

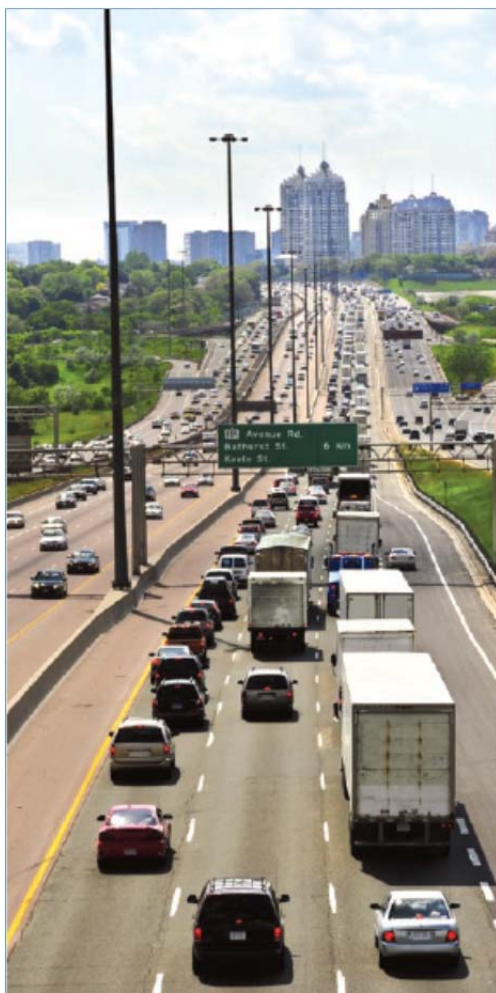


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# Human Health Risk Assessment for Ambient Nitrogen Dioxide



Canada 

# **Human Health Risk Assessment for Ambient Nitrogen Dioxide**

Water and Air Quality Bureau  
Safe Environments Directorate  
Healthy Environments and Consumer Safety Branch  
Health Canada

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Évaluation des risques pour la santé humaine du dioxyde d'azote ambiant

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## List of Abbreviations and Acronyms

$\Delta$	increment
$\alpha$	attenuation factor
$\beta$	regression coefficient
ACS	American Cancer Society
AER	air exchange rate
AHR	airway hyperresponsiveness
AHSMOG	Adventist Health and Smog study
APHEA	Air Pollution and Health: A European Approach
AQCD	Air Quality Criteria Document
AQHI	Air Quality Health Index
AQMS	Air Quality Management System
avg	average
BALF	bronchoalveolar lavage fluid
BAMSE	Children, Allergy, Milieu, Stockholm, Epidemiology survey
BC	black carbon
BLIERS	base-level industrial emissions requirements
BMD	bone mineral density
BPD	biparietal diameter
BS	black smoke
BTEX	aromatic hydrocarbons (benzene, toluene, ethylbenzene, <i>m/p</i> -xylene, and <i>o</i> -xylene)
C	peak concentration
CAAQS	Canadian Ambient Air Quality Standards
CAD	coronary artery disease
CCME	Canadian Council of Ministers of the Environment
CEPA 1999	<i>Canadian Environmental Protection Act, 1999</i>
CHD	coronary heart disease
CHF	congestive heart failure
CHS	Children's Health Study
CI	confidence interval
CIMT	carotid intimal medial thickness
CO	carbon monoxide
COPD	chronic obstructive pulmonary disease
CP	cardiopulmonary
CRP	C-reactive protein
CTS	California Teachers Study
Cu/Zn-SOD	Cu/Zn-superoxide dismutase
CVD	cardiovascular disease
DEARS	Detroit Exposure and Aerosol Research Study
DL	detection limit
DM	diabetes mellitus
EBC	exhaled breath condensate
EC	elemental carbon
ECG	electrocardiogram
ECP	eosinophilic cationic protein
ECRHS	European Community Respiratory Health Survey
ELF	epithelial lining fluid (of the lung)
eNO	exhaled nitric oxide
eNOS	endothelial nitric oxide synthase

EPIC	European Prospective Investigation into Cancer and Nutrition study
EPRI	Electric Power Research Institute
ERVs	emergency room visits
ET-1	endothelin-1
ETS	environmental tobacco smoke
FEF <sub>25–75%</sub>	forced expiratory flow at 25–75% of forced vital capacity
FeNO	fraction of exhaled nitrogen oxide
FEV <sub>0.5</sub>	forced expiratory volume in 0.5 second
FEV <sub>1</sub>	forced expiratory volume in 1 second
Finf	infiltration factor
FMD	flow-mediated vasodilation
FPM	fine particulate matter
FVC	forced vital capacity
GAM	generalized additive model
GEE	generalized estimating equation
GINIplus	German Infant Nutritional Intervention birth cohort
GIS	geographic information system
GLM	generalized linear model
GPx	glutathione peroxidase
GS <sup>•</sup>	thiyl radical
GSH	glutathione
GSR	glutathione reductase
GSSG <sup>•</sup>	glutathione disulphide
H <sub>2</sub> O <sub>2</sub>	hydrogen peroxide
HDL	high-density lipoprotein
HDMA	house dust mite allergen
HF	high frequency
HNO <sub>3</sub>	nitric acid
HR	hazard ratio
HRV	heart rate variability
IBD	inflammatory bowel disease
ICAM-1	intercellular adhesion molecule-1
ICS	inhaled corticosteroid
IgE	immunoglobulin E
IHD	ischemic heart disease
IDW	inverse-distance weighting
IL	interleukin
INMA	Spanish Children's Health and Environment (Infancia y Medio Ambiente) project
I/O	indoor/outdoor ratio
IQR	interquartile range
ISA	Integrated Science Assessment
ISAAC	International Study of Asthma and Allergies in Childhood
IUGR	intrauterine growth restriction
IVF	<i>in vitro</i> fertilization
k	NO <sub>2</sub> decay rate
LBW	low birth weight
LF	low frequency
LISApplus	a prospective birth cohort study on the influence of life-style factors on the development of the immune system and allergies in East and West Germany
LRTI	lower respiratory tract infection
LU	large urban (NAPS station)

LUR	land-use regression
MAL	maximum acceptable level
max	maximum
MEF <sub>25</sub>	maximal expiratory flow at 25% of FVC
MEF <sub>50</sub>	maximal expiratory flow at 50% of FVC
MI	myocardial infarction
min	minutes
MMEF	maximal (mid-) expiratory flow
MMP	matrix metalloproteinase
Mn-SOD	manganese superoxide dismutase
ms	millisecond
MU	medium urban (NAPS station)
NAAQOs	National Ambient Air Quality Objectives
NAPS	National Air Pollution Surveillance
NMMAPS	National Morbidity, Mortality, and Air Pollution Study
NLCS	Netherlands Cohort Study on Diet and Cancer
NN	normal-to-normal intervals in heart rate i.e. beat-to-beat intervals
NN50	the number of pairs of successive NNs that differ by more than 50 ms
$\cdot\text{NO}_2$	nitrogen dioxide free radical notation
NO	nitrogen (nitric) oxide
NO <sub>2</sub>	nitrogen dioxide
NO <sub>2</sub> <sup>-</sup>	nitrite ion
NO <sub>3</sub> <sup>-</sup>	nitrate ion
NOS	nitric oxide synthase
NO <sub>x</sub>	nitrogen oxides
NO <sub>z</sub>	oxidized N species
NPRI	National Pollutant Release Inventory
NU	non urban (NAPS station)
O <sub>2</sub> <sup>•-</sup>	superoxide anion
O <sub>3</sub>	ozone
8-OHdG	8-hydroxy-2'-deoxyguanosine
OC	organic carbon
OECD	Organisation for Economic Co-operation and Development
OR	odds ratio
<i>P</i>	penetration coefficient
<i>p</i>	probability value
PAARC	Pollution Atmosphérique et Affections Respiratoires Chroniques study
PAHs	polycyclic aromatic hydrocarbons
PAN	peroxyacetylnitrate
PAO <sub>2</sub>	pressure of oxygen in arterial blood
PCO	protein carbonyl
PEACE	Pollution Effects on Asthmatic Children in Europe study
PEF	peak expiratory flow
PIAMA	Prevention and Incidence of Asthma and Mite Allergy study
PM	particulate matter
PMN	polymorphonuclear cell
PM <sub>2.5</sub>	PM of 2.5 µm or less in median aerodynamic diameter
PM <sub>10</sub>	PM of 10 µm or less in median aerodynamic diameter
PM <sub>10-2.5</sub>	PM with a median aerodynamic diameter between 2.5 and 10 µm
pNN50	the proportion of NN50, i.e. the number of pairs of successive NNs that differ by more than 50 ms, divided by the total number of NNs

ppb	parts per billion
ppm	parts per million
QTc	time between start of Q wave and end of T wave in the heart's electrical cycle
r	correlation coefficient
R <sup>2</sup>	coefficient of determination
RANCH	Road Traffic and Aircraft Noise Exposure and Children's Cognition and Health
RBC	red blood cell
RHINE	the prospective Respiratory Health in Northern Europe cohort study
ROS	reactive oxygen species
RR	relative risk
$r_s$	Spearman rank correlation coefficient
Rt	total airway resistance
S	potentially industrial source-influenced (NAPS station)
SABA	short-acting beta-agonist
SAPALDIA	Study on Air Pollution and Lung Disease in Adults
SDNN	standard deviation of normal-to-normal intervals
Se	selenium
SE	standard error
SES	socioeconomic status
SIDS	Sudden Infant Death Syndrome
SGA	small for gestational age
SO <sub>2</sub>	sulphur dioxide
SO <sub>4</sub> <sup>2-</sup>	sulphate ion
SRaw	airway resistance
ST	part of an electrocardiogram immediately following the QRS complex and merging into the T wave
SU	small urban (NAPS station)
T site	transportation-influenced (NAPS station)
T wave	repolarization (or recovery) of the ventricles
TBARS	thiobarbituric acid reactive substances
TIMP	tissue inhibitor of metalloproteinase
TNF-α	tumour necrosis factor-α
TNF-RII	tumour necrosis factor-α soluble receptor-II
TP	total power
TRAPs	traffic-related air pollutants
TSP	total suspended particles
µg/m <sup>3</sup>	microgram/cubic metre
UFPs	ultrafine particles
URTI	upper respiratory tract infection
US	United States
US EPA	United States Environmental Protection Agency
VCAM-1	vascular cell adhesion molecule-1
VEGF	vascular endothelial growth factor
VOCs	volatile organic compounds
VSGA	very small for gestational age
Vtg	volume of thoracic gas
WHO	World Health Organization

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# Executive Summary

This *Human Health Risk Assessment for Ambient Nitrogen Dioxide* (NO<sub>2</sub>) is a comprehensive review of the most relevant health- and exposure-related science for this air pollutant, prepared by the Air Quality Assessment Section of Health Canada. This review is intended to support the development of Canadian Ambient Air Quality Standards (CAAQS) for NO<sub>2</sub>; these standards are one of the elements of the federal-provincial-territorial Air Quality Management System.

## Emissions

Nitrogen oxides (NO<sub>x</sub>) are emitted predominantly from combustion sources. Most emissions of NO<sub>x</sub> are as nitric oxide (which is rapidly converted to NO<sub>2</sub>), along with lesser quantities of NO<sub>2</sub> itself. Based on the National Pollutant Release Inventory (NPRI), in 2011 the major ambient releases of NO<sub>x</sub> in Canada were from mobile sources (50% of total emissions), mostly from off-road and on-road diesel engines. Substantial amounts were also emitted from industrial sources (30%), the majority from upstream oil and gas. Lesser quantities were released by the non-industrial category (12%, most of this from combustion-generated electrical power), and by natural sources (7%, the majority from microbial activity in fertilized agricultural soils). The trend in NO<sub>x</sub> emissions as reported to the NPRI from 1985 to 2011 has been generally downward for mobile, non-industrial, and natural sources, whereas industrial emissions have increased over the same time period, largely because of emissions from the upstream oil and gas sector.

## Ambient Levels

Information on ambient NO<sub>2</sub> concentrations in Canada is provided primarily by the National Air Pollution Surveillance (NAPS) network of monitoring stations. Data from this network indicate that NO<sub>2</sub> levels display marked variations in space and time on several scales, often reflecting the important influence of traffic emissions on exposure.

With respect to spatial variation, the highest concentrations of ambient NO<sub>2</sub> occur at transportation- and potentially industrial source-influenced sites. NO<sub>2</sub> levels at other NAPS site types are lower and appear proportional to the degree of urbanicity, probably a function of the parallel changes in emissions from mobile sources, residential heating, and other population-related sources. Various concentration metrics (daily 1-hour maximum (1-h max), 24-hour average (24-h avg), and annual average) demonstrate similar relationships in this regard. There is also large spatial variability in NO<sub>2</sub> in relation to markers of traffic emissions, including distance from roads, traffic volumes, and road length.

Concerning temporal variation, both daily 1-h max and annual avg ambient NO<sub>2</sub> concentrations at various NAPS site types nationwide decreased steadily between 1997 and 2011, attributable to NO<sub>x</sub>-specific regulatory controls on the mobile sector and fossil-fueled electric power generation. All site types also exhibited a common pattern by season, with wintertime maxima and summertime minima, the latter consistent with increased mixing heights and photochemical oxidation of NO<sub>2</sub> and decreased emissions from residential heating compared with winter. Concentrations of ambient NO<sub>2</sub> also vary throughout the day, with two peak concentrations corresponding to morning and afternoon/evening rush hours. NO<sub>2</sub> levels on the weekend are generally lower than those on weekdays and the diurnal peaks are shorter on weekends, likely as a combined result of reduced traffic (especially diesel truck traffic) and the lack of rush hour traffic on weekends.



## Exposure to NO<sub>2</sub> from Ambient Sources

The entire population is exposed to NO<sub>2</sub> originating from ambient sources, both when people are outdoors and when they are in indoor environments into which ambient NO<sub>2</sub> has infiltrated. As they go through their day, some people also spend time in locations that have higher NO<sub>2</sub> concentrations as a result of releases from non-ambient sources (e.g. indoors in homes with gas stoves).

This assessment is being conducted to support the development of an ambient standard for NO<sub>2</sub>, and is based in large part on the extensive epidemiological evidence linking ambient concentrations of NO<sub>2</sub> to a wide range of health effects. In this context, a key issue is the ability of NO<sub>2</sub> concentrations measured by the monitoring network to serve as an indicator of personal exposure to NO<sub>2</sub> of *ambient origin*, as opposed to the total personal exposure to NO<sub>2</sub> from all sources that is measured in most exposure assessment studies.

Studies of the relationship between personal exposures to NO<sub>2</sub> and concentrations measured by ambient monitoring networks have generally shown positive and often statistically significant correlations or regressions between short-term ambient concentrations and total personal exposures. Usually the ambient component of personal exposure to air pollutants is not directly measurable, but the total personal exposure can be regarded as the personal exposure of ambient origin if there are no indoor sources. In those studies where indoor sources of NO<sub>2</sub> were absent, the correlation of personal exposure with ambient concentrations was moderate to strong, and was increased 2- to 3-fold compared with that observed in the presence of indoor sources. In addition, the association between total personal exposures and ambient NO<sub>2</sub> was greater in the warm season (when people are generally more exposed to ambient air pollutants because building infiltration and time spent outdoors are greater) in a number of studies. These findings were confirmed in a recent meta-analysis of a large number of exposure assessment studies.

Overall, the results of these studies indicate that, although the concentrations measured by the ambient monitoring network may not account for differences between individuals in exposure to NO<sub>2</sub> of ambient origin, they appear to be a reasonable surrogate for exposure at a population level. In addition, day-to-day variations in exposure of the population to NO<sub>2</sub> of ambient origin are likely to track changes in the concentrations measured at a central site/sites. It is these variations over time and the ability to represent population average personal exposure, rather than the absolute magnitude of the exposure itself, that are the basis for the associations between ambient NO<sub>2</sub> levels and the health effects reported in short-term epidemiological studies. Therefore, ambient concentrations are a useful and appropriate exposure measure for epidemiological studies of the health effects of NO<sub>2</sub> air pollution.

## Traffic as a Source of Exposure to NO<sub>2</sub>

Vehicle emissions are an important source of NO<sub>2</sub> in urban environments. Large horizontal gradients in NO<sub>2</sub> concentrations near major roadways have been observed in a number of studies; levels on or near roads or in vehicle cabins were several times greater than urban background levels in these studies. Traffic variables are also often significant predictors of ambient NO<sub>2</sub> in land use regression models, and of personal exposure to NO<sub>2</sub>. Near-road NO<sub>2</sub> also displays a negative vertical gradient, with concentrations being increased nearer to the road surface.

Localized emission from roadway sources leads to variability in NO<sub>2</sub> concentrations that is not captured by the existing regional air quality monitoring network. This variation affects population-level exposure estimates and adds exposure measurement error to epidemiology studies that rely on ambient concentrations as indicators of exposure. Elevated concentrations

of NO<sub>2</sub> on or near roadways also increase the exposure of anyone who spends substantial amounts of time in such locations.

### **Correlations of Other Pollutants with Ambient NO<sub>2</sub>**

The associations between ambient NO<sub>2</sub> and co-pollutants released from the same sources need to be considered in interpreting the results of epidemiological studies of NO<sub>2</sub>-related health effects. Causal attribution to NO<sub>2</sub> is challenging because epidemiological associations can potentially reflect correlations with other pollutants rather than true causal association with NO<sub>2</sub>. In most studies, ambient levels of NO<sub>2</sub> are moderately to strongly correlated with traffic-related pollutants such as carbon monoxide (CO) and fine particulate matter (PM<sub>2.5</sub>), and less so with pollutants that are more regional in nature (e.g. ozone (O<sub>3</sub>)) or that originate from other sources (e.g. sulphur dioxide (SO<sub>2</sub>)). Correlations are also moderate between personal NO<sub>2</sub> and ambient or personal exposure to other combustion-related pollutants within urban areas, most notably CO, PM<sub>2.5</sub>, polycyclic aromatic hydrocarbons, certain volatile organic compounds such as benzene and 1,3-butadiene, and PM constituents such as elemental carbon (EC) and organic carbon (OC).

### **General Approach to Assessing Weight of Evidence for Health Effects**

The health effects of NO<sub>2</sub> have been extensively examined in a very large number of studies, including epidemiological studies of health effects associated with NO<sub>2</sub>, controlled human exposure studies in volunteers exposed to NO<sub>2</sub> in experimental chamber studies, and toxicology studies of animals exposed to NO<sub>2</sub> in the laboratory.

In this assessment, epidemiological studies of ambient NO<sub>2</sub> have been weighted more heavily than animal toxicological or controlled human exposure studies for several reasons: 1) epidemiological studies provide the most direct approach for assessing the health effects of “real world” complex mixtures of air pollutants to which people are exposed; 2) human populations are highly heterogeneous as compared with laboratory animal populations and encompass a large range of susceptibilities, disease/illness status and exposures; and 3) no species extrapolation is necessary.

However, the results from animal toxicological studies and especially controlled human exposure studies are still quite relevant and shed light on results from epidemiological studies, particularly with respect to pathophysiological mechanisms underlying observed effects.

In addition, the epidemiology studies are observational rather than experimental, and hence there can be uncertainty as to whether the effects reported in the epidemiology studies are in fact due to ambient NO<sub>2</sub> alone. The NO<sub>2</sub> may be a marker (in whole or in part) for other air pollutants, or the observed association may even be the result of some other factor.

To evaluate the weight of evidence that the epidemiological associations between health outcomes and ambient NO<sub>2</sub> are causal, it is necessary to examine the various lines of evidence in combination and to assess the collective evidence using established criteria for causal determination. In this assessment, the evidence for various categories of health outcomes is reviewed in an integrated fashion, by reporting together the findings from the available epidemiological, controlled human exposure, and/or animal toxicological studies. This collective evidence is then evaluated for various categories of health outcomes in light of considerations that have traditionally been used to form judgments as to whether the observed associations are causal; likely to be causal; suggestive, but not sufficient to infer a causal relationship; etc.

These considerations include:

- the *strength of association*, including the magnitude and precision of the risk estimates and their statistical significance;
- the *robustness* of the associations to model specifications and adjustment for potential confounders such as weather, temporal trends, and co-occurring pollutants;
- the *consistency* of reported associations across studies and study designs conducted by different researchers in different locations and times;
- the *coherence* of the relationship between exposure to NO<sub>2</sub> and related endpoints within and across animal toxicology, controlled human exposure, and various types of epidemiological studies; and
- the *biological plausibility* of the associations in light of what is known regarding NO<sub>2</sub> dosimetry and the types of effects observed and the associated potential mechanisms of action, based largely on animal toxicology and controlled human exposure studies.

## Short-term Respiratory Effects

In short-term controlled studies of asthmatic adults, exposure to near-ambient levels of NO<sub>2</sub> elicited a range of adverse respiratory effects, including decreased lung function, increased airway hyperresponsiveness (AHR), and airway inflammation. Most of these effects, as well as increases in asthma-related respiratory symptoms, were also associated with ambient NO<sub>2</sub> in epidemiological studies of asthmatic children. Respiratory symptoms in asthmatic children were also related to indoor NO<sub>2</sub> in several epidemiological studies, and interventions to reduce NO<sub>2</sub> from gas appliances in classrooms decreased respiratory symptoms. The mechanisms by which these effects occur have been investigated in both humans and animals and provide biologically plausible pathways for these effects.

Ambient NO<sub>2</sub> concentrations were significantly and independently associated with increased respiratory and asthma hospitalizations and asthma emergency room visits (ERVs) in numerous population-based epidemiology studies. These findings are strongly coherent with the experimental and epidemiological evidence for lung function decrements, increased respiratory symptoms, airway inflammation, and increased AHR in asthmatics, and they provide an indication of the public health impacts at a population level arising from the effects on the airways seen in experimental and epidemiological studies.

Thus several lines of evidence indicate that ambient NO<sub>2</sub> is associated with asthma exacerbations. The epidemiological associations with asthma-related endpoints exhibit strength of association, consistency, robustness, and coherence. In conjunction with the experimental findings in animals and humans, the overall evidence indicates that **there is a causal relationship** between short-term exposure to ambient NO<sub>2</sub> at current levels and increased asthma-related morbidity (including airway inflammation and AHR, increases in respiratory symptoms, and asthma hospitalizations and ERVs).

## Short-term Cardiovascular Effects

In population-based epidemiological studies, there were consistent and significant associations of ambient NO<sub>2</sub> with increased cardiovascular mortality, hospitalizations, and ERVs, but these morbidity outcomes were often also related to other pollutants. In addition, the NO<sub>2</sub>-related risks were often attenuated by adjustment for co-pollutants or no co-pollutant models were conducted.

In some panel studies and controlled human exposure studies, there were NO<sub>2</sub>-related decreases in heart rate variability, changes in ventricular repolarization, and increases in inflammatory and/or coagulatory biomarkers. However, the findings in this small dataset were

somewhat inconsistent, and the spectrum of NO<sub>2</sub>-related cardiovascular effects has not been well characterized.

Given the questions surrounding the independence of the NO<sub>2</sub>-related effects and the limited supporting data, the overall evidence is **suggestive, but not sufficient to infer a causal relationship** between short-term exposure to ambient NO<sub>2</sub> and cardiovascular effects.

### Mortality Related to Short-term Exposure

In numerous epidemiological studies of various designs, short-term ambient NO<sub>2</sub> was independently associated with increases in total non-accidental, cardiopulmonary, cardiovascular, and respiratory mortality. These associations were observed in cities from various regions of the world, encompassing different climatic regimes, pollutant mixes, and socioeconomic conditions. However, the coherence of the epidemiological findings with respect to NO<sub>2</sub>-related morbidity that could give rise to mortality from cardiovascular and respiratory causes is somewhat limited, though there is evidence for several elements in the sequences of events that could give rise to increased cardiovascular and respiratory mortality. There are also indications of plausible (albeit non-specific) mechanisms of action for mortality from major cardiovascular and respiratory causes (myocardial infarction, chronic obstructive pulmonary disease (COPD), and respiratory infections).

Overall, the associations with total non-accidental, cardiopulmonary, and to a lesser extent cardiovascular and respiratory mortality display strength of association, consistency, and robustness, but lack some aspects of coherence; it is concluded that there is **likely a causal relationship** between short-term exposure to ambient NO<sub>2</sub> at current levels and these categories of mortality.

### Long-term Respiratory Morbidity

In epidemiological studies, long-term exposure to ambient NO<sub>2</sub> was associated with adverse respiratory effects, especially in children, including reduced measures of lung function and reduced lung function growth. In children, several cohort studies also showed relationships between long-term exposure to NO<sub>2</sub> and the development of asthma and/or allergic responses. Long-term exposure to NO<sub>2</sub> levels appears to increase the incidence of asthma in adults as well. However, some uncertainty remains about the possible role of other co-occurring pollutants in the NO<sub>2</sub>-related respiratory effects.

The epidemiological associations with respiratory health endpoints exhibit consistency, strength of association, and coherence across disciplines, as well as some indication of robustness and biological plausibility. However, considering the questions surrounding the possible role of co-pollutants, the overall evidence indicates that there is **likely a causal relationship** between long-term exposures to current levels of ambient NO<sub>2</sub>/NO<sub>x</sub> and respiratory effects related to the development of asthma or allergic-related disease.

### Other Long-term Effects

Overall, the limited available evidence is **suggestive, but not sufficient to infer a causal relationship** between long-term exposure to ambient NO<sub>2</sub> and each of cardiovascular effects, cancer and related effects, mortality, and reproductive and developmental endpoints. For each of these, there are significant ambient NO<sub>2</sub>-related associations in some epidemiology studies, but the database is lacking in a number of respects, and more research is needed.

### Emerging Effects

A number of other emerging NO<sub>2</sub>-related effects warrant further examination, including those on the central nervous system and on other morbidity outcomes (diabetes, appendicitis, inflammatory bowel disease, otitis media, osteoporosis and rheumatoid arthritis) to determine

whether such effects are consistently observed and occur at relevant concentrations. The emerging evidence that polymorphisms in some genes can influence the association between air pollutant exposures and morbidity effects indicates a potential role for such genetic effect modification in some at-risk populations, and additional research in this area would contribute to a fuller understanding of these gene–environment interactions.

### Potential Confounding by Co-pollutants

The presence of other co-pollutants, especially those that arise from the same sources as NO<sub>2</sub>, such as traffic, complicates the interpretation of the results of epidemiological studies of NO<sub>2</sub>-related health effects. It also makes causal attribution to NO<sub>2</sub> challenging because epidemiological associations can potentially reflect correlations with other pollutants rather than true causal association with NO<sub>2</sub>. For those health effects for which the weight of evidence is relatively strong (i.e. causal or likely to be causal), NO<sub>2</sub>-related risks were generally robust to adjustment for co-pollutants. This was observed most often in models with common air pollutants including PM<sub>10</sub>, O<sub>3</sub>, and SO<sub>2</sub>. However, effect estimates for NO<sub>2</sub> were also generally not sensitive to adjustment for traffic-related pollutants including CO, PM<sub>2.5</sub>, and (in a small number of studies) NO, ultrafine particles, EC/BC or particulate metals, though traffic-related pollutants have not been extensively studied in this regard.

### Subgroups with Increased Sensitivity or Exposure to Ambient NO<sub>2</sub>

Individuals with certain pre-existing diseases appear to be sensitive to exposure to ambient NO<sub>2</sub>. Several lines of evidence from controlled human exposure and epidemiological studies indicate that asthmatics are a susceptible subgroup. There is some evidence (albeit more limited than for asthma) indicating that people with COPD also appear to be more sensitive to NO<sub>2</sub>.

Age is also clearly related to susceptibility. The results of epidemiological studies indicate that children, especially asthmatics, are more at risk of respiratory health outcomes from both short- and long-term exposure to NO<sub>2</sub>. Older adults appear to be more sensitive to short-term effects of NO<sub>2</sub> on respiratory hospital admissions, ERVs and other medical visits, as well as all-cause and respiratory mortality. Older adults also had increased risks for cardiovascular mortality and morbidity in epidemiological studies.

Concentrations of ambient NO<sub>2</sub> are increased near local sources, especially in on-road, near-road, and in-vehicle microenvironments for roadways with heavy traffic. People who spend substantial amounts of time in such locations can have elevated exposures to NO<sub>2</sub>. These would include people who spend a long time in vehicles commuting or during the course of their work (e.g. truck drivers), who work or commute in proximity to major roadways (e.g. roadway construction workers, cyclists), or who reside, work, attend school, etc. in buildings near such roadways.

People engaged in vigorous physical activity would also inhale greater amounts of NO<sub>2</sub>.

### Implications of Exposure Measurement Error

The relationship between ambient concentrations and personal exposure to NO<sub>2</sub> of ambient origin will vary as a result of the influence of a number of factors, including spatial and temporal variability in NO<sub>2</sub> concentrations, time-activity patterns, building ventilation, and perhaps measurement artifacts and analytical methods. The influence of these factors results in exposure measurement error and potential bias in the risk estimates of epidemiology studies that are based on ambient concentrations. The bias can be either upward or downward, though it is expected to most often underestimate risks and make it more difficult to detect a health effect. Several studies performed in Atlanta, GA, investigated the potential bias from using fixed area monitors on the resulting estimates of short-term risk for cardiovascular disease ERVs.



Their results indicated that the spatial heterogeneity of air pollutants was a much greater source of measurement error than instrument imprecision. For NO<sub>2</sub>, most results suggested that this measurement error markedly attenuated the risk estimates, sometimes even resulting in a loss of statistical significance.

Therefore, this source of uncertainty should not change one of the principal conclusions of this assessment, based largely on epidemiological studies, that several categories of adverse health effects are consistently and independently associated with ambient NO<sub>2</sub> concentrations.

## Public Health Impacts

The effects associated with NO<sub>2</sub> have been observed in epidemiological studies in Canada and in other countries at NO<sub>2</sub> concentrations that occur in Canada, well below existing ambient air quality objectives and standards. For those health outcomes for which the weight of evidence and statistical power are greatest (i.e. mortality, respiratory/asthma hospitalizations and asthma-related ERVs for short-term ambient NO<sub>2</sub> exposure, and respiratory morbidity for long-term ambient NO<sub>2</sub> exposure), the mean or median ambient levels at which effects are observed overlap those measured at all NAPS site types, ranging from non urban to transportation- and potentially industrial source-influenced sites. Therefore, adverse health effects in epidemiological studies are occurring at ambient NO<sub>2</sub> concentrations that are commonly experienced in Canada.

In most of the studies that examined the shape of the concentration–response relationship for short-term NO<sub>2</sub>-related mortality or medical visits, there was an approximately linear relationship, with no clear evidence of a threshold. Overall, the current evidence indicates that if a general population threshold exists for the health effects of NO<sub>2</sub>, it is likely to be near the lower limit of ambient NO<sub>2</sub> concentrations. Consequently, the available evidence indicates that any increment in concentrations of ambient NO<sub>2</sub> presents an increased risk for serious health effects, up to and including mortality.

Although the risks for ambient NO<sub>2</sub>-related health effects are relatively small by traditional epidemiological standards, the entire population is exposed, and the subpopulations that have increased sensitivity or exposure to NO<sub>2</sub> (including children, older adults, individuals with asthma or COPD, people engaged in vigorous physical activity, and those spending substantial amounts of time near major roadways) comprise a considerable proportion of the population. In addition, the health impacts that have been the focus of most assessments, including mortality, hospitalizations, and ERVs, represent serious outcomes. Further, these are just the “tip of the iceberg” in the pyramid of health effects associated with ambient NO<sub>2</sub>, and the unmeasured morbidity also has important public health impacts and costs. As a result, the public health impacts of ambient NO<sub>2</sub> are substantial and are expected to remain important as the population ages and the pool of older adults increases, especially given the higher underlying death and disease rates in this age group.

## Exposure Duration

With respect to the durations of exposure that are associated with health effects, the types of health effects, the estimated risks for these effects, and the consistency of the findings are much the same for daily 1-h max and 24-h avg ambient NO<sub>2</sub>. There is also some indication of the same kinds of health effects for other sub-daily averages (e.g. 3-h). These similarities are not unexpected, given that these various short-term exposure metrics are highly correlated with one another. In addition, the overlap between ambient levels in Canada and the concentrations associated with health effects in the epidemiological studies is similar for daily 1-h max, 24-h avg, and even annual or longer-term average NO<sub>2</sub>. In short, the information on health effects associated with ambient NO<sub>2</sub> does not itself provide strong support for any one short-term exposure metric over the other as the basis for the form of the CAAQS. However, the

differences between the types of health effects that are related to short-term versus long-term ambient NO<sub>2</sub> suggest that standards are needed for each of these durations to protect against the associated health effects.

