2003	Randomised Cross over	H, N	21-32	12	2	N	240	Y	Y	IL-5, IL-6, IL-8, IL-10, IL-13, CXCL1, NF-κB, sICAM-1, TNF-α, GM-CSF, Eot	↑ IL-5, IL-10, IL-13, sICAM-1
Solomon 2000	Randomised Cross over	H, N	24-40	15	2	N	240	Y	Y	E, N, M, L, LSP, Le	↑ %N; ↓% some LSP
Azadniv 1998	Randomised Cross over	H, N	22-35	12	2	N	360	N	Y	E, M, L, LSP, PMN, MC, VirA	↑ % PMN; ↓ CD8+ T lymphocytes and T lymphocytes expressing neither CD4 nor CD8
Rasmussen 1992	Randomised Cross over	H,N	22-66	14	2.3	N	300	N	N	Ap, GSH, GPx, Se	↓GPx, Ap
Goings 1989	Randomised control trial	H,N	18-35	152(Total)	3	N	120	N	N	IgG, IgA	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

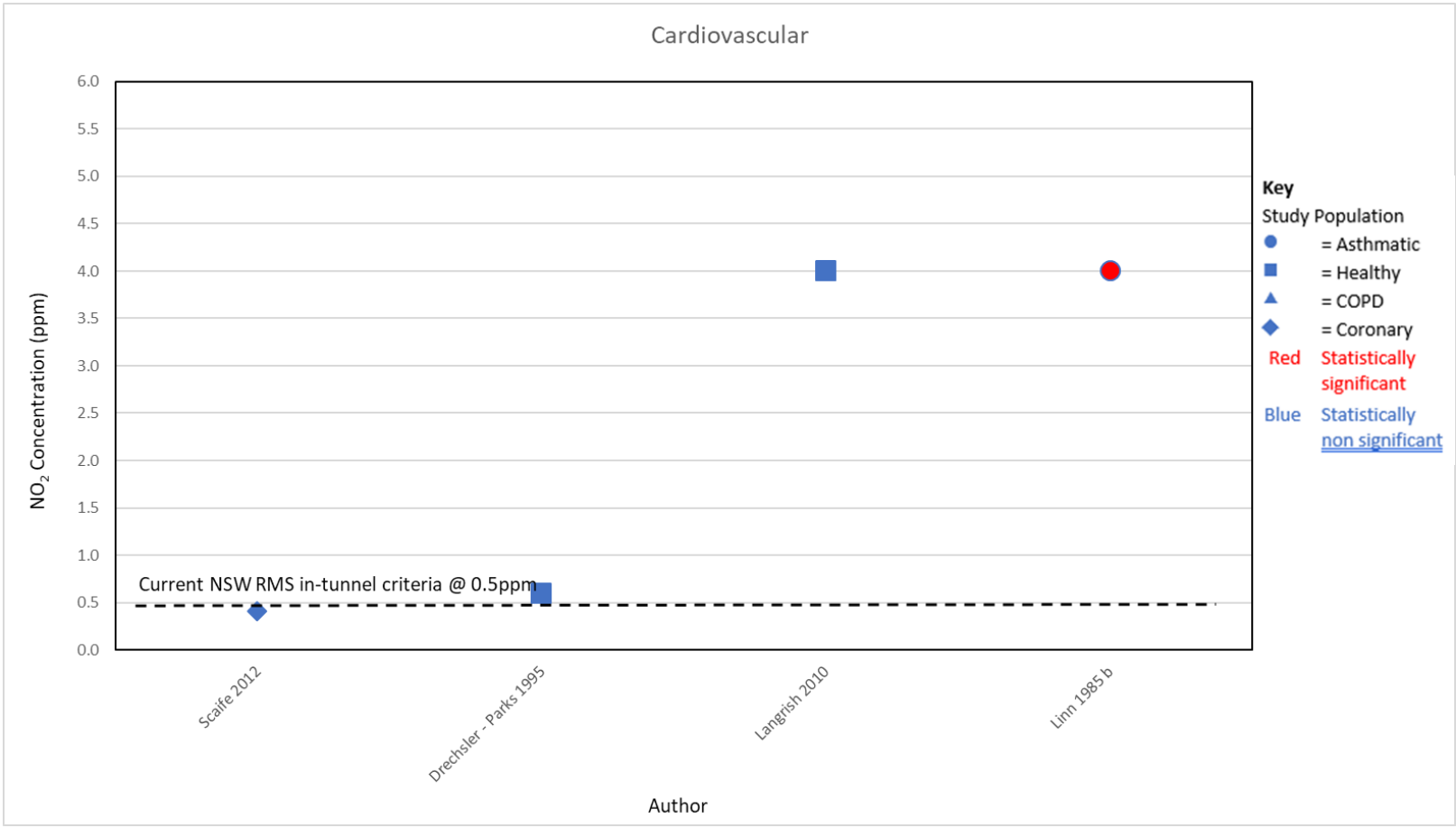

Figure B.10 Graph of studies examining exposures to NO_2 and cardiovascular outcomes, ranked by NO_2 concentration

