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AIR QUALITY IN ROAD TUNNELS

HEALTH EFFECTS OF NITROGEN DIOXIDE

AND ASPECTS ON CO-POLLUTANTS

LUFTKVALITET I TUNNLAR

HÄLSOEFFEKTER AV KVÄVEDIOXID
OCH ASPTEKTER PÅ ANDRA LUFTFÖRORENINGAR

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Rapporten beskriver kunskapsläget för hälsoeffekter av kvävedioxid och möjlig interaktion med andra komponenter i vägtunnelmiljö.

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Förord

Det är av fundamental betydelse att Vägverkets tunnlar kan användas utan att någon negativ hälsopåverkan uppstår. En tunnels ventilationsanläggning måste därför utformas, drivas och underhållas så att tunnelluften hela tiden har acceptabel kvalitet. En dimensioneringsförutsättning är, bland andra, vilka gränsvärden för föroreningshalterna som kan användas.

Denna rapport har framtagits av Vägverket för att få bättre kunskapsunderlag om hälsoeffekterna av tunnelluftens kvävedioxider och en eventuell interaktion med andra luftföroreningskomponenter. Resultatet kommer att användas i det fortsatta arbetet med att förbättra underlagen för dimensioneringen av en tunnels ventilationsanläggning.

Inom Vägverkets ramprojekt Miljöanpassade tunnlar har detta FoU-projekt genomförts av Thomas Sandström, Bertil Forsberg och Gunnar Bylin.
För studien värdefulla synpunkter har framförts vid ett seminarium i Stockholm den 14 maj 2002.
Vägverkets projektledare har varit Bernt Freiholtz och Ove Sundmark. Bo Bjerre har medverkat som medicinsk rådgivare.

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SAMMANFATTNING

Denna rapport har författats på uppdrag av Vägverket för bedömning av luftkvalitet i vägtunnlar. Projektet var avsett att beskriva kunskapsläget för hälsoeffekter av kvävedioxid (NO_2) i vägtunnelmiljö, samt eventuell interaktion med andra luftföroreningskomponenter. De effekter som skulle beaktas skulle kunna uppträda vid koncentrationer och maximal exponeringstid i svenska vägtunnlar (högst 15-30 min).

Rapporten beskriver det aktuella kunskapsläget baserat på epidemiologiska befolkningsstudier samt experimentella studier på människor. Det framkommer att personer med astma, vilka i Sverige utgör 6-8 % av den vuxna populationen, är betydligt känsligare vad gäller negativa hälsoeffekter av luftföroreningar som NO_2 än friska personer. Personer med astma kan då reagera med luftrörssammandragning, samt ökad känslighet i luftrören för kontakt med irritanter och allergen som tex pollen. Effekter av denna art har påvisats vid exponering för NO_2 samt även i svensk tunnelmiljö (Söderledstunneln) och kan leda till astmaförsämring och astmaattacker.

Det noteras att det finns ett påtagligt forskningsbehov beträffande effekter hos andra grupper av potentiellt känsliga individer än vad som hittills studerats. Detta kan röra sig om barn, personer med svårare astma, kroniskt obstruktiv lungsjukdom (KOL) samt individer med hjärt-kärlsjukdom. Därtill saknas betydande forskningsinformation om effekter av andra luftföroreningskomponenter i vägtunnel miljö vid sidan av NO_2 , t ex olika partikelkomponenter. Effekter av mer än enstaka exponeringstillfällen saknas i stort sett helt, varför långtidseffekter är svåra att bedöma.

Beträffande gränsvärdessättning tar man ofta i beaktande effekter hos mer känsliga individer. Som nämnts ovan finns idag begränsad information vad gäller astmatiker, medan det i stort sett saknas helt för övriga potentiellt känsliga grupper såsom barn, personer med KOL samt personer med hjärt-kärlsjukdom. Det går därför inte att i nuläget föreslå någon exakt och säker gräns, utan ett eventuellt beslut måste baseras på en kompromiss baserad på bästa tillgängliga data. Även om andra komponenter i tunnelmiljön potentiellt sett kan vara lika eller mer skadliga än NO_2, så finns det inte tillräcklig information för att föreslå någon annan parameter att basera ett gränsvärde på. I den engelskspråkiga huvudrapporten anges därför grad av säkerhet, för- och nackdelar med fyra alternativa gränsvärden, samt på vilka grunder de föreslås.

Alternativ 1: 1000 µg NO_2/m^3

Alternativ 2: 500 µg NO_2/m^3

Alternativ 3: 300 µg NO_2/m^3 under 30 min

Alternativ 4: 150-180 µg NO_2/m^3 under 15-30 min

Abbrevations

CO	Carbon monoxide
ECP	Eosinophil cathionic protein
ELF	Epithelial lining fluid
FEV_1	Forced expiratory volume in first second
FVC	Forced vital capacity
ICAM-1	Intracellular adhesion molecule-1
IgA	Immunogloublin A
IL-4	Interleukin-4
IL-5	Interleukin-5
IL-8	Interleukin-8
IL-10	Interleukin-10
MPO	Myeloperoxidase
NO_2	Nitrogen dioxide
O_3	Ozone
PC20	Provocative concentration giving 20 % decrease in FEV_1
PEF	Peak expiratory flow rate
PM2.5	Particulate matter less than 2.5 μm
PM10	Particulate matter less than 10 μm
ROFA	Residual oil fly ash
sRAW	Specific airway resistance

PREFACE

This report was initiated by initiative from the The Swedish National Road Administration (SNRA, Vägverket) in order to better identify the health effects associated with road tunnel exposure to nitrogen dioxide. NO_2 was to be studied in depth, while some aspects on co-pollutants such as particles, was needed for comparison but not in central focus. The current research knowledge has been described within the frames of the exposure conditions occurring in Swedish road tunnels, with comparisons with higher exposure doses where needed in order to give a broader perspective. The work has not been intended to represent a full criteria document.

Contact person at SNRA/Vägverket has been Ove Sundmark who have directed this project. His support is greatly acknowledged. Additionally the undersigned want to thank Ragnberth Helleday and Anders Blomberg Umeå for assistance in this work.

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PROJECT DESCRIPTION

The following is the project description regarding the work on a criteria document for air quality in tunnels. The Swedish National Road Administration (SNRA, Vägverket) assigned the performance of a study within the research area of "Air Quality in Tunnels in Medical Perspective". The aim of the project was to increase the knowledge regarding how air quality in tunnels affects health status in humans. In this context a document was to be developed. This was expected to give recommendations for the determination of exposure limit values for air pollutants in tunnels.

The project was intended to clarify the current knowledge as regards the medical effects of different air pollutants at exposure times which can occur in Swedish road tunnels (usually maximally 15-30 minutes), both as single exposure as well as daily repeated exposures. The results were expected to demonstrate which types of air pollutants, which with today's knowledge are harmful, and should be limited, and furthermore in which way they are harmful and at which concentration. Special regard should be given to individuals with decreased lung function and/or increased sensitivity in the airways.

The study was to cover nitrogen dioxide and possible interaction with other agents. Additionally, for particles to give an outline description of the current knowledge, a preliminary evaluation of harmful concentrations and exposure times and suggestions for future research needs.