### Support for Development of New CAAQS

This risk assessment was conducted to inform the development of new CAAQS for NO<sub>2</sub> to replace the current National Ambient Air Quality Objectives (NAAQOs). It is recommended that new CAAQS be developed for ambient NO<sub>2</sub> with consideration of the following key conclusions from the health risk assessment:

- there is strong evidence that ambient NO<sub>2</sub> causes both short-term and long-term respiratory effects, and short-term mortality, as well as suggestive evidence linking it to a wide range of other adverse health outcomes;
- these effects have been observed in epidemiological studies at NO<sub>2</sub> concentrations that commonly occur in Canada, well below the levels of existing NAAQOs and other ambient standards, such as provincial/territorial guidelines and the US National Ambient Air Quality Standards;
- in studies examining the shape of the concentration–response curve, there is an approximately linear relationship between ambient NO<sub>2</sub> concentrations and health effects, with no clear evidence of a threshold; hence, based on the balance of the evidence it should be assumed that any increment in levels of ambient NO<sub>2</sub> presents an increased risk for health effects, up to and including mortality;
- the health evidence supports the establishment of both short-term and long-term standards to protect against the full suite of health effects associated with ambient NO<sub>2</sub>.

# 1. Introduction

This *Human Health Risk Assessment for Ambient Nitrogen Dioxide* (NO<sub>2</sub>) is a comprehensive review of the most relevant health- and exposure-related science for this air pollutant. It was prepared by the Air Quality Assessment Section of the Air Health Effects Assessment Division in the Safe Environments Directorate of Health Canada. This review was initiated in 2012 and is intended to support the development of updated Canadian Ambient Air Quality Standards (CAAQS) for NO<sub>2</sub>.

The goals of this assessment are to

- update the available information on adverse effects of NO<sub>2</sub> on human health
- determine Canadian exposure levels
- inform the revision or development of ambient air quality objectives/standards, including, if considered appropriate, proposed health-based benchmarks for NO<sub>2</sub>
- identify knowledge gaps and areas for further research on NO<sub>2</sub>

The following sections describe the context for development of this risk assessment, the process by which it was developed, the organization of its content, and its objectives.

## 1.1 Canadian Air Quality Policy Context

Ambient air quality management in Canada is a shared responsibility among provincial, territorial, federal, and in some cases municipal governments, made possible through an array of commitments and initiatives that have evolved over many decades.

Although provincial governments have the primary responsibility for many aspects of air pollution control, federal actions are integrated with those of the other levels of government. The *Canadian Environmental Protection Act, 1999* (CEPA 1999) provides the federal government with broad authority to address atmospheric emissions of substances that have negative impacts on health and the environment. The *Canadian Environmental Protection Act* was first promulgated in 1988 and was revised and renewed as a new Act in 1999. The focus of CEPA 1999 is pollution prevention and the protection of the environment and human health as a means to promote sustainable development. Health Canada and Environment Canada share responsibility under CEPA 1999 to assess and manage the threats that pollutants may pose; Health Canada focuses on risks to human health, while Environment Canada focuses on risks to the environment, as well as monitoring emissions to and contaminants in the environment.

Federal, provincial and territorial governments work together in partnership under the framework of the Canadian Council of Ministers of the Environment (CCME). In 2012, Canadian Environment Ministers agreed to take further action to protect the health of Canadians and the environment with measures to improve air quality in Canada through a comprehensive new Air Quality Management System (AQMS) (CCME, 2014). The AQMS includes the establishment of CAAQS for air pollutants of concern and new base-level industrial emissions requirements (BLIERs) for major industrial sectors and equipment groups. It also provides for the management of air quality at local and regional levels and a collaborative process to address mobile source emissions. Where CAAQS are exceeded, governments may require further emission reductions from industrial and non-industrial sources of pollution to manage air quality issues. Management levels (levels set below the CAAQS) have also been or are being established to provide indicators of deteriorating air quality, and they may be used by jurisdictions in their own air quality management frameworks. The AQMS will also address the



transboundary movement of air pollutants between provinces and territories and between Canada and the United States.

## 1.2 National Ambient Air Quality Objectives for NO<sub>2</sub>

Governments at various levels in Canada can assess air pollutants and set ambient air quality objectives or standards. These are benchmark levels representing goals for outdoor air quality that protect public health, the environment, or aesthetic properties of the environment. Ambient air quality objectives or standards are primarily effects-based, but they are also reflective of technological, economic and societal considerations. They guide federal, provincial, territorial and municipal governments in making risk management decisions related to air quality concerns.

With respect to NO<sub>2</sub> in particular, in the 1970s the Subcommittee on Air Quality Objectives of the Federal-Provincial Committee on Air Pollution developed recommendations for National Ambient Air Quality Objectives (NAAQOs) for NO<sub>2</sub> and several other common air pollutants (The Subcommittee on Air Quality Objectives, 1976). NAAQOs were developed for various averaging times and for different types of intervention levels, including the maximum acceptable level (MAL) and the maximum desirable level (MDL). For NO<sub>2</sub>, MALs were developed for 1-h, 24-h, and annual averaging times, while an MDL was derived for an annual average (Table 1.1). These objectives were prescribed under the *Clean Air Act*, and when it was subsumed into the first *Canadian Environmental Protection Act* in 1988, the NAAQOs for NO<sub>2</sub> and other common air pollutants were incorporated into the new Act (*Canada Gazette*, 1989).

**Table 1.1: Selected existing NAAQOs for NO<sub>2</sub>**

Standard*	Concentration of NO <sub>2</sub> (ppb)	Concentration of NO <sub>2</sub> (µg/m <sup>3</sup> )
Maximum acceptable level:** 1-h avg	213	400
Maximum acceptable level:** 24-h avg	106	200
Maximum acceptable level:** annual avg	53	100
Maximum desirable level:** annual avg	32	60

\* At 25°C, and 760 mmHg

\*\* The MAL is intended to provide adequate protection against effects on the environment and human health, and represents the realistic objective for all parts of Canada at the time. The MDL defines the long-term goal for air quality and provides a basis for an anti-degradation policy for the unpolluted parts of the country and for the continuing development of control technology.

Under the new AQMS (described in the previous subsection), CAAQS will be established as objectives under CEPA 1999, and will replace existing Canada-wide standards, including the NAAQOs. Initial standards for fine particulate matter (PM<sub>2.5</sub>, or FPM) and ground-level ozone (O<sub>3</sub>) were established in May of 2013. Sulphur dioxide (SO<sub>2</sub>) and NO<sub>2</sub> were identified as the next pollutants for which CAAQS were to be developed under the new system.

## 1.3 Approach for Identification and Inclusion of Assessment Information

This *Human Health Risk Assessment for Ambient Nitrogen Dioxide* presents a concise synthesis and evaluation of the most policy-relevant science regarding exposure and health effects associated with ambient NO<sub>2</sub> in Canada. In conjunction with other relevant information, the assessment is intended to provide the scientific foundation for the development of an updated ambient air quality standard for NO<sub>2</sub>.

This review was initiated in the spring of 2012. Scientific publications to be reviewed were located through an extensive literature search in July 2012. The following databases were searched, from 2007 to the present: *Medline*, *Embase*, *Global Health*, *Scopus* and *Toxline*. The subject areas were kept broad; they included environmental levels, exposure, toxicity, epidemiology, and various health endpoints such as cardiovascular diseases, respiratory diseases, cancer and low birth weights. Additional publications were identified through reference lists of reviewed papers and other recent assessments (notably those by the United States Environmental Protection Agency (US EPA, 2008, 2013) and the World Health Organization (WHO, 2010)), as well as through ongoing Google Alerts. Generally, only information that had undergone scientific peer review and had been published or accepted for publication was considered. In addition, information on emission sources and ambient levels in Canada was obtained from the National Pollutant Release Inventory (NPRI) and the National Air Pollution Surveillance (NAPS) Network databases, respectively.

As discussed in Chapters 8, 9 and 10, the epidemiological literature concerning the health effects of ambient NO<sub>2</sub> is large and expanding rapidly. The epidemiological sections of this report that describe studies of ambient NO<sub>2</sub> use the US EPA's 2008 *Integrated Science Assessment for Oxides of Nitrogen–Health Criteria* (US EPA ISA) as a starting point for the review.<sup>1</sup> The sections initially summarize the findings of this earlier assessment to cover the earlier epidemiological literature for ambient NO<sub>2</sub>, and then critically review original papers that have been published subsequent to this assessment up to and including April 2013. In order to retain the substantial knowledge base covered in the 2008 US EPA ISA as part of the overall weight of evidence, the summary of this earlier literature derived from the US EPA assessment is fairly detailed, and where of utility in illustrating important issues, includes individual references to key earlier papers, i.e. those published prior to 2008. Further, as discussed in Chapters 8, 9 and 10, the various epidemiological sections are focused on studies from countries that are judged to be most relevant to a Canadian assessment with respect to the air pollutant mixture, standard of living, health care, climate, etc. Hence, the discussion of the epidemiological studies concentrates on studies conducted in Canada and the US, as well as in Europe and Australia.

In addition, Health Canada has recently reviewed much of the relevant literature during the course of an assessment of indoor NO<sub>2</sub> to support the development of the Residential Indoor Air Quality Guideline for this pollutant (Health Canada, 2015). Although ambient and indoor sources of NO<sub>2</sub> and related pollutants are thought to be substantially different, and as a result the exposures to and health effects of the respective pollutant mixtures may differ, there are nonetheless a number of subject areas of common interest to these two assessments.

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<sup>1</sup> The US EPA has released two external review drafts of an updated *Integrated Science Assessment for Oxides of Nitrogen* (US EPA, 2013, 2015). These were released for comment well after the cutoff date for inclusion of information in this Canadian assessment, and were labelled as "DRAFT: Do Not Cite or Quote." Consequently, they are not discussed in this assessment, though the 2013 draft ISA was used to identify additional studies that were published within the review period.

Therefore, several of the sections in this report on ambient NO<sub>2</sub> are based on those from the indoor air review. These include the sections on controlled human exposure studies, toxicological studies in animals, toxicokinetics, and mechanisms of action, as well as the summary of epidemiological studies of indoor NO<sub>2</sub>.

## 1.4 Content of the Assessment

This assessment builds upon the 2008 US EPA ISA and critically evaluates relevant information that has become available since that assessment up to the end of April 2013. Evaluation and interpretation of this information is essential to establishing the weight of evidence for the various health effects associated with NO<sub>2</sub> exposure and for determining whether population health impacts can be expected from current ambient exposures.

Following the introductory and physical-chemical properties chapters, the environmental sources and ambient levels of NO<sub>2</sub> in Canada are presented in Chapter 3. Chapter 4 examines the current knowledge regarding exposure of Canadians to NO<sub>2</sub>, with a particular focus on exposure to NO<sub>2</sub> of ambient origin. The dosimetry and toxicokinetics of inhaled NO<sub>2</sub> in the respiratory tract are presented in Chapter 5. Chapter 6 examines the results of toxicological studies in animals, including the mechanisms of action of this pollutant, while Chapter 7 summarizes the results of controlled studies of human volunteers exposed to NO<sub>2</sub>. Chapters 8 through 10 review the epidemiological literature on health effects associated with ambient NO<sub>2</sub>, encompassing studies of each of short-term and long-term exposure, including reproductive and developmental endpoints. A brief summary of the health effects associated with exposure to indoor NO<sub>2</sub> is presented in Chapter 11. The report finishes with a risk characterization (Chapter 12) that integrates key information from the prior chapters to summarize and draw conclusions with respect to: the weight of evidence that the relationship between ambient NO<sub>2</sub> and various categories of health effects is causal; subgroups with increased sensitivity or exposure to ambient NO<sub>2</sub>; ambient sources, levels and exposure; concentration–response (C–R) relationships between ambient NO<sub>2</sub> and key health effects; and key uncertainties in this health assessment.

## 1.5 Objectives of the Assessment

The information and conclusions presented in this document are intended to provide scientific guidance to decision-makers in the review and/or development of air quality policies, including CAAQS for this pollutant. By identifying NO<sub>2</sub>-related human health risks and supporting the establishment of ambient standards to protect against these risks, it will facilitate the development of strategies to reduce the risk NO<sub>2</sub> poses to the health of Canadians. The knowledge gaps identified in this assessment are intended to provide direction for future scientific research so that the available information expands to better support future policy decisions.

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## 2. Physical-Chemical Properties of NO<sub>2</sub>

Nitrogen dioxide is a reddish-brown gas with an acrid, ammonia- or bleaching solution-like odour. It is corrosive and a highly oxidizing agent. An odour threshold range of 200–800 µg/m<sup>3</sup> (~100–400 ppb) is generally reported (WHO, 2010), although one investigation reported an odour threshold of 75 µg/m<sup>3</sup> (40 ppb) in healthy (non-asthmatic) subjects (Bylin et al., 1985). Table 2.1 lists the physical and chemical properties of NO<sub>2</sub>.

Nitrogen dioxide is poorly soluble in water ( $3.7 \times 10^{-2}$  mL/mL) (Table 2.1) and has a very low probability of adsorbing to water surfaces (uptake coefficient  $\gamma \approx 10^{-8}$ ) (Lee and Schwartz, 1981). There are limited solubility data for NO<sub>2</sub> in other solvents, but Signorelli et al. (2011) recently estimated a partition coefficient for NO<sub>2</sub> of 2.7 between n-octanol and water, and 1.5 between lipid membranes and water, indicating that NO<sub>2</sub> is a moderately hydrophobic molecule.

Nitrogen oxides are a large and complex family of compounds, including NO<sub>2</sub>, many of which readily interconvert in both indoor and outdoor air. A similarly complex array of endogenous nitrogen oxides are formed *in vivo* (Guidotti, 1978).

Nitrogen dioxide is a free radical (one unpaired electron), which is key to its reactivity in the environment (WHO, 1997), its role in physiological processes, and its toxic properties (Augusto et al., 2002). For simplicity, the free radical notation (<sup>•</sup>NO<sub>2</sub>) is not used in this report except in chemical reaction equations.

**Table 2.1: Physical and chemical properties of NO<sub>2</sub>**

Property	Value
<b>Molecular formula</b>	NO <sub>2</sub>
<b>CAS number</b>	10102-44-0
<b>Molecular weight</b>	46.01 g/mol
<b>Melting point<sup>a</sup></b>	–11.2°C
<b>Boiling point<sup>a</sup></b>	21.2°C
<b>Solubility in water at 27°C<sup>b</sup></b>	$3.7 \times 10^{-2}$ mL/mL
<b>Diffusion coefficient in water<sup>b</sup></b>	$2.73 \times 10^{-5}$ cm <sup>2</sup> /s
<b>Absorption constant (R)<sup>b</sup></b>	$1.7 \times 10^5$
<b>Vapour pressure<sup>c</sup></b>	720 mm Hg at 20°C
<b>Vapour density<sup>a,c</sup></b>	Relative: 1.58 (air = 1); absolute: 1.449 g/L at 20°C
<b>Henry's Law constant<sup>d</sup></b>	0.01 M/atm at 298 K
<b>Conversion: ppm → mg/m<sup>3</sup><sup>a</sup></b>	1 ppm = 1.88 mg/m <sup>3</sup> ; 1 mg/m <sup>3</sup> = 0.53 ppm at 25°C

<sup>a</sup> From Guidotti (1978) and Graham et al. (1997)

<sup>b</sup> From Tsujino et al. (2005)

<sup>c</sup> From [www.nap.edu/openbook.php?record\\_id=6205&page=147](http://www.nap.edu/openbook.php?record_id=6205&page=147)

<sup>d</sup> From Lee and Schwartz (1981)

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## 3. Environmental Sources and Concentrations of Ambient NO<sub>2</sub>

### 3.1 Introduction

This chapter contains a general discussion of the primary anthropogenic and natural sources of ambient NO<sub>2</sub> in Canada, as well as measured ambient levels, including both recent and historical trend data. It begins with a brief description of the emission of oxides of nitrogen and their interconversion, followed by National Pollutant Release Inventory (NPRI) estimates for Canadian emissions of NO<sub>x</sub> from various anthropogenic and natural sources, which are discussed with respect to the most recent data considered for this assessment (2011). Next, ambient NO<sub>2</sub> data acquired via the NAPS monitoring network, as well as a brief description of the monitoring technique, are presented and discussed. Historical as well as more recently acquired data are categorized in terms of monitoring location with respect to population size (large urban (LU), medium urban (MU), small urban (SU), and non urban (NU)) as well as transportation- and potentially industrial source-influenced stations (T and S). As the short-term exposure indices most commonly employed in the epidemiological literature are the maximum 1-hour average in a 24-hour period (daily 1-h max) and the 24-hour average (24-h avg), data for these indices as well as the annual average, which is the exposure metric employed for chronic epidemiological studies, are presented. Summary statistics of data are presented for these three averaging times across the NAPS site types to illustrate the patterns in ambient NO<sub>2</sub> concentrations measured in Canada on various time scales (seasonal, diurnal, and day of the week) as well as long-term trends (from 1997 to 2011), .

### 3.2 Sources and Emissions of Ambient NO<sub>x</sub>

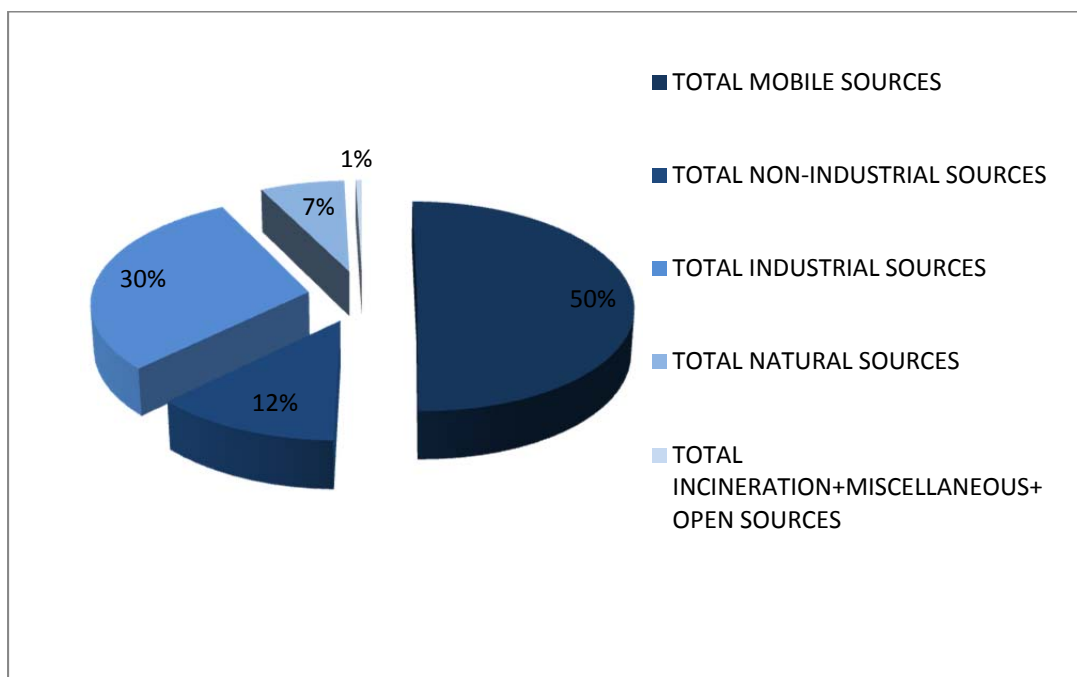
Nitrogen oxides (NO<sub>x</sub>) are emitted primarily as the result of combustion activities and can originate from both anthropogenic and natural processes, though anthropogenic sources dominate the inventory (Figure 3.1). NO<sub>x</sub> is a mixture of two species; nitric oxide (NO) and NO<sub>2</sub>, with the majority of primary emissions being in the form of NO. The two species are grouped together under the heading NO<sub>x</sub> because they are emitted together; however, NO is rapidly converted to NO<sub>2</sub> (a time scale on the order of a minute). While exposure to both can be significant, especially in close proximity to sources, it is NO<sub>2</sub> that is the primary focus of research regarding health effects.

Canadian emission estimates for numerous pollutants including NO<sub>x</sub>/NO<sub>2</sub> are compiled in the NPRI, Canada's legislated, publicly accessible inventory of pollutant releases (to air, water and land), disposals and transfers for recycling. It comprises facility-reported data collected under the authority of CEPA 1999. The NPRI also presents emission summaries and trends for key air pollutants, including NO<sub>x</sub>/NO<sub>2</sub>, based upon facility-reported data and emission estimates for such other sources as motor vehicles, residential heating, forest fires and agriculture.



Figure 3.1 provides a breakdown of the 2011 Canadian NO<sub>x</sub> emissions by the broadest NPRI categories. At a national level mobile sources (transportation) are the dominant NO<sub>x</sub> source, at 50% of the total, with industrial sources contributing a further 30%. Non-industrial (e.g. electrical power generation, commercial fuel combustion) and natural sources combined contributed slightly less than 20% of 2011 NO<sub>x</sub> emissions, with incineration, miscellaneous and open sources together contributing the remaining 1%. Mobile sources are even more important from a human health perspective than this breakdown would suggest, considering that most of the Canadian population lives in urban areas where the bulk of NO<sub>x</sub> emissions are from transportation and to a lesser extent consumer/residential sources (e.g. residential fuel combustion, residential wood combustion); such areas tend to be removed from natural and industrial sources.

**Figure 3.1: 2011 NPRI NO<sub>x</sub> emissions by broad source category\***

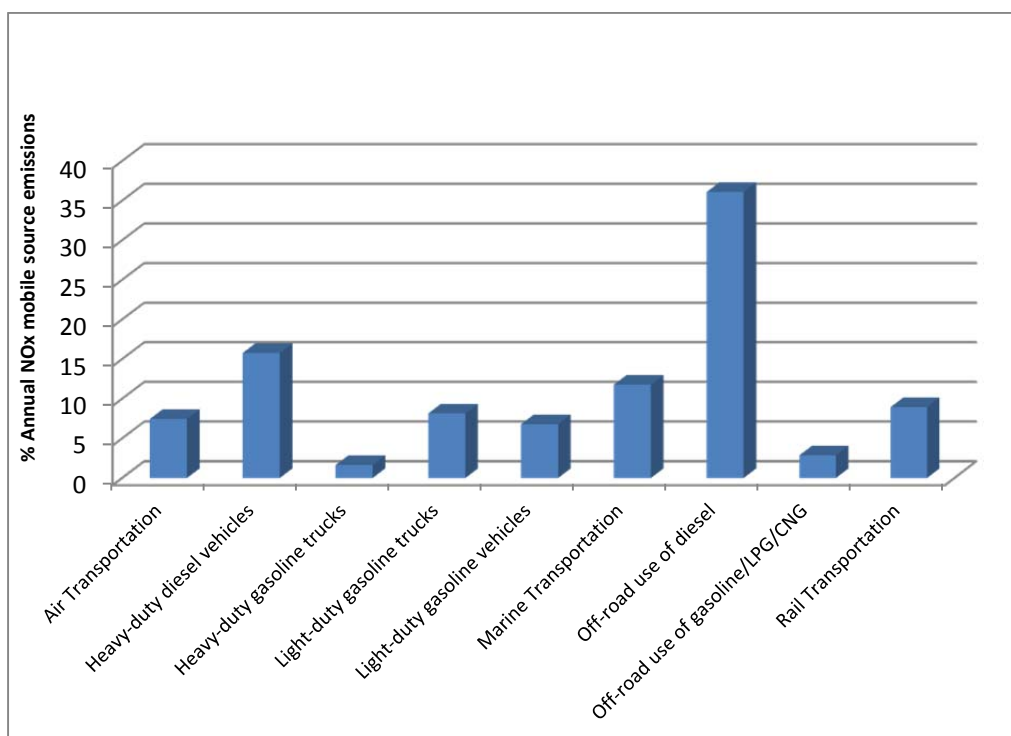


\*From NPRI, <https://www.ec.gc.ca/inrp-npri/>

Figure 3.2 presents the breakdown of 2011 mobile source NO<sub>x</sub> emissions as listed on NPRI. More than 50% of the emissions from this source category arose from the combustion of diesel fuel. Off-road diesel (e.g. mining, construction equipment) was the highest single NO<sub>x</sub> emitting mobile source at 36%, followed by heavy-duty diesel (e.g. transport trucks) at 16% and marine transportation at 12%. Rail transportation, air transportation, light-duty gasoline trucks and light-duty gasoline vehicles (regular passenger vehicles) each contributed between 7% and 9%. Within a typical Canadian city, then, most of the mobile sources listed in Figure 3.2 would be present and contributing to urban ambient NO<sub>x</sub> levels in roughly similar proportions to those outlined above.



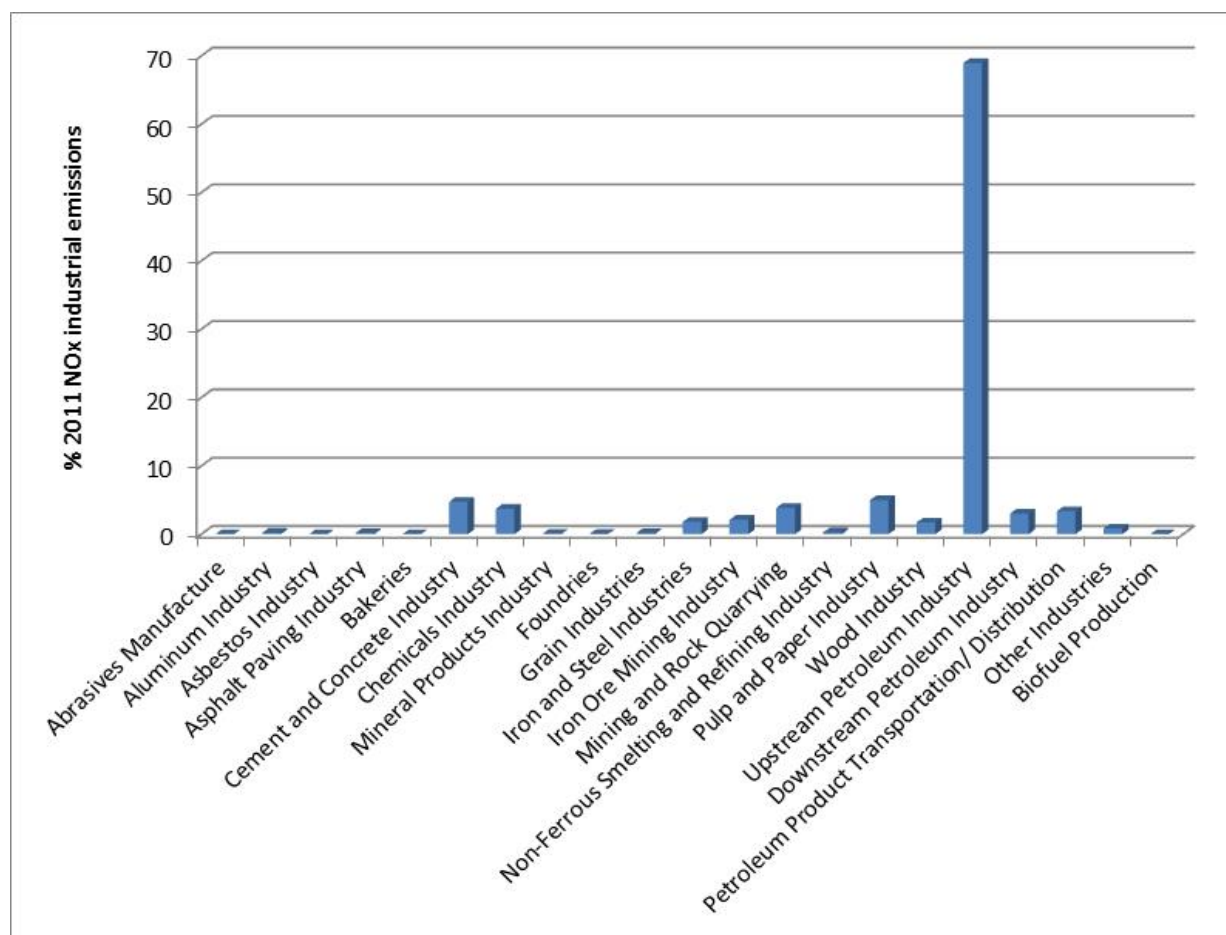
**Figure 3.2: 2011 NPRI mobile source NO<sub>x</sub> emissions by sub-category\***



\*From NPRI, <https://www.ec.gc.ca/inrp-npri/>

With respect to industrial NO<sub>x</sub> emissions (Figure 3.3), upstream petroleum (exploration, production and basic processing) was by far the largest single source category at almost 70% of reported emissions, with the pulp and paper and cement and concrete industries being the next highest contributors at approximately 5% each. The remaining 20% was scattered among the other 18 distinct industrial sectors as defined in the NPRI database (Figure 3.3). Hence, with the exception of upstream petroleum operations, the ambient NO<sub>x</sub> contributions by each of various industrial sectors operating within Canada and reporting to NPRI are relatively small and approximately equal (between <1% and 5% of the total).

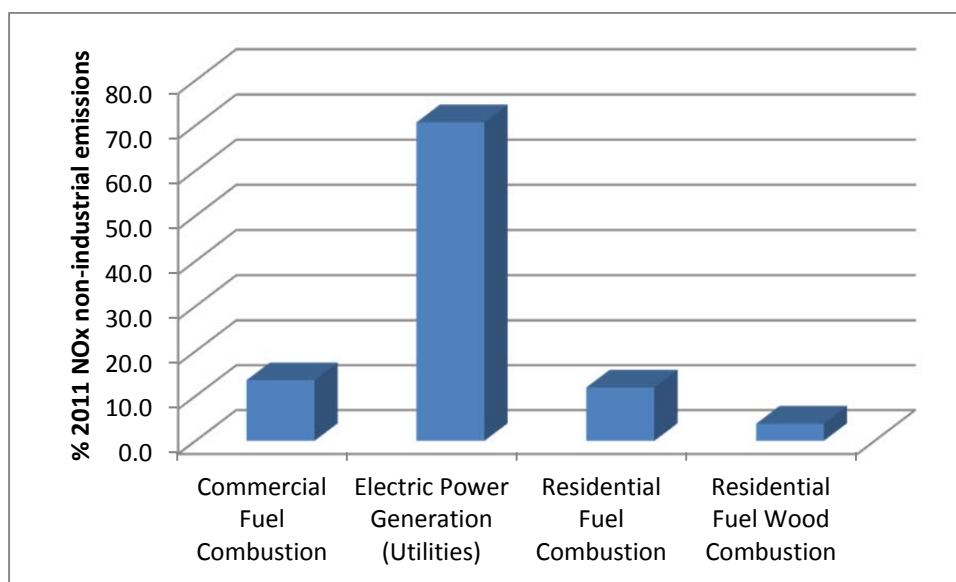
**Figure 3.3: 2011 NPRI industrial source NO<sub>x</sub> emissions by sub-category\***



\*From NPRI, <https://www.ec.gc.ca/inrp-npri/>

With respect to non-industrial Canadian NO<sub>x</sub> emissions (Figure 3.4), one sub-category, electric power generation, dominates at almost 70%, with commercial and residential fuel consumption following at approximately 10% each and residential fuel wood combustion contributing approximately 3% to the category total. Electric power generation is further subdivided (not shown) on the NPRI database into coal, natural gas and other. Coal-fired electricity generation is listed as the dominant, non-industrial NO<sub>x</sub> emissions source of the category at approximately 75%, with the other two types together contributing approximately equally to the remaining 25%. As of 2011, then, coal-powered electricity generation was the dominant non-industrial NPRI-listed source of NO<sub>x</sub> emissions in Canada. Since this time, several coal-fired electricity facilities have closed (Ontario phased out coal-powered electricity generation entirely as of 2014) so it is expected that some changes in electricity-generation-related NO<sub>x</sub> emissions have taken place since 2011.