Table B.10 Studies examining exposures to NO_2 and cardiovascular outcomes, ranked by NO_2 concentration*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Scaife 2012	Randomised Cross over	Coronary heart disease with reduced LV systolic function, N	56-76	18	0.4	N	60	N	N	HR, BP, Lcc, HRv	
Drechsler - Parks 1995	Randomised Cross over	H, N	56-85	8	0.6	N	120	N	Y	VR, HR, Rf, O2, CO	
Langrish 2010	Randomised Cross over	H,N	22-28	10	4	N	60	N	Y	Forearm blood flow	
Linn 1985 b	Randomised Cross over	A, H, NS	18-34(A), 20-36(H)	23(A), 25(H)	4	N	75	N	Y	BP,HR, SkC	Healthy only - ↓ systolic pressure

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

Appendix C – Traffic pollution experimental studies

Table C.1 Traffic pollution experimental studies examining exposures to NO_2 and lung function, ranked by NO_2 concentration and stratified by exposure time*

Author	Study design	Study population	Age	N	Pollutant Source	NO_2 (ppm)	$PM_{2.5}$ ($\mu g/m^3$)	PM_{10} ($\mu g/m^3$)	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Studies ≤ 30 minutes													
Svartengren 2000	Non-randomised cross over	A, N, ES	19-55	20	Road tunnel Sweden	0.15[$]	95	170	30	N	N	sRaw, TGV, FEV1	
Huang 1991	Experimental exposure	A,N	10 to 14	6	Road tunnel Taipei city	0.45-0.5 NOx	N/A	N/A	5	N	N	FVC, FEV1, Raw, MMF, PEFR, FEF	
Studies >30 to ≤ 60 minutes													
Rudell 1994	Experimental exposure	H,N	19-27	8	Idling Scania diesel lorry	1.6	N/A	N/A	60	N	Y	FVC, FEV1, FEF25-75, MTT	
Studies > 60 minutes													
Klepczynska 2012	Randomised Cross over	A	18-52	16	Subway - Sweden	0.01[$]	71	232	120	N	Y	VC, FEV1, FVC, PEF, exhaled NO	
Klepczynska 2010	Randomised Cross over	H	18-46	20	Subway - Sweden	0.012[$]	76	237	120	N	Y	VC, FEV1, FVC,	

Author	Study design	Study population	Age	N	Pollutant Source	NO_2 (ppm)	$PM_{2.5}$ (µg/m^3)	PM_{10} (µg/m^3)	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
												PEF, eNO	
McCreanor 2007	Randomised Cross over	A,N	19-55	60	London UK - street	0.075$	28	125	120	N	Y	FEV1, FVC, FEF, PEF, eNO	↓FEV1, FVC
Avol 1988		A		36		0.086	N/A	N/A	120	N			
Larsson 2007	Randomised Cross over	H,N	19-59	16	Road tunnel Sweden	0.12$	64	176	120	N	Y	FEV1, FVC	
Larsson 2010	Randomised Cross over	A, N	18-55	14	Road tunnel Sweden	0.14$	80	183	120	N	Y	VC, FEV1, PEF, FVC, exhaled NO	↓ PEF
Gong 2005	Semi randomised cross over	H, COPD	mean 68(H), 72(COPD)	6(H), 18(COPD)	Harvard/EPA fine particle concentrator - ambient Los Angeles USA air	0.4	185	N/A	120	N	Y	MMEF, FEV1, FVC	
Huang 2012	Semi randomised cross over	H,N	20-36	23	Harvard/EPA fine particle concentrator - ambient Chapel Hill USA air	0.5	74	N/A	120	N	Y	FVC, FEV1, FEF25-75	
Stenfors 2004	Randomised Cross over	A, H, N	22-52(A), 19-42(H)	15(A), 25(N)	Idling Volvo diesel engine	0.7	N/A	108	120	N	Y	FEV1, FVC, sRaw	↑sRaw

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

Table C.2 Traffic pollution experimental studies examining exposures to NO_2 and airway responsiveness, ranked by NO_2 concentration and stratified by exposure time*