NITROGEN DIOXIDE

Nitrogen dioxide (NO_2) is a common environmental air pollutant. The individual exposure pattern to environmental NO_2 is often characterized by relatively long periods of exposure to low or moderate levels of NO_2 alternating with short periods of high exposure. Exposure to such peaks of NO_2 may occur in a street with heavy traffic or when passing a road tunnel. NO_2 levels varied between 313 and 462 ug/m^3 (30 min averages values) in a road tunnel during morning rush hours (Svartengren, 2000). Concentrations and conditions in the Söderleden tunnel in Stockholm were well described in an earlier report from Vägverket (Jugnelius & Svartengren , 2000).

In the major road tunnels in the Alps and in Norway the NO_2 levels are still higher, and have been reported to exceed 1000 ug/m^3 (Indrehus O & Vassbotn P, 2001).

Air quality standards (limits) and air quality guidelines (expert recommendations) may play an important role in the implementation of control strategies. In terms of guidelines for ambient air quality, the lead agency is the World Health Organization (WHO, 2000a). The objective of WHO's Guidelines for Air Quality is to help countries derive their own national air quality standards, to help protect human health from air pollution. The guidelines are technologically feasible and considering socio-economic and cultural constraints. They provide a basis for protecting public health from the adverse effects of air pollution, and for eliminating or reducing to minimum, air pollutants likely to be hazardous to human health. The guidelines are statements of levels of exposure at which, or below which, no adverse effects can be expected. This does not imply that as soon as a guideline is exceeded adverse effects occur, but rather that the likelihood of such effects occurring would be increased. Based on human clinical data, a one-hour guideline of 200 µg/m^3 is proposed by WHO (2000a);

"At double this recommended guideline (400 µg/m^3), there is evidence to suggest possible small effects in pulmonary function of asthmatics. Should the asthmatic be exposed either simultaneously or sequentially to NO_2 and an aero-allergen, the risk of an exaggerated response to the allergen is increased."

National ambient air quality standards in Sweden have different basis and force of law behind them. The new type of legally binding standards "miljökvalitetsnormer", have been introduced for nitrogen dioxide to be fully established by the year 2006. The 1-hour 98^{th} percentile is 90 µg/m^3. The older limit value (1-hour 98^{th} percentile for October – March) is 110 µg/m^3.

Toxicity, solubility and deposition

NO_2 is a highly reactive, nitrogen-centred free radical and the most toxic of the nitrogen compounds (Morrow et al 1975). It is a poorly water-soluble gas and is therefore deposited far more peripherally in the airspaces as compared to the highly water-soluble sulphur dioxide (SO_2), for example (Miller et al 1992). NO_2 is absorbed along the entire respiratory tract (Moshenin et al 1994). Previous human and animal studies have suggested that the major target sites for the action of NO_2 are the terminal bronchioles. Since NO_2 is a potent oxidant, its main mechanism of pulmonary toxicity has been suggested to involve lipid peroxidation in cell membranes and various actions of free radicals on structural and functional molecules. The airway epithelium is covered by a thin layer of fluid (epithelial lining fluid, ELF) rich in antioxidant defences such as glutathione, uric acid, ascorbic acid and α-tocopherol. NO_2 is not likely to diffuse in unreacted form through the surface lining layer. Under normal circumstances, the oxidative injury of the respiratory epithelium by NO_2 is minimised by antioxidant reactions within the ELF (Blomberg 1999). Antioxidants such as ascorbic acid and α-tocopherol appear to play a role in protecting the organism from the effects of NO_2 (Moshenin et al 1994).

In comparison with another oxidative air pollutant ozone (O_3), which is found in ambient air, nitrogen dioxide is a less potent oxidant. Even though NO_2 is in itself a free radical this has not for certainty been demonstrated to be associated with any enhanced action among the strong oxidants.

NO_2 and bronchoconstriction

Individuals with asthma are by far more sensitive to develop lung function impairment after NO_2 exposure than healthy subjects, according to results from controlled human exposure studies. The main effect of NO_2 in these studies has been on bronchial responsiveness.

In healthy subjects, some studies have reported that exposure of 2400-9000 ug/m^3 NO_2 significantly increases airway resistance (Beil 1974, Stresemann 1980), whereas other studies were unable to find any effects on lung function, in spite of an exposure dose as high as 7000 ug/m^3 (Linn 1985). Short exposures to high ambient levels of NO_2 have been shown to increase exercise-induced bronchospasm (Bauer 1986). It has been concluded that exposure to NO_2 concentrations of below 1 ppm (1800 ug/m^3) do not produce any significant effect on lung function in normal subjects (Bylin 1985, Folinsbee 1978).

In terms of airway reactivity, a meta-analysis of all the published reports on bronchial responsiveness after exposure to NO_2 have shown a statistically significant increase in bronchial responsiveness at concentrations of above 1800 ug/m^3 in healthy subjects and above 180 ug/m^3 in asthmatics (Folinsbee 1992).

In patients with chronic obstructive pulmonary disease (COPD), NO_2 at a concentration of 540 ug/m^3 has been shown to decrease lung function and the NO_2-induced reduction in FEV_1 in normal elderly subjects was shown to be greater among smokers than never-smokers (Morrow 1992).

NO_2 and airway inflammation

Bronchoscopy with BAL after controlled chamber exposures has produced the opportunity to assess the airway effects of air pollutants in man.

Dividing the lavage samples into two fractions has been found to be useful in distinguishing between airway and alveolar inflammation. The use of separate analyses of the instilled aliquots demonstrated an increase in the number of neutrophils in the proximal airways after exposure to an industrial concentration of NO_2 of 6300 ug/m^3 (Helleday 1994). In another study was a small

yet significant increase in neutrophils found after a very high total exposure dose (3600 ug/m^3 for 6 h) (Frampton 1991).

The dose response and time kinetics of the NO_2-induced airway inflammation have been investigated in non-smoking healthy subjects. Dose-dependent increases in mast cells and lymphocytes were found in BAL fluid after a single exposure to the occupational levels 3-9000 ug/m^3 of NO_2 and the airway inflammation resolved within 72 hours after exposure (Sandström 1990, 1991). At NO_2 concentrations below 2 ppm, changes in cell numbers have often not been found. Frampton and co-workers showed significantly increased levels of the antiprotease α_2-macroglobulin together with decreased inactivation of influenza virus by alveolar macrophages collected by bronchoalveolar lavage 3.5 hours after a 3 hour exposure to 1080 ug NO_2/m^3 (Frampton et al 1987).

When using the BAL technique, information can only be obtained about changes in the airways and the alveoli and they might indirectly reflect events in the airway walls.

In a recent study, exposure to 3600 ug/m^3 of NO_2 for 4 hours induced an early (1.5 hours after exposure) increase in Interleukin (IL)-8 in a small volume lavage, bronchial wash (BW) followed by a 2.5-fold increase in neutrophils at 6 hours. In contrast to the BW findings, no signs of inflammatory cell recruitment or upregulation of the expression of vascular endothelial adhesion molecules were seen in endobronchial mucosal biopsies from more proximal airways. Other novel findings here were decreases in total protein, albumin, IgA and soluble ICAM-1 in BW (Blomberg 1997). It was suggested that the increase in neutrophils was most likely due to the enhanced IL-8 secretion, but the mechanism behind the increase in IL-8 and its cellular origin, are still unclear. It is plausible that increase oxidant stress during the exposure to NO_2 is involved. The neutrophilic inflammation in the BW, but no signs of inflammatory cell recruitment in the biopsies, suggests that the major transit of inflammatory cells in the airways following a *single* exposure to NO_2 is restricted to the smaller airways and probably the terminal bronchioles.