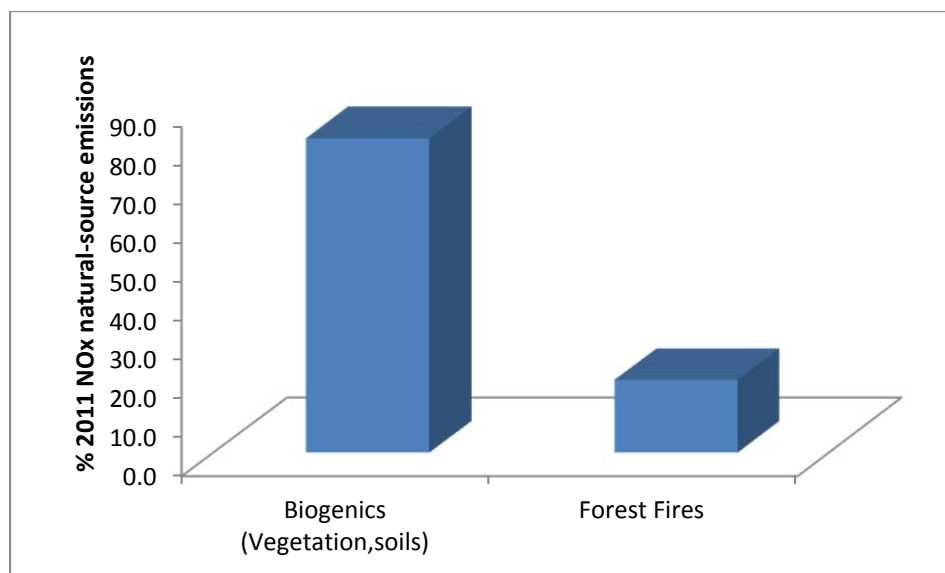
**Figure 3.4: 2011 NPRI non-industrial source NO<sub>x</sub> emissions by sub-category\***



\*From NPRI, <https://www.ec.gc.ca/inrp-npri/>

With respect to the minor contribution of emissions from natural sources of NO<sub>x</sub> emissions, the two sources listed in Figure 3.5, biogenic (produced by living organisms) and forest fires, contributed approximately 75% and 25%, respectively, in 2011. Biogenic NO<sub>x</sub> is produced as a result of microbial activity in fertilized agricultural soils.

**Figure 3.5: 2011 NPRI NO<sub>x</sub> emissions from natural sources\***



\*From NPRI, <https://www.ec.gc.ca/inrp-npri/>

Because of the inherent proximity of roads and construction to human habitation, this source may take on a greater importance for exposure and risk than the simple percentage of emissions indicates. While industrial and non-industrial sources are not as closely associated

with human populations as mobile sources, for those regions where such sources exist, the population exposure can be significant.

The trend in NO<sub>x</sub> emissions as reported to NPRI from 1985 to 2011 has been generally downward, particularly for three of the dominant broad source categories—mobile sources, natural sources, and non-industrial sources—by between 7% (natural sources) and 34% (mobile sources). Industrial NO<sub>x</sub> emissions have increased over the same time period by approximately 13%, largely because of increased emissions from the upstream petroleum sector. Due to the importance of the mobile sources for NO<sub>x</sub> emissions, there has been an overall decrease in emissions nationally, though this is not true in all regions as a result of the differing importance of various sectors.

### 3.3 Ambient Concentrations of NO<sub>2</sub> in Canada

Data on ambient concentrations of NO<sub>2</sub> across Canada are collected under the NAPS program. The NAPS network was established in 1969 to monitor and assess the quality of ambient air in the populated regions of Canada and provide accurate and long-term air quality data of a uniform standard across the country. The network is managed using a cooperative agreement among the federal, provincial, territorial and some municipal governments. Today there are 286 sites in 203 communities located in every province and territory at which NO<sub>2</sub> and/or other criteria air contaminants (O<sub>3</sub>, FPM, SO<sub>2</sub>, and carbon monoxide (CO)) are being continuously monitored. Approximately 160 monitors of the NAPS network collect data regarding Canadian ambient levels of NO<sub>2</sub>. Provinces also maintain other monitors that, while they may be NAPS-compliant in terms of technical standards, are not part of the national system. Other entities, such as industrial companies, may also maintain monitors that measure NO<sub>x</sub> and other substances.

NO<sub>2</sub> is generally not measured directly, but by subtraction following a separate measurement of the total of NO + NO<sub>2</sub> and of NO itself monitored via the chemiluminescence technique, the reference method recommended by the US EPA and European legislation. This method involves the reduction of NO<sub>2</sub> to NO using heated molybdenum oxide surfaces and a subsequent reaction between NO and O<sub>3</sub>, which forms an electronically excited \*NO<sub>2</sub> molecule that emits light when returning to the ground state. This light emission is measured as it is proportional to the NO concentration. The concentration of NO<sub>2</sub> is determined as the difference between the air sample that is passed over the molybdenum oxide substrate (the total NO<sub>2</sub> + NO) and the air sample that has not passed over the substrate (NO alone).

The reduction reaction of NO<sub>2</sub> to NO on the molybdenum substrate is not specific to NO<sub>2</sub>, and so the chemiluminescence method can be interfered with by the presence of other oxidized nitrogen compounds, primarily peroxyacetylnitrate (PAN) and nitric acid (HNO<sub>3</sub>), which are often referred to collectively as NO<sub>z</sub> compounds. Several studies have investigated the degree to which the NO<sub>2</sub> is overestimated as a result of NO<sub>z</sub> interference (e.g. Steinbacher et al., 2007). It appears that this interference is approximately 10% during the day in winter, but can be higher at some locations during the afternoon in summer due to enhanced photochemical creation of NO<sub>z</sub> species. It is also important to note that urban monitors are generally less affected by interference on a relative basis due to their proximity to strong NO<sub>x</sub> sources (vehicles), whereas monitors located more geographically removed from sources are more affected by NO<sub>z</sub> interference because the air mass has had a longer time for chemical reactions to form the interfering species.

### 3.3.1 Monitored NO<sub>2</sub> by NAPS Station Type

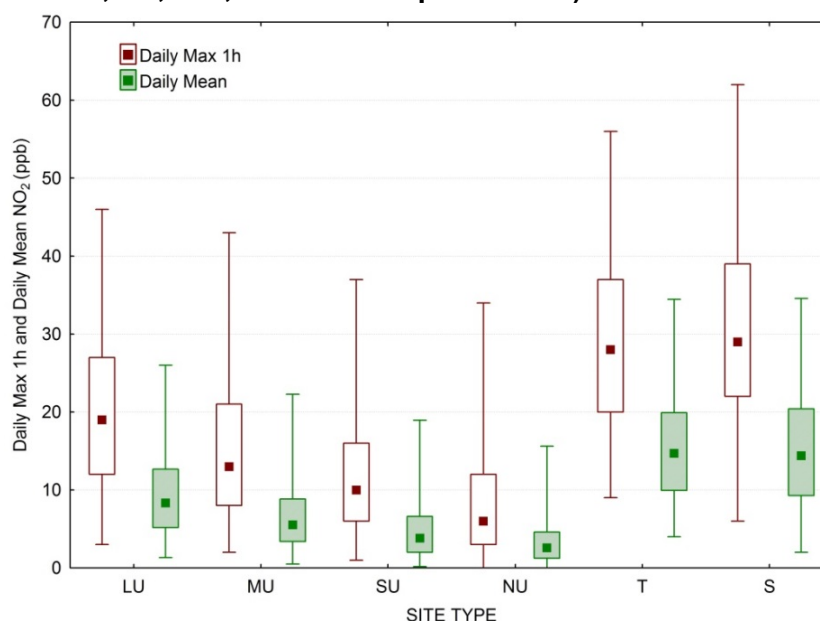
A detailed analysis of NAPS data for NO<sub>2</sub> was undertaken for Health Canada and Environment Canada under contract (Health Canada, 2013, unpublished). Data from all NAPS stations monitoring NO<sub>2</sub> were compiled, statistics were calculated and multi-year, seasonal and diurnal trends were examined. For the purposes of the analysis, monitoring stations were categorized into the following station-type categories used in the NAPS program (Table 3.1).

**Table 3.1: NAPS monitoring station types**

Code ID	Station type
<b>LU</b>	Stations in population centres with population ≥ 100,000
<b>MU</b>	Stations in in population centres with population ≥ 30,000 < 100,000
<b>SU</b>	Stations located in population centres with population ≥ 1,000 < 30,000
<b>NU</b>	Stations in areas not classified as urban (above)
<b>T</b>	Traffic- or transportation-influenced stations near major roadways. The definition of major roadways is all roads classed as arterial, expressway/highway, and freeway, as defined in the National Road Network ( <a href="http://www.geobase.ca/geobase/en/data/nrn/index.html">http://www.geobase.ca/geobase/en/data/nrn/index.html</a> )
<b>S</b>	Stations potentially influenced by industrial sources

To characterize short-term ambient levels for each of the site types, the distributions of daily 1-h max and 24-h avg NO<sub>2</sub> measurements for 2011 were plotted by site type and are presented in Figure 3.6.

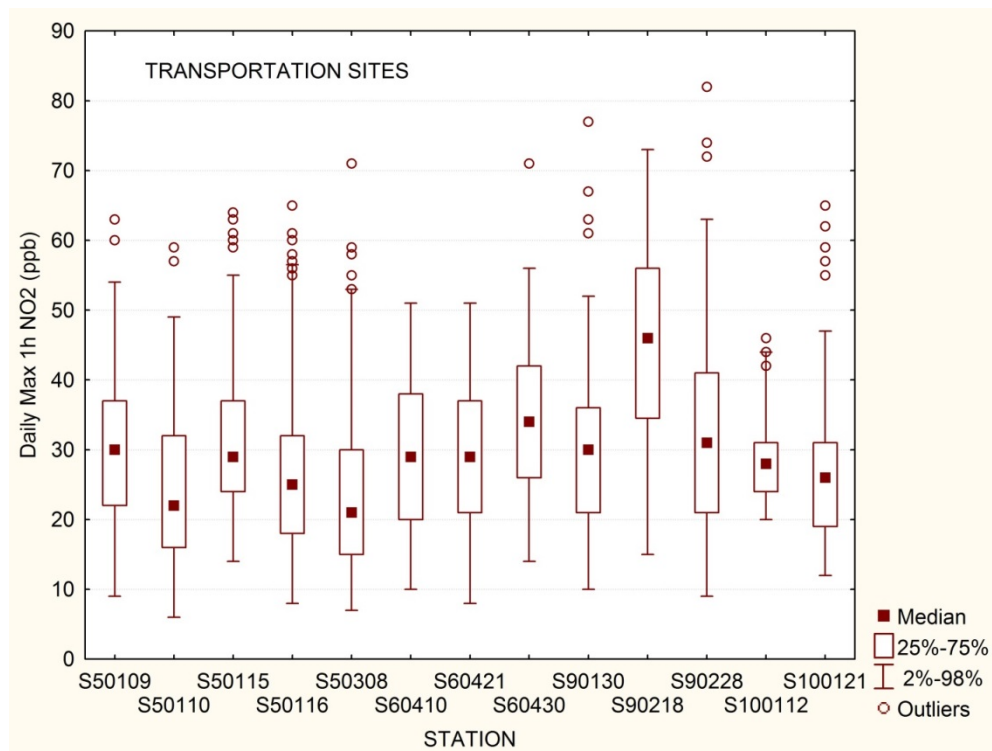
**Figure 3.6: Distribution of daily 1-h max and 24-h avg NO<sub>2</sub> by NAPS site type (2011) (median, 2<sup>nd</sup>, 25<sup>th</sup>, 75<sup>th</sup> and 98<sup>th</sup> percentiles)**



Measured daily 1-h max and 24-h avg NO<sub>2</sub> concentrations decreased as population size decreased, moving from LU through MU to SU and then NU monitoring sites, most likely as a result of the correspondingly smaller emissions from traffic, residential heating, and other population-dependent sources. Data from T- and S-influenced sites indicated substantially higher daily 1-h max and 24-h NO<sub>2</sub> avg concentrations than even the LU stations, but very similar to each other, with the median daily 1-h max values approaching 30 ppb and median 24-h means being approximately 15 ppb for both types of sites.

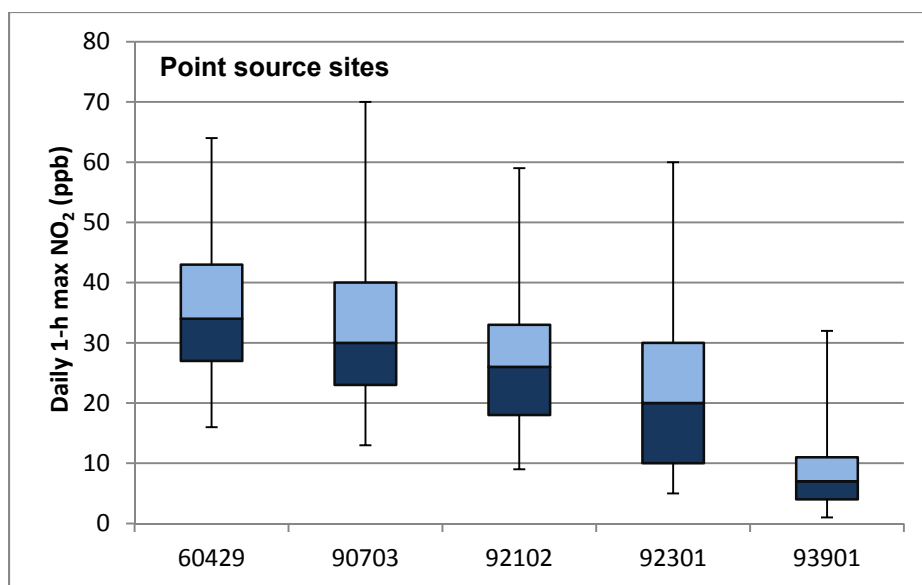
Figure 3.7 shows detailed statistics for all 13 individual NAPS stations considered to be substantially influenced by transportation-related emissions. Median daily 1-h max concentrations ranged from approximately 20 ppb to 40 ppb; 98<sup>th</sup> percentile values ranged from approximately 50 ppb to 72 ppb, with some more extreme values of up to more than 80 ppb. Hence, these results illustrate the importance of traffic emissions in influencing the spatial distribution of ambient NO<sub>2</sub> concentrations, with levels consistently increased at a number of traffic-related sites in various locations. In addition, the observation in many health studies of distance to roadway being a risk factor for adverse outcomes could be related to the spatial distribution of NO<sub>2</sub> and/or associated spatial patterns (e.g. other mobile pollutants, socioeconomic factors).

**Figure 3.7: Distribution of daily 1-h max NO<sub>2</sub> by individual T-type NAPS site (2011)**



Only five NAPS sites at which NO<sub>2</sub> is measured are classified as the “point source” S category, though they are not all true point source sites, with some being T-type sites (e.g. rail yard, haul road). The monitored data statistics for four of the stations are very consistent with one another (and with the T sites), with medians of the daily 1-h max ranging between 20 and 34 ppb and 98<sup>th</sup> percentiles ranging from 59 to 70 ppb (Figure 3.8).

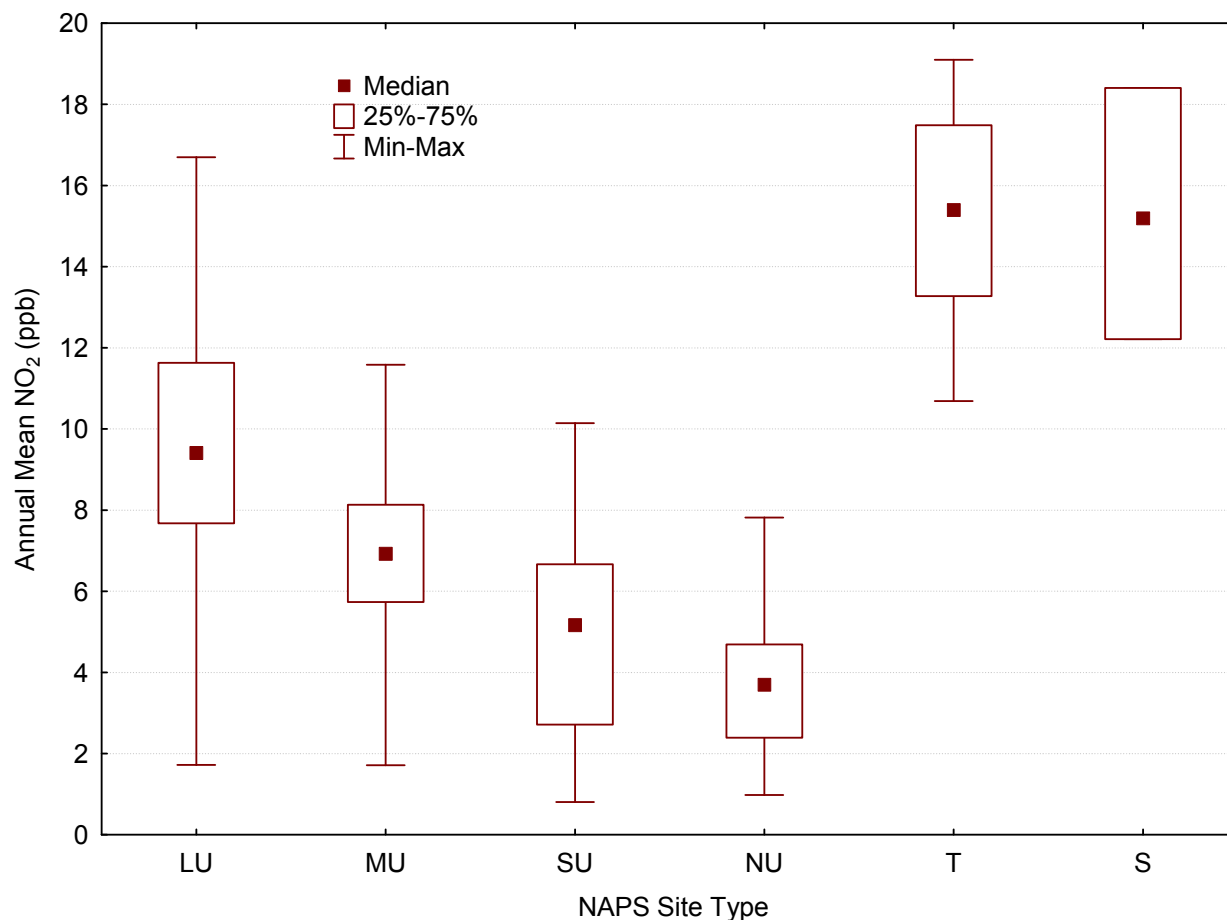
**Figure 3.8: Distribution of daily 1-h max NO<sub>2</sub> by individual S-type NAPS site (2009–2011) (5<sup>th</sup>, 25<sup>th</sup>, median, 75<sup>th</sup> and 98<sup>th</sup> percentiles)**



With respect to the measured annual means for the different site types (Figure 3.9), once again the median values decreased in an almost linear or monotonic fashion according to population size at the sites classified by urbanicity, from the largest urban sites at approximately 9 ppb to the NU sites at approximately 4 ppb. The data also became less variable, moving from the LU sites to NU sites, as indicated by the tighter whiskers (2<sup>nd</sup> and 98<sup>th</sup> percentiles). Once again, the T- and S-influenced site data generally indicated considerably higher annual NO<sub>2</sub> concentrations than at the other site types and very similar values to each other (medians = ~15 ppb).



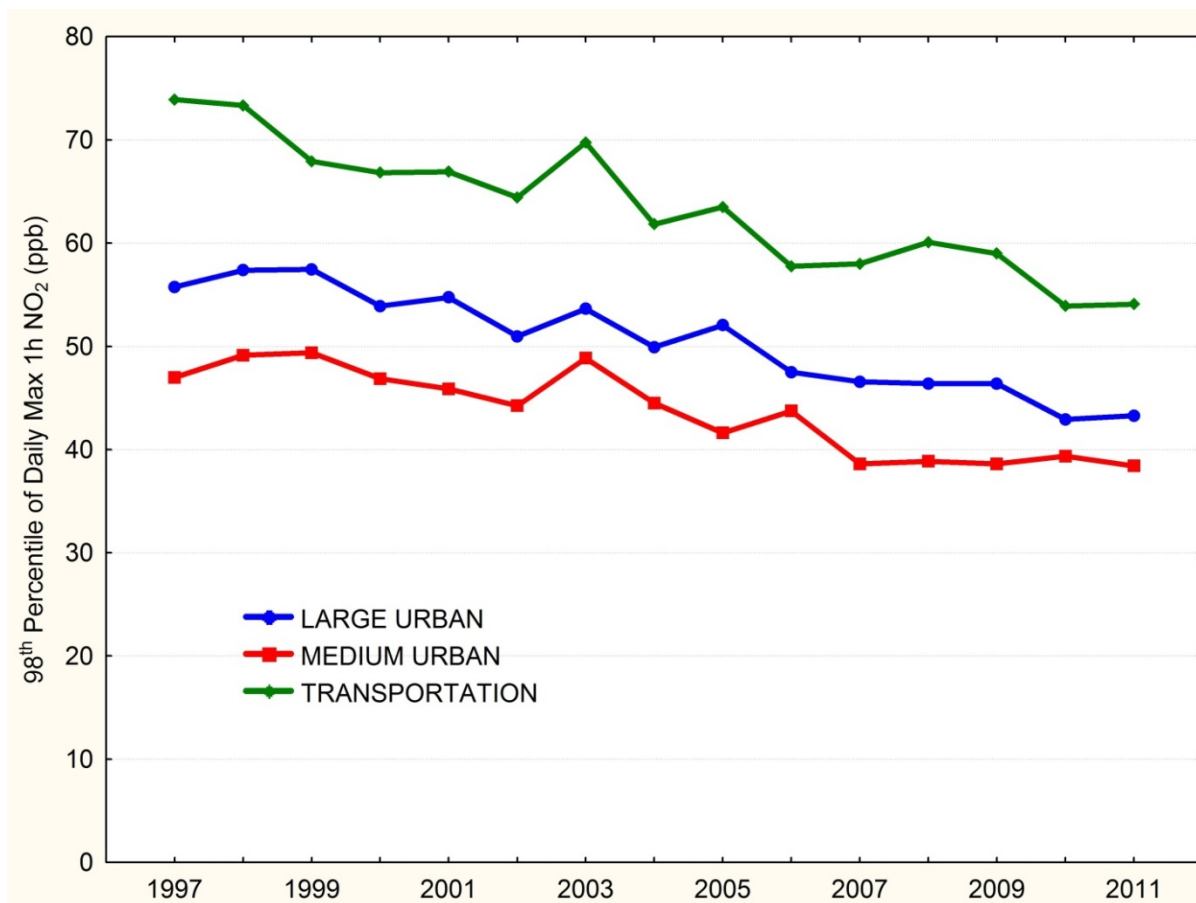
**Figure 3.9: Distribution of annual mean NO<sub>2</sub> by NAPS site type (2011)**



### 3.3.2 Trends in NO<sub>2</sub> Measured Over Time

The trend in the 98<sup>th</sup> percentile of the daily 1-h max measured NO<sub>2</sub> for the years 1997–2011 at LU, MU and T sites is illustrated in Figure 3.10, which presents the national mean of this statistic for each year by site type. There were insufficient SU, NU or S trend sites to compute a composite trend. All site types shown have a significant negative trend at the 99.9% confidence level ( $p = 0.001$ ), with the slope for the transportation sites being somewhat steeper than that for the others. These trends likely reflect the significant emission reductions (>98%) instituted for mobile sources since the initial 1970-era controls, with NO<sub>x</sub> being specifically targeted in order to reduce secondary pollutant formation (primarily O<sub>3</sub>, but also PM<sub>2.5</sub>). The LU and MU sites probably reflect this decrease as well, due to the large NO<sub>x</sub> emission signal originating with the mobile sources, and the strong relationship of population to the mobile sector.

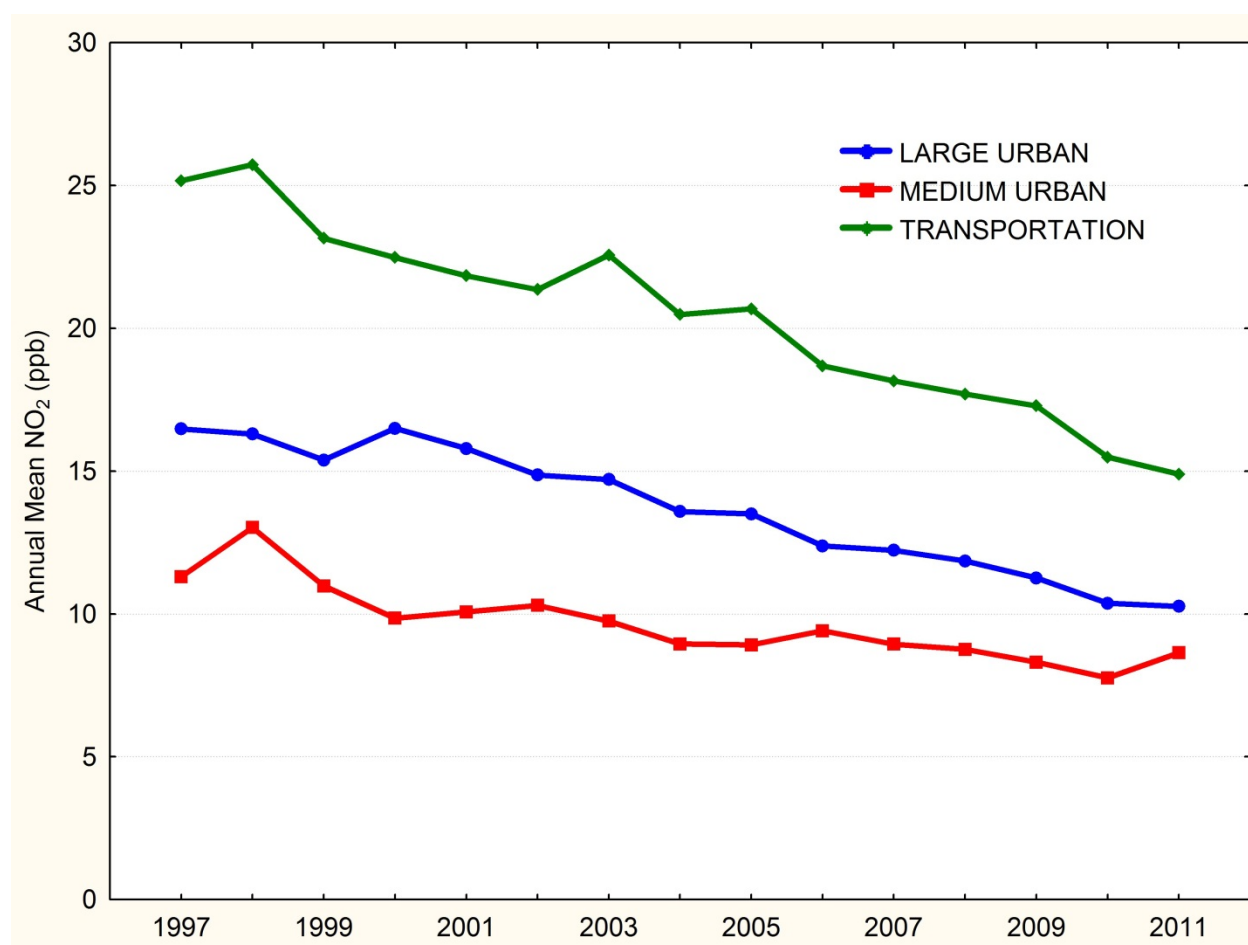
**Figure 3.10: Trend in 98<sup>th</sup> percentile of daily 1-h max NO<sub>2</sub> for various NAPS site types (1997–2011)**



The degree of decline in the 98<sup>th</sup> percentile of daily 1-h max in each of the environments described in Figure 3.10 was similar, though the greatest decline (27%) occurred for T-related sites, followed by LU (23%) and MU (17%), most likely reflecting the degree to which the mobile source influences each.

Annual mean NO<sub>2</sub> for the years 1997–2011 is presented in Figure 3.11 for consistent groups of LU, MU and T sites, and it also indicates a declining trend. Again, there were insufficient SU, NU and S trend sites to compute a composite trend. All site types shown have a significant negative trend at 99.9% confidence level ( $p = 0.001$ ). For both daily 1-h max and annual concentrations, the measured NO<sub>2</sub> concentrations decrease steadily from the T-influenced sites, followed by the LU sites, then the MU monitoring stations.

**Figure 3.11: Trend in annual mean NO<sub>2</sub> for various NAPS site types (1997–2011)**



Similarly to what is illustrated in Figure 3.10, the slope of the trend line is somewhat steeper for the T sites than for the others, again likely reflecting the relative importance of the decrease in mobile source NO<sub>x</sub> emissions to the T sites as compared to the LU and MU ones. The degree of reduction is much more pronounced for annual averages than for the daily 1-h max measures, with reductions of 40%, 36% and 33% for T, LU, and MU respectively. Annual average concentrations may be more reflective of emission decreases, since peak values (such as the 98<sup>th</sup> percentile of daily 1-h max) are more influenced by such factors as weather and, in the case of the mobile sector, urban congestion and infrastructure.

### 3.3.3 Seasonal Trends

NAPS data were also analyzed by season. Analyses were carried out using 3 years of data for the periods 2009–2011 and 1997–1999 to examine whether the seasonal trends have remained consistent over the years 1997–2011.