Author	Study design	Study population	Age	N	Pollutant Source	NO_2 (ppm)	$PM_{2.5}$ ($\mu g/m^3$)	PM_{10} ($\mu g/m^3$)	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Studies ≤ 30minutes													
Svartengren 2000	Non-randomised cross over	A, N, ES	19-55	20	Road tunnel Sweden	0.15$	95	170	30	N	N	sRaw, TGV, FEV1	After allergen - ↑ sRaw, TGV
Huang 1991	Experimental exposure	A,N	10 to 14	6	Road tunnel Taipei city	0.45-0.5 NOx	N/A	N/A	5	N	N	FVC, FEV1	
Studies > 60 minutes													
McCreanor 2007	Randomised Cross over	A,N	19-55	60	London UK - street	0.075$	28	125	120	N	Y	FEF 25-75	
Avol 1988		A		36		0.086	N/A	N/A	120	N			
Larsson 2010	Randomised Cross over	A, N	18-55	14	Road tunnel Sweden	0.14$	80	183	120	N	Y	VC, FEV1, PEF, FVC	

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease
$ = conversion from $\mu g/m^3$ to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

Table C.3 Traffic pollution experimental studies examining exposures to NO_2 and inflammatory, cellular and biochemical markers, ranked by NO_2 concentration and stratified by exposure time*

Author	Study design	Study population	Age	N	Pollutant Source	NO_2 (ppm)	$PM_{2.5}$ ($\mu g/m^3$)	PM_{10} ($\mu g/m^3$)	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Studies >30 to ≤ 60 minutes													
Pourazar 2005	Randomised Cross over	H, N	21-28	15	Idling Volvo diesel engine	1.6	N/A	300	60	N	Y	NF-κB,AP-1, p38, JNK, Tys	↑NF-κB,AP-1, p38, JNK, Tys
Salvi 2000	Randomised Cross over	H, N	21-28	15	Idling Volvo diesel engine	1.6	N/A	300	60	N	Y	IL-4, IL-5, IL-8, IL-1β, IFNγ, TNF -α, GM-CSF, CXCL1	↑IL-8, CXCL1
Studies > 60 minutes													
Klepczynska 2012	Randomised Cross over	A	18-52	16	Subway - Sweden	0.01[$]	71	232	120	N	Y	Blc, Fi, Le, N, E, B, L, LSP, Mo, M, IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, PAI-1	↑ LSP activity

Author	Study design	Study population	Age	N	Pollutant Source	NO_2 (ppm)	$PM_{2.5}$ (µg/m^3)	PM_{10} (µg/m^3)	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Klepczynska 2010	Randomised Cross over	H	18-46	20	Subway - Sweden	0.012[$]	76	237	120	N	Y	Blc, Fi, Le, N, E, B, L, LSP, Mo, M, IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α, PAI-1	↑ Fi, some LSP
Steenhof 2014	Experimental exposure	H, N, ES	19-26	31	Underground train station, traffic, farm, urban background in Netherlands	0.02 (GM) 0.009 - 0.034 (Range)	39 (GM) 8 - 167 (Range)	76 (GM) 18 - 450 (Range)	300	N	Y	Wc, N, Mo, L, E, B	Regression analysis - ↓L,E associated with NO2 exposure
McCreanor 2007	Randomised Cross over	A,N	19-55	60	London UK - street	0.075[$]	28	125	120	N	Y	N, E, MPO, IL-8, ECP	↑MPO
Larsson 2007	Randomised Cross over	H,N	19-59	16	Road tunnel Sweden	0.12[$]	64	176	120	N	Y	E, N, B, AM, L, LSP, MC, Fib	↑ L, some LSP, AM
Larsson 2010	Randomised Cross over	A, N	18-55	14	Road tunnel Sweden	0.14[$]	80	183	120	N	Y	E, N, B, L, Mo, , IL-1B, IL-6, IL-8, IL-10, TNF-α, IL-12p70.	↑ IL-10, TNF-α, IL-12p70

Author	Study design	Study population	Age	N	Pollutant Source	NO_2 (ppm)	$PM_{2.5}$ ($\mu g/m^3$)	PM_{10} ($\mu g/m^3$)	Exposure time (mins)	Repeat exposure	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Gong 2005	Semi randomised cross over	H, COPD	mean 68(H), 72(COPD)	6(H), 18(COPD)	Harvard/EPA fine particle concentrator - ambient Los Angeles USA air	0.4	185	N/A	120	N	Y	Ep, Mo, N, E	
Huang 2012	Semi randomised cross over	H,N	20-36	23	Harvard/EPA fine particle concentrator - ambient Chapel Hill USA air	0.5	74	N/A	120	N	Y	LDH, IL-6, IL-8, A1AT, CoF, Tchol, HDL-chol	↑ LHD
Stenfors 2004	Randomised Cross over	A, H, N	22-52(A), 19-42(H)	15(A), 25(N)	Idling Volvo diesel engine	0.7	N/A	108	120	N	Y	N, L, MC, E, ECP, IL-1β, IL-6, IL-8, IL-10, IFNɣ, CXCL1, TNF -α, NF-κB, CCL5, LSP, VCAM-1, sICAM-1	For healthy - ↑ N, L, LSP, IL-6, IL-8, VCAM-1; For asthmatics - ↑ IL-10; ↓E
Channell 2012	Non-randomised cross over	H	25.3 (mean)	7	Diesel exhaust from a Cummins engine	0.8[$]	106	N/A	120	N	Y	ICAM -1, VCAM-1, IL-8, MCP-1	↑ VCAM-1