The kinetics of the NO_2-induced antioxidant reactions were examined in a study in which healthy subjects were exposed to 3600 ug/m^3 of NO_2 for 4 hours and assigned to bronchoscopy at three different time points, 1.5 hours, 6 hours or 24 hours after each exposure. In both BW and BAL, exposure to NO_2 resulted in rapid losses of ascorbate and uric acid with concentrations returning to baseline levels at 6 and 24 hours respectively. In contrast, GSH levels increased at both 1.5 and

6 hours in BW after exposure to NO_2 and subsequently returned to control levels by 24 hours. The data suggest that antioxidants present in the lung ELF react with, and hence modulate the impact of, NO_2 on the airways (Kelly 1996).

The effects of repeated exposure to NO_2 have been investigated in four studies. Rubinstein *et al.* exposed healthy volunteers to 1080 ug/m^3 (0,6 ppm) of NO_2 for two hours on four separate days within a six-day period. The only significant change was a slight increase in the percentage of NK-cells (Rubinstein 1991). In contrast, repeated exposure to the more occupational levels of 2700 and 7200 ug/m^3 of NO_2 for 20 minutes every second day produced a different cell response in BAL after six exposures. The amounts of lavaged B-cells and NK-cells decreased and the CD4+/CD8+ cell ratio was altered, but no effect on lymphocyte or mast cell numbers was found (Sandström 1992a,b). Recently, the effects of repeated exposure to NO_2 on lung function, airway antioxidant status, inflammatory cell and mediator response were examined in healthy subjects exposed to 3600 ug/m^3 of NO_2 for four hours on four consecutive days. Data from this study revealed significant decrements in FEV_1 and FVC after the first exposure to NO_2 but that these attenuated with repeated exposures. Repeated NO_2 exposure also resulted in a decrease in neutrophil numbers in the bronchial epithelium. Signs of a neutrophilic airway inflammation together with an increase in myeloperoxidase was found in the BW indicative of both migration and activation of neutrophils in the airways. Antioxidant status was unchanged after the forth NO_2 exposure (Blomberg 1999). Taken together, these data suggest that sequential exposure to NO_2 results in a neutrophilic airway inflammation in the absence of significant persistent changes in pulmonary function and depletion of antioxidants.

NO_2 and allergen responses

The effects of NO_2 exposure (and NO_2 combined with SO_2 exposure) on allergen response in allergic asthmatic and allergic rhinitis subjects have been investigated.

Nasal allergen challenge following an exposure to 800 ug/m^3 of NO_2 for six hours significantly increased levels of ECP in nasal lavage fluid, as compared with air exposure in subjects with allergic rhinitis (Wang 1995).

Pre-exposure to NO_2 can more importantly also enhance the asthmatic response to a following inhalation of an airborne allergen. Exposure to NO_2, 800 ug/m^3 for 1 hour, enhanced the asthmatic response to inhaled mite allergen in subjects with allergic asthma (Tunnicliffe, 1994). A similar effect was seen when subjects with allergic asthma were exposed to NO_2 500ug/m^3 for half an hour followed by inhalation of pollen allergen (Strand, 1997). This amplifying effect on the asthmatic response has also been reported after repeated exposure to NO_2 500 ug/m^3 in combination with low, non symptom-causing doses of allergen (Strand, 1998), suggesting that NO_2 may exert this effect commonly on asthma patients. NO_2 enhanced both the early phase (within 15 minutes) and late phase reaction (3 –10 hours after allergen) to allergen. The circumstance that effects may persist long, up to 48 hours has been shown. NO_2 given at a concentration of 720 ug/m^3 in combination with SO_2 7000 ug/m^3 enhanced airway response to inhaled allergen to reach a maximum at 24 hours after exposure but remaining the following day. (Ruznak 1996, Devalia 1994). The combination exposure makes it difficult to more precisely position the NO_2 effect but the principle of maintained effect is important to consider.

Twenty subjects were exposed in a road tunnel for 30 min in order to study the effects of air pollution from traffic on allergic asthma (Svartengren 2000). Four hours later the subjects inhaled an allergen dose and the asthmatic reaction was measured. Subjects exposed to tunnel NO_2 levels $\geq$ 300 µg/m^3 had a significantly greater early reaction following allergen exposure, as well as lower lung function and more asthma symptoms during the late phase compared to control exposure. Subjects exposed to PM2.5 $\geq$ 100 µg/m^3 had also a slightly increased early reaction compared to control. It is likely that the enhancing effect of the automotive air pollution on the asthmatic response to allergen was causally related to NO_2 and PM2.5. But these substances may also have been markers for the concentrations of other toxic compounds in the exhaust, which were not measured in the study.

The mechanisms for this enhancing effect of NO_2 on the asthmatic reaction to allergen are not well understood. There are only two reports in the literature on the effects of NO_2 on separate cells and mediators in the allergic reaction. NO_2 increased eosinophil activation in the early-phase response to nasal allergen provocation in allergic rhinitis (Wang, 1995). Thirty minutes exposure to NO_2 (500 µg/m^3*)* followed by allergen inhalation caused increased levels of eosinophil cationic

protein (ECP) and of neutrophils in bronchial lavage fluid during the late phase of the allergic reaction (Barck, 2002).

In order to mimic the short exposure to NO_2 for a person passing a road tunnel subjects with allergic asthma were exposed to NO_2 (500 µg/m^3) for 15 minutes in the morning one day (Barck 2000). The following day the subjects were exposed to NO_2 for 2 x 15 minutes with two hours between the exposures. The exposures to NO_2 were both days followed same hours later by inhalation of a low dose of pollen allergen. NO_2+allergen increased the levels of ECP more in both sputum and serum than after the control exposure with air+allergen. Moreover, the neutrophil counts in serum were higher after NO_2+allergen than after air+allergen and so were the myeloperoxidase (MPO) levels in serum. Symptoms and pulmonary function were not affected by NO_2. The pre-exposures to NO_2 for 15 minutes thus enhanced the allergen induced inflammation in the bronchi without giving any obvious clinical symptoms after two days of combined exposure. However, the sub clinical airway inflammation caused by repeated, short exposures to NO_2 followed by allergen could increase the risk for chronic lesions of the lung. ECP released from eosinophils may contribute to the remodelling of the airways seen in patients with asthma. Furthermore, increased numbers of neutrophils in the bronchi seems to be associated with more severe forms of asthma and the finding of an enhanced neutrophil response after NO_2+allergen thus merits attention.

EPIDEMIOLOGICAL STUDIES

Epidemiological studies of short-term exposure to NO_2 - especially as a vehicle exhaust indicator in ambient air

The main advantage of the epidemiological studies is that they reflect the integrated real world exposure mixture, with a few pollutants used as indicators. This also results in a number of

limitations (Forsberg & Bylin, 2001). Traffic exhaust is the main source of nitrogen dioxide (NO_2) in most areas. In such environments a close correlation is expected between nitrogen oxides and other components in vehicle exhaust, *e.g*. exhaust particulates (soot) and carbon monoxide (especially with peak hour levels). In the absence of data on ultra fine particulates and carbon monoxide, nitrogen oxides could be used as an indicator also of those pollutants. Similarly, in epidemiological analyses where nitrogen dioxide is used without control for correlated pollutants, *e.g.* particulates, it should be seen as a pollution indicator. Thus, an observed effect of nitrogen dioxide may not be caused by nitrogen dioxide alone, and if not, the effect estimate could be seen as "biased". Since most epidemiological studies of health effects from ambient levels of nitrogen dioxide did not include other components in vehicle exhaust, the role of nitrogen dioxide alone cannot today be well enough separated from the effects of co-pollutants. Studies reporting effects of particles could in the same way be biased. However, in many studies PM10 and PM2.5 include a large proportion of secondary particles, *e.g.* sulphates, why the correlation with carbon monoxide and nitrogen oxides may be low.