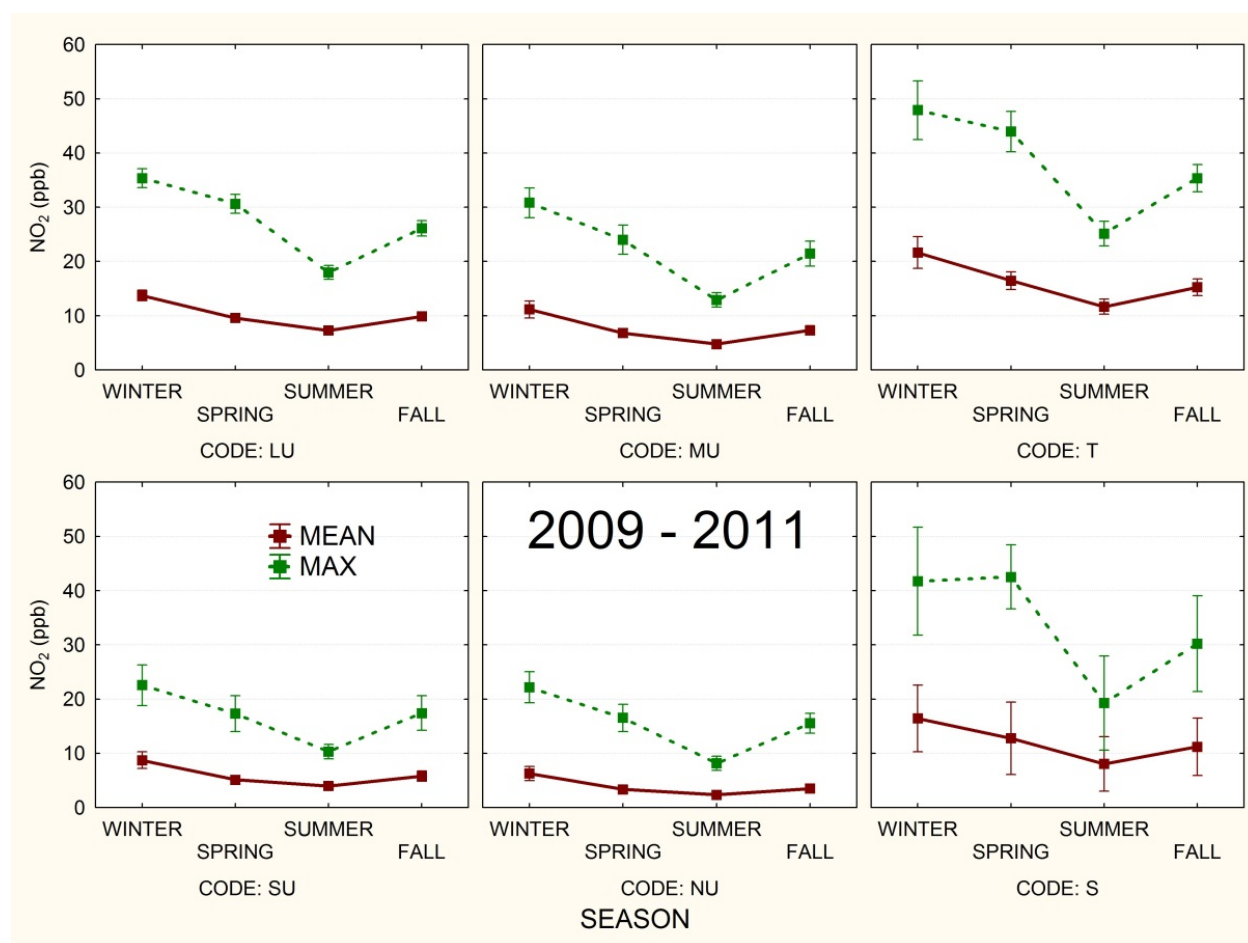
Seasons were defined as follows:

- Winter (December 1 to February 28/29)
- Spring (March 1 to May 31)

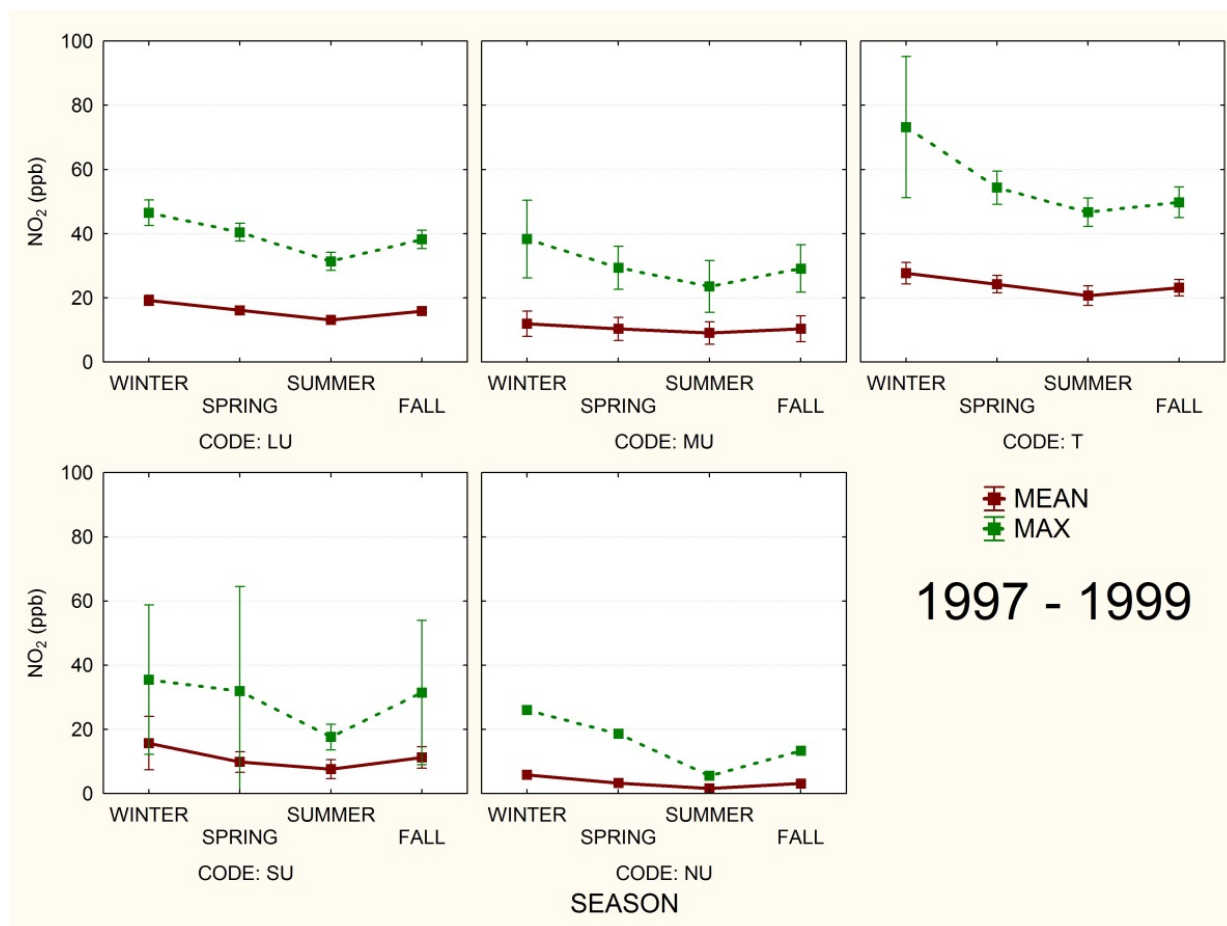
- Summer (June 1 to August 31)
- Fall (September 1 to November 30)

All site types exhibit the same seasonal trend, with levels being at their highest during the winter, trending downward through spring and into summer and trending upward once more in the fall. The low summer levels are consistent with higher mixing layer heights, increased rates of photochemical oxidation of  $\text{NO}_2$  to  $\text{NO}_x$  species, and decreased emissions from residential heating, as compared to winter (US EPA, 2008). These patterns are evident in both the 2009–2011 and 1997–1999 periods (Figures 3.12, 3.13). While seasonal trends are similar at all sites, NU sites show no concentration trend between years, remaining essentially unchanged. All other site types demonstrate declines in all seasons, likely reflecting the importance of mobile sources and regulatory efforts over that time period. Of interest, the decline in winter concentrations was more pronounced for all site types except NU, with winter declines ranging from 10% to 40% greater than those for summer, though there is no obvious explanation for this.

**Figure 3.12: Seasonal trends in measured  $\text{NO}_2$  for all site types (2009–2011)**



**Figure 3.13: Seasonal trends in measured NO<sub>2</sub> for all site types (1997–1999)**

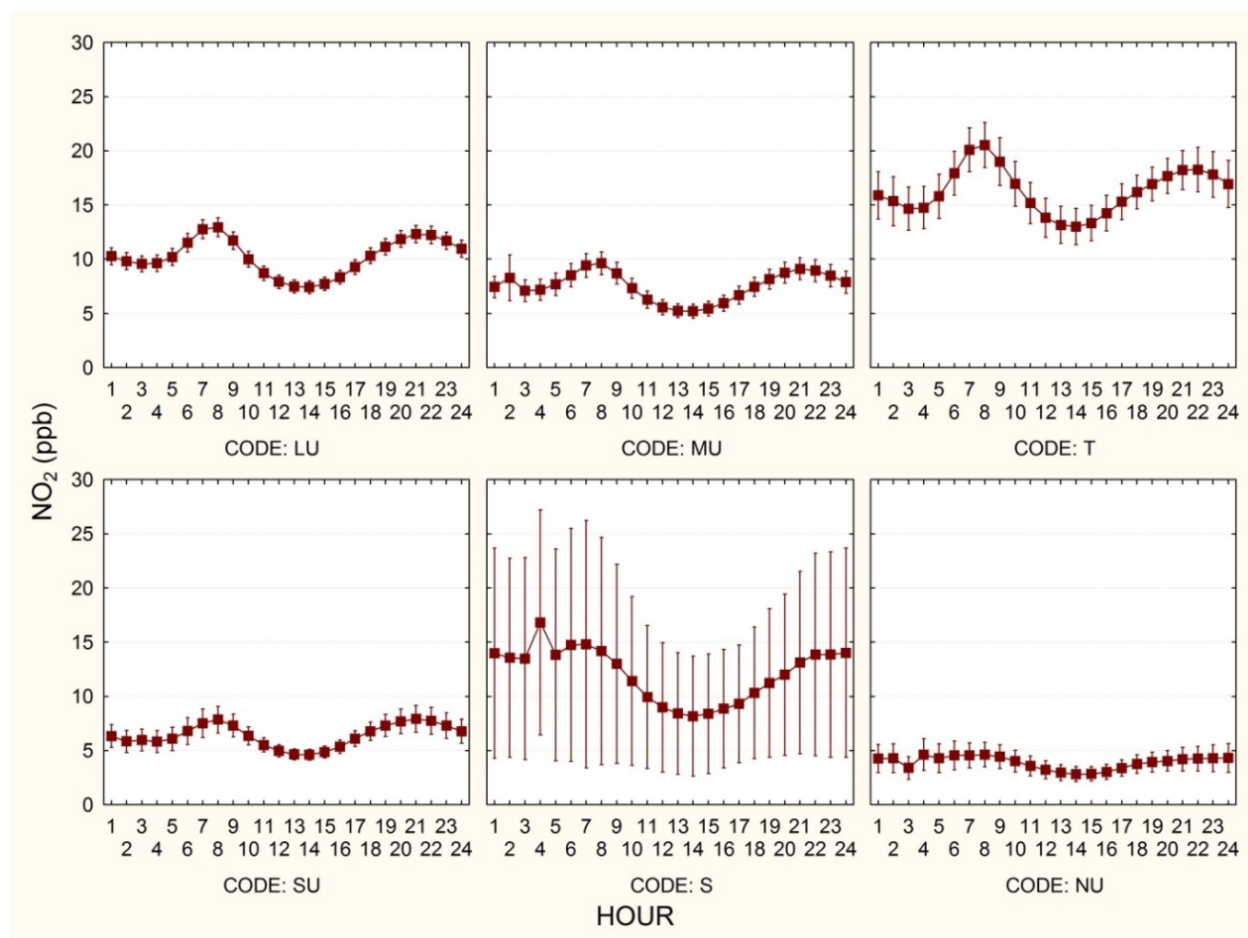


These seasonal trends are in some contrast to other major air pollutants. O<sub>3</sub>, for reasons related to atmospheric conditions, markedly peaks in the warm season in many regions, while PM shows a less pronounced, though still noticeable, warm-season increase. Such differences may be important in epidemiological studies, which largely but not always take into account such seasonal trends.

### 3.3.4 Diurnal Trends

The diurnal distributions of the 1-h concentrations were computed for each site type for the 3-year period 2009–2011, as shown below.

**Figure 3.14: Diurnal variation in composite mean hourly average NO<sub>2</sub> by NAPS site type (2009–2011) (mean and the 90% CI are plotted)**



The diurnal trend in monitored NO<sub>2</sub> concentrations holds for all site types, though the NU pattern is much less pronounced than that for the others. NO<sub>2</sub> levels increase through the morning hours, peaking at approximately 8 a.m. and then gradually decreasing until early afternoon, before rising again until early evening. This pattern coincides with increased traffic levels during morning and evening rush hours, with the morning peak NO<sub>2</sub> levels being slightly higher than the evening peak levels. As well, the morning peak is narrower (in terms of hours) than the evening peak. The S category stations exhibit the same trend, presumably as a result of traffic-related emissions being superimposed on those from industrial sources, though the variability in the data is significantly greater.

### 3.3.5 Weekday/Weekend Trends

The NAPS data analysis commissioned for this assessment did not include an analysis of weekday versus weekend diurnal trends; however, other jurisdictions (US EPA, 2008) have compared NO<sub>2</sub> concentrations for data acquired from the same monitoring sites during weekdays and weekends. The weekend concentrations are generally found to be lower than



those measured on weekdays and the diurnal cycles are more compressed on weekends versus weekdays, likely as a combined result of the decreased traffic overall (especially diesel truck traffic) and the resulting lack of specific rush hour traffic patterns on weekends.

### 3.3.6 Near-road NO<sub>2</sub> Concentration Gradients

Numerous studies have shown that measured NO<sub>2</sub> concentrations exhibit a strong gradient near traffic corridors, i.e. monitors placed at the sides of major roads measure significantly higher NO<sub>2</sub> concentrations than monitors located more distant from roadways from which mobile-source NO<sub>2</sub> originates. NO<sub>2</sub> concentrations generally decrease with increasing distance from the roadway, reaching urban background levels within 100–500 m, though some studies show elevated levels over greater distances. Meteorology is also important, with wind direction and speed being important factors affecting the concentration gradients of NO<sub>2</sub> concentrations near roadways. This issue is discussed in somewhat more detail in subsection 4.4.1.

## 3.4 Summary and Considerations

Emissions of NO<sub>x</sub> in Canada are dominated by the mobile and industrial sectors, which emit about 80% of the national total. The ubiquitous nature of the mobile sector in relation to populations indicates that this source may be the most important for human exposure. Off-road diesel (e.g. construction equipment) is the largest single source within the mobile sector (35%), indicating that greater knowledge and attention to this source is warranted when seeking to reduce exposures. Within the industrial sector, upstream oil and gas dominate the emissions profile (70% of industrial emissions). Because of the regional nature of that industry, it is likely that this source represents a significant source of exposure for those populations resident in certain parts of the country. Since this is the only sector whose emissions are increasing, its importance for population exposure in some regions is expected to increase. The non-industrial category contributed 12% of overall NO<sub>x</sub> emissions, most of which were as a result of electricity-generating combustion activities. The remaining 7% of Canadian NO<sub>x</sub> emissions in 2011 originated from natural sources, mainly biogenic in origin.

Ambient NO<sub>2</sub> levels display marked variations in space and time on several scales, often reflecting the important influence of traffic emissions on exposure. With respect to spatial variation, the highest concentrations of ambient NO<sub>2</sub> occur at transportation- and potentially industrial source-influenced sites. NO<sub>2</sub> levels at other NAPS site types are lower and appear proportional to the degree of urbanicity, likely a function of the parallel changes in emissions from mobile sources, residential heating, and other population-related sources. Various concentration metrics (daily 1-h max, 24-h avg, annual avg) demonstrate similar relationships in this regard. There is also large spatial variability in NO<sub>2</sub> in relation to markers of traffic emissions, including distance from roads, traffic volumes, and road length (Section 4.6).

Concerning temporal variation, both daily 1-h max and annual avg ambient NO<sub>2</sub> concentrations at various NAPS site types decreased steadily between 1997 and 2011, attributable to NO<sub>x</sub>-specific regulatory controls on the mobile sector and fossil-fueled electric power generation. All site types also exhibited a common pattern by season, with wintertime maxima and summertime minima, the latter consistent with increased mixing heights and photochemical oxidation of NO<sub>2</sub>, and decreased emissions from residential heating compared with winter. Concentrations of ambient NO<sub>2</sub> also vary throughout the day, with two peak concentrations corresponding to morning and afternoon/evening rush hours. NO<sub>2</sub> levels on the weekend are generally lower than those on weekdays and the diurnal peaks are shorter on weekends, likely as a combined result of reduced traffic (especially diesel truck traffic) and the lack of rush hour traffic on weekends.



The relative paucity of NAPS sites classed as potentially industrial source-influenced limits our knowledge of this sector to some degree, though the available information indicates that exposure to ambient NO<sub>2</sub> for those living near such sources is similar to that for roadways. Since many health studies find proximity to roadways to be a useful exposure term in examining effects, population relationships with stationary sources may also be important, though these are more difficult to study because of such things as the small populations near many large point sources and the dominance and ubiquity of traffic as a source of NO<sub>2</sub>.

Although ambient concentrations of NO<sub>2</sub> have been decreasing over time, like PM and O<sub>3</sub>, the levels remain significant. Moreover, the entire population, including sensitive subgroups, is exposed to NO<sub>2</sub> of ambient origin, due to the pervasive presence of nearby combustion sources.

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## 4. Exposure to Ambient NO<sub>2</sub>

### 4.1 Introduction

As described in the 2008 US EPA ISA for Oxides of Nitrogen (US EPA, 2008), human exposure to an airborne pollutant consists of contact between the human and the pollutant at a specific concentration for a specified period of time. People spend various amounts of time in different microenvironments characterized by different pollutant concentrations. The integrated exposure of a person to a given pollutant is the sum of the exposures over all time intervals for all microenvironments in which the individual spends time. Microenvironments in which people are exposed to air pollutants such as NO<sub>2</sub> typically include residential indoor environments, other indoor locations, near-traffic outdoor environments, other outdoor locations and in vehicles.

It is important to note that this assessment is being conducted to support the development of an ambient standard for NO<sub>2</sub>. There is also extensive epidemiological evidence (summarized in Chapters 8, 9 and 10) linking ambient concentrations of NO<sub>2</sub> to a wide range of health effects. In this context, a key issue is the ability of NO<sub>2</sub> concentrations measured in the monitoring network to serve as an adequate indicator of personal exposure to NO<sub>2</sub> *of ambient origin*, as opposed to the total personal exposure to NO<sub>2</sub> from all sources that is measured in most exposure assessment studies. In general, the relationship between personal exposures to pollutants of ambient origin and ambient concentrations can be modified by microenvironments (US EPA, 2008). During infiltration indoors, ambient pollutants can be lost through chemical and physical processes; therefore, the ambient component of a pollutant's concentration in an indoor microenvironment is not the same as its ambient concentration but rather the product of the ambient concentration and the infiltration factor. In addition, exposure to non-ambient microenvironmental sources such as gas stoves modifies the relationship between total personal exposures and ambient concentrations. The following sections of this chapter describe personal monitoring methods for NO<sub>2</sub>, followed by a discussion of the relationship between ambient NO<sub>2</sub> concentrations and personal exposure to NO<sub>2</sub>, including several factors that modify this relationship. After this, the correlations between personal and ambient levels of NO<sub>2</sub> and other co-occurring pollutants are summarized. Finally, a brief treatment of some methods of exposure assessment that are used in epidemiological studies of health impacts of NO<sub>2</sub> exposure is undertaken.

### 4.2 Personal NO<sub>2</sub> Monitoring

The US EPA defines personal exposure as being a general term for the average personal exposure over some specified time period, quantified as the concentration at the oral or nasal contact boundary (US EPA, 2004). A large number of studies have examined the magnitude and determinants of personal exposure through the use of personal exposure monitors. These monitors are sampling devices worn on the human body to estimate an individual's exposure to air pollutants. Personal exposures to NO<sub>2</sub> are most often measured by passive samplers, the most commonly used types of which are Palmes tubes (Palmes et al., 1976), Ogawa samplers (Ogawa and Co.), Yanagisawa badges (Yanagisawa and Nishimura, 1982), and radial diffusive samplers (Cocheo et al., 1996). Passive sampling relies on diffusion or small-scale turbulence to transport NO<sub>2</sub> to a sorbent, which can be either physically sorptive (e.g. active carbon) or chemisorptive (e.g. triethanolamine). Passive samplers for NO<sub>2</sub> are chemisorptive; i.e. a reagent coated on a support (metal, mesh, filter) chemically reacts with and captures NO<sub>2</sub>. The sorbent is extracted and analyzed for one or more reactive derivatives. A number of factors can affect the performance of passive samplers:

- variability in environmental conditions, which might cause variations in sampling rates throughout the sampling period
- chemical reactions between NO<sub>2</sub> and O<sub>3</sub>, especially at roadsides
- differential sensitivity to other NO<sub>x</sub> species (e.g. PAN)
- possible lack of specificity to uptake of NO<sub>2</sub>.

Notwithstanding the above-noted factors, however, studies indicate that passive samplers are quite precise, generally within 5% (Gair et al., 1991; Gair and Penkett, 1995; Kirby et al., 2001; Plaisance et al., 2004). Nevertheless, an important limitation of passive sampling is that passive samplers give relatively long time-averaged concentrations (days–weeks) with higher detection limits over short sampling periods. The long averaging time and high detection limits of passive samplers preclude their use for estimating short-term, peak exposures to NO<sub>2</sub>.

### 4.3 Personal–Ambient Exposure Relationships

The entire population is exposed to NO<sub>2</sub> of ambient origin, both when people are outdoors and when they are in indoor environments into which ambient NO<sub>2</sub> has infiltrated. A number of studies have examined the short-term temporal relationship between personal exposure to NO<sub>2</sub> and the corresponding concentration of ambient NO<sub>2</sub> measured at a central site monitor, which is often used as a surrogate for community exposure to ambient air pollutants in epidemiological studies. As reported in the 2008 US EPA ISA, most of these studies found that personal NO<sub>2</sub> exposure was related to ambient NO<sub>2</sub>, but the strength of the association ranged from poor to good.

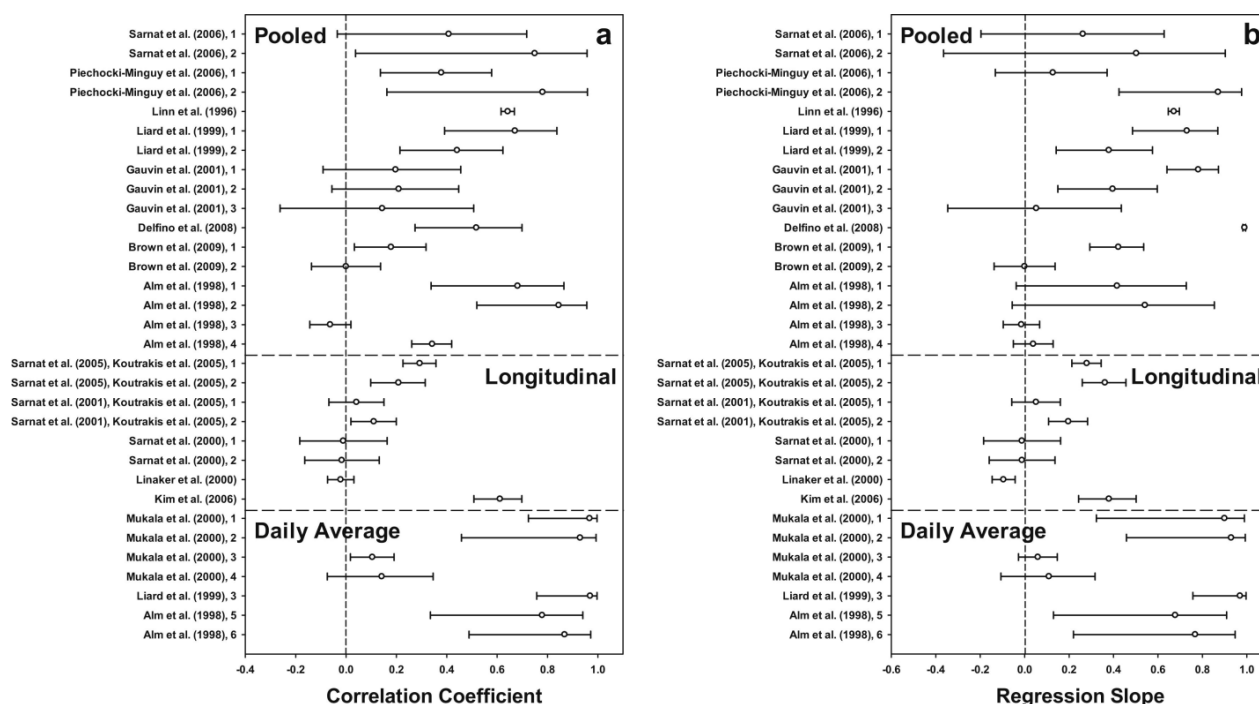
Some of the variability in this relationship is illustrated in the findings of four earlier Canadian studies. Silverman et al. (1982) reported that among 12 asthmatics and 11 healthy non-asthmatics from the Toronto area monitored daily for 2–4 weeks (wk) in summer and winter, personal NO<sub>2</sub> exposure was significantly correlated with concentrations at a central site ( $r = 0.49$ ). The personal versus city-wide average ambient correlation was lower but also significant ( $r = 0.358$ ) in a study of 33 healthy older adults from Hamilton who were monitored for a total of 60 72-h periods in winter, spring and summer (Sahsuvaroglu et al., 2009). In contrast, personal and ambient NO<sub>2</sub> were non-significantly correlated ( $r = 0.31$ ) in a study of 19 Toronto asthmatics who were followed 8 h/weekday for 2 weeks in each of the heating and non-heating seasons (Hosein et al., 1991). However, a somewhat higher personal–ambient NO<sub>2</sub> correlation ( $r = 0.57$ ) was observed in another Toronto area study of 28 CAD patients monitored 1 d/wk for 5–10 weeks (Kim et al., 2006). The relatively high correlation in the latter study may be the result of the limited mobility of the cardiac-compromised subjects (reducing exposure measurement error) and the fact that most of the sampling was in summer (a time of increased infiltration of and exposure to NO<sub>2</sub> of ambient origin). Factors affecting the strength of the personal–ambient NO<sub>2</sub> relationship are discussed in Section 4.4.

Studies from elsewhere in the world confirm the generally positive but variable relationship between personal and ambient NO<sub>2</sub>. Figure 4.1 presents the correlation coefficients ( $r$ ) and regression slopes relating personal NO<sub>2</sub> exposure to ambient NO<sub>2</sub> concentrations in a number of US and European studies included in a recent meta-analysis by Meng et al. (2012a, Figure 1). These authors performed a random effects meta-analysis of 15 studies that calculated slopes and correlations between personal NO<sub>2</sub> measurements and ambient NO<sub>2</sub> concentrations for 32 sample populations (17 from pooled analyses, 8 from longitudinal analyses, and 7 from daily average analyses). **Pooled correlations** are calculated when a study involves one/a few measurements per subject and when different subjects are studied on subsequent days. They combine individual-subject/individual-day data for the calculation of correlations, and describe the relationship between daily personal NO<sub>2</sub> exposure and daily ambient NO<sub>2</sub> concentration

across all subjects. **Longitudinal correlations** are calculated when data from a study include consecutive multiple measurements for each subject (longitudinal study design). Longitudinal correlations describe the temporal relationship between personal NO<sub>2</sub> exposure and ambient NO<sub>2</sub> concentration for the same subject. A longitudinal correlation coefficient between personal exposures and ambient concentrations is relevant to the design of some of the panel epidemiologic studies discussed in Section 8.4. **Daily average correlations** are calculated by averaging exposure across subjects for each day. Daily average correlations then describe the relationship between the daily average exposure and daily ambient NO<sub>2</sub> concentration. This type of correlation (i.e. the association between community average exposures and ambient concentrations) is more directly relevant to community time-series epidemiologic studies, discussed in Sections 8.2, 8.3 and 8.4, in which ambient concentrations are used as a surrogate for community average exposure to NO<sub>2</sub> of ambient origin).

In this study, ambient NO<sub>2</sub> concentrations were found to be related to personal NO<sub>2</sub> exposure, with generally positive findings that were mostly statistically significant in individual studies (Figure 4.1). Overall correlation coefficients were 0.42 for pooled analyses, 0.16 for longitudinal analyses, and 0.72 for daily average analyses, all of which were statistically significant and robust to correction for publication bias. The differences in the magnitudes of these three types of correlation coefficients can be understood in terms of the different components of the variance in physical factors influencing the strength of the personal–ambient associations that they incorporate (US EPA, 2008). These include factors such as infiltration, indoor and other local sources (including traffic), and the time people spend in different microenvironments with different NO<sub>2</sub> concentrations (Section 4.4). Longitudinal correlation coefficients reflect the intra-personal variations in these physical factors, while pooled correlations incorporate both inter- and intra-personal variations. In the case of daily average correlation coefficients of the association between community average exposures and ambient concentrations, inter-personal variations in these physical factors are reduced by averaging personal exposures across a community. Hence, daily means of personal exposures across subjects are better indicators of personal exposure to outdoor pollution because they average out the more variable individual exposures to non-ambient sources influencing the total personal exposure (Mage et al., 1999).

**Figure 4.1: (a) Correlation coefficients and their 95% confidence limits for each study grouped by different types of correlation coefficients; (b) regression slopes and their 95% confidence limits for each study\***



\*From Meng et al. (2012a, Figure 1)

The relatively low personal–ambient correlations for longitudinal analyses do not necessarily mean that ambient concentrations are not a good surrogate for personal exposures to NO<sub>2</sub> of ambient origin, because the weak associations could have resulted from the day-to-day variation in the non-ambient component of total personal exposure. Further, in each of the longitudinal studies reviewed by the US EPA (2008), correlations were strong for a number of individuals in the study, even when the correlation for all subjects combined was relatively weak. Meng et al. (2012a) also reported that some of the longitudinal studies had a high frequency of samples below detection limits or had a long sampling duration that might introduce extra sampling errors.

For determining the health effects of ambient air pollutants on human health, the association between the ambient component of personal exposures and the ambient concentrations is more relevant than the association between personal total exposures (ambient + non-ambient components) and ambient concentrations. Usually the ambient component of personal exposure is not directly measurable, although it can be estimated by exposure models; alternatively the personal total exposure can be regarded as the personal exposure of ambient origin if there are no indoor or non-ambient sources.

Personal exposures were clearly stratified by the presence/absence of indoor sources in four studies evaluated by the US EPA in 2008 (Alm et al., 1998; Linaker et al., 2000; Piechocki-Minguy et al., 2006; Sarnat et al., 2006). Only two of these studies (Alm et al., 1998; Piechocki-Minguy et al., 2006) compared the association between total personal exposures and ambient concentrations as well as the association between the ambient component of personal

exposures (i.e. in the absence of indoor sources) and ambient concentrations. A stronger association was observed between the ambient component of personal total exposures and ambient concentrations (as has been seen for other pollutants). In addition, the correlation between personal total and ambient NO<sub>2</sub> was stronger in the warm season (when people are generally more exposed to ambient air pollutants because building infiltration and time spent outdoors are greater) in these and a number of other studies.

For example, Alm et al. (1998) found a greater personal–ambient NO<sub>2</sub> correlation among preschool child subjects from downtown Helsinki who resided in houses that had electric stoves ( $r = 0.42$ ) than in those living with gas stoves ( $r = 0.16$ ), and among subjects without exposure to environmental tobacco smoke (ETS) ( $r = 0.47$ ) compared with those exposed to such smoke ( $r = 0.23$ ). In addition, the personal–ambient correlation was strong in the spring ( $r = 0.64$ ) and absent in the winter ( $r = -0.06$ ). Similarly, in a study from Lille, France, Piechocki-Minguy et al. (2006) reported that the at-home-personal versus ambient NO<sub>2</sub> correlation was much stronger when restricted to summer measurements for subjects in houses with electrical stoves and heating ( $r = 0.78$ , converted from the  $R^2$  values reported in the paper) than it was among all study subjects in both winter and summer campaigns ( $r = 0.30$ ). Some other studies that did not stratify by indoor sources nonetheless reported that the association between ambient or outdoor concentrations and personal exposure to NO<sub>2</sub> was strengthened after control for indoor sources such as gas appliances and ETS, with  $R^2$  values increasing by 10% to 40% (US EPA, 2008, pp. 2–50). Hence, these findings are consistent with the expectation that the association between ambient concentrations and the ambient component of personal exposures would be stronger than the association between ambient concentrations and personal total exposures.

## 4.4 Factors Affecting Personal–Ambient NO<sub>2</sub> Relationships

A number of factors are known to affect the relationship between ambient and personal NO<sub>2</sub>, including proximity to traffic, building infiltration and factors that affect building air exchange, indoor sources of NO<sub>2</sub>, variations in time-activity patterns, and analytical limitations. Each of these is briefly discussed below.

### 4.4.1 Proximity to Traffic

Vehicle emissions are an important source of NO<sub>2</sub> in urban environments, and a number of studies have investigated spatial heterogeneity in NO<sub>2</sub> levels in relation to major roadways.

Large horizontal gradients in NO<sub>2</sub> concentrations near roadways have been observed in several studies, and NO<sub>2</sub> concentrations have been found to be correlated (or inversely correlated) with traffic-related variables, including distance from the roadway, traffic volume, road length, open space, and population density (US EPA, 2008). For example, Gilbert et al. (2003) reported that 7-d passive measurements of NO<sub>2</sub> near a major expressway in Montreal, QC, were strongly related to distance from the road and wind direction ( $R^2 = 0.97$ ). Mean concentrations were elevated more than 2-fold over urban background levels immediately over the road, decreased monotonically with increasing distance from it, and were greater downwind than upwind. The decrease was most pronounced in the first 200 m, but levels were still increased 1310 m downwind of the highway. Similarly, Beckerman et al. (2008) observed that NO<sub>2</sub> concentrations measured over 1 week were elevated up to 2-fold over background levels adjacent to a Toronto, ON-area expressway, and decayed with increasing distance, mostly within 300 m, with the decline being steeper on the upwind side of the expressway. In a more recent study of another Toronto-area expressway (Gordon et al., 2012), real-time levels of NO<sub>2</sub> measured by a mobile laboratory while driving transects on a side road perpendicular to the expressway were again



increased 2- to 3-fold within 50 m of the roadway compared with background levels, and the levels fell off with increasing distance in a similar manner to that reported in the earlier studies. However, while this pattern was seen in morning and evening periods, it was absent around sunrise, when background NO<sub>2</sub> concentrations were high, likely because of the relatively low boundary layer at this time of day.