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

Appendix D – Repeat exposure experimental studies

Table D.1 Repeat exposure experimental studies examining exposures to NO_2 and lung function, ranked by NO_2 concentration and stratified by exposure time*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Studies ≤ 30minutes										
Koenig 1987	Randomised Cross over	A, H	11 to 19	10(A), 10(H)	0.12	N	30	N/Y	FEF50, FEF75, FEV1, FVC	
Koenig 1987	Randomised Cross over	A, H	11 to 19	10(A), 11(H)	0.18	N	30	Y	FEF50, FEF75, FEV1, FVC	
Ezratty 2014	Randomised Cross over	A,N	20-69	19	0.2	N	30	N	FEV1, PEF	
Barck 2005 a	Randomised Cross over	A, NS, ES	23-48	18	0.26$	N	15	N	FEV1, sRaw, TGV	
Strand 1998	Randomised Cross over	A, N, ES	21-52	16	0.26$	N	30	N	sRaw, TGV, FEV1	
Ezratty 2014	Randomised Cross over	A,N	20-69	19	0.6	N	30	N	FEV1, PEF	
Studies > 60 minutes										
Rubinstein 1991	Randomised Cross over	H, N	18-45	5	0.6	N	120	Y	sRaw, FEV1, FVC	
Blomberg 1999	Non-randomised cross over	H, N	21-32	12	2	N	240	Y	FEV1, FVC	↓ FEV1 ,FVC

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

Table D.2 Repeat exposure experimental studies examining exposures to NO_2 and airway responsiveness, ranked by NO_2 concentration and stratified by exposure time*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Studies ≤ 30minutes										
Barck 2005 a	Randomised Cross over	A, NS, ES	23-48	18	0.26$	N	15	N	FEV1, sRaw, TGV	
Strand 1998	Randomised Cross over	A, N, ES	21-52	16	0.26$	N	30	N	sRaw, TGV, FEV1	↓FEV1

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.

Table D.3 Repeat exposure experimental studies examining exposures to NO_2 and inflammatory, cellular and biochemical markers, ranked by NO_2 concentration and stratified by exposure time*

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Studies ≤ 30minutes										
Ezratty 2014	Randomised Cross over	A,N	20-69	19	0.2	N	30	N	E, N, M, ECP	
Barck 2005 a	Randomised Cross over	A, NS, ES	23-48	18	0.26[$]	N	15	N	E, N, ECP, MPO	After allergen challenge, ↑ ECP; ↓ MPO
Ezratty 2014	Randomised Cross over	A,N	20-69	19	0.6	N	30	N	E, N, M, ECP	↑ %E; ↑ ECP compared to baseline measure
Studies > 60 minutes										
Rubinstein 1991	Randomised Cross over	H, N	18-45	5	0.6	N	120	Y	L, LSP	
Blomberg 1999	Non-randomised cross over	H, N	21-32	12	2	N	240	Y	M, L, N, MC, LSP, ICAM-1, NE, MCT, Tp, Alb, M-his, IL-8, MPO, HLA, GSH, GSSG, AsA, UA	↑N, MPO; ↓N, Alb

Author	Study design	Study population	Age	N	NO_2 (ppm)	NO_2 exposure as part of traffic pollution	Exposure time (mins)	Intermittent Exercise	Endpoint (outcome measure)	Values driving statistical significance
Pathmanathan 2003	Randomised Cross over	H, N	21-32	12	2	N	240	Y	IL-5, IL-6, IL-8, IL-10, IL-13, CXCL1, NF-κB, sICAM-1, TNF-α, GM-CSF, Eot	↑ IL-5, IL-10, IL-13, sICAM-1
Solomon 2000	Randomised Cross over	H, N	24-40	15	2	N	240	Y	E, N, M, L, LSP, Le	↑ %N; ↓% some LSP

*Studies in red signify a statistically significant result only (clinical relevance not considered); #See Key Terms – **Appendix A** for outcomes; ↑,↓ = significant increase or decrease; ^ A = asthmatics, N = non smokers, ES = exsmokers, S = smokers, H = Healthy, COPD = Chronic Obstructive Pulmonary Disease

$ = conversion from µg/m^3 to ppm using UK Air Pollution Information System converter for NO_2 http://www.apis.ac.uk/unit-conversion, Temperature 25 degrees Celsius.