Epidemiological studies are often categorised based on different dimensions, such as the directionality of time in design. Cross-sectional (Prevalence) studies and Follow-up (Cohort) studies usually build on geographical differences in the urban background long-term air pollution concentrations. However, most longitudinal air pollution studies focus on effects of "short-term exposure" (fluctuations in urban background concentrations) in a population or a recruited study panel. In these studies "short-term exposure" usually means that the 24-hour averages are used. For nitrogen dioxide, carbon monoxide and ozone, also the daily 8-hour or 1-hour maximum could be used. Since the major part of studies use the urban background pollution concentration as exposure information locally emitted pollutants, such as exhaust gases, may close to busy streets on average occur in more than twice the urban background concentration. In street canyons and road tunnels these concentrations may during rush hours be several times higher than the urban background concentration. On the other hand, most people seldom spend more than short periods of time in such "hot spots". However, personal exposure or activity patterns cannot be studied in the typical population studies, *e.g.* register studies of hospital admissions. Thus, the impacts of these peaks in exposure are not separately evaluated in the epidemiological literature.

In fact, most people spend only a few hours outdoors each day, which means that they may receive their major exposure to pollutants from outdoor sources, e.g. nitrogen dioxide, indoors. Especially

elderly people and persons with chronic diseases may be even less exposed in outdoor environments.

The above-mentioned facts show that epidemiological results are difficult to use for a risk assessment focussing on the short exposures to vehicle exhaust in road tunnels, using nitrogen dioxide as a marker.

Short-term exposure in panel studies

Short-term effects of ambient air pollution on respiratory health have been studied in many panel studies, often on children with asthma or respiratory problems. An association between nitrogen dioxide and symptoms in these studies is not generally established. However, some panel studies with adult asthmatics found symptoms associated with very moderate 24-hour levels of exhaust related pollutants such as black smoke (Forsberg et al, 1993) and nitrogen dioxide (Forsberg et al, 1998).

There is more support from the panel studies for an effect on lung function of around 1-2 % decrease of the forced vital capacity per 100 $\mu g/m^3$ increase in the daily mean of NO_2 concentration (Ackermann-Liebrich & Rapp, 1999). Ultra fine particles (number concentration) have up till now not been measured in many epidemiological studies. However, in a Finnish panel study of 57 asthmatics, the effect on peak expiratory flow (PEF) of nitrogen dioxide could not definitely be separated from the effect of other traffic generated pollutants, such as ultra fine particles (Penttinen P et al, 2001).

Short-term exposure in respiratory hospital admission studies

As a basis for the impact modelling in the SHAPE Study, Bellander et al (1999) made an overview of hospital admission studies where the effect of nitrogen dioxide was calculated with a simultaneous control for particles (PM10). They found only five studies, with different diagnose groupings, and relative risks ranging between 0,997 and 1,037 per 10 $\mu g/m^3$. For respiratory admissions in elderly over 65 years, two studies gave a weighted estimate 1.1 % (0.2-2.1) per 10

μg/m^3 in the urban background 24-hour mean, which is of the same magnitude as the typical effect of PM10 on respiratory hospital admissions (usually not adjusted for nitrogen dioxide). One common conclusion seems to be that there have not been enough studies on short-term effects of nitrogen dioxide to allow the calculation of relationships for distinct health outcomes (Ackermann-Liebrich & Rapp, 1999, WHO, 2000a). However, in the WHO AirQ programme (WHO, 2000b) default values for relative risk estimates are given for nitrogen dioxide, assuming that double-counting (by adding effects of PM10) will be avoided. For respiratory admissions in elderly (65+) the value is 0.38 % per 10 μg/m^3 as the 24-hour mean and 0.10 % per 10 μg/m^3 as the daily 1-hour maximum. For asthma admissions 15-64 years the value is 0.58 % per 10 μg/m^3 as the 24-hour mean and 0.22 % per 10 μg/m^3 as the daily 1-hour maximum.

Some additional studies from the last years are now available, and the recent paper from APHEA2 is perhaps the most relevant (Atkinson et al, 2001). This study shows that it is difficult to separate the effects between PM10 and nitrogen dioxide, and the study found large changes in the effect estimates when both pollutants were included simultaneously in the analysis.

Short-term exposure in studies of cardiovascular effects

In the SHAPE Study, Bellander et al (1999) made an overview also of cerebrovascular disease (CVD) admission studies where the effect of nitrogen dioxide was calculated adjusted for particles (PM10). They found only three studies and with different diagnose groupings. One study of acute heart disease found a relative risk of 1,042 per 10 μg/m^3 as the 24-hour mean, which is about half of the typical effect of PM10 on heart disease admissions (usually not adjusted for nitrogen dioxide). The two other discussed studies found effects close to zero. Some later admission studies from *e.g*. London and Valencia have reported effects of nitrogen dioxide.

In one panel study from Boston life-threatening arrhythmia had a stronger relation to ambient levels of nitrogen dioxide and black carbon, (24-hour mean, 5 day mean), respectively, than to particles (Peters et al, 2000). The authors concluded that they might be better markers for local traffic related pollution. The previous day 24-hour mean nitrogen dioxide and 8-hour maximum CO concentration was associated with an increase in blood fibrinogen in office workers in London (Pekkanen et al, 2000). This may indicate that short-term exposure to vehicle exhaust increase the risk of cardiovascular events.

Short-term effects on daily mortality

A meta-analysis from European cities in the APHEA study estimated a relative risk of 1.013 per 50 μg/m^3 increase in the daily 1-hour maximum of NO_2 (1.015-1.018 if three Swiss studies were included). Adjusted for particles (black smoke) the effect was halved (Touloumi et al, 1996). Similar results have been reported from other parts of the world. In the WHO AirQ programme (WHO, 2000b) default values for relative risk estimates are given for nitrogen dioxide, assuming that double-counting (by adding effects of PM10) will be avoided. The relative risk is value is 1.003 per 10 μg/m^3 increase in the daily 1-hour maximum.

Occupational Exposure

In occupational epidemiology the effects of long-term exposure is usually studied, why the effect peak exposure versus long-term exposure to vehicle exhaust is not possibly to separate.
A study of pulmonary function and respiratory symptoms in men collecting tolls and directing traffic found tunnel workers to have significantly lower FEV_1 (forced expiratory volume at one second) and FVC (forced vital capacity), more respiratory symptoms, and higher carboxyhemoglobin levels than the bridge workers (Evans et al, 1988). Bridge and tunnel officers working over 20 years had the lowest mean pulmonary function values, the steepest slopes, and the most respiratory symptoms. Another study reports a higher prevalence of all acute symptoms in garage workers than in taxi drivers, and lower spirometric tests in the exposed garage workers than in taxi drivers (Bener et al, 1998).