The importance of traffic in determining the spatial heterogeneity of ambient NO<sub>2</sub> is confirmed by the results of land-use regression (LUR) models. (This technique is discussed in Section 4.6). In LUR models developed for Montreal (Gilbert et al., 2005, 2007) and Toronto (Jerrett et al., 2007), traffic-related variables (such as traffic counts, distance from expressways or major roads, and nearby lengths of expressways/major roads), were significant predictors of NO<sub>2</sub> concentrations.

No Canadian studies investigating the relationship between proximity to traffic and personal exposure to NO<sub>2</sub> were identified, but a number of studies in other countries found that personal NO<sub>2</sub> was increased in relation to traffic and/or urbanicity. Proximity of traffic to home and/or school was a significant predictor of personal exposure to NO<sub>2</sub> in studies carried out in three French metropolitan areas (Cauvin et al., 2001), in Helsinki, Finland (Rotko et al., 2001), and in Genoa, Italy (Galleli et al., 2002). Personal exposure to NO<sub>2</sub> was also higher in urban than in less urbanized areas, perhaps as a result of greater traffic density, in studies in Switzerland (Monn et al., 1998), and in Helsinki (Alm et al., 1998).

Pollutants that are emitted by sources at or just above ground level have negative vertical gradients: concentrations are greatest near the ground and decrease with increasing height. Because vehicle emissions are an important source of NO<sub>2</sub>, this pollutant displays such a gradient. In a study conducted in New York City (Restrepo et al., 2004), 24-h avg concentrations of NO<sub>2</sub> were about 2.5 times greater 4 m above the roadway than at a height of 15 m. (This range of elevations is within that recommended for NAPS monitoring sites (CCME, 2011)). In a pilot study conducted to assist US air agencies in selecting potential sites for a near-road NO<sub>2</sub> monitoring network (STI, 2011) passive sampling was conducted near heavily trafficked roads in five metropolitan areas. Concentrations of NO<sub>2</sub> at 1.8 or 2 m above the roadway were greater than those measured at heights of 3.8 or 7 m, though the difference was small (roughly 1–3 ppb 7-d NO<sub>2</sub>). The use of ambient monitors positioned at elevation would therefore underestimate human exposure to NO<sub>2</sub> (though to what extent is somewhat uncertain).

The strong horizontal and vertical gradients in ambient NO<sub>2</sub> concentrations near roadways discussed above result in considerable spatial heterogeneity in NO<sub>2</sub> concentrations. In the 2008 US EPA ISA, analyses of correlations among monitoring sites in a region for various air pollutants showed that NO<sub>2</sub> was more spatially variable than the more secondary pollutants PM<sub>2.5</sub> or O<sub>3</sub>. Lower bounds on inter-site correlation coefficients for PM<sub>2.5</sub> and O<sub>3</sub> tended to be much higher than for NO<sub>2</sub> in the same areas. Coefficients of divergence (CODs) for PM<sub>2.5</sub> were much lower than for O<sub>3</sub>, whereas CODs for NO<sub>2</sub> tended to be the largest among these three pollutants. (A COD of 0 indicates that there are no differences between concentrations at paired sites (spatial homogeneity), while a COD approaching 1 indicates extreme spatial heterogeneity.) This greater spatial variability could lead to larger exposure measurement error for NO<sub>2</sub> in epidemiological studies, particularly those that rely on ambient concentrations at a central site as a surrogate for population exposure.

Not unexpectedly, vehicle emissions can result in elevated on-road levels of NO<sub>2</sub>. For example, NO<sub>2</sub> concentrations were increased about 4-fold on a Los Angeles freeway with heavy diesel truck traffic compared with levels in residential areas (Westerdahl et al., 2005). NO<sub>x</sub> concentrations were roughly 7-fold greater at a San Francisco tunnel exit than at the entrance (Kean et al., 2000). Similarly, personal NO<sub>2</sub> concentrations were increased a few times over



urban background levels for taxi, bus, or lorry drivers in Stockholm, Sweden (Lewne et al., 2006), and in-vehicle concentrations were elevated to a similar or greater degree in California school buses, especially if they had windows open and were following diesel buses with visible exhaust (Sabin et al., 2005).

Although traffic is a major source in urban areas, industrial point sources also contribute significantly to ambient NO<sub>2</sub> in some locales, though there is only limited information to characterize the role of point sources in this regard. In rural areas where traffic is sparse, other sources could dominate, including agriculture, wildfires and residential wood burning. NO<sub>2</sub> may therefore not always be a reliable indicator for traffic pollution.

#### 4.4.2 Infiltration

Most people spend an average of about 90% of their time indoors, principally at home (Health Canada, 2013a). Consequently, the extent of infiltration of ambient NO<sub>2</sub> into indoor environments is an important determinant of personal exposure to NO<sub>2</sub> of ambient origin.

Nitrogen dioxide indoors is subject to removal by a variety of chemical processes, including gas-phase reactions with O<sub>3</sub> and various free radicals, and by surface-promoted hydrolysis and reduction reactions (US EPA, 2008). As a result of such removal processes, indoor levels of NO<sub>2</sub> of ambient origin are less than those outdoors. In the absence of indoor sources, indoor levels are roughly half of those outdoors (US EPA, 2008), although the difference is often greater in winter and less in summer (when ventilation is generally increased by open windows and doors). For example, in Canadian studies of residential exposure to NO<sub>2</sub> during winter, median indoor and outdoor levels in homes with electric stoves were 2.49 and 5.98 ppb, respectively, in Halifax, NS, in 2008–2009 (Health Canada, 2012), and 3.46 and 13.1 ppb in Edmonton, AB, in 2010 (Health Canada, 2013b). The indoor versus outdoor levels differed in a similar manner at 4.16 and 11.6 ppb in a Hamilton, ON, study undertaken in the spring of 2007 (Health Canada, 2010), but the difference was less pronounced in summer studies: e.g. 4.40 vs. 7.76 ppb in Toronto in 2006–2007 (Health Canada, 2010) and 3.19 vs. 3.56 ppb in Edmonton in 2010 (Health Canada, 2013b). (In these studies, the indoor/outdoor (I/O) ratios in some houses were greater than 1, suggesting that indoor sources of NO<sub>2</sub> were present.)

Home ventilation in particular is an important factor modifying the relationship between personal and ambient/outdoor NO<sub>2</sub>; one would expect it to be strongest for subjects spending time indoors with open windows or other indications of increased ventilation (US EPA, 2008). This is indeed the case in most studies, though not all, in that the associations are most often stronger in the warm season or in relation to various measures of increased ventilation (e.g. Quackenboss et al., 1986; Alm et al., 1998; Sarnat et al., 2005, 2006; Piechocki-Minguy et al., 2006; Sahsuvaroglu et al., 2009; Meng et al., 2012a, b).

For example, in a recent study of strong design (Meng et al., 2012b), personal–ambient associations and determinants were investigated in 140 randomly selected participants in the Detroit Exposure and Aerosol Research Study (DEARS). In analyses for those residences without indoor sources of NO<sub>2</sub>, the slope relating personal exposure to ambient concentration was greater and statistically significant in summer but not in winter. In investigations of exposure determinants using mixed-effect models, the slope of the personal–ambient association ( $\alpha$ ) was significantly affected by high air exchange rate (AER) (>1.3 air changes per hour), no central air conditioning, use and non-use of window fans, and window opening (non-significantly increased) in summer but not in winter.

Seasonality also figured prominently in the meta-analysis by Meng et al. (2012a) of 32 subpopulations from 15 US and European studies that was described in Section 4.3. In this study, the results of random effects meta-regressions indicated that personal–ambient

associations were stronger in spring and fall than in winter, which the authors suggested might be due to window opening in the warmer seasons. The associations for summer were intermediate between spring/fall and winter; this finding was not discussed, but could conceivably be related to reduced ventilation as a result of the use of air conditioning in summer. Personal–ambient associations were also greater in homes without indoor sources, as well as in people with pre-existing cardiopulmonary (CP) disease (attributed to decreased mobility, so that impacts of local or indoor sources were less variable in this group), and in adults as compared to children (not significantly).

Another aspect of the relationship between personal and ambient NO<sub>2</sub> is the contribution of ambient NO<sub>2</sub> to personal exposure (US EPA, 2008). The infiltration factor (*Finf*) and alpha ( $\alpha$ ) (also known as the attenuation factor or the ambient exposure factor) are keys to evaluate exposure to personal NO<sub>2</sub> of ambient origin. The *Finf* of NO<sub>2</sub> is the fraction of ambient NO<sub>2</sub> found in the indoor environment, and is determined by the NO<sub>2</sub> penetration coefficient (*P*), AER (*a*), and the NO<sub>2</sub> decay rate (*k*). The attenuation factor  $\alpha$  for NO<sub>2</sub>, which is the ratio of personal ambient exposure concentration to the ambient concentration, is a function of *Finf* and the time people spend outdoors. Specific *P*, *a*, and *k* are not reported for study homes in most studies. Other means to estimate the *Finf* are the I/O ratio of NO<sub>2</sub> in the absence of indoor sources. The population-averaged *Finf* can also be estimated as the slope of the indoor concentrations versus the outdoor concentrations across study homes. The  $\alpha$  can be estimated as the slope of the regression of personal versus ambient NO<sub>2</sub> across study subjects.

The US EPA (2008) summarized the results of a number of studies that had estimated these parameters; it reported that most of the infiltration factors ranged from 0.4 to 0.7, while most values for  $\alpha$  were between 0.3 and 0.6. In the DEARS study by Meng et al. (2012b) described earlier in this section,  $\alpha$  for those homes without indoor sources of NO<sub>2</sub> was 0.13 in summer, with similar or greater values for the subset of these homes with high AER (0.38), without central air conditioning (0.20), with use of window fan (0.60), and with open windows (0.16, not significant). There was no significant association in winter.

#### 4.4.3 Indoor Sources

A Science Assessment Document in support of the Residential Indoor Air Quality Guideline for NO<sub>2</sub> (Health Canada, 2015) that deals in detail with sources and exposure to NO<sub>2</sub> in indoor environments has been prepared by the Indoor Air Contaminants Assessment Section of the Water and Air Quality Bureau of Health Canada. Given the focus of the present assessment on ambient NO<sub>2</sub>, these topics are discussed only briefly in this report; readers requiring more information on indoor sources and exposure are referred to the indoor air assessment document.

Total personal exposure to NO<sub>2</sub> is not only to NO<sub>2</sub> from ambient sources, but also includes NO<sub>2</sub> from sources indoors. Indoor emissions of NO<sub>2</sub> principally arise from the combustion of fossil fuels (e.g. gas stoves, clothes dryers, or oil and gas furnaces) and biomass (e.g. wood-burning appliances, tobacco smoking, and candles) (US EPA, 2008).

Many studies have noted the importance of unvented gas cooking and heating appliances as indoor sources of NO<sub>2</sub> and determinants of indoor levels (US EPA, 2008). Homes with gas stoves had indoor concentrations 50–400% greater than those with electrical stoves, depending on geographic location, season, other sources of NO<sub>2</sub> and household characteristics (e.g. whether a range hood is typically used while cooking). Gas stoves with pilot lights emit more NO<sub>2</sub> and add roughly 10 ppb to indoor levels over those from gas stoves with more modern electric ignition systems. Gas space heaters and hot water heaters also produce increased levels of indoor NO<sub>2</sub> if they are not adequately vented. On the other hand, mean/median concentrations of NO<sub>2</sub> indoors were relatively low in a study of Canadian homes with vented gas

furnaces, and were not increased in Canadian and US studies of wood-burning fireplaces and stoves, indicating that these are not likely to be major sources of NO<sub>2</sub> to indoor environments.

ETS can also be an important indoor source of NO<sub>2</sub> in homes where smoking occurs indoors; most studies, though not all, found positive associations between indoor NO<sub>2</sub> and ETS. Personal NO<sub>2</sub> was also associated with time exposed to burning candles in a study in Copenhagen, Denmark (US EPA, 2008).

These indoor combustion sources are also significant determinants of total personal exposure to NO<sub>2</sub> in many studies, as a combined result of their contribution to indoor concentrations and the considerable time spent indoors. For example, Levy et al. (1998) measured wintertime personal, indoor and outdoor NO<sub>2</sub> concentrations and collected household and activity information for 568 participants from 18 cities in 15 countries from the northern hemisphere (including Ottawa, ON). For all countries combined, personal NO<sub>2</sub> exposures were more strongly related to household indoor concentrations ( $r_s = 0.75$ ) than those outdoors ( $r_s = 0.57$ ). In log-linear multivariate regression, the strongest predictors of increased personal exposure were the presence of gas stoves, kerosene heaters, and elevated outdoor concentrations; smaller contributions came from the presence of smokers and gas space heaters, and commuting for greater than 1 h. These findings highlight the contribution of both indoor and outdoor sources to total personal exposure to NO<sub>2</sub>.

As discussed above, indoor sources of NO<sub>2</sub> are important determinants of personal exposure. They introduce error into the relationship between total personal exposure and ambient concentrations of NO<sub>2</sub>; as discussed in Section 4.3, this relationship is stronger in the absence of indoor sources. However, indoor sources of NO<sub>2</sub> should not affect the correlation between ambient NO<sub>2</sub> concentrations and personal exposure to NO<sub>2</sub> of *ambient origin*.

#### 4.4.4 Other Factors

**Time-Activity:** There are complex human activity patterns across the general population that affect the relationship between ambient concentrations and personal NO<sub>2</sub> exposure. People engage in diverse activities and spend variable time in different microenvironments that are characterized by different levels of NO<sub>2</sub>. For example, the time that people spend in microenvironments that have relatively high NO<sub>2</sub> concentrations, such as in vehicles or adjacent to major roadways, can make a substantial contribution to their daily exposure to NO<sub>2</sub> of ambient origin. People engaged in vigorous physical activity would also have increased inhalation exposure to NO<sub>2</sub> by virtue of their increased minute volume and oral breathing. Data on the time spent doing various activities are available from the Canadian Human Activity Pattern Survey 2, a national time-activity survey of adults and children from urban centres and rural areas conducted in 2010–2011 (Health Canada, 2013a). In this survey, time-activity patterns differed by such factors as age, gender, season and rurality. For example, across age class, adults 20–59 years of age and infants <1 year old were at opposite ends of the spectrum with respect to the average amount of time spent indoors at home (66.8% vs. 89.2%, respectively), at other indoor locations (21.4% vs. 4.8%), and in vehicles (6.3% vs. 2.0%). The mean time spent in these microenvironments was intermediate for young children aged 1–4, children 5–11, adolescents 12–19, and seniors 60 or older. Infants <1 year of age also spent the least time outdoors on average (4.0%), while young children (1–4) spent the most time in this microenvironment (7.6%).

**Socioeconomic Status (SES):** Microenvironmental exposures to ambient NO<sub>2</sub> can also be influenced by other individual-specific factors such as SES. Across the indicators of SES examined, there is some evidence indicating higher exposure to NO<sub>2</sub> of ambient origin among low SES groups in the population, though these relationships are not uniformly observed. For example, in studies in Toronto (Buzzelli and Jerrett, 2007) and in Montreal (Crouse et al.,

2009a), NO<sub>2</sub> levels were often greater, as expected, in neighbourhoods with low SES indicators including low incomes, low education, and a higher proportion of people living alone. However, some deprived neighbourhoods had low levels of pollution, and some affluent neighbourhoods in the downtown core were also characterized by high NO<sub>2</sub> levels.

**Analytical Issues:** A number of analytical aspects can also affect the personal–ambient relationship for NO<sub>2</sub>. An important limitation of passive samplers is that they have relatively high detection limits (DLs) over shorter periods, potentially leading to an increase in non-detectable personal NO<sub>2</sub> concentrations. The fraction of data below the DL might be of concern in some studies, and would bias the associations low. In a number of personal exposure studies reviewed in the 2008 US EPA ISA, the majority of measurements were less than the DL and in all studies some personal measurements were below the DL. In addition, depending on the sorbent material, personal NO<sub>2</sub> samplers are subject to biases as a result of interference from other oxidized nitrogen species and high relative humidity (US EPA, 2008). Such measurement artifacts and differences in analytical capabilities among different research groups could have contributed to the mixed results in personal–ambient NO<sub>2</sub> relationships (US EPA, 2008). Instrument precision also contributes to exposure measurement error, but has a small effect relative to that from spatial heterogeneity in ambient NO<sub>2</sub> concentrations (subsection 8.3.2.3; Goldman et al., 2010, 2011).

## 4.5 Personal and Ambient Exposure Relationships between NO<sub>2</sub> and Other Pollutants

The associations between ambient NO<sub>2</sub> and ambient co-pollutants released from the same sources need to be considered in interpreting the results of epidemiological studies of NO<sub>2</sub>-related health effects. As reviewed in the 2008 US EPA ISA (US EPA, 2008), in most studies short-term measurements of ambient levels of NO<sub>2</sub> are moderately to strongly correlated with CO and PM<sub>2.5</sub> over time, whereas the correlations with O<sub>3</sub> and SO<sub>2</sub> were most often weaker and more variable and sometimes negative in the case of O<sub>3</sub>. For example, in a number of epidemiology studies in Canada, the correlation coefficients between daily 1-h max or 24-h avg ambient concentrations of NO<sub>2</sub> and co-pollutants ranged from 0.63 to 0.74 for CO; from 0.44 to 0.71 for PM<sub>2.5</sub>; from -0.51 to 0.40 for O<sub>3</sub>; and from 0.18 to 0.63 for SO<sub>2</sub> (Burnett et al., 2000; Kim et al., 2006; Schildcrout et al., 2006; Brook et al., 2007; Villeneuve et al., 2007; Liu et al., 2009). In a few studies that examined more specific traffic-related pollutants, relatively high correlations were found with elemental carbon (EC) (0.93 in Hochadel et al., 2006) and with combustion-related organics, including several aromatic hydrocarbons (BTEX) (0.45–0.6) and hopanes (0.67–0.8) (Brook et al., 2007).

Personal exposure to NO<sub>2</sub> is often most significantly correlated with, either at the ambient or personal level, PM<sub>2.5</sub> and other combustion-related pollutants (e.g. CO, EC, organic carbon (OC), certain polycyclic aromatic hydrocarbons (PAHs) and some volatile organic compounds (VOCs) such as benzene and 1,3-butadiene) (US EPA, 2008). Higher correlations were typically observed between ambient measurements of NO<sub>2</sub> and other traffic-related pollutants compared to the correlations for personal NO<sub>2</sub> versus personal/ambient levels of the other pollutants. For example, in a study in Toronto (Kim et al., 2006), the 24-h avg correlations between ambient NO<sub>2</sub> and ambient CO and PM<sub>2.5</sub> (0.72 and 0.44, respectively) were greater than those for personal NO<sub>2</sub> with ambient co-pollutants (0.20 and 0.30) or for personal NO<sub>2</sub> with personal co-pollutants (0.12 and 0.41). This reduced association most likely arises because personal exposure to NO<sub>2</sub> and other traffic-related pollutants is also the result of emissions from indoor and other outdoor sources, and these differ somewhat by pollutant; in addition, these various

pollutants are subject to different transportation and transformation processes in indoor and outdoor environments.

## 4.6 Spatially Resolved Approaches to Exposure Assessment of Ambient NO<sub>2</sub>

The concentration of NO<sub>2</sub> measured at a central site monitor or monitors is typically used as the measure of community population exposure in epidemiological studies of health effects associated with ambient NO<sub>2</sub>. However, a variety of alternative exposure assessment approaches with differing degrees of sophistication have been applied to try to take account of the substantial traffic-related spatial heterogeneity that exists for ambient NO<sub>2</sub> (subsection 4.4.1). Several of the methods that have been employed in the epidemiological studies of NO<sub>2</sub>-related health effects reviewed in Chapters 8, 9 and 10 are discussed below.

Some of the methods used to try to reflect the spatial heterogeneity of ambient NO<sub>2</sub> are fairly simple in nature. For example, epidemiology studies often use the NO<sub>2</sub> concentrations measured at the monitor closest to individual subjects or neighbourhoods as the exposure measure, rather than those at a central monitor or than the average of all monitors in a community. However, Sarnat et al. (2010) examined the effect of proximity to the monitoring station on the risks for circulatory disease emergency room visits (ERVs) in Atlanta, GA. For urban monitors risks were similar regardless of the monitor used within 20 miles (whether for the population as a whole or when analyses were restricted to subpopulations within 5 miles of a given monitor), but were reduced and non-significant using data from rural sites, which were at least 30 miles distant. Concentrations at the urban and residential sites were all well correlated with one another ( $r = 0.75$  to  $0.80$ ), but more poorly with the rural agricultural sites ( $r = 0.09$  to  $0.53$ ). Hence, at least in this setting where urban/residential sites were well correlated with each other, use of local monitoring data as opposed to other urban monitoring data did not significantly modify exposure estimation for NO<sub>2</sub> and other pollutants.

For traffic-related pollutants like NO<sub>2</sub>, some studies use measures of proximity to traffic (e.g. distance of residence from roadways, traffic counts) as indicators of a person's exposure to traffic-related air pollution. For example, in a study in Hamilton, ON, Jerrett et al. (2005) reported a significantly increased risk for asthma symptoms in women residing within 50 m of a major road, with a pattern of declining risk with increasing distance from the road. Similarly, in Canadian study of birth outcomes in Vancouver, BC (Brauer et al., 2008), residing within 50 m of a highway was associated with increased risks for low birth weight (LBW) and small for gestational age (SGA) infants. While relatively simple to apply, such approaches are somewhat rudimentary and do not take into account the total influence of multiple roadways or meteorology on exposures, and they are an indirect measure of NO<sub>2</sub> and related traffic pollutants. Additionally, traffic counts may not be widely available or are of variable quality (if comparing across cities) and there are some indications that the type of traffic (truck versus car, congested versus free-flowing) is as important as the quantity. In a review of intraurban air pollution exposure models, Jerrett et al. (2005) discussed the strengths and limitations of this approach to characterizing exposure.

For some panel epidemiology studies where individual subjects can be followed intensively, NO<sub>2</sub> concentrations outside of the subjects' residences can be measured. A number of these studies are reviewed in Section 8.4. These levels capture the small-scale spatial heterogeneity of ambient NO<sub>2</sub> at a location where people spend much of their time, but their validity as an exposure measure is affected by the time that subjects spend away from home (especially in high-pollutant microenvironments), and the labour- and resource-intensive nature of such monitoring generally limits its use to small populations and limited periods of observation.



In more sophisticated approaches to exposure assessment, computational models are used to estimate NO<sub>2</sub> levels at locations and/or times when measurements are not available to estimate spatial and temporal variations in concentrations within communities. These methods include dispersion models, spatial interpolation between monitors through statistical techniques, and LUR models; a brief discussion of each of these follows.

Dispersion models are a widely used tool to estimate population exposures to air pollutants. These models predict the transport and dispersion of an air pollutant emitted from a source by solving an equation that estimates the spread of pollutant to follow a Gaussian curve that is a function of distance from source. These models utilize input data such as pollutant emissions (quantity, location, height), meteorological conditions, topography and background pollutant levels to generate spatially resolved estimates of air pollution concentrations. From the inputs, the model computes the pollutant concentrations at the predefined receptor locations for the study area. This type of modelling has been widely employed in epidemiological studies of ambient NO<sub>2</sub>/NO<sub>x</sub>, most often in studies of effects associated with long-term exposure (Chapters 9, 10). In particular, a number of these studies investigated the association of modelled long-term NO<sub>2</sub>/NO<sub>x</sub> with the development of asthma (Modig et al., 2009; Oftedal et al., 2009; McConnell et al., 2010; Gruzieva et al., 2013). For example, Modig et al. (2009) reported that increased NO<sub>2</sub> levels estimated from air dispersion models were associated with dose-related increased risks for asthma in young adults from three Swedish cities. Dispersion models have the advantage of incorporating both spatial and temporal variations of air pollution within a study area without the need for dense monitoring networks. However, the available dispersion models have a number of limitations, including the need for considerable computing power (an issue when larger areas are being modelled and when higher spatial resolution is desired), the need for detailed input data to allow the model to estimate pollutant levels with an acceptable level of accuracy, and a limited ability to model the production of secondary pollutants and (for some) to model releases from a line source (e.g. roadway) or at a fine enough scale.

Another exposure assessment approach is geostatistical interpolation modelling, including such methods as kriging, splines, and inverse distance weighting (IDW). These are interpolation techniques in which the surrounding measured values are weighted to derive a predicted value for an unmeasured location. Weights are based on the distance between the measured points, the prediction locations, and the overall spatial arrangement among the measured points. Kriging can be used with whatever monitoring data are available (fixed, network monitors and/or monitoring campaign data). Monitoring data for the pollutant of interest are input and concentration values for locations for which no monitoring data exist are interpolated by the model, creating a pollutant concentration surface for the entire study area. For example, in a study in Vancouver, BC, of traffic-related air pollution impacts on birth outcomes (Brauer et al., 2008), residential exposures to NO<sub>2</sub> and other pollutants (estimated using IDW of concentrations from study area monitors) were associated with increased risks for LBW and SGA. Interpolative modelling requires a dense network of monitors to provide input data for the best interpolation results. The Canadian network of fixed monitors is relatively sparse, and monitoring campaigns may be required to provide adequate input data, especially for spatially heterogeneous pollutants such as NO<sub>2</sub>. This and other limitations of interpolation techniques are discussed by Jerrett et al. (2005).

Another exposure modelling approach of considerable interest is LUR modelling, which has been widely employed to characterize air pollution exposure (including NO<sub>2</sub>) and health effects for individuals residing within urban areas (Jerrett et al., 2005). LUR modelling utilizes geographically resolved data for a population (e.g. residence locations) and incorporates various parameters (such as land use and traffic characteristics) to explain the spatial variation in measured pollution concentrations. This is then used to estimate the pollution concentration

at unmeasured locations in order to allow estimation of the pollutant exposure of the population under study. LUR models generally require extensive monitoring as an important step in their development, though some are developed utilizing existing data from fixed-site network monitors. Typically, however, monitoring campaigns are undertaken specifically to gather information to aid in the development of the models. A typical monitoring campaign lasts 7–14 d (continuous) and is designed to cover the city of interest sufficiently to reflect the predictor variables to be incorporated into the model. Predictor variables in LUR models are normally computed for circular zones around each monitoring site; thus the variables are described with respect to buffer zones (i.e. “within X m”). The validity of the model predictions strongly depends upon the quality of the geographic predictor data, the selected monitoring sites, the complexity of the airshed, the inherent variability in concentrations and the accuracy of measurements at given sites (Wang et al., 2012). It is also important to note that current LUR models do not predict short-term concentrations, but rather produce estimates of seasonal and/or annual averages.

Many Canadian studies incorporating LUR have been carried out (Gilbert et al., 2005, 2007; Luginaah et al., 2006; Henderson et al., 2007; Jerrett et al., 2007; Atari et al., 2008; Marshall et al., 2008; Su et al., 2008, 2009, 2010; Wheeler et al., 2008; Sahsuvaroglu et al., 2009; Crouse et al., 2009b; Poplawski et al., 2009; Allen et al., 2011; Oiamo et al., 2012; Parenteau and Sawada, 2012). There is a large variation in performance across the models, with calculated  $R^2$  values between monitored and LUR-modelled  $\text{NO}_2$  concentrations ranging from 0.32 to 0.93, though most are between 0.6 and 0.8. Many of the significant predictor inputs are related to roadways and/or traffic, confirming the important influence of vehicle emissions on the spatial heterogeneity of ambient  $\text{NO}_2$ . Some factors (roadway data) are consistently significant inputs across all the models, whereas others are unique to the city for which the model has been developed (e.g. distance to the Ambassador Bridge for the Windsor, ON, models by Wheeler et al. (2008) and Luginaah et al. (2006)).

Some work has been done on the transferability of models to different urban areas; for example, Poplawski et al. (2009) employed the Henderson et al. (2007) Vancouver model in a Victoria, BC study. The Poplawski Vancouver model  $R^2$  increased from 0.51 to 0.61 after optimisation for use in Victoria. Similarly, Allen et al. (2011) developed LUR models for Winnipeg, MB, and Edmonton, AB, and transferred them between the cities (with some features of the “transferable” models being altered/deleted from the originally developed ones). The transferable models performed less well, with  $R^2$  values of 0.70 and 0.77 for Edmonton and Winnipeg, respectively, compared with 0.81 and 0.84 for the “best” models. These two studies indicate that LUR models do not appear to perform optimally “as is” and give better results if modified to tailor the variables to the city being studied (in addition to requiring monitoring data for the city to which the LUR model is to be applied). A few studies have assessed the temporal stability of LUR models over periods of several years (Eeftens et al., 2011; Wang et al., 2012; Cesaroni et al., 2012). These studies found good agreement between measured  $\text{NO}_2$  concentrations and the performance of LUR models across periods of between 7 and 12 years, and results supported the use of LUR models in epidemiological studies based on the findings that LUR models predicted spatial contrast well in both forecasting and hindcasting. Variations have been incorporated into LUR models (e.g. meteorological models, time-activity diaries, source-area factors) to improve the models’ ability to estimate the exposure of populations to the pollutant of interest in epidemiological studies, and work continues on their development.

The use of satellite remote sensing, rather than direct monitoring campaigns, is another method of collecting surface concentration data to examine spatial heterogeneity and estimate exposure to various pollutants, including  $\text{NO}_2$ . This technique can be especially useful in areas where



surface monitors are sparse. As described in a review by Martin (2008), satellite remote sensing does not explicitly resolve surface concentrations, but instead measures an integrated column amount over a vertical thickness that usually exceeds a few kilometres. Relating column amounts to ground-level concentrations requires information on the vertical structure of the atmosphere. Column observations of NO<sub>2</sub> contain large contributions from the boundary layer, due to strong surface sources, short lifetimes, and the increase in the NO/NO<sub>2</sub> ratio with altitude that is driven by the temperature dependence of the NO + O<sub>3</sub> reaction.

Hystad et al. (2011) created national air pollution models for NO<sub>2</sub>, PM<sub>2.5</sub>, benzene, ethylbenzene and 1,3-butadiene using satellite remote sensing data to estimate annual average pollutant concentrations, as well as LUR variables, NAPS data and census data to estimate population exposure. The LUR model performed quite well in the case of NO<sub>2</sub>, with an R<sup>2</sup> of 0.73 with NAPS monitoring data and explaining 43% of within-city NO<sub>2</sub> variation, well within the range of the LUR models developed via monitoring campaigns within the specific cities for which they were developed. The work of Hystad et al. (2011) provides an example of an alternative to city-specific monitoring campaigns to provide inputs into LUR models. As spatial (finer than the 10 × 10 km) and temporal resolution (annual average NO<sub>2</sub> concentrations are currently available via this method) improves in satellite remote sensing, there is likely to be an increase in use of this technique (probably in combination with traditional ground monitoring and LUR modelling) to estimate exposure to air pollutants such as NO<sub>2</sub>.

Much work has been done to develop exposure models that reasonably reflect the actual exposures of people to NO<sub>2</sub> for the purpose of determining the relationships between ambient NO<sub>2</sub> exposure and various health endpoints. Improvements in such exposure assessments are expected to be made as monitoring and modelling techniques continue to advance.

## 4.7 Summary and Considerations

The entire population is exposed to NO<sub>2</sub> originating from ambient sources, both when people are outdoors and when they are in indoor environments into which ambient NO<sub>2</sub> has infiltrated. As they go through the day, some people also spend time in locations that have higher NO<sub>2</sub> concentrations as a result of releases from non-ambient sources (e.g. indoors in homes with gas stoves).

This assessment is being conducted to support the development of an ambient standard for NO<sub>2</sub>, and is based in large part on the extensive epidemiological evidence linking ambient concentrations of NO<sub>2</sub> to a wide range of health effects (Chapters 8, 9, 10). In this context, a key issue is the ability of NO<sub>2</sub> concentrations measured by the monitoring network to serve as an indicator of personal exposure to NO<sub>2</sub> of *ambient origin*, as opposed to total personal exposure to NO<sub>2</sub> from all sources that is measured in most exposure assessment studies.