Exhaust From Ice Resurfacing Machines In Arenas

A number of investigated gas accidents caused by dysfunctional gas-fuelled ice resurfacing machines and poorly ventilated ice-skating arenas have estimated the nitrogen dioxide concentrations to 2000 – 20000 μg/m^3 for hours. Also CO levels from ice resurfacing machines have been very high in some accidents (Grevsten & Bergdahl, 2002). These events have been associated with cough, haemoptysis, chest pain, shortness of breath and headache, often with a delayed onset, in persons generally exposed more than one hour (Rosenlund & Bluhm, 1999, Grevsten & Bergdahl, 2002). Since the exposed individuals usually have been heavily exercising with very high minute ventilation in extremely high gas concentrations (nitrogen oxides and/or

CO), it is difficult to draw conclusion about dose-response relations at lower exposure levels. Additionally the mechanisms mediating effects may be very different between road concentrations and extremely high accidental concentrations

Gas cookers and indoor nitrogen dioxide at home

Studies of respiratory illness associated with exposure to gas cookers and stoves at home have often focussed on school children and usually assessed the nitrogen dioxide exposure as weekly averages, not short-term levels. Thus, it has been difficult to separate and quantify the effect of the peak exposure. It is likely that the risk associated with exposure to gas stoves could not be fully accounted for by mean NO_2 measurements, and that the peak NO_2 exposure may be the critical factor associated with the toxicological and adverse respiratory effects. This hypothesis was proposed in a study, which showed that the use of gas stoves was a significant risk factor for respiratory symptoms in children even after adjusting for NO_2 levels (Garret 1998). This is an obvious problem when trying to extrapolate data to road tunnel situation.

It has also recently become clear that gas combustion in homes may generate high concentrations of other pollutants such as particles in terms of numbers as well as mass, suggesting that NO_2 may not alone be the causal exposure in studies of gas use (Wallace 2001). However, this pollutant mixture may be very different from the traffic generated in road tunnels. As a consequence these studies in homes may not necessarily be considered as single pollutant studies with NO_2.

Some large and powerful prevalence studies have not found an association of the presence of a gas cooker or level of indoor nitrogen dioxide with respiratory disease in children, others found an increased risk of lower respiratory illness. A meta-analysis including 11 studies on the health effect of NO_2 found that the relative risks of lower respiratory tract illnesses in children associated with exposure to a 30 $\mu g/m^3$ increase in NO_2 ranged from 0.63 to 1.53 with an overall estimate of 1.18 (95% CI 1.1 to 1.3) (Hasselblad 1992), but some more recent studies have shown no or weaker effects. In a very large survey from ISAAC in Great Britain, involving more subjects than were available for the meta-analysis, the use of gas for cooking was associated with slightly raised odds ratios, of which only one was significantly different from unity (Burr 1999).

Adult women, who use the cooker the most, may be the group at greatest risk. The European Community Respiratory Health Survey (ECRHS) which analysed data collected from young

adults aged 20-44 years living in 23 centres in 11 countries showed no association of gas cooking with wheeze in men but an overall positive association in women (ECRSH 1998).

The relationship of adult respiratory symptoms to cooking fuel used in childhood has been investigated in a cohort study. No association was found between the incidence or prognosis of asthma, allergic sensitisation, and the use of gas for cooking in childhood or adulthood. However, the use of gas for cooking in adulthood was associated with reduced ventilatory function in men (Moran 1999).

Ng et al investigated the immediate airflow response to acute exposure from single episodes of gas cooking, and peak airflow variability from continued exposure to repeated episodes of gas cooking in a small group of non-smoking asthmatic women in Singapore (Ng 2001). The acute short-term level of nitrogen dioxide (NO_2) during gas cooking episodes and the mean exposure to NO_2 from repeated gas cooking episodes were measured over a 2-week period. The short-term exposure to NO_2 during cooking ranged from 0.03 $\mu g/m^3$ to 490.9 $\mu g/m^3$ with a mean level of 121.2 $\mu g/m^3$. The duration of cooking ranged from 5 to 34 minutes, with a mean of 15 minutes. The mean NO2 exposure level during the 2-week study period was 80.5 $\mu g/m^3$, with minimum and maximal concentrations of 37.3 µg/m3 and 135.6 $\mu g/m^3$. The frequency of cooking over a 2-week period was positively correlated ($r=0.53$) with the mean exposure to NO_2, suggesting that gas cooking contributes about 28% of the ambient exposure to NO_2 in these women. The acute short term NO_2 level during cooking was significantly correlated with the fall in PEFR (Peak expiratory flow rate) ($r=-0.579$; $p=0.019$). The mean fall in PEFR after cooking was -3.4%. Continued exposure to NO_2 over a 2-week period was negatively associated with PEFR variability and respiratory symptom severity score, probably due to the masking effects of increased bronchodilator treatment.

In summary, the studies of nitrogen dioxide from gas cookers and stoves have usually assessed the exposure levels in terms of weekly averages. The studies suggest an increase in respiratory illness but unfortunately it is difficult to determine the concentrations in relation to the duration of exposures to really compare with road tunnel situations. Additionally, other components like gases, hydrocarbons and particles formed during the use of the stoves or heaters may be responsible for an uncertain amount of the observed effects. Thus, gas stove and cooker exposures in homes appear not an ideal model for determining exposure limits for traffic situations, where traffic related PM components additionally play a role.

CONCLUSIONS FROM EPIDEMIOLOGICAL STUDIES ON NO_2 EFFECTS

In the epidemiological studies "short-term exposure" usually means that the 24-hour averages or the daily 8-hour or 1-hour maximum are used. Most studies use the urban background concentration as exposure information, why impacts of peaks in exposure, *e.g.* in rush hour traffic, are not separately evaluated. Locally emitted pollutants often have a close correlation. In such situations the effects of nitrogen oxides, exhaust particulates and carbon monoxide cannot be well separated. In the absence of data on ultra fine particulates (or soot) and carbon monoxide, nitrogen oxides could act as an indicator also of those pollutants. Since many epidemiological studies of health effects from ambient levels of nitrogen dioxide or PM10 build on one-pollutant models (did not control for other components), the effect may result also from co-pollutants. However, in many areas PM10 and PM2.5 include a large proportion of secondary particles, *e.g.* sulphates, why their relation to vehicle exhausts components may be weak. A common approach in modern epidemiological studies of short-term exposure is to assume a no threshold linear effect in regression analyses. With this assumption a large part of these studies have reported positive associations with 24-hour average concentrations, especially for particles and with lung function, admissions and mortality. This indicates that an increase in exposure at all levels seems to contribute to adverse effects, and that the dose-contribution could be used as a crude marker of the increase in risk.

CONCLUSIONS FROM EXPERIMENTAL STUDIES ON NO_2 EFFECTS

From experimental studies with nitrogen dioxide it is clear that asthmatic individuals, who in Sweden make up to 6-8% of the population, are more susceptible than healthy people. Individuals with asthma may respond with increased bronchial responsiveness to non-specific irritants and even increased responsiveness to allergen after exposure to nitrogen dioxide at concentrations occurring in Swedish tunnel milieu. Exposure duration has been 30 minutes or more to produce these effects. It can be argued that shorter exposures may potentially not elicit these effects and shorter time (such as 15 minutes) exposures have not been evaluated in published reports yet. However, there are preliminary data showing that three repeated, 15 minutes long exposures to NO_2 during two days, followed some hours later by low doses of allergen, augment the allergic inflammation in the lower airways. It is possible that more frequent and repeated exposures, especially in combination with pollen or other allergens such as from fur animals, may potentially enhance effects. Likewise, the asthmatic individuals in the studies have been relatively mild and people with more severe asthma may potentially be more responsive.

Subjects with more severe lung disease such as chronic obstructive pulmonary disease (COPD) have so far been little investigated, and not in relation to effects suggested from epidemiological studies. Endpoints related to their increased morbidity have not been enough investigated. The same applies for subjects with cardiovascular disease who in epidemiological studies appear sensitive, but have not yet been well investigated as regards experimental exposure situations similar to road tunnels.

DISCUSSION OF THE ROLE OF PARTICULATES AND OTHER COMPONENTS IN ROAD TUNNELS

Particulate composition

Particulates in ambient air and in road tunnels have a very complex composition, as well as different origins. Apart from the exhaust particles from engines there are particles, which have been shed from the road surface, quartz particles, rubber from tyres, break pad particles, as well as metals from tyre spikes and other parts from vehicles. Diesel and gasoline engine particles show differences. Diesel engines normally produce many fold higher numbers of ultra fine particles compared with gasoline engines. The exhaust particles commonly have a carbon core, which can be of differing size. Size has per se being suggested to be involved in the toxic effects of particulates, with smaller particles, particularly ultra fine, being more toxic. Particles have also been proposed to accommodate different toxicity whether they are single particles, twins or clusters or aggregates. Additionally, a range of different hydrocarbon species covers the surface of exhaust particles, with diesel being less refined fuel, resulting in hydrocarbons suggested to be more harmful (Pooley 1999).

Metal content and metal valences have been shown to be important. Certain experimental studies and also human experimental studies have proposed certain metal profiles to be associated with a pronounced ability for cell and protein damage. Sulphur particles have in some studies been shown to be of importance, as have sulphur and nitric compounds on particles (Ghio 1999).

Even though a large of proportion of particles in a road tunnel can be assumed to have been generated within the tunnel or vicinity, particulates from ambient air from other sources may also add to the substantial amounts of PM in the tunnel environment as well as road canyons. Hence, particles may have been generated from combustion and industrial processes in Sweden as well as from far distant sources, transported from the wind. Vehicles and wood burning are other sources suggested to be of importance.

In summary particles are an extremely heterogeneous entity as regards size, numbers and physicochemical characteristics, which may account for very different reactivity and toxicity, as shown in range of different experimental in vitro, animal and some human experimental studies.

Particles and epidemiology

Much of the epidemiological research has been focused on the effects of short-term exposure because of the relative availability of time-series data sets from hospital admission registers and mortality registers. Fluctuations in daily ambient particle concentrations have rather consistently been associated with daily non-accidental mortality (approximately 0,8% per 10 $\mu g/m^3$ PM10), hospital admissions for lung- and heart disease (RR:s approximately in the same range as for mortality) and emergency visits for asthma and respiratory problems (Pope & Dockery,1999, Nyberg et al, 2000).

Most mortality studies have reported association between daily deaths and pollution concentrations, on the same day or a day or two before. In early studies, the "best" lag was often chosen in each analysis. This has been criticized as possibly tending to an overestimate of the effect. However, new studies have shown that mortality counts on a given day are impacted by air pollution levels for several preceding days and some papers have also presented the shape of the distributed lag (Zanobetti et al, 2002). It has been speculated that the air pollution hastens the deaths of persons who would die in a few days anyway. Such "harvesting" implies a negative correlation between pollution concentrations and daily deaths at longer lags. These new analyses show that the effect of air pollution is not short-term harvesting, since the effect estimate for particles more than doubles (the APHEA2 estimate becomes 1,6 % per 10 $\mu g/m^3$ PM10) when longer term effects are considered.

The acute effects of particle exposure are also confirmed from a variety of panel based symptom and morbidity studies (Pope & Dockery,1999, Nyberg et al, 2000). However in all these kinds of short-term studies, RR:s are generally for exposure estimates based on the urban background 24-hour mean concentration or longer averages.

The epidemiologic support for particles as an important risk factor for lung- and heart disease and cardiopulmonary mortality is stronger than for other exhaust components such as nitrogen dioxide and carbon monoxide. This may reflect the biological linkages, but may also be related to the difficulties to have representative exposure information. It is well known that especially secondary particles, often a major fraction of PM2.5 (and of PM10 in low pollution areas) show a very homogenous spatial pattern, why variations in population exposure is fairly easy to describe from single monitoring sites. Nitrogen oxides, carbon monoxide and ultra fine particles show stronger

local gradients, and thus the exposure in population studies may be less well described from a few monitoring stations. The epidemiological effects of different pollution components need to be investigated in the light of their ability to represent variations in the population exposure. Modelling of exposure may be one way to have more comparable exposure data.

PM2.5 has generally shown a stronger effect on morbidity and mortality than has PM10 (Pope & Dockery,1999, Nyberg et al, 2000 WHO, 2000b). The explanations may be both methodological (see above) and toxicological. Apart from the size aspects, numbers and resulting differences in cumulative surface area of PM, the detailed chemical composition may be very different. This is currently an area of considerable focus in air pollution research.
However, the health effects of resuspended road dust have hardly been investigated at all. Road dust is to a varying degree included in the common mass measurements of PM10 (and partly in PM2.5), and may explain the major part of the highest recordings. It is likely that the biological effects of such particles may differ from the effect of finer combustion particles and of secondary particles such as sulphates. This need to be investigated both with epidemiological and experimental studies.

EXPERIMENTAL STUDIES

The role of nitrogen dioxide and relationship as regards reactivity and toxicity has been evaluated in a range of experimental studies including in-vitro research, animal studies as well as some human experimental studies. NO_2 has as regards oxidative stress and free radical activity been shown to be less potent than another common oxidative air pollutant, namely ozone. Additive effects have been described. Allergen reaction enhancing effects have not only been described for NO_2, but more also with ozone, and in a large number of animal studies with diesel engine exhaust.

Among particles, carbon black, suggested to be inert and very small, often ultrafine, particles have been shown clearly to be toxic in animal models as well as in vitro cell cultures. Diesel engine exhaust particles have commonly been more reactive, with residual oil fly ash (ROFA) even more so. For ROFA this has mainly been suggested to be due to the metal content, which is very high in residual oil. Differences in hydrocarbon profiles have also been suggested to be importance, with

less refined hydrocarbons often being more reactive. Gasoline particles have so far been so little studies that it can not be determined whether they are toxic and in any extent comparable to ROFA or diesel particles. Proinflammatory effects are not known to any established extent, which puts gasoline particles in a special situation. Earlier studies were mainly directed at exploring potentially mutagenic effects (Richardson et al 1986). Additionally, particles found in tunnel environments such as from the road surface, tyres, break pads and other constituents in road dust have not been explored.