Studies of the relationship between personal exposures to NO<sub>2</sub> and concentrations measured by ambient monitoring networks have generally shown positive and often statistically significant correlations or regressions between short-term ambient concentrations and total personal exposures. Usually the ambient component of personal exposure to air pollutants is not directly measurable, but the total personal exposure can be regarded as the personal exposure of ambient origin if there are no indoor sources. In those studies where indoor sources of NO<sub>2</sub> were absent, the correlation of personal exposures with ambient concentrations was moderate to strong, and was increased 2- to 3-fold compared with that observed in the presence of indoor sources. In addition, the association between total personal exposure and ambient NO<sub>2</sub> was greater in the warm season (when people are generally more exposed to ambient air pollutants because building infiltration and time spent outdoors are greater) in a number of studies. These

findings were confirmed in a recent meta-analysis of a large number of exposure assessment studies.

Overall, the results of these studies indicate that, although the concentrations measured by the ambient monitoring network may not account for differences between individuals in exposure to NO<sub>2</sub> of ambient origin, they appear to be a reasonable surrogate for exposure at a population level. In addition, day-to-day variations in exposure of the population to NO<sub>2</sub> of ambient origin are likely to track changes in the concentrations measured at a central site/sites. It is these variations over time and the ability to represent population average personal exposure, rather than the absolute magnitude of the exposure itself, that are the basis for the associations between ambient NO<sub>2</sub> levels and the health effects reported in short-term epidemiological studies. Therefore, ambient concentrations are a useful and appropriate exposure measure for epidemiological studies of the health effects of NO<sub>2</sub> air pollution.

Vehicle emissions are an important source of NO<sub>2</sub> in urban environments. Large horizontal gradients in NO<sub>2</sub> concentrations near major roadways have been observed in a number of studies; levels on or near roads or in vehicle cabins were several times greater than urban background levels in these studies. Traffic variables are also often significant predictors of ambient NO<sub>2</sub> in LUR models, and of personal exposure to NO<sub>2</sub>. Near-road NO<sub>2</sub> also displays a negative vertical gradient, with concentrations being increased nearer to the road surface.

Localized emission from roadway sources leads to variability in NO<sub>2</sub> concentrations that is not captured by the regulatory monitoring network. This variation affects population-level exposure estimates and adds exposure measurement error to epidemiology studies that rely on ambient concentrations as indicators of exposure. Elevated concentrations of NO<sub>2</sub> on or near roadways also increase the exposure of anyone who spends substantial amounts of time in such locations. These would include people who spend a long time in vehicles commuting or during the course of their work (e.g. truck drivers), who work or commute in proximity to major roadways (e.g. roadway construction workers, cyclists), or who reside, work, attend school, etc. in buildings near such roadways.

The relationship between ambient concentrations and personal exposure to NO<sub>2</sub> of ambient origin will vary as a result of the influence of a number of factors, including spatial and temporal variability in NO<sub>2</sub> concentrations, time-activity patterns, building ventilation, and perhaps measurement artifacts and analytical methods. The influence of these factors results in exposure measurement error and potential bias in the risk estimates in epidemiology studies that are based on ambient concentrations. The bias can be either upward or downward, though it is expected to most often underestimate risks and make it more difficult to detect a health effect. Several studies performed in Atlanta, GA, that investigated the potential bias from using fixed area monitors on the resulting estimates of short-term risk for cardiovascular disease (CVD) ERVs indicated that the spatial heterogeneity of air pollutants was a much greater source of measurement error than instrument imprecision. For NO<sub>2</sub>, most results suggested that this measurement error markedly attenuated the risk estimates, sometimes even resulting in a loss of statistical significance.

The associations between ambient NO<sub>2</sub> and co-pollutants released from the same sources need to be considered in interpreting the results of epidemiological studies of NO<sub>2</sub>-related health effects. Causal attribution to NO<sub>2</sub> is challenging because associations can potentially reflect correlations with other pollutants rather than true causal association with NO<sub>2</sub>. In most studies, ambient levels of NO<sub>2</sub> are moderately to strongly correlated with CO and PM<sub>2.5</sub>, and less so with O<sub>3</sub> and SO<sub>2</sub>. Correlations are also moderate between personal NO<sub>2</sub> and ambient or personal exposure to other combustion-related pollutants within urban areas, most notably CO, PM<sub>2.5</sub>, PAHs, certain VOCs such as benzene and 1,3-butadiene, and PM constituents such as EC and

OC. Other sources (e.g. industrial point sources, fires) may contribute significantly to exposure to NO<sub>2</sub> and other pollutants in some settings.

The concentration of NO<sub>2</sub> measured at central site monitors is typically used as the measure of community population exposure to ambient NO<sub>2</sub> in epidemiology studies of health effects. However, a variety of alternative approaches to exposure assessment with differing degrees of sophistication have been developed to try to take account of the important spatial heterogeneity that exists for ambient NO<sub>2</sub>. These methods are expected to continue evolving and improving exposure assessments.

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## 5. Toxicokinetics

As mentioned in Chapter 1, much of the relevant literature on the toxicokinetics of NO<sub>2</sub> has recently been reviewed by Health Canada during the course of an assessment of indoor NO<sub>2</sub> to support the development of the Residential Indoor Air Quality Guideline for this pollutant (Health Canada, 2015). For subject areas that are of common interest to these two assessments, several of the chapters in this report of the assessment of ambient NO<sub>2</sub> are based on those from the indoor air review, including this current chapter.

The absorption of inhaled NO<sub>2</sub> in the body is governed primarily by the initial reactions in the epithelial lining fluid (ELF) of the lung, which are considered key in the production of toxic effects in the lung. Systemic uptake of NO<sub>2</sub> is generally in the form of the nitrite and nitrate ions NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>, which contributes to the NO<sub>2</sub><sup>-</sup>/NO<sub>3</sub><sup>-</sup> pool in the bloodstream. While mechanisms of action for systemic effects of inhaled NO<sub>2</sub> have been proposed (Section 6.6), any discussion of the distribution, metabolism, and excretion of nitrite/nitrate in the bloodstream would need to be considered in the larger context of the considerably greater contributions from dietary sources and endogenous production of NO<sub>2</sub><sup>-</sup> and NO<sub>3</sub><sup>-</sup>. As most toxicological and epidemiological investigations of inhaled NO<sub>2</sub> considered in this assessment have focused on respiratory effects, the discussion of toxicokinetics presented below is limited to absorption and the reactions occurring at the level of the ELF.

NO<sub>2</sub> is a highly reactive, nitrogen-centred free radical with limited aqueous solubility (Denbigh and Prince, 1947). Due to this low aqueous solubility, there is considerable resistance to NO<sub>2</sub> interfacial transfer through the aqueous surface film lining of the respiratory tract.

While some NO<sub>2</sub> is absorbed in the nasal cavity, contributing to nasal tissue dose, modelling of pulmonary uptake in rats indicates that the greatest tissue dose of NO<sub>2</sub> likely occurs deeper in the lung airways, in the proximal alveolar region (Overton and Graham, 1995). In the lung, inhaled NO<sub>2</sub> first dissolves to form a solute gas, which then reacts with a reduced substrate in the ELF (e.g. glutathione (GSH), and ascorbic and uric acids). In rat models, GSH and ascorbic acid are the primary absorption substrates (Velsor and Postlethwait, 1997), and there is some evidence that in human ELF, reactions with NO<sub>2</sub> deplete ascorbic and uric acid levels but not GSH levels (Kelly and Tetley, 1997).

Absorption of NO<sub>2</sub> into the ELF occurs primarily by reactive uptake; this process is rate limited by the conversion of NO<sub>2</sub> to its metabolites rather than gas solubility and is thus a saturable process (Postlethwait et al., 1995; Kelly and Tetley, 1997; Velsor and Postlethwait, 1997; US EPA, 2008). As the driving force for net absorption is dependent on the solute NO<sub>2</sub>–substrate reaction rather than the initial rate of formation of solute NO<sub>2</sub>, the rate of renewal of reactants in the ELF may be a significant determinant of NO<sub>2</sub> uptake, and the relationship between exposure concentration and cytotoxicity may not be linear.

The rate of renewal of reactants (such as GSH, and ascorbic and uric acids) in the ELF may be a significant determinant of NO<sub>2</sub> uptake. Given a sufficient supply of reduced reactants, even if the exposure concentration and NO<sub>2</sub> absorption are moderately high, the initial reaction products (reactive oxygen species (ROS), thiyl, and ascorbate radicals) will be partially quenched (often due to self-quenching reactions), and cytotoxicity may be minimal. Conversely, where the initial concentration of reduced reactants is lower or depleted by ongoing exposure, both absorption and quenching also decrease, and a breakthrough of unreacted NO<sub>2</sub> may occur; the resulting cytotoxicity may therefore be relatively greater than where absorption was higher. Similarly, where NO<sub>2</sub> concentrations are very high, NO<sub>2</sub> diffusion may exceed reaction, thereby permitting direct interaction with underlying cellular membranes (as reviewed in

Postlethwait and Bidani, 1994; Velsor and Postlethwait, 1997). Therefore, the relationships between exposure concentration, absorption of primary NO<sub>2</sub>, and cytotoxic effects, cannot be assumed to be linear. Furthermore, there is potential for significant interspecies and individual differences in uptake as a result of differing concentrations of reactive substrates (e.g. ascorbate and urate) within the ELF (Loria et al., 1998; Cahill et al., 2009) and in ELF antioxidant concentrations (Slade et al., 1993).

Most inhaled NO<sub>2</sub> is converted to the relatively stable nitrite ion NO<sub>2</sub><sup>-</sup> by reactions in the pulmonary ELF. Other major products of NO<sub>2</sub> reactions in the ELF appear to include ascorbyl (A<sup>•</sup>) and thiyl radicals (GS<sup>•</sup> and/or its recombination product GSSG<sup>•</sup>), and the ROS superoxide (O<sub>2</sub><sup>•-</sup>) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) (Velsor et al., 2003). Ascorbyl and thiyl radicals have a greater reducing potential than the parent antioxidants and are able to initiate a reaction cascade leading to oxidation of cellular membranes underlying the aqueous phase in *in vitro* models (Velsor and Postlethwait, 1997).

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## 6. Animal Toxicology

Experimental animal research provides valuable information about the biological effects of NO<sub>2</sub> and the underlying mechanisms of action. Quantitative extrapolation from high-dose animal assays to low-dose human exposures would, however, be associated with significant uncertainty about the sensitivity of the respiratory system to NO<sub>2</sub>. Nonetheless, similarities between health effects observed in animal studies and those observed in human studies may strengthen the biological plausibility of a relationship between pollutant exposure and observed outcomes and may contribute to an understanding of the mechanisms by which toxic effects are induced.

As noted in Chapter 1, the sections of this chapter that deal with toxicological studies of respiratory effects in experimental animals and the mechanisms of action for various effects are based on the summary of the literature that Health Canada has conducted to support the development of the Residential Indoor Air Quality Guideline for NO<sub>2</sub> (Health Canada, 2015).

The following sections provide an overview of respiratory effects with respect to host defences, respiratory tract morphology and function, and antioxidant defences. While the focus is on respiratory effects, as these endpoints are most relevant to the effects observed in controlled human exposure and epidemiological studies, neurodevelopmental, genotoxic, carcinogenic, cardiovascular and reproductive/developmental effects have also been reviewed and are discussed. The evidence for potential mechanisms of action involved is also summarized. A more detailed and comprehensive synthesis of toxicological studies of NO<sub>2</sub> is also available through assessments by various agencies (US EPA, 1993, 2008; WHO, 1997, 2010) and a review chapter by Schlesinger (2009).

### 6.1 Respiratory Effects

Over the years several experimental animal studies have investigated various respiratory effects, particularly with respect to host and antioxidant defences, as well as respiratory function and morphology, following either short- or long-term exposure to NO<sub>2</sub>. Possible mechanisms of action of NO<sub>2</sub> toxicity, including oxidative stress and inflammation, hematological and tumour-promoting effects, have been proposed; they are based primarily on evidence from *in vitro* (human and animal cell line) and *in vivo* (experimental animal) studies.

There is considerable experimental and epidemiological evidence linking exposure to NO<sub>2</sub> with asthma-related outcomes in humans. However, asthma is a disease that is unique to humans and is characterized by a complex pathology. While a variety of animal models have been developed to mimic different respiratory symptoms associated with asthma, none can encompass all aspects of the disease as it occurs in humans (Szelenyi, 2000; Holgate, 2011; Holmes et al., 2011), thus making quantitative estimations of risks in humans that are based on animal studies particularly uncertain. The most common model of asthma in the rat, the ovalbumin-induced asthma phenotype, is known to display significant differences between rat strains; it is only useful for evaluating acute inflammatory effects, rather than chronic effects, due to adaptive responses (Kucharewicz et al., 2008).

#### 6.1.1 Effects on Host Defences

A reduction in ciliary activity and weak eosinophil accumulation were observed in the nasal mucosa of Hartley guinea pigs exposed to 3 ppm NO<sub>2</sub> (6 h/d, 6 times/wk) for 2 weeks (Ohashi et al., 1994; Kakinoki et al., 1998). The effects were greater at a higher concentration (9 ppm). Similar observations were made with respect to the trachea and bronchi, including a reduction in

ciliary activity in both guinea pigs and New Zealand white rabbits at the same two concentrations (Ohashi et al., 1994; Kakinoki et al., 1998), and an increase in the number of eosinophils in the tracheal mucosa of guinea pigs at 9 ppm and 18 ppm (Ohashi et al., 1994; Papi et al., 1999). Following subchronic exposure to a high concentration of NO<sub>2</sub> (4 ppm for 12 consecutive weeks), Fujimaki and Nohara (1994) observed an immunoglobulin E (IgE)-mediated release of histamine from mast cells isolated from guinea pigs but not from those isolated from rats.

The effects of varying exposure times (repeated bursts to 20-d continuous) to concentrations of NO<sub>2</sub> ranging from 5 ppm to 500 ppm on bronchoalveolar lavage fluid (BALF) were characterized by the following:

- an increase in total proteins in rats, rabbits, and sheep (Januszkiewicz and Mayorga, 1994; Lehnert et al., 1994; Meulenbelt et al., 1994; Kleeberger et al., 1997; McElroy et al., 1997; Garn et al., 2003; Müller et al., 2003)
- an increase in the number of sloughed epithelial cells in rats and sheep (Januszkiewicz and Mayorga, 1994; Elsayed et al., 2002)
- an increase in the number of eosinophils and neutrophils in guinea pigs, rats, and rabbits (Meulenbelt et al., 1994; Papi et al., 1999; Garn et al., 2003)
- an increase in the number of macrophages in rats and mice (Lehnert et al., 1994; Holroyd et al., 1997; Kleeberger et al., 1997; van Bree et al., 2000; Garn et al., 2003)
- a decrease in the number of macrophages at a very high concentration (200 ppm and 500 ppm) in rats and sheep (Januszkiewicz and Mayorga, 1994; Elsayed et al., 2002)
- an increase in the number of polymorphonuclear (PMN) leukocytes in rats (Lehnert et al., 1994; Pagani et al., 1994)

These numerous effects are characteristic of pulmonary inflammation.

Generally, as indicated above, dose-related increases in macrophage numbers in rats exposed acutely or subchronically (up to 15 wk) occur at NO<sub>2</sub> concentrations above 5 ppm (Schlesinger, 2009). However, functional properties may be affected at lower concentrations. Baboons exposed to an NO<sub>2</sub> concentration of 2 ppm (8 h/d, 5 d/wk) for 6 months were less responsive to migration inhibitory factor, a lymphokine mediating cell movement (Greene and Schneider, 1978). Depressed phagocytosis of macrophages was found in mice exposed to NO<sub>2</sub> at 0.5 ppm (3 h/d, 5 d/wk) for 2 months, although the opposite effect was observed in rats in a 7-d exposure at 40 ppm (Sone et al., 1983).

In AKR/cum and C57BL/6J mice exposed to 0.25 ppm NO<sub>2</sub> for 7 weeks and 0.35 ppm NO<sub>2</sub> for 12 weeks, respectively, the percentages of all T-lymphocyte subpopulations (mature T-lymphocytes [Lyt-1<sup>+</sup>], T-helper/inducer lymphocytes [L3T4<sup>+</sup>], T-cytotoxic/suppressor lymphocytes [Lyt-2<sup>+</sup>], and natural killer cells [asialo GM1<sup>+</sup>]) were lower in spleens of mice exposed to NO<sub>2</sub> than in spleens of filtered-air controls (Richters and Damji, 1988). In AKR/cum mice exposed to 0.25 ± 0.05 ppm NO<sub>2</sub> (7 h/d, 5 d/wk) for up to 181 d, the T-lymphocyte subpopulations (and T-helper/inducer [CD4<sup>+</sup>] lymphocytes in particular) were significantly lower in NO<sub>2</sub>-exposed animals following 37 d and 181 d of exposure (Richters and Damji, 1990).

Fujimaki et al. (1998) found that NO<sub>2</sub> exposure (1 or 2 ppm for 3 months) before sensitization resulted in a reduction in IgG- and IgE- specific antibody production in mice. Gilmour et al. (1996), Hubbard et al. (2002) and Proust et al. (2002) demonstrated that exposures ranging from 0.7 to 20 ppm NO<sub>2</sub> immediately after antigen provocation can attenuate allergic reactions through a reduction in eosinophil levels in mouse BALF at 0.7, 5, 7, and 20 ppm NO<sub>2</sub>, and a reduction in IgG- and IgA-specific antibodies in rat BALF after a 3-h exposure to 5 ppm. Proust

et al. (2002) explain that the process whereby allergic responses are attenuated with exposure to concentrations of NO<sub>2</sub> of 5 and 20 ppm is not known, but would be related to pharmacodynamic alterations that NO<sub>2</sub> induces in pulmonary defence mechanisms. These studies support the notion that NO<sub>2</sub> modulates the immune response to inhaled allergens through alterations in eosinophil levels and/or activity, as suggested by the results of controlled human exposure studies (Section 7.2); however, unlike the results of human studies, animal studies generally show a decrease in eosinophil levels following allergen exposure. The reason for this discrepancy is unclear.

Studies investigating the combination of allergen sensitization, provocation by the same agent, and exposure to NO<sub>2</sub> concentrations from 1 to 87 ppm showed hypersensitivity and an increase in antigen-induced allergic responses in rats, mice, and guinea pigs (Kobayashi and Shinozaki, 1990; Kitabakate et al., 1995; Kobayashi and Miura, 1995; Gilmour et al., 1996; Siegel et al., 1997; Mi et al., 2002).

The effect of NO<sub>2</sub> exposure on resistance to infectious agents has been investigated in a number of studies, with results depending on the specific pathogen, concentrations of NO<sub>2</sub>, and exposure pattern and duration. For example, exposure of mice to 0.5 ppm NO<sub>2</sub> for 3 h/d for 3 months increased mortality to *Streptococcus sp.*, while exposure to 0.5–1.5 ppm NO<sub>2</sub> continuously over 3 months produced no effect on mortality due to *Klebsiella pneumoniae*. Exposure to this same pathogen and 5 ppm NO<sub>2</sub> continuously for 3 d did, however, increase mortality (Schlesinger, 2009). Similar results were noted in monkeys, where continuous exposure to 5 ppm of NO<sub>2</sub> for 2 months showed increased markers of infection and mortality to *Klebsiella pneumoniae* (Henry et al., 1970).

Miller et al. (1987) investigated in mice the effects of spikes of NO<sub>2</sub> (two 1-h spikes of 0.80 ppm, 5 d/wk) superimposed upon chronic exposure at 0.20 ppm, in relation to antibacterial lung defences. This exposure pattern was designed to correspond with urban exposure to NO<sub>2</sub> where diurnal peaks are superimposed on a continuous baseline exposure. After 16, 32, and 52 weeks of exposure the pulmonary host defence of the mice was assessed through exposure to *Streptococcus zooepidemicus*. Mortality was followed over a 14-d period during which the mice were exposed to clean air. The mortality of mice in the spiked exposure regimen was significantly greater than in mice only exposed to 0.20 ppm or in the control group (clean air). In addition, there was a significant trend of increasing mortality with the exposure duration. No difference in mortality was observed between the chronic exposure group (0.20 ppm) and the control group. Schlesinger (2009) noted that the importance of the effect of spiked exposures on host defences in other studies varies with both spike duration and time between spikes. In addition, while both exposure duration (T) and peak concentration (C) influence the effect of NO<sub>2</sub> on mortality due to bacterial infection, peak concentration appears to have a greater effect on the overall potency of NO<sub>2</sub> (i.e. fixed C × T values) than exposure duration.

### 6.1.2 Respiratory Morphology and Function

Aguggini et al. (1994) studied the effects of NO<sub>2</sub> on the respiratory system of pigs exposed to 100 or 400 ppm NO<sub>2</sub> for 30 minutes (min). They observed that NO<sub>2</sub> exposure alters certain properties of the respiratory system and lungs (increased flow resistance and reduced lung elasticity). These effects were far more marked in pigs exposed to an NO<sub>2</sub> concentration of 400 ppm. The authors concluded that such changes are likely to be caused by tissue damage resulting from exposure to that concentration of NO<sub>2</sub>; characteristically, they consist of changes in the viscoelastic properties of the lung resulting from NO<sub>2</sub>-induced parenchyma alterations.

Meulenbelt et al. (1994) exposed rabbits to different concentrations of NO<sub>2</sub> (125–800 ppm for 10 min), and observed that effects increased in relation to concentration. These effects were characterized by an increase in lung weight as well as microscopic histological changes, such



as desquamation of bronchiolar epithelium. Severe catarrh (acute or chronic inflammation of the airways, accompanied by mucous gland hypersecretion and epithelial cell desquamation) and hemorrhagic pneumonia were observed following exposure to 600 ppm NO<sub>2</sub> for 10 min.

Elsayed et al. (2002) exposed male rats to an NO<sub>2</sub> concentration of 200 ppm for 15 min. Minute volume decreased by 59% during the period of exposure, and lung weight increased by 40% (animals euthanized 1 h after exposure). An electron paramagnetic resonance study of lung tissue uncovered hemoglobin oxidation and carbonated radical formation. A reduction in the quantity of vitamin E, vitamin C, and uric acid was also observed in lung tissues, along with an increase in lipid peroxidation. However, total protein and lipid rates did not show any variation when compared to controls. This study indicated that brief exposure to high levels of NO<sub>2</sub> is sufficient to induce significant injury.

Januszkiewicz and Mayorga (1994) studied the effects of NO<sub>2</sub> in sheep; they found that exposure to 500 ppm NO<sub>2</sub> for 15–20 min induced a decrease in tidal volume and an increase in breathing rate. Changes in pulmonary function were measured by an increase in pulmonary resistance and a decrease in pulmonary compliance. A histological study of the lungs performed 24 h after exposure revealed the presence of patchy pulmonary edema.

The region of the respiratory tract most sensitive to NO<sub>2</sub> includes the terminal and respiratory bronchioles, and the adjacent alveolar ducts and alveoli. The primary cellular targets are ciliated cells of the bronchiolar epithelium, and type I cells of the alveolar epithelium (Schlesinger, 2009). In the lower respiratory tract of rats, the effects of different concentrations of NO<sub>2</sub> (5–250 ppm) were characterized by the alteration and degeneration of type I cells (McElroy et al., 1997; Elsayed et al., 2002), and by the proliferation of type II cells (Barth et al., 1994a; Müller et al., 1994). Changes typical of type II cells and their metabolism were also observed, including reduced surfactant secretion, increased phospholipid synthesis, cell hyperplasia, increased choline absorption, and growth in the number and size of lamellar bodies (Müller et al., 1994). No significant difference in proliferation of type II cells was observed with an exposure to 0.80 ppm NO<sub>2</sub> for 1 and 3 d (Barth et al., 1994a).

In an investigation using mice, Miller et al. (1987) evaluated the effects that spikes of NO<sub>2</sub> (two 1-h spikes of 0.80 ppm, 5 d/wk) superimposed upon chronic exposure to 0.20 ppm NO<sub>2</sub> had on pulmonary function variables. Although some significant differences in some tests (end expiratory volume, vital capacity) were found between the different treatment groups (chronic + spike, chronic alone, and control), these changes were small (e.g. a 5–7% decrease in vital capacity was observed in chronic + spike exposure versus chronic exposure).

Schlesinger (2009) noted the difficulty of establishing a threshold for morphological endpoints, due to the complexity of changes and the large interspecies differences in response. He concluded that long-term exposures to levels of NO<sub>2</sub> greater than 2 ppm are generally required to produce extensive or permanent damage in experimental animals. The uncertainty of extrapolating from different species to humans with respect to morphological and functional changes, especially at high exposure levels, was also underlined by Elsayed et al. (2002) in the study report described above. These researchers found significant interspecies differences in dosimetry and species-specific responses when comparing rats with sheep, and they suggested that sheep may be the better model for human risk assessment.

### **6.1.3 Effects on Antioxidant Defences**

Nitrogen dioxide is a reactive free radical and thus would be expected to elicit the lung's antioxidant defences. Sagai and Ichinose (1987) investigated lipid peroxidation and changes in antioxidative protective enzymes in lungs of rats exposed to NO<sub>2</sub> at concentrations of 0.04, 0.4, and 4 ppm for 9, 18, and 27 months. Lipid peroxidation, measured as ethane concentrations in



the breath of rats and thiobarbituric acid reactive substances (TBARS) in lung homogenates, decreased on d 1 and then increased to a maximum at d 3. In the subacute study, after the initial increase, ethane exhalation levels had decreased to near initial levels by 4 weeks and then proceeded to increase gradually from 8 to 16 weeks in a dose–response relationship, with a similar pattern being observed in the levels of TBARS. In the case of chronic exposure, the amounts of TBARS increased significantly in the two higher dose groups (0.4 and 4 ppm). Elevated ethane exhalation increased significantly in a dose–response fashion in the groups exposed at 0.04 and 0.4 ppm. In the highest dose group, ethane exhalation was similar to controls, but pathological examination showed an increase in the thickness of the alveolar wall and lung fibrosis, perhaps because of a decrease in ventilatory capacity.

In a parallel study in rats exposed continuously to 0.4, 1.2, and 4 ppm NO<sub>2</sub> for 16 weeks, Sagai and Ichinose (1987) examined changes in the protective enzymes glutathione peroxidase (GPx), glutathione reductase (GSR), glucose-6-phosphate dehydrogenase, superoxide dismutase (SOD), and disulphide reductase in lungs. Maximum levels of these enzymes were observed at the fourth week and tended to decrease thereafter, inversely following changes in lipid peroxidation.

## 6.2 Cardiovascular Effects

The effect of short-term exposures to NO<sub>2</sub> on various hematological parameters in animals has been investigated in a number of studies. Changes in red blood cell membranes of experimental animals following NO<sub>2</sub> exposures were reported. In guinea pigs, following exposure to 0.36 ppm NO<sub>2</sub> for 1 week, Mersch et al. (1973) reported increased red blood cell D-2,3-diphosphoglycerate, which is indicative of low tissue oxygenation. An increase in red blood cell sialic acid, indicative of a younger population of red blood cells, was reported in rats exposed to 4 ppm NO<sub>2</sub> continuously for 1–10 d (Kunimoto et al., 1984). In another study performed by Mochitate and Miura (1984), however, exposure to the same concentration of NO<sub>2</sub> resulted in a decrease in red blood cell number. In the only study evaluating methemoglobin formation following exposure concentration of less than 5 ppm, it was reported that the amount of methemoglobin was not increased in mice exposed to 0.8 ppm NO<sub>2</sub> for 5 d (Nakajima and Kusumoto, 1968; in US EPA, 1993). This is in contrast to some, but not all *in vitro* and *in vivo* studies using higher NO<sub>2</sub> concentrations, which have found methemoglobin effects (Bloch et al., 1973; Case et al., 1979; Oda et al., 1981; US EPA, 1993).

More recent laboratory animal studies with short-term exposures to NO<sub>2</sub> identified slight histological changes in the heart, including inflammatory infiltration in Wistar rats exposed to 2.5, 5 or 10 ppm for 1 week. Markers of oxidative stress were decreased (copper/zinc-superoxide dismutase (Cu/Zn-SOD)) or increased (manganese-superoxide dismutase (Mn-SOD), malondialdehyde (MDA), protein carbonyl (PCO)) with NO<sub>2</sub> exposures. Protein and messenger ribonucleic acid (mRNA) levels of markers of vasoactivity (endothelin-1 (ET-1), endothelial nitric oxide synthase (eNOS)), inflammation (tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), intracellular adhesion molecule-1 (ICAM-1)) and cardiac myocyte apoptosis (p53, bax:bcl-2, mean number of TUNEL-positive myocytes) were increased with NO<sub>2</sub> exposures compared with controls (Li et al., 2011).

Apo E<sup>-/-</sup> mice on a high-fat diet exposed to 0.2 or 2 ppm NO<sub>2</sub> for 7 d had a significant decreasing trend in a marker of oxidative stress (aortic heme oxygenase-1 (HO-1)) but did not display significant changes in markers of vascular remodelling (matrix metalloproteinase-9 (MMP-9), ET-1 or tissue inhibitor of metalloproteinase-2 (TIMP-2)) or in assays for lipid peroxidation or gelatinase activity (Campen et al., 2010).

Wistar rats on a low- or high-selenium (Se) diet were exposed to 0 or 5 ppm NO<sub>2</sub> for 5 d or 0 or 50 ppm NO<sub>2</sub> for 30 min. Markers related to oxidation were examined in blood (chemiluminescence), plasma (Se, GPx, TBARS), and red blood cells (RBCs) (GPx, SOD). Blood parameters (cell counts, hemoglobin) were also examined. Plasma Se and GPx levels decreased in the NO<sub>2</sub>-exposed rats on the high-Se diet, while GPx increased in RBCs in NO<sub>2</sub>-exposed rats in both diet groups. Glutathione S-transferase levels increased in NO<sub>2</sub>-exposed rats in the 5-d exposure groups. Decreased blood hematocrit and hemoglobin were observed in the high-Se diet NO<sub>2</sub> 5-d-exposed groups (DeBurbure et al., 2007).

Limited toxicology data also exist on the effect of long-term exposure to NO<sub>2</sub> on the cardiovascular system. In the older literature, alterations in vagal responses were observed when rats were exposed to 10 ppm NO<sub>2</sub> for 24 h, but not with 0.4 ppm NO<sub>2</sub> exposure for 4 weeks (Tsubone and Suzuki, 1984). A significant reduction in the pressure of oxygen in arterial blood (PaO<sub>2</sub>) was found in rats exposed to 4 ppm NO<sub>2</sub> for 3 months, whereas there was no effect following exposure to 0.4 ppm NO<sub>2</sub> over the same period (Suzuki et al., 1981; in US EPA, 1993). In a subsequent study (Suzuki et al., 1984; in US EPA, 1993), the investigators observed a reduction in heart rate in mice exposed to both 1.2 and 4 ppm NO<sub>2</sub> for 1 month. It is, however, unknown whether these effects were the direct result of NO<sub>2</sub> exposure or secondary responses to lung edema and changes in blood hemoglobin content. Changes in blood triglycerides, high-density lipoprotein (HDL), and HDL/total cholesterol ratios in response to a 24-week exposure of 0.16 ppm NO<sub>2</sub> were seen in an obese rat strain (Takano et al., 2004). In squirrel monkeys exposed to 1 ppm NO<sub>2</sub> for 16 months (Fenters et al., 1973) or in dogs exposed to ≤5 ppm NO<sub>2</sub> for 18 months (Wagner et al., 1965) no changes on hematocrit and hemoglobin levels were observed. Furiosi et al. (1973), however, reported polycythemia as well as an increased ratio of PMN leukocytes to lymphocytes in the blood of rats exposed to 2 ppm NO<sub>2</sub> for 14 months.