Human experimental exposure studies have been carried out using an exposure chamber, which has been validated giving controlled challenges (Rudell 1990,1994,1996). A range of inflammatory effects has been demonstrated in healthy and asthmatic subjects investigated with nasal lavages, bronchoalveolar lavages, bronchial mucosal biopsies, blood tests. The inflammatory response containing markers of oxidative stress, neutrophilic, mast cell, lymphocytic cellular infiltration of the airways together with enhanced adhesion molecule expression in the bronchial mucosa and activation of the bronchial epithelium to produce a multitude of inflammatory cytokines were identified after exposure to diesel exhaust with concentrations 100 $\mu g/m^3$ with NO_2 1000 μg and 300 $\mu g/m^3$ PM_{10} with 2 700 μg NO_2 (Rudell 1996, 1999b, Salvi 1999,2000). Notably none of these effects were identified to any extent similarly even after single and repeated exposures to NO_2 at doses 8-40 times greater (Blomberg 1997, 1999). An additive or synergistic effect of NO_2 in the diesel engine exhaust is clearly possible, but as regards inflammation development this cannot to a major extent be attributed to NO_2, but rather the particulate component. Additional support for this is the observation by Nightingale and co-workers who exposed healthy volunteers for two hours to resuspended earlier collected diesel particles 200 $\mu g/m^3$ PM_{10} and found changes in exhaled CO and sputum neutrophils and myeloperoxidase (MPO) six hours post exposure (Nightingale 1996) .

As regards bronchoconstriction due to diesel engine exhaust this has been identified at a similar level both in healthy and asthmatic volunteers as reflected by increase in airway resistance (sRaw). Notably FEV_1 and FVC have remained unchanged (Rudell 1996, 1999, Nordenhäll 2000). An interesting observation came from a study where asthmatics treated with inhaled corticosteroids and had a good asthma control, were exposed to diesel engine exhaust with PM_{10} 300 $\mu g/m^3$. Twenty-four hours after exposure metacholine tests were performed and the PC_{20} had decreased from 3.4 to 1.7 mg. This is a difference in doubling concentrations, which is at clinically important

level and means that the asthmatic despite their corticosteroid treatment became very clearly hyperresponsive, which is in response associated with asthma deterioration and exacerbation. The finding such may therefore reflect an association with epidemiological findings. Additionally the healthy and asthmatic subjects have deviated from each other as regards inflammatory response in terms of inflammation and cytokine expression especially IL-10 which yields additional support that asthmatics are more sensitive than healthy to particulate air pollutants (Nordenhäll 2001).

RESEARCH NEEDS

The scientific background for determining whether simply NO_2 or particulate matter or a combination of different air pollutant agents should be used for exposure limits is relatively meagre. The study model used by Svartengren and co-workers exposing subjects in a real tunnel milieu has been shown to be useful. Additional research is needed with different sensitive populations and larger number of individuals. This study model gives the main advantages by studying effects in real road tunnel environments. Study parameters could be extended to cover a wider range of both respiratory and cardiovascular end points. Difficulty, which could be overcome, is to not only study acute effects but cumulative effects over a longer period of time. This could also be addressed in cohort studies over time.

As regards the hypothesis that NO_2 is a major component in the road traffic environment in causing adverse health effects, is supported by a variety of studies by Bylin et al, Strand et al, Barck et al. The challenge studies using environmental chamber exposures have been proven to be useful and could clearly be extended both as regards determining possible threshold concentrations, but also for studying larger sets of populations with different degrees of susceptibility. Just like discussed above, a range of respiratory and cardiovascular endpoints could be used for a more complementary and wide appreciation of NO_2 effects. Additionally longer term studies with repeated exposures can be advocated.

Additionally complementary studies could be directed towards identifying the possible addition of toxic effects of a large variety of both gases and particulate components from the combustion process, lubricants, engine surfaces, break systems, tyres, spikes, the road surface, minerals and dusts of other origin.

Enhanced toxicological understanding of effects in individuals would be welcome. Interventive aspects in reducing air pollution components in road tunnel environments would clearly be beneficial.

The health effects of different pollution components need to be investigated in the light of their ability to represent variations in the population exposure. Modelling of short-term exposure in time-series studies may be one way to have more comparable exposure data. Also in

epidemiological studies particle pollution need to be further characterised in terms of size and composition.

CONSEQUENCES OF USING AN EXPOSURE LIMIT FOR NO_2 FOR REGULATION OF AIR QUALITY IN SWEDISH ROAD TUNNELS

When considering consequences of an exposure limit it is usually taken into account what populations of subjects that are particularly sensitive. As regards responses to automotive associated air pollutants in general, children, asthmatics and individuals with COPD and cardiovascular disease are considered especially at risk. Therefore it seems logical to consider such populations when estimating an exposure limit. For NO_2 there is so far a considerable amount of research that is lacking and the issues of the mentioned populations is far from well addressed. Currently there is only more reasonably good data from studies in asthmatics. These individuals are apparently sensitive to exposure in road tunnel milieu. Today it cannot be excluded that subjects from the other mentioned groups may be similarly or more sensitive as they have not been reasonably well studied. Therefore the discussion below of possible exposure limit does not rest on a very firm foundation, in the light of lack of much needed scientific research, but on compromises based on the best available data.

As given in fig 1 a range of different responses have been identified in experimental studies with asthmatic subjects, which have used a variety of NO_2 concentrations and exposure time. Clear dose response relationships have not been clarified which may partly be due to the relatively low number of studies performed. The exposure duration has been varying in the relatively few studies and it must therefore be emphasised that there is not substantial data that can allow for detailed comparisons of differences in effects over a larger range of exposure times, that may be relevant for road tunnel travel.

As transit through road tunnels is expected to be limited in time, issues like increased bronchial sensitivity to inhaled allergen, airway inflammation and eosinophil activation are the end points which have been shown to be increased after brief exposures of 30 minutes or less. Effects have thus been identified by single exposure of 300 $\mu g\ NO_2/m^3$ and above. Since lower concentrations of NO_2 have not been studied, thresholds for effects have not been identified. Neither has it been well clarified to what extent PM from different sources found in road tunnels (or homes with gas stoves or heaters) add to the toxicological effects that have been linked with NO_2 exposure. Therefore there is not possible to discuss co-variation aspects between PM from different sources and with different characteristics with NO_2 levels in order to achieve some kind of balanced exposure limits, which would take toxicological effects of multiple pollutants into account. This is

unfortunate as there currently is a major focus on the adverse health effects found to be associated with PM pollution world wide.

With the relatively little information available it is consequently not possible to determine ideal exposure limits based on firm scientific data.

Table 1 presents four different alternatives as an outline of possible alternative exposure limits for NO2 in road tunnels and arguments can be raised in favour for and against these alternatives.

Alternative 1: NO_2 1000 μg/m^3

Such high concentrations clearly contain no margin of protection as increased bronchial hyperresponsiveness and bronchoconstriction have clearly been demonstrated. Additionally complementary effects from particulates may potentially enhance effects, as is also the case for the lower NO_2 concentrations discussed below.

Alternative 2: NO_2 500 μg/m^3

An NO_2-exposure for four days in an exposure chamber increased the sensitivity to allergen inhalation in asthmatics. Exposure to the same concentration three times in two days enhanced the allergic inflammation in the bronchi. This relates to worsening of asthma and increased responsiveness to allergen factors in the environment. As the NO_2 level has not been studied in combination with particulates or other road environment agents it is possible that those would add to the effects. The level is interpreted as not giving a protective margin for sensitive individuals.