Several recent studies of effects of long-term inhalational exposure to NO<sub>2</sub> on cardiovascular health in laboratory animals were identified. In one study middle-aged AKR/J mice were exposed to filtered ambient air or unfiltered ambient air from Baltimore for 40 d. Heart rate, heart rate variability (HRV) parameters (standard deviation of normal-to-normal intervals (SDNN), the square root of the mean of the sum of the squares of the successive differences between adjacent normal-to-normal intervals (rMSSD), total power (TP), low frequency (LF), high frequency (HF), LF:HF ratio), deep body temperature and body weights were measured. Mean levels of NO<sub>2</sub> were 0.021 ppm in the filtered air and 0.036 ppm in the unfiltered air. When the two groups were directly compared, the physiological parameters were generally higher, but not significantly so, for the mice in the low-pollution chamber as compared with those mice in the high-pollution chamber, with the exception of the LF:HF ratio. Data were also analyzed longitudinally. An increase of 0.01 ppm NO<sub>2</sub> was associated with a decrease in heart rate at lag 3 d (central estimate of the association = -7.7; 95% CI -12.6, -2.8) and with the 7-d cumulative lag (central estimate of the association = -8.3; 95% CI -15.9, -0.7) in multi-pollutant models, adjusted for CO, PM, air temperature, air relative humidity and a time variable (Ramos-Bonilla et al., 2010). Significant declines in heart rate were also observed with CO (lag 3 d and the 7-d cumulative lag) and PM (lag 3 d) exposures.

Wistar rats on a low- or high-Se diet were exposed to 0, 1 or 10 ppm NO<sub>2</sub> for 4 weeks. Markers related to oxidation were examined in blood (chemiluminescence), plasma (Se, GPx, TBARS), and RBCs (GPx, SOD). Blood parameters (cell counts, hemoglobin) were also examined. Plasma Se levels decreased in the NO<sub>2</sub>-exposed rats on the high-Se diet, while GPx increased in RBCs in NO<sub>2</sub>-exposed rats in both diet groups. Glutathione S-transferase levels increased in NO<sub>2</sub>-exposed rats in both diet groups. Decreased blood hematocrit and hemoglobin were observed in the high-Se diet NO<sub>2</sub>-exposed groups. An increase in TBARS was observed only in the 1-ppm 28-d-exposure groups (DeBurbure et al., 2007).

ApoE<sup>-/-</sup> mice on a high-fat diet were exposed to filtered air or dilutions of diesel or gasoline exhaust, wood smoke or coal emissions for 50 d (Seilkop et al., 2012). They were assessed for potential relationships between specific air components including NO<sub>2</sub> and markers of cardiovascular responses in the proximal aorta using a data mining technique called multiple additive regression trees analysis. NO<sub>2</sub> was a strongly predictive component for changes in some markers in pathways leading to atherosclerosis, including oxidative stress (TBARS), endothelial dysfunction (ET-1) and inflammation (tissue inhibitor of metalloproteinase2, or TIMP2), whereas little association was observed for other markers related to atherosclerosis, including those for oxidative stress (OH-1), inflammation (MMP3, MMP7, MMP9) or angiogenesis (vascular endothelial growth factor, or VEGF) (Seilkop et al., 2012).

## 6.3 Genotoxicity and Carcinogenicity

The significance of the few positive genotoxicity results for NO<sub>2</sub> is unclear, in part because of the difficulty in separating direct and indirect effects. Overall, it appears that NO<sub>2</sub> has the ability to induce genotoxic effects *in vitro*, both in bacterial and eukaryotic models, and in some cases genotoxicity is observed at quite low concentrations (e.g. in nasal epithelial cells (Koehler et al., 2010, 2013)). The few *in vivo* studies in *Drosophila* and rodents have generally provided negative results (at concentrations ranging from 0.1 to 7000 ppm) (US EPA, 2008). Positive findings were, however, noted by Isomura et al. (1984), in which both chromosome aberrations and mutations in rat lung cells were observed after NO<sub>2</sub> inhalation *in vivo* (8–29 ppm NO<sub>2</sub>), and by Walles et al. (1995), in which single strand breaks were produced at very high exposure concentrations (30 ppm NO<sub>2</sub>) in the lungs of mice.

The chemical reactivity of NO<sub>2</sub> suggests that it may have the potential to be involved in pulmonary carcinogenesis; however, the experimental and epidemiological evidence indicate it is unlikely that NO<sub>2</sub> is able to act as a direct pulmonary carcinogen. The only evidence for tumour induction has been obtained in the lung tumour-prone A/J mouse model (Adkins et al., 1986) and after intermittent exposure in the NMRI mouse prone to skin fibroadenomas (Henschler and Ross, 1966; Ross and Henschler, 1968). In co-exposure studies, NO<sub>2</sub> appeared to have co-carcinogenic effects when combined with other inhaled oxidants, but not with chemical carcinogens administered parenterally (except for liver tumours in hamsters administered N-nitrosodiethylamine (Witschi et al., 1993)), and such effects required fairly high NO<sub>2</sub> concentrations (>3.7 ppm NO<sub>2</sub>). NO<sub>2</sub> may therefore potentially act as a tumour promoter rather than as a complete carcinogen (as reviewed in Witschi, 1988).

A relationship between NO<sub>2</sub> exposure and the formation of carcinogenic nitrosamines has been demonstrated in mice exposed to 4–4.5 ppm NO<sub>2</sub> for 1 h (Rubenchik et al., 1995). Co-exposure studies indicate the tumour-promoting activity of NO<sub>2</sub> may be a function of free radical generation and lipid peroxidation (Ichinose et al., 1991; Ichinose and Sagai, 1992). There is no clear explanation of the increased frequency of pulmonary adenomas in NMRI mice intermittently exposed to NO<sub>2</sub>, given that continuous exposure reduced tumour frequency; however, the findings appear to indicate that some form of pulmonary adaption may occur during continuous exposure (Henschler and Ross, 1966; Ross and Henschler, 1968).

A critical effect of NO<sub>2</sub> exposure may be to increase dissemination and/or proliferation of tumour metastases (Richters and Damji, 1990), depending on the circumstances of exposure and the tumour involved (Richters and Kuraitis, 1981, 1983, 1985). It is the formation and growth of metastatic tumours that usually makes cancer a fatal disease. The lung, in addition to being a target of inhaled toxicants, is one of the organs where metastases are most frequently located (Witschi, 1988).

Several explanations for the findings of increased metastases have been suggested: NO<sub>2</sub> may

increase capillary permeability and endothelial cell injury, adversely affect immunologic host defence systems, or cause alterations in clotting mechanisms (Henschler and Ross 1966; Richters and Kuraitis, 1983).

Evidence for increased cell permeability and endothelial injury includes a study in which C57Bl/6J mice were exposed to  $0.35 \pm 0.05$  ppm  $\text{NO}_2$  for 6 weeks; the main lesions identified in lung capillaries by light and electron microscopy were microthrombi and injury to capillary endothelial cells. After infusion of B16 melanoma cells, the incidences of microthrombi, endothelial cell injury, and lung metastasis were increased in exposed animals (Richters and Richters, 1989). Endothelial dysfunction in association with evidence of oxidative stress has also been noted in the Wistar rat heart after 7-d exposure to 2.7–10.6 ppm  $\text{NO}_2$ , as indicated by an increase in eNOS expression with no obvious change in ET-1 expression at 2.7 ppm, and a significantly increased ET-1 and reduced eNOS at 10.6 ppm (Li et al., 2011).

In Sprague-Dawley rats exposed to 10.1 ppm  $\text{NO}_2$  for 7 d, histological examination of lung tissue found increased inflammation in respiratory bronchioles and alveoli, loss of cilia in the epithelium of small airways, and ectasia of alveolar capillaries, but there was no evidence of microvascular leakage in the larynx, trachea, main bronchi, or intrapulmonary airways (Chitano et al., 1996).

As discussed in subsection 6.1.1, studies of experimental animals exposed to  $\text{NO}_2$  at a range of doses and exposure times have produced varying results with respect to alteration of immune system parameters.  $\text{NO}_2$  exposure may enable metastases under certain conditions by facilitating arrest, extravasation, and growth of circulating tumour cells in the lung, but under other conditions may also inhibit metastatic processes (Witschi, 1988). These effects may be a function of the central role of NO and related endogenous  $\text{NO}_x$  in the immune defence, which may influence carcinogenesis (as reviewed in Tamir and Tannenbaum, 1996).

## 6.4 Neurodevelopmental Effects

Evidence for the neurodevelopmental effects of  $\text{NO}_2$  has been demonstrated in a limited number of studies in experimental animals. Neurobehavioural deficits were observed in the offspring of mice and rats, and these results are discussed in subsection 6.5.2. Li et al. (2012) exposed adult male Wistar rats to 2.7, 5.3, and 10.6 ppm  $\text{NO}_2$  (6 h/d for 7 d) and observed decreases in brain to body weight ratios and an increase in the number of apoptotic cells in the brain. These changes were accompanied by alterations in the levels of oxidative stress markers (Cu/Zn-SOD, Mn-SOD, GPx, NO and PCO), and increases in both protein and gene expression levels of oncogenes (c-fos and c-jun) and apoptosis-related genes (p53 and bax).

## 6.5 Reproductive and Developmental Effects

Relatively few animal toxicology studies have focused on potential effects of  $\text{NO}_2$  on reproduction and development, and no study published after the 1990s was identified. A very small number of studies examined the effects of  $\text{NO}_2$  on sperm and on fertility. Pre- and postnatal development animal studies have investigated whether maternal exposure to  $\text{NO}_2$  can affect the developing fetus, resulting in low fetal and birth weight, neurobehavioural deficits and changes in some biochemical parameters in the livers of pups. Also, studies of postnatal exposures have examined potential adverse respiratory effects in neonates.

### **6.5.1 Gametogenesis and Reproductive Function**

Kripke and Sherwin (1984) studied the testes of groups of six rats exposed to 1 ppm NO<sub>2</sub> for 7 h/d, 5 d/wk over 3 weeks. Histological examination revealed that exposed rats did not show alterations in spermatogenesis, germinal cells or interstitial cells.

Shalamberidze and Tsereteli (1971) showed that long-term exposure to NO<sub>2</sub> could lower fertility in female albino rats. The animals were exposed to NO<sub>2</sub> alone (0.07 or 1.25 ppm) or to NO<sub>2</sub> and SO<sub>2</sub> (0.6 ppm and 1.3 ppm, respectively) for 12 h/d over 3 months. The authors reported disturbances in the estrual cycle (expressed as increased duration) and decreases in the number of normal and total cycles at 1.25 ppm NO<sub>2</sub> or 0.6 ppm NO<sub>2</sub> and 1.3 ppm SO<sub>2</sub>.

### **6.5.2 Prenatal and Postnatal Development**

Shalamberidze and Tsereteli (1971) studied pregnant albino rats exposed to 0.07 or 1.25 ppm NO<sub>2</sub> alone or to 0.6 ppm NO<sub>2</sub> and 1.3 ppm SO<sub>2</sub> combined, for 12 h/d during 3 months prior to breeding. These exposures reduced the weights of fetuses, as well as causing decreases in birth weights that persisted to postnatal d 4 and 12.

Tabacova et al. (1985) reported, in Wistar rat progeny up to the age of 3 months, dose-dependent neurobehavioural deficits that included retarded neuromotor development, disturbances in motor coordination, or retarded locomotor patterns after exposure (6 h/d during gestation) to 0.05, 0.53 and 5.3 ppm NO<sub>2</sub> but not to 0.03 ppm. A dose-dependent delay in eye opening and incisor eruption was observed at 0.53 and 5.3 ppm exposures. Also, at the age of 1 month the authors found significant decreases in biochemical parameters in the liver, such as the activities of cytochrome P450 at 5.3 ppm exposure.

Singh (1988) showed that continuous exposure of CD-1 mice to 22 or 45 ppm of NO<sub>2</sub> from gestation d 7–18 significantly decreased birth weight and altered the righting reflex and aerial righting score of the pups on postnatal d 1 and 12, respectively. However, exposure to NO<sub>2</sub> did not affect the mean number of live pups/litter or negative geotaxis.

Di Giovanni et al. (1994) used the rate of calling to examine alterations in ultrasonic vocalization, a measure of emotional state, in Wistar rat offspring exposed continuously to 1.5 or 3 ppm NO<sub>2</sub> from d 0 to d 20 of pregnancy. Only exposure to 3 ppm during gestation elicited a significant decrease in the duration of ultrasonic signals in 10- and 15-d-old male pups when removed from their nest.

Chang et al. (1986) exposed 1-d-old and 6-wk-old male Fisher 344 rats to 0.5 ppm NO<sub>2</sub> for 23 h/d, 7 d/wk for 6 weeks. Subsequently, these groups of then-juvenile and -adult rats were exposed to 0.5 ppm NO<sub>2</sub> for 23 h/d, 7 d/wk for 6 weeks, with two 1-h spikes to 1.5 ppm applied 5 d/wk. Exposure to NO<sub>2</sub> did not affect the body weights of either juvenile or adult rats, but did cause alveolar epithelial injury, seen as spreading and hypertrophy of type II epithelial cells, followed by differentiation into type I cells in both the juvenile and the adult rats.

Azoulay-Dupuis et al. (1983) found that rat and guinea pig neonates were less susceptible than adults to lung damage following 3-d exposures to NO<sub>2</sub>. At 2 ppm exposure, only adult guinea pigs had histological alterations in the lung, such as thick alveolar walls infiltrated by inflammatory mononuclear cells and alveolar edema. At 10 ppm NO<sub>2</sub>, slight histological modifications, such as extinction of cilia in tracheal and bronchiolar epithelia, were only seen in rat litters from 45 d until adult age and not in neonates, although in guinea pigs this disappearance of cilia was observed in both neonates and adults. After the 10 ppm NO<sub>2</sub> exposure both species showed decreased SOD activity in alveolar macrophages of both neonates and adult animals.



## 6.6 Mechanisms of Action

Inhaled  $\text{NO}_2$  and its major absorption product,  $\text{NO}_2^-$ , can produce both local pulmonary and systemic effects, the potential mechanisms of which are summarized as follows.

**Oxidative stress and inflammation:** Local effects induced by  $\text{NO}_2$  may be caused by direct reactions with the airway ELF or epithelial cells and by induction of oxidative/nitrosative stress. These effects have been associated with exacerbation of asthma symptoms and declines in lung function. Toxicity is likely mediated by conversion of  $\text{NO}_2$  to toxic reaction products and by the generation of free radicals that interact with pulmonary tissues. For example, inhaled  $\text{NO}_2$  is capable of altering surfactant and ELF structural/biochemical properties, turnover, and synthesis (Wright et al., 1982; Finlayson-Pitts et al., 1989; Müller et al., 1992, 1994, 1998, 2001, 2003; Mengel et al., 1993; Ayyagari et al., 2004; Aufderheide, 2005).  $\text{NO}_2$  can also result in increased local (and possibly systemic) nitrosative and oxidative stress, including S-nitrosylation and tyrosine nitration (Persinger et al., 2002), which may affect critical proteins such as cytochrome P450 (Maples et al., 1991) and Mn-SOD (Surmeli et al., 2010). Conversion of  $\text{NO}_2$  to  $\text{NO}_2^-$  in the ELF can also induce secondary formation of ROS ( $\text{O}_2^{\cdot -}$  and  $\text{H}_2\text{O}_2$ ) as well as  $\text{A}^{\cdot -}$  and thiol radicals ( $\text{GS}^{\cdot -}$  and  $\text{GSSG}^{\cdot -}$ ) (Velsor and Postlethwait, 1997), which may contribute to increased pulmonary (and possibly systemic) oxidative stress (Kelly and Tetley, 1997).

Nitrogen dioxide may also initiate fatty acid/lipid peroxidation and/or thiol oxidation (Posin et al., 1978; Sagai et al., 1982, 1983, 1984, 1987; Ichinose et al., 1983, 1988; Sagai and Ichinose, 1987; Ichinose and Sagai, 1989).  $\text{NO}_2$  may also trigger inflammation or modulate ongoing inflammatory processes. Such effects include changes in epithelial permeability and ELF inflammatory cell numbers and activity (Spannhake et al., 2002; Ayyagari et al., 2004, 2007), histamine-dependent epithelial inflammation (Devalia et al., 1993), microedema of the bronchial or interstitial epithelium and/or bronchoconstriction (von Niding et al., 1971). Inhaled  $\text{NO}_2$  may also activate antioxidant defences (Johnston et al., 2000, 2001; van Bree et al., 2000), and has been shown to mobilize antioxidants such as vitamin E to the lung (Elsayed, 2001). This may occur in response to the increase in oxidative stress and inflammation and/or may be a protective effect of  $\text{NO}_2$ .

Increased endogenous NO synthesis may also occur as a result of inflammation induced by inhaled  $\text{NO}_2$  (Giroux and Ferrières, 1998; Ayyagari et al., 2004, 2007). Increased NO may, in turn, play a role in bronchiolar cytotoxicity (Kallio et al., 1997), may also influence pulmonary cyclic guanosine monophosphate concentrations (Kobayashi et al., 1982), induce sustained pulmonary vasodilation (Hunter et al., 2004), and modulate mitochondrial ROS formation (Lundberg et al., 2008). Elevated NO levels may also result in cytotoxicity and oxidative stress in other organs, including the heart (Li et al., 2011).

**Hematological effects:** Exposure to inhaled  $\text{NO}_2$  causes increased  $\text{NO}_2^-$  concentrations in the lung (Postlethwait and Bidani, 1989), circulation, and other tissues (Parks et al., 1981; Ohta et al., 1982). Nitrite or other downstream metabolites that become absorbed into pulmonary capillaries may also affect pulmonary vasculature, circulating RBCs, blood pressure, or other tissues. Nitrite may also interact with either oxygenated or deoxygenated hemoglobin (oxyHb or deoxyHb) to produce  $\text{NO}_3^-$ /methemoglobin or  $\cdot\text{NO}$ , respectively (Gladwin et al., 2009).

Nitrite is also capable of inhibiting nitric oxide synthase (NOS) activity and NO concentrations locally (Gladwin et al., 2000), also resulting in sustained pulmonary vasodilation (Hunter, 2004), and altered systemic blood pressure and RBC chemistry (Bryan et al., 2005; Dejam et al., 2007).



**Tumour-promoting effects:** Inhalation of NO<sub>2</sub> may also increase the production of potentially carcinogenic nitrosamines (Iqbal et al., 1980; van Stee et al., 1983; Rubenchik et al., 1995), which may contribute to its possible tumour-promoting activity. NO<sub>2</sub> exposure may also increase tumour metastases by increasing capillary permeability and endothelial cell injury, inhibiting immunologic host defence systems, and/or causing alterations in clotting mechanisms.

**Implications for dose–response:** The severity of the effect of inhaled NO<sub>2</sub> may be a function of timing and pattern of exposure. Intermittent peak exposures may have greater effects than long-term low-level exposures (to which the body may adapt by adjusting long-term storage pools), although total exposure (concentration × duration) may also influence the outcome (Gardner et al., 1979). As such, NO<sub>x</sub> homeostasis may be transiently disrupted if exposure to higher concentrations occurs very rapidly, or may be chronically disrupted if elevated exposure is prolonged. In animals, epithelial damage after acute exposures is thought to be due to radical formation and lipid peroxidation (Barth et al., 1994b), which may stimulate antioxidative enzyme activation during the early weeks to months of ongoing NO<sub>2</sub> exposure. However, these protective effects are lost in later phases of chronic exposure (as reviewed in Chitano et al., 1995), and emphysema may be produced secondary to an imbalance of the pulmonary protease/anti-protease system (Barth et al., 1994b).

The severity of effects of inhaled NO<sub>2</sub> may also be a function of the status of pulmonary and systemic NO<sub>x</sub> pools, which may vary significantly between individuals. This potential variability in response, coupled with non-linear toxicokinetics, results in considerable uncertainty in NO<sub>2</sub> dose–response prediction. Under normal circumstances (i.e. in the absence of inhaled NO<sub>2</sub>), the body's pool of systemic endogenous NO<sub>x</sub> is primarily driven by dietary intake of NO<sub>3</sub><sup>−</sup> and/or NO<sub>2</sub><sup>−</sup> and endogenous synthesis of NO, both of which may vary significantly, leading to variation in tissue pools, although plasma concentrations of NO<sub>2</sub><sup>−</sup> generally remain stable (Bryan et al., 2005). Vegetables are the main dietary source of NO<sub>3</sub><sup>−</sup>; however, some NO<sub>3</sub><sup>−</sup> may also be present in some water supplies, particularly groundwater sources. Nitrite is also added to some foodstuffs during preparation (as reviewed in Lundberg and Weitzberg, 2010). Other factors such as exercise and disease status may also affect the severity of effects induced by inhaled NO<sub>2</sub>. For example, regular exercise increases eNOS expression and activity, resulting in higher plasma NO<sub>3</sub><sup>−</sup> concentrations. Chronic inflammation, as occurs in asthma or chronic obstructive pulmonary disease (COPD), may alter NOS activity in the lung; in systemic inflammatory disorders such as severe gastroenteritis, massive iNOS activation leads to greatly increased plasma NO<sub>3</sub><sup>−</sup> and NO<sub>2</sub><sup>−</sup> concentrations. Conversely, in CVDs, eNOS activity is reduced, and plasma NO<sub>3</sub><sup>−</sup> and NO<sub>2</sub><sup>−</sup> concentrations are often low (as reviewed in Lundberg et al., 2008).

## 6.7 Summary and Considerations

Experimental animal studies have shown that short- or long-term exposure to NO<sub>2</sub> leads to a wide range of biological effects, especially within the respiratory system. Increased AHR, a hallmark of asthma in humans, was observed in guinea pigs following both short- or long-term exposure to NO<sub>2</sub>. Inhalation of NO<sub>2</sub> at greater than ambient levels can induce pulmonary inflammation, increased lipid peroxidation, increased markers of oxidative stress and alterations in the GSH antioxidant pathway. Exposure to NO<sub>2</sub> induced morphological and functional changes in the respiratory tract of different species and impairment of components of the lung host defence system, including mucociliary activity/transport and alveolar macrophages. Findings in several animal studies showed that NO<sub>2</sub> can modulate the immune response to inhaled allergens. Subchronic exposure to high concentrations of NO<sub>2</sub> increased IgE-mediated mast cell histamine release in guinea pigs, though not in mast cells from rats. Acute exposure to NO<sub>2</sub> increased the number of eosinophils in the tracheal mucosa of guinea pigs; eosinophil

activation was also observed in other rodent species, though not all. Some animal toxicological studies demonstrated that inhalation of NO<sub>2</sub> increased susceptibility to pulmonary infection, where increased mortality was found in both mice and monkeys following short-term exposure to NO<sub>2</sub> combined with bacterial or viral challenge.

A number of animal toxicological studies also demonstrated extra-pulmonary effects and suggested potential mechanisms of action leading to these various health effects following exposure to NO<sub>2</sub>.

A limited number of experimental studies have shown that exposure to NO<sub>2</sub> at relatively high levels may induce cancer-related health effects. The few *in vivo* genotoxicity studies were generally negative, with some isolated positive findings in lung cells of rats and mice exposed to higher-than-ambient concentrations. In some recent experimental studies at relatively low concentrations, exposure to NO<sub>2</sub> induced genotoxic and mutagenic effects in nasal epithelial cells *in vitro*. In single studies, increased pulmonary tumours and skin adenomas were also observed following exposure to NO<sub>2</sub> in mouse strains that are prone to develop these lesions. Some studies also demonstrated a relationship between acute exposure to NO<sub>2</sub> and the formation of carcinogenic nitrosamines, as well as an increased lipid peroxidation and dissemination and/or proliferation of tumour metastases. The mechanisms for these cancer-related effects are currently unclear.

Short-term exposure to NO<sub>2</sub> caused a range of effects in the cardiovascular system in experimental animals, including slight histological changes in the heart and decreased hematocrit and hemoglobin. Changes in markers of oxidative stress, inflammation, vasoactivity and cardiac myocyte apoptosis were also observed. The older literature (<2008) identified effects on RBCs, including changes in D-2,3-diphosphoglycerate and sialic acid and decreases in numbers of cells. Effects on methemoglobin were not observed after exposures to 0.8 ppm NO<sub>2</sub> but have been noted in some studies with higher exposures. Consistent with results reported in the older literature (<2008), a NO<sub>2</sub>-related decrease in heart rate of mice was observed in a more recent study following chronic exposure to ambient air. A decrease in hematocrit and hemoglobin in rats on a high-Se diet was reported. Older studies also identified polycythemia in rats but no effects on hematocrit or hemoglobin in monkeys or dogs. Changes in markers of oxidative stress and inflammation were observed in the more recent studies and have been proposed as potential mechanisms of action for hematological effects.

Toxicological studies employing rodent models have shown that exposure to NO<sub>2</sub> at environmentally relevant concentrations can adversely affect pre- and postnatal development. Prenatal exposure to concentrations as low as 0.07 ppm and 0.05 ppm reduced the weights of rat fetuses and progeny, respectively. *In utero* exposure of rats to NO<sub>2</sub> at 0.05, 0.53 and 5.3 ppm resulted in such neurobehavioural deficits as retarded neuromotor development or disturbances in motor coordination. Neurological effects, such as decreases in brain to body weight ratios and increases of apoptotic cells in the brain, were also observed in a single study of adult rats following acute exposure to NO<sub>2</sub>. Postnatal exposures adversely affected the respiratory system of neonates at concentrations as low as 0.5 ppm.

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## 7. Controlled Human Exposure Studies

Controlled human exposure studies document the health effects associated with inhalation of specific concentrations of pollutants under laboratory conditions. Quantitative studies on humans, when carefully controlled, complement epidemiological investigations, in that they provide direct evidence for the type of health outcomes associated with exposure to the pollutant and can provide dose–response data for short-term exposure. In addition, such experiments provide the opportunity to examine interactions with other variables, such as exercise and exposure to co-pollutants. Potentially susceptible populations can also be studied directly.

Controlled human exposure studies have limitations:

- for practical and ethical reasons, they must be limited to small groups, which may not be representative of the general population
- individuals with a pre-existing disease usually have a well-controlled or mild disease
- there may be fewer studies of susceptible populations
- exposure must be limited to a short duration and to concentrations of pollutants that are expected to produce mild and transient responses
- exposures are often limited to a single pollutant or to a very limited pollutant mix, which never replicates the complex mixture to which populations are actually exposed

Furthermore, the extent to which transient responses in clinical studies predict more chronic and persistent effects is unknown.

As noted in Chapter 1, the sections of this chapter that deal with controlled human exposure studies of respiratory effects are based on the literature review that Health Canada has conducted to support the development of the Residential Indoor Air Quality Guideline for NO<sub>2</sub> (Health Canada, 2015).

An extensive literature of controlled human exposure studies (over 50 investigations) is available, in which subjects were exposed to NO<sub>2</sub> concentrations (0.1–3.5 ppm) for short periods (20 min to 6 h). The controlled NO<sub>2</sub> exposure studies considered in this review were those with human inhalation exposures of 1 ppm or less; concentrations that are not far removed from, and in some instances overlap, short-term peak ambient NO<sub>2</sub> concentrations. Most were single-exposure studies, with a few repeated exposure studies lasting less than a week. Each subject acted as his/her own control in randomized single- or double-blind crossover studies, wherein NO<sub>2</sub> effects were determined after comparison to effects of filtered air alone, with 1–4 weeks in between NO<sub>2</sub> exposures. Investigations included both healthy subjects (Section 7.1) and those with respiratory diseases (asthma, COPD, or allergies) (Section 7.2) under a variety of conditions (with or without exercise, and/or followed by provocation with cold, allergens, cholinergic agents, or histamine). Cold air can be a non-specific trigger for airway hyperresponsiveness (AHR) that can be reasonably expected to occur under normal circumstances, in contrast to studies where AHR was induced with chemicals or allergens at levels designed to aggravate the subjects' AHR. Thus studies with cold air challenge were not grouped together with the artificial induction with agents.

The outcomes assessed varied according to the study, but often included respiratory symptoms and lung function, and sometimes also cardiovascular, immunological, or inflammatory reaction, or bronchial responsiveness. Some experiments on resistance to viral infections were also completed. Conclusions were drawn primarily along consistency and extent of responses, but



as there were often high intraspecies variations and small sample sizes, statistically significant changes in individuals with strong responses were also considered.

Medication use varied prior to and during the studies. Most asthmatics used medication (corticosteroids, anti-inflammatories, and/or bronchodilators), but mild to severe asthmatics refrained from medication use for at least 8 h prior to exposure, depending on the medication. By contrast, only a few studies testing asthmatics with allergies who were challenged with allergens followed a similar explicit scenario (Barck et al., 2005; Witten et al., 2005). Almost all older adults with COPD used medication, which they were allowed to use during two out of the three studies (Morrow et al., 1992; Gong et al., 2005).

Available studies that included exposures to 1 ppm NO<sub>2</sub> or less are described below.

## 7.1 Respiratory Effects in Healthy Subjects

### 7.1.1 Exposure to NO<sub>2</sub> with or without Exercise

In 10 studies, healthy adults (defined as non-smokers with no history of CVD, lung disease, or asthma) were exposed to a single or four repeated exposures of 0.18 to 1 ppm NO<sub>2</sub> for 0.5 to 3 h, mostly with intermittent exercise. One study assessed older adults (Gong et al., 2005). In another study, an increased symptom score was observed with exposure to 0.3 ppm NO<sub>2</sub> (Vagaggini et al., 1996), but in general respiratory symptoms were not increased in healthy subjects exposed to NO<sub>2</sub> under the conditions tested (Folinsbee et al., 1978; Adams et al., 1987; Rubinstein et al., 1991; Jorres et al., 1995; Gong et al., 2005). Lung function (e.g. forced expiratory volume in 1 second (FEV<sub>1</sub>), forced vital capacity (FVC), volume of thoracic gas (V<sub>tg</sub>), airway resistance (S<sub>Raw</sub>)) was also not significantly affected during or after exercise in these studies (Folinsbee et al., 1978; Adams et al., 1987; Frampton et al., 1989a, 1989b, 2002; Kim et al., 1991; Rubinstein et al., 1991; Jorres et al., 1995; Vagaggini et al., 1996; Gong et al., 2005). Also, cardiovascular function was not affected by NO<sub>2</sub> under the conditions tested (Folinsbee et al., 1978; Adams et al., 1987; Gong et al., 2005).

Some 3-h studies used *in vitro* tests or examined enzyme biomarkers to elucidate the lung immune response and inflammation resulting from NO<sub>2</sub> exposure. Increased α<sub>2</sub>-macroglobulin protein levels in BALF in some subjects exposed to 0.6 ppm NO<sub>2</sub> may indicate increased antiprotease activity, although this was not observed with higher continuous (1.5 ppm) or peak concentrations (0.05 ppm interspersed with three 15-min peaks of 2 ppm, total NO<sub>2</sub> equalling 0.6 ppm) (Frampton et al., 1989a). This occurred in the absence of changes in lung function, with or without a carbachol challenge. Another study suggested that NO<sub>2</sub> increased susceptibility to viral infections in four out of nine subjects at 0.6 ppm NO<sub>2</sub>, based on reduced ability to inactivate influenza virus in a plaque-forming assay *in vitro* when mixed with BALF, although this was not observed with higher continuous (1.5 ppm) or peak concentrations (0.05 ppm interspersed with three 15-min peaks of 2 ppm, total NO<sub>2</sub> equalling 0.6 ppm) (Frampton et al., 1989b). Moreover, this was not replicated in another study (Frampton et al., 2002) by the same authors with the same methodology. At the higher dose of 1.5 ppm NO<sub>2</sub>, there was increased lactate dehydrogenase in BALF exposed to Rous sarcoma virus and influenza virus, results that the authors suggested were indicative of increased cell permeability, but there were no changes in viral susceptibility (Frampton et al., 2002).

After a 3-h exposure to 0.6 ppm NO<sub>2</sub>, there was suggestive evidence of adverse hematological effects (<5% decreased hematocrit and hemoglobin, <25% decreased lymphocytes; increased PMN leukocytes in BALF at a higher dose) (Frampton et al., 2002). Inflammatory cells in venous blood and BALF were not affected by repeated (4 times over 6 d) 2-h exposures of 0.6 ppm NO<sub>2</sub>, though there was a significant increase in natural killer cells (Rubinstein et al., 1991). The

small number of subjects (five) and lack of functional testing are important limitations of this study. In another study, there was no cellular indication of inflammation or any effect on differential cell counts or inflammatory mediator levels (including leukotrienes, histamine and prostaglandin) in the BALF of healthy adults exposed for 3 h to 1 ppm NO<sub>2</sub> with intermittent exercise (Jorres et al., 1995).