Alternative 3: NO_2 300 μg/m^3 for 30 min

The Svartengren study in the Södertunneln road tunnel in Stockholm found an increase in the sensitivity to allergen inhalation in a population of allergic asthmatics. The effect is related to risk of worsening of asthma and which could result in exacerbations. It can be noted that the effects occurred with PM2.5 100 μg/m^3 in the tunnel. If higher PM concentrations would occur in tunnel environment it is possible that a lower NO_2 concentration could be sufficient to produce the identified effects. It can be concluded that a single 30 minutes exposure to 300 μg/m^3 in the Stockholm road tunnel environment did not contain any safety margin for allergic asthmatic

individuals. It can be argued that the majority of the asthmatics could be expected to be less responsive to allergen than the investigated individuals. On the other hand there is clearly a population of asthmatics, which would be even more responsive to allergen, especially if other factors such as virus infection ("common cold") or other provocative event would enhance their airway inflammation and responsiveness. It can also be argued that a more brief exposure such as 10-15 minutes might give an NO_2 dose at a sub threshold level, but this remains to be proven. More susceptible individuals may potentially still respond.

Alternative 4: NO_2 concentration 150-180 μg/m^3 for 15-30 min

The level is based on the calculation that this would be half the concentration mentioned above from the Svartengren study with a 30-minute exposure time. It could therefore potentially contain a margin of safety, even though the actual concentration and time has not yet been investigated in asthmatic or other potentially sensitive subjects.

With a shorter exposure time like 15 minutes this would representative a further step down in exposure dose with potentially reduced risk for sensitive individuals.

It can still be argued that in the case of higher PM levels or other gaseous and particulate components a possible protective margin could be less or non-existent. An argument supporting a level of NO_2 150-180 μg/m^3 at could be that this concentration range would roughly represent twice the concentration and 30 minutes would represent half the exposure time in the proposed Swedish exposure limit for outer air (NO_2 90 μg/m^3 for one hour suggested by Swedish EPA for 2006).

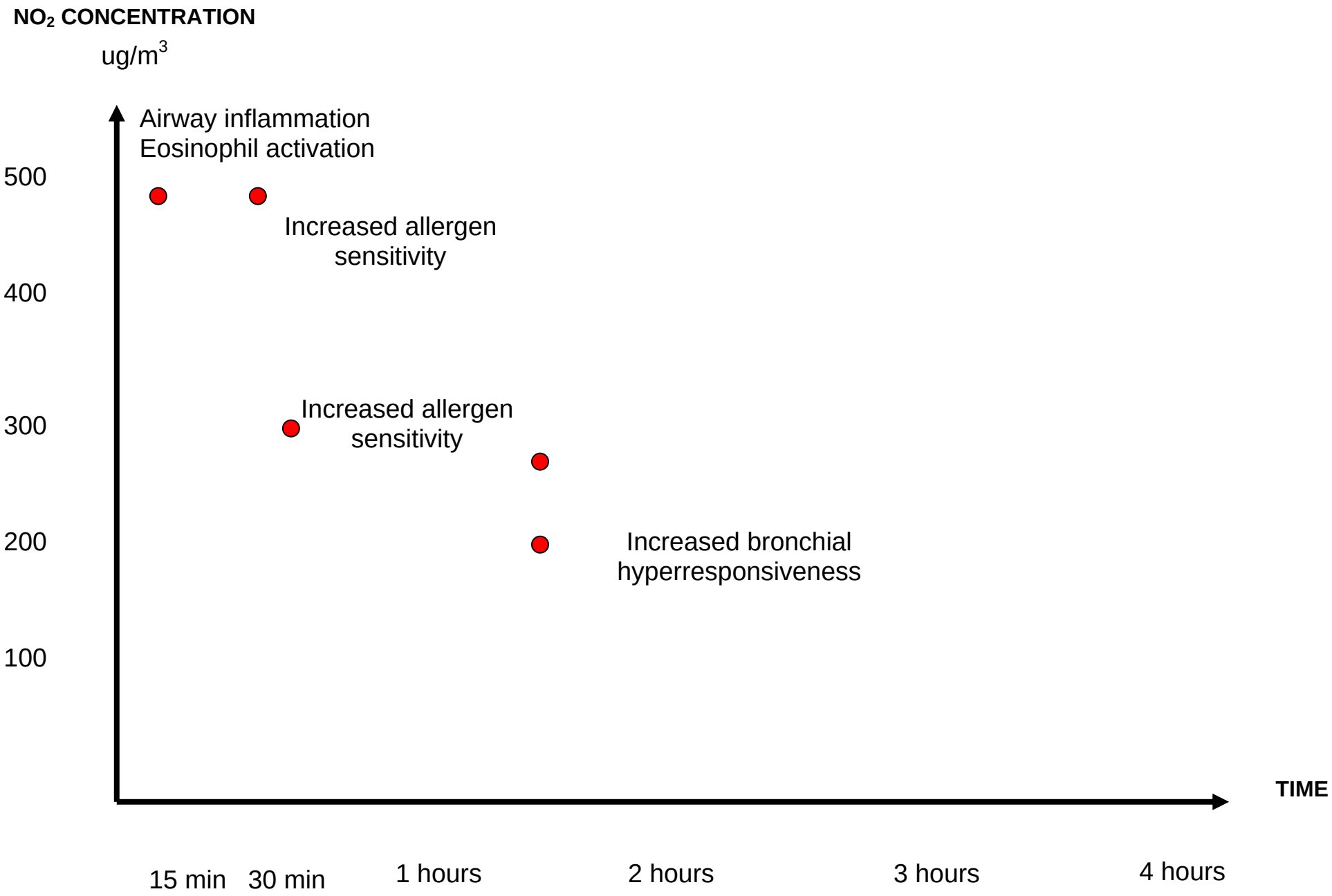
The toxicological and biomedical data are scarce in representing road tunnel environments. In the absence of a more firm foundation for determining an exposure limit, it can be argued that a compromise decision based on an NO_2 concentration given in the alternatives above is motivated. From a purely scientific point of view complementary research is highly motivated if a well-balanced exposure limit is expected.

Table 1

PROPOSED CONCENTRATION LIMIT	EXPOSURE SITUATION	EXP. TIME	EFFECT AND CONSEQUENCE	NOTE	REF
1000ug/m^3	Exposure chamber	30 min – 6 hours	Increased bronchial hyperresponsiveness, bronchoconstriction, cell effects	No particulates included	Folinsbee Bylin Frampton
500 ug/m^3 30 min mean	Exposure chamber	30min x 4 days 15 min x3, 2 days	Increased sensitivity to allergen inhalation. Relationship to asthma worsening Increased allergic inflammation	No particulates included	Strand Barck
300 ug/m^3 30 min mean	Road tunnel	30 min	Increased sensitivity to allergen inhalation. Relationship to asthma worsening	Occurs with PM 2.5 100 ug/m3 in tunnel. If higher PM, lower NO_2 may possibly be sufficient for effect.	Svartengren
150-180 ug/m^3 15-30 min mean	Calculation only		Exposure corresponds to half the concentration, which was identified to give increased sensitivity to allergen in study above. Exposure also corresponds to twice the concentration and half the exposure time as in the proposed Swedish exposure limit for outdoor air 90 ug/m^3 for 1 hour.	Relates to PM 2.5 100 ug/m3 in tunnel. If higher PM, lower NO_2 may possibly be sufficient for effect PM not calculated in model	Svartengren Swedish EPA for 2006

Fig 1

RESPONSES IN ASTHMATICS REPORTED AFTER NO_2 EXPOSURE IN RELATIONSHIP TO EXPOSURE TIME AND CONCENTRATION



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