### **7.1.2 Investigations with Bronchial Challenge Using Histamine, Carbachol or Methacholine**

In four studies, healthy adults were exposed to a minimum of 20 min of 0.1 ppm NO<sub>2</sub>, sometimes with intermittent exercise, followed by bronchial challenges (Hazucha et al., 1983; Bylin et al., 1985; Frampton et al., 1991; Morrow et al., 1992). One of these studies included older adults, with subgroups of former and current smokers (Morrow et al., 1992).

Overall, the lung function of the average healthy person was not affected after a single exposure to NO<sub>2</sub> up to 0.6 ppm under the conditions tested, with a few exceptions. Eight subjects at rest demonstrated increased SRaw during 20 min of exposure to 0.24 ppm NO<sub>2</sub> but decreased SRaw at 0.48 ppm, and no effect during a histamine challenge (Bylin et al., 1985). This transiently impaired lung function was not observed in other studies evaluated at 0.24 ppm NO<sub>2</sub> exposure. There was no decreased lung function after 3 d of repeated 1-h exposures to 0.1 ppm, nor following a methacholine and forced random noise challenge, suggesting no detectable impairment on small airway function (Hazucha et al., 1983). Following exposures to 0.3 ppm NO<sub>2</sub> or more and a carbachol challenge, healthy adults were not significantly affected in symptoms or lung function, although a subgroup of older smokers demonstrated slight transient decreased FEV<sub>1</sub> and FVC (<3%, relative to air alone) with 0.3 ppm NO<sub>2</sub> alone (Morrow et al., 1992). Healthy adults did not demonstrate similarly decreased lung function until exposure to 1.5 ppm NO<sub>2</sub> and a carbachol challenge, with the exception of 1 healthy individual out of 15, who had significantly decreased FEV<sub>1</sub> (20%, relative to air alone) following exposure to 0.05 ppm interspersed with three peak exposures of 2 ppm (concentration equivalent to 0.6 ppm continuous exposure) and a carbachol challenge (Frampton et al., 1991).

Subjects were not always able to detect an increase in NO<sub>2</sub> concentration by its odour. Among subjects able to perceive an increase, there was no correlation between the subjects' ability to notice an odour and their increased respiratory susceptibility to NO<sub>2</sub> (Bylin et al., 1985; Frampton et al., 1991). As such, increased odour detection may be an indicator of exposure, but it was not associated with decreased respiratory function.

## **7.2 Respiratory Effects in Subjects with Asthma, Chronic Obstructive Pulmonary Disease, or Allergies**

### **7.2.1 Investigations with Subjects with or without Exercise, or with Cold Air Challenge**

Three studies were identified that tested older adults (mean age 58–72) with COPD (Morrow et al., 1992; Vagaggini et al., 1996; Gong et al., 2005). Asthmatic adults with asthma ranging in severity from mild to severe were investigated in five studies (Bauer et al., 1986; Avol et al., 1988; Roger et al., 1990; Jorres et al., 1995; Vagaggini et al., 1996). One other study assessed children with asthma and allergies (Avol et al., 1989). Bronchial reactivity was tested by a cold air challenge in some of these studies (Bauer et al., 1986; Avol et al., 1988, 1989).

Older adults with COPD, exercising intermittently and exposed to 0.3 ppm NO<sub>2</sub> for 1 or 4 h, had decreased lung function (decreased FEV<sub>1</sub> or FVC, 8–9%, relative to air) after a lag period of several hours (Morrow et al., 1992; Vagaggini et al., 1996), but not after a 2-h exposure to 0.4 ppm NO<sub>2</sub> with intermittent exercise (Gong et al., 2005). There were no observed changes in heart function (Gong et al., 2005). The symptom score (not attributed to a particular symptom type) increased in one of these three studies 2 h after exposure, but the extent of change was similar with air or NO<sub>2</sub> exposure; thus it may be related to exercise (Vagaggini et al., 1996). No changes were observed in inflammatory cells in nasal lavage and/or saline-induced sputum (Vagaggini et al., 1996; Gong et al., 2005), or after a bronchial challenge with isoproterenol (Morrow et al., 1992).

Asthmatic adults exposed to 0.3 ppm NO<sub>2</sub> or more for up to 3 h with intermittent exercise had mixed results in terms of lung effects in five studies. In two studies, asthmatics exposed to 0.3 ppm NO<sub>2</sub> demonstrated transiently decreased lung function within 0.5 h (decreased FEV<sub>1</sub> in both studies; decreased FVC, increased SRaw, and/or decreased partial expiratory flow rates at 60% of total lung capacity) (Bauer et al., 1986; Roger et al., 1990). By contrast, lung function impairment was not observed at 0.3 ppm or higher in a larger study reported in the Roger et al. (1990) paper or in two other studies (Vagaggini et al., 1996; Avol et al., 1988). It is recognized that some individuals may be more sensitive to the effects of NO<sub>2</sub>, as evidenced in at least one study where 1 out of 15 adults developed dyspnea with decreased FEV<sub>1</sub> and withdrew from the study (Bauer et al., 1986).

The only available study of asthmatic children, almost all with upper respiratory allergies, challenged with cold air (n = 34) also demonstrated transiently decreased lung function in the first hour while exercising at 0.3 ppm NO<sub>2</sub> for 2 h. The change was small (<4% FEV<sub>1</sub> or FVC, <6% peak expiratory flow (PEF) rate, relative to clean or ambient air), and only statistically significant relative to ambient air, not clean air (Avol et al., 1989). The effects were transient, and the authors attributed the observed changes to exercise. Since decreased lung function was demonstrated in some asthmatic adults (n = 13–15/study) at 0.3 ppm (Bauer et al., 1986; Roger et al., 1990), there was no clear evidence that children were more sensitive than asthmatic adults.

In a preliminary study of 13 mildly asthmatic adults exposed to 0.3 ppm NO<sub>2</sub> for 2 h with intermittent exercise, symptoms (increased transient cough and dry throat) were observed in the first 10 min of exercise only, but not in the second or third 10-min interval of exercise (Roger et al., 1990). Further, symptoms were not observed in a larger study with 0.15 to 0.6 ppm NO<sub>2</sub> exposure for 75 min and intermittent exercise (Roger et al., 1990), in a 3-h exposure to 1 ppm NO<sub>2</sub> with intermittent exercise (Jorres et al., 1995), or in studies without exercise at a similar exposure (Avol et al., 1988; Vagaggini et al., 1996). Asthmatic children who intermittently exercised with 0.3 ppm NO<sub>2</sub> for 2 h had a delayed increase in self-reported symptoms (day and week after exposure) (Avol et al., 1989). Interpretation of results was confounded because there was also an increase in symptoms the week prior to NO<sub>2</sub> exposure; thus the events could be unrelated (Avol et al., 1989). Therefore, 0.3 ppm NO<sub>2</sub> may transiently increase some respiratory symptoms in exercising asthmatic adults, but the weight of evidence suggests that it will not do so. The evidence in exercising asthmatic children remains unclear.

A cold air challenge did not increase bronchial reactivity an hour following NO<sub>2</sub> exposure in severely asthmatic adults (Avol et al., 1988) or in asthmatic children with allergies (Avol et al., 1989). Conversely, adults with moderate asthma demonstrated bronchial distress (decreased FEV<sub>1</sub>) following 0.5 h of 0.3 ppm NO<sub>2</sub> regardless of a cold air challenge, but only with increased ventilation rates (Bauer et al., 1986). This suggested that prior exposure to 0.3 ppm NO<sub>2</sub> may increase cold air hyperventilation in exercising asthmatics.

Asthmatic adults exposed to 0.3 ppm NO<sub>2</sub> for 1 h with intermittent exercise did not show any cellular signs of inflammation in nasal lavage or sputum (Vagaggini et al., 1996). Conversely, NO<sub>2</sub> mediated inflammation in the BALF of asthmatic adults exposed for 3 h to 1 ppm NO<sub>2</sub> while intermittently exercising (Jorres et al., 1995). The latter conclusion was based on increased endobronchial appearance (erythema, edema, friability) as well as increased bronchodilator (thromboxane B<sub>2</sub>, prostaglandin D<sub>2</sub>) and decreased bronchoconstrictor (6-keto-prostaglandin F<sub>1α</sub>) levels, and a slight increase in some leukotrienes (such as LTB<sub>4</sub>) (Jorres et al., 1995). Other inflammatory mediators (prostaglandin E<sub>2</sub>, prostaglandin F<sub>2α</sub>, and histamine) did not change significantly. Thus inflammation could be induced in exercising asthmatic adults at 1 ppm NO<sub>2</sub> exposure, but not at 0.3 ppm.

### ***7.2.2 Investigations with Bronchial Challenge Using Allergens, Cholinergic Agents, or Histamine***

Seventeen studies were identified that involved asthmatics with or without allergies (or, in the case of one study, individuals without asthma but with seasonal allergic rhinitis) exposed to NO<sub>2</sub> followed by bronchial challenge. More specifically, the studies involved exposures ranging from 0.1 to 0.6 ppm NO<sub>2</sub> for up to 6 h, followed by a challenge with an allergen, a cholinergic agent (such as carbachol or methacholine), or histamine. Study subjects were either at rest or intermittently exercising during the NO<sub>2</sub> exposure period.

Eight of the 17 studies involved bronchial challenge with a house dust mite allergen (HDMA) (Tunnicliffe et al., 1994; Jenkins et al., 1999; Witten et al., 2005), pollen (Wang et al., 1995a, 1995b; Strand et al., 1997; Barck et al., 2002, 2005), or cat allergen (Riedl et al., 2012). These studies examined doses of NO<sub>2</sub> as low as 0.26 ppm, and the allergen challenge followed either immediately or within 4 h of the NO<sub>2</sub>.

The results of a number of these studies demonstrated an effect of NO<sub>2</sub> on lung function measurements and pulmonary inflammatory markers following allergen challenge, as compared to air exposure. In 18 adults with mild atopic asthma, after a 30-min exposure to 0.26 ppm NO<sub>2</sub> followed 4 h later by pollen allergen challenge, PEF was on average 6.6% lower than after an allergic challenge following air exposure (Strand et al., 1997). In addition, Jenkins et al. (1999) observed that a significantly lower dose of HDMA was required to cause a 20% decrease in FEV<sub>1</sub> in 10 atopic asthmatics when allergen exposure followed 3 h of 0.4 ppm NO<sub>2</sub> with intermittent exercise. Furthermore, in a study of 10 adult asthmatics, 1-h exposure to 0.4 ppm NO<sub>2</sub> followed immediately by exposure to HDMA resulted in a significant reduction in FEV<sub>1</sub>, both within 2 h after exposure to the allergen and later, during a subsequent decrease in FEV<sub>1</sub> after returning to baseline (Tunnicliffe et al., 1994).

In three allergen challenge studies, NO<sub>2</sub> exposure resulted in significant effects on inflammatory markers in the lung. In a study by Barck et al. (2002) of 13 mild asthmatics, exposure to 0.26 ppm NO<sub>2</sub> for 30 min at rest resulted in an increase in the percentage of neutrophils as well as the level of eosinophilic cationic protein (ECP) in the bronchi following challenge with birch or timothy pollen. More specifically, ECP levels in bronchial wash fluid were 2.5 times greater following exposure to NO<sub>2</sub> and allergen as compared to air and allergen (9.0 µg/L vs 3.60 µg/L). In a subsequent study of 18 adults with mild asthma and allergy to pollen, Barck et al. (2005) observed a significant increase in ECP in sputum and blood following a 2-d exposure to NO<sub>2</sub> (15 min on d 1 and 2 × 15 min on d 2) and a challenge with birch or timothy pollen. Consistent with these results in asthmatics, a group of 16 adults with seasonal allergic rhinitis exposed for 6 h to 0.4 ppm NO<sub>2</sub> followed by mixed grass pollen allergen challenge experienced an increase in ECP levels in nasal lavage fluid (Wang et al., 1995a, 1995b). These results suggest an effect of relatively brief exposure to low-level NO<sub>2</sub> on eosinophil numbers, neutrophil inflammation, and activity in lungs of susceptible populations. It should be noted that lung function (i.e. FEV<sub>1</sub>, V<sub>t</sub>g,

SRaw) was measured but was not significantly affected in these studies, potentially indicating an enhanced allergic inflammatory reaction without overt pulmonary dysfunction (Barck et al., 2002).

By contrast, a number of allergen challenge studies in mild asthmatics at similar NO<sub>2</sub> concentrations and duration did not measure an adverse effect; they in fact observed somewhat of a “protective” effect of NO<sub>2</sub> on lung function or pulmonary inflammation following allergen challenge in some studies. In two studies examining exposure to 0.26 ppm NO<sub>2</sub> for similar short durations (30 min or 15 min followed the next day by two 15-min exposures) no effect was noted following birch or timothy pollen challenge (Barck et al., 2002, 2005). After exposing subjects to 0.4 ppm NO<sub>2</sub> for 3 h followed by HDMA challenge, Witten et al. (2005) did not observe an effect on FEV<sub>1</sub> among 15 adults with mild to moderate atopic asthma, but they did measure a decrease in eosinophil concentration in sputum. In a recently conducted study, NO<sub>2</sub> appeared to attenuate allergen-induced effects on airway responsiveness and inflammation (Riedl et al., 2012). In 30 asthmatics, exposure to 0.35 ppm NO<sub>2</sub> for 2 h resulted in a lower mean percentage change in FEV<sub>1</sub> following cat allergen challenge. Finally, in a study of 18 mild asthmatics exposed for 30 min to 0.26 ppm NO<sub>2</sub> followed 4 h later by allergen challenge, although lung function was significantly decreased (discussed above), an increase in total eosinophil count following air and allergen was no longer significant following NO<sub>2</sub> and allergen (Strand et al., 1997).

Considered together, these studies provide some evidence indicating that NO<sub>2</sub> can potentiate allergen-induced airway response in asthmatics at concentrations as low as 0.26 ppm, although results remain inconsistent and effects appear to be small. The evidence also shows that exposure to NO<sub>2</sub> at this level may enhance the inflammatory response to inhaled allergen, possibly through alterations in eosinophil number and/or activity. The magnitude of these effects appeared to be larger than that of the lung function decrements (i.e. 2- to 6-fold increases in eosinophil concentrations and ECP levels). Recruitment and activation of eosinophils into the airways is considered to be a contributing causative agent in the histopathology and lung dysfunction in asthma (Jacobsen et al., 2007).

Although evidence for inflammation and decrements in pulmonary function exists at concentrations as low as 0.26 ppm, not all studies examining these endpoints found adverse effects due to NO<sub>2</sub>. These conflicting results are observed in bronchial allergen challenge studies examining similar NO<sub>2</sub> concentrations and dosing durations, and thus no dose–response relationship can be derived from this dataset. In fact, in certain studies, the adverse effect(s) of the allergen was attenuated by NO<sub>2</sub>. Exercise during NO<sub>2</sub> exposure appears to have little effect, and no effects on symptoms were observed, consistent with the majority of asthmatics that were not challenged (subsection 7.2.1). It should be noted that NO<sub>2</sub> levels below 0.26 ppm were not examined. In addition, one study considered a potential stratification of asthmatics as “responders” versus “non-responders” (Witten et al., 2005); this issue is discussed further in the following paragraphs on cholinergic and histamine challenges following NO<sub>2</sub>.

Twelve studies involving bronchial challenge with a cholinergic agent, including carbachol (Orehek et al., 1976), methacholine (Kleinman et al., 1983; Mohsenin, 1987; Roger et al., 1990; Jorres and Magnussen 1991; Riedl et al., 2012), or with histamine (Bylin et al., 1985, 1988; Tunnicliffe et al., 1994; Salome et al., 1996; Strand et al., 1996) were identified. Contrary to the dataset for allergen challenge, these studies examined doses of NO<sub>2</sub> as low as 0.1 ppm. They were also conducted either at rest or with intermittent exercise, and the bronchial challenge followed either immediately (within 5 h of the NO<sub>2</sub> exposure), or at a later point (up to 7 d) post-NO<sub>2</sub>.



A number of studies observed an effect on lung function of NO<sub>2</sub> exposure at levels as low as 0.26 ppm following a bronchial challenge with a cholinergic agent or histamine. Strand et al. (1996) observed a significant increase in bronchial responsiveness to histamine in 19 asthmatics, 5 h post-exposure to 0.26 ppm NO<sub>2</sub> for 30 min, as well as a decrease in lung volume 20 min and 7 d post-exposure. Similarly, in a group of 20 mild asthmatics, a 30-min exposure to 0.27 ppm NO<sub>2</sub> also resulted in an increased bronchial responsiveness to histamine (Bylin et al., 1988). These results remain consistent in studies examining NO<sub>2</sub> at slightly higher concentrations. Bylin et al. (1985) also observed an increase in bronchial reactivity in a group of eight asthmatics exposed to 0.48 ppm NO<sub>2</sub> for 20 min, while Mohsenin (1987) reported a significant potentiation of airway reactivity in 10 adults with asthma exposed for 1 h to 0.5 ppm NO<sub>2</sub>, followed by methacholine challenge. Finally, 9 adults and 11 children with moderate to severe asthma exposed to 0.6 ppm NO<sub>2</sub> for 1 h followed by histamine challenge demonstrated increased AHR as compared to air exposure (with no difference being observed between children and adults) (Salome et al., 1996).

In addition to decrements in lung function, two studies of mild asthmatics demonstrated variable signs of inflammation from NO<sub>2</sub> exposure. Strand et al. (1996) observed an increased expression of Mac-1 on granulocytes after exposure to 0.26 ppm NO<sub>2</sub> followed by histamine challenge. Contrary to these results, Riedl et al. (2012) observed a significant decrease in mean IgG4 levels in sputum post-exposure to 0.35 ppm NO<sub>2</sub> for 2 h followed by methacholine challenge among 15 asthmatics with allergy.

An emerging theme in this dataset is a differing susceptibility among asthmatics to the effects of NO<sub>2</sub>. As with the allergen challenge dataset, a considerable proportion of studies did not observe an effect of NO<sub>2</sub> on lung function following bronchial challenge with a cholinergic agent or histamine (Orehek et al., 1976; Kleinman et al., 1983; Roger et al., 1990; Jorres and Magnussen, 1991). For example, in a study of 18 mild asthmatics with timothy grass or birch pollen allergies, a 30-min exposure to 0.26 ppm followed by allergen and histamine challenges had no effect on lung function (Strand et al., 1997). Although the absorption of NO<sub>2</sub> is expected to be greater with exercise, asthmatics exposed to approximately 0.3 to 0.6 ppm NO<sub>2</sub> for 1 h did not demonstrate a clear difference in lung function between those intermittently exercising (e.g. Roger et al., 1990) and those asthmatics exposed at rest (e.g. Salome et al., 1996).

In the majority of these studies, although a statistically significant effect as a whole was not observed, some asthmatic subjects were more responsive. A 30-min exposure of 11 adults with mild asthma to 0.25 ppm did not affect the methacholine challenge outcome overall, but the authors discussed the results of other studies to suggest differing responsiveness of individual asthmatic patients to NO<sub>2</sub> (severity of the disease, baseline airway calibre, stimulus for evaluating AHR) (Jorres and Magnussen, 1991). For instance, in a study examining exposures ranging from 0.15 to 0.6 ppm NO<sub>2</sub> for 75 min followed by methacholine challenge, there was an indication in two of the subjects of a concentration-related increase in AHR due to NO<sub>2</sub> (Roger et al., 1990). Kleinman et al. (1983) did not report increased symptoms or decreased lung function relating to NO<sub>2</sub> among 31 asthmatics exposed to 0.2 ppm NO<sub>2</sub> for 2 h followed by methacholine, but individual responses did vary. In 20 out of 31 subjects, there was a slight increase in bronchial reactivity after NO<sub>2</sub> exposure (-4.8% in absolute area of FEV<sub>1</sub>, not statistically significant; no change in FVC or total resistance to breathing (R<sub>t</sub>)), with 5 of them being more responsive (percentage of absolute change not reported). A 1-h exposure to 0.1 ppm NO<sub>2</sub> did not affect lung function; nor did it increase bronchial hyperresponsiveness or resistive properties of the respiratory system (assessed by random noise excitation) when followed by methacholine challenge in 15 adults with mild asthma as a group (Hazucha et al., 1983). However, NO<sub>2</sub> exposure resulted in a 2-fold increase in the bronchconstrictor effect of the methacholine carbachol in 3 of the 15 subjects (i.e. the cumulative dose of methacholine



causing 100% of initial SRaw was 3.4 mg for air vs. 1.8 mg for NO<sub>2</sub> (Hazucha et al., 1983). Similarly, Orehek et al. (1976) examined the effect of a 1-h exposure to 0.1 ppm NO<sub>2</sub> followed by carbachol challenge in a group of 20 subjects with mild asthma. In 13 of the 20 subjects, NO<sub>2</sub> resulted in a 2-fold increase in the bronchoconstrictor effect of the carbachol (i.e. the mean dose of carbachol causing 100% of initial SRaw was 0.66 mg for air vs. 0.36 mg for NO<sub>2</sub>).

The existence of a dose–response relationship of the effects of NO<sub>2</sub> on bronchial challenge with cholinergic agents or histamine was examined in a small number of studies. In two studies (Bylin et al., 1985; Salome et al., 1996) utilizing NO<sub>2</sub> exposure concentrations ranging from 0.12 to 0.6 ppm followed by histamine challenge, pulmonary effects were only observed at the highest concentration in each study (i.e. 0.48 and 0.6 ppm). As discussed above, in a study of 21 mild asthmatics exposed to concentrations of NO<sub>2</sub> ranging from 0.15 to 0.6 ppm for 75 min, a concentration-related increase in AHR to methacholine due to NO<sub>2</sub> exposure appeared to exist in a small proportion of the subjects (Roger et al., 1990). On the other hand, Bylin et al. (1988) exposed 20 mild asthmatics to 0.14, 0.27, and 0.53 ppm NO<sub>2</sub>; the decrease in histamine threshold was only observed at 0.27 ppm and not at the highest dose studied.

It can be concluded that the controlled human studies indicate a relatively consistent relationship between low concentrations of NO<sub>2</sub> and impacts on AHR to cholinergic agents and histamine. The majority of effects are seen at concentrations of NO<sub>2</sub> at or above 0.26 ppm, though very few studies have examined lower concentrations. Data also exist indicating that a subset of the asthmatic population is sensitive to the effects of NO<sub>2</sub> (thus defined as “responders”); significant adverse effects of NO<sub>2</sub> on AHR following a bronchial challenge have been reported at levels as low as 0.1 ppm. Again, it should be noted that NO<sub>2</sub> concentrations below 0.1 ppm have not been examined in the reviewed literature. Limited and not entirely consistent data exist of a dose–response relationship and the development of adverse effects other than AHR. Exercise during NO<sub>2</sub> exposure appears to have little effect. With the exception of one preliminary NO<sub>2</sub> study of adults with a transient increase in dry mouth and cough in the initial but not subsequent exercising intervals, symptoms were not observed in adults below 0.26 ppm NO<sub>2</sub>. As results from studies evaluating delayed symptom onset with NO<sub>2</sub> exposure in exercising children are variable, no conclusions can be made on this effect.

## 7.3 Meta-Analyses and Systematic Reviews of Respiratory Effects in Controlled Human Exposure Studies

A meta-regression analysis of 41 exposure scenarios from 28 controlled NO<sub>2</sub> exposure studies investigating AHR in slight to severe asthmatics and non-asthmatics was conducted by Goodman et al. (2009) by combining AHR from nonspecific and specific airway challenges. There was no dose–effect relationship following NO<sub>2</sub> exposures from 0.1 to 0.6 ppm. Analyses were conducted on the basis of various metrics, including fraction of asthmatics with greater AHR, difference between airway challenge provocative dose, or difference between the change in FEV<sub>1</sub> induced by an airway challenge. No clear dose–effect relationship for these metrics was found in increasing exposure categories between 0.1 and 0.6 ppm NO<sub>2</sub>, although statistically significant results had been observed in individual studies at varying levels of exposure. Adversity was defined by the authors as a 10% or more change in FEV<sub>1</sub> (with NO<sub>2</sub>, relative to air) or a 50% or more reduction in provocative dose (resulting from NO<sub>2</sub>, relative to air). The size of the effects on AHR at this range of exposure was small, and the authors considered that the effects, even though statistically significant, were unlikely to be clinically relevant. Overall, the authors of this meta-analysis concluded that controlled NO<sub>2</sub> exposure studies do not provide evidence for adverse effects on AHR in asthmatics at concentrations up to 0.6 ppm.

A systematic review of more than 50 controlled short-term NO<sub>2</sub> exposure studies was carried out by Hesterberg et al. (2009), looking at a wide range of effects (AHR, pulmonary inflammation, and susceptibility to viral infection) on healthy and mild-to-severe asthmatic subjects. The authors of this review found that asthmatic individuals generally did not show NO<sub>2</sub>-induced AHR below 0.6 ppm. Occasional changes in lung function tests or symptoms were small in extent and transient, and NO<sub>2</sub> dose–response relationships were poor; thus they were considered of questionable clinical relevance. Orehek et al. (1976) suggested that some individuals (“responders”) may be particularly sensitive and experience effects at levels as low as approximately 0.1 ppm. These results should be considered with the lack of dose–response with a 2-fold higher dose in the same study, but the weight of evidence in the database supports intraspecies variation. With respect to lung immune responses and inflammation, the authors concluded that there was no consistent evidence for effects below 1 ppm exposure in healthy individuals or below 0.6 ppm in asthmatics. They considered there to be weak evidence for pulmonary effects in asthmatics, in the range of 0.2–0.6 ppm NO<sub>2</sub> exposure. Only some of the investigations found effects in this range, and the magnitude of those effects was small (e.g. changes of less than 10% in lung function tests). They did not find evidence for NO<sub>2</sub>-induced susceptibility to viral infection at less than 2 ppm.

In its *Integrated Science Assessment for Oxides of Nitrogen–Health Criteria*, the US EPA (2008) conducted a meta-analysis of controlled short-term studies of NO<sub>2</sub>-induced AHR in asthmatics, which included most but not all of the studies considered by Goodman et al. (2009). Differences in the US EPA analysis (relative to Goodman et al., 2009) were that the following were included: individual data (versus mean), separate assessments for specific and nonspecific challenges for AHR, and the fraction of affected asthmatics (versus quantification of magnitude of change). AHR increased in asthmatics with 0.1 ppm NO<sub>2</sub> exposure after 1 h and with 2- to 3-fold higher exposures after 0.5 h; in healthy people the effect occurred with 5-fold higher doses after 3 h. Overall, the US EPA concluded that exposure to NO<sub>2</sub> at levels less than 0.3 ppm, and as low as 0.1 ppm, for 1 h was linked to nonspecific AHR in people with mild asthma.

The US EPA (2008) also reviewed controlled exposure studies with respect to other health endpoints (airway inflammation, immunological function) in healthy volunteers. The review found that, for healthy individuals, NO<sub>2</sub> exposures of less than 1 ppm for periods of less than several hours do not generally lead to changes in markers of airway inflammation. Frampton et al. (2002) indicated that exposure to 0.6 ppm NO<sub>2</sub> for 3 h may decrease host resistance to viral challenge. However, examination of Frampton’s (2002) *in vitro* study demonstrated equivocal evidence, since it was not replicated and only decreased with one of two viruses.

The World Health Organization (WHO, 2010), in reviewing the controlled short-term exposure studies as well as the Goodman et al. (2009) meta-analysis, agreed with the US EPA (2008) conclusion that in healthy individuals, NO<sub>2</sub> exposures of less than 1 ppm generally do not lead to measurable changes in lung function, in bronchial responsiveness, or in markers of inflammation. They did note that a 3-h exposure to 0.6 ppm NO<sub>2</sub> inhibited alveolar macrophage response to viral infection (Frampton et al., 2002). In the case of asthmatics or individuals with mild COPD, the WHO (2010) concluded that, although the findings are highly variable, NO<sub>2</sub> exposures as low as 0.2 to 0.3 ppm for periods of 1 h or longer have, in some studies, produced a range of responses within the lung, suggestive of airway inflammation and alteration in immune defences in asthmatics. They also noted that the results of recent systematic reviews and meta-analyses provided suggestive evidence that controlled exposures to as low as 0.1 to 0.2 ppm NO<sub>2</sub> are associated with small increases in airway reactivity to a range of stimuli in asthmatics.

## 7.4 Cardiovascular Effects

The 2008 US EPA ISA for NO<sub>x</sub> reported that a few controlled human exposure studies had indicated effects of NO<sub>2</sub> exposures on cardiac output, blood pressure and circulating RBCs, but noted that the results required further confirmation. Drechsler-Parks (1995) observed a decrease in cardiac output after exposures to 0.6 ppm NO<sub>2</sub> and 0.45 ppm O<sub>3</sub> combined for 2 h with intermittent exercise. No significant changes were observed for cardiac output, stroke volume or heart rate after exposures to 0.6 ppm NO<sub>2</sub> alone. Folinsbee et al. (1978) also did not identify significant changes in heart rate, cardiac output or blood pressure in young healthy males exposed to 0.6 ppm NO<sub>2</sub> for 2 h with intermittent exercise. Linn et al. (1985) did not identify a change in heart rate in patients with COPD exposed to up to 2 ppm NO<sub>2</sub> for 1 h with intermittent exercise. Gong et al. (2005) identified a slight increase in heart rate, but no significant changes in blood pressure or minute volume in healthy and COPD subjects exposed to 0.4 ppm NO<sub>2</sub> for 2 h with intermittent exercise. Frampton et al. (2002) reported a concentration-related reduction in hematocrit and hemoglobin among healthy subjects exposed to 0.6 or 1.5 ppm NO<sub>2</sub> for 3 h with intermittent exercise, confirming the findings of an earlier study conducted by Posin et al. (1978), which had exposures to 1 or 2 ppm NO<sub>2</sub> for 3 h with intermittent exercise.

More recent controlled human exposure studies with short-term exposures to NO<sub>2</sub> included one study of 18 patients with coronary heart disease (CHD) exposed to 0 or 0.4 ppm NO<sub>2</sub> for 1 h. No effects were observed on heart rate, HRV (rMSSD, SDNN, PNN50 (i.e. the number of pairs of successive NNs that differ by more than 50 ms, divided by the total number of NNs), HF, LF, LF:HF ratio, TP) or frequency of ectopic beats or arrhythmias (Scaife et al., 2012).

In another study, 14 healthy volunteers were exposed to 0.5 ppm NO<sub>2</sub> for 2 h with intermittent exercise. NO<sub>2</sub> exposures did not alter blood cell components or coagulation factors. However, increased HDL cholesterol in peripheral blood, a decreased measure of HRV (high frequency domain normalized for heart rate) and a decreased marker of cardiac repolarization (QT variability index) (an increase is associated with arrhythmias) were observed after NO<sub>2</sub> exposures (Huang et al., 2012).

Riedl et al. (2012) studied 15 subjects with mild asthma, 15 subjects with mild asthma and cat allergy, and 4 healthy subjects exposed to 0.02 or 0.36–0.39 ppm NO<sub>2</sub> for 2 h with intermittent exercise. NO<sub>2</sub> exposures did not alter serum biochemistry (immunoglobulins, interleukin-6 (IL-6) or ICAM), coagulation factors (factor VII, fibrinogen), blood pressure, oxygen saturation or cardiovascular symptom scores.

To examine whether vascular toxicity of inhaled NO<sub>2</sub> may be caused by soluble factors released into the systemic circulation, plasma from seven humans exposed to 0 or 0.5 ppm NO<sub>2</sub> for 2 h was incubated with commercially available primary human coronary artery endothelial cells. Increases were observed in some cell inflammatory markers (ICAM-1, VCAM-1, IL-8) but not others (MCP-1) (Channell et al., 2012).

## 7.5 Summary and Considerations

Human controlled exposure studies demonstrate a relationship between exposure to NO<sub>2</sub> and adverse respiratory effects in asthmatics or COPD subjects. Unfortunately, the dose–response relationship at concentrations below 1 ppm NO<sub>2</sub> is unclear. Inconsistencies may be the result of intraspecies (i.e. inter-individual) variability, the limited number of exposure levels tested in specific studies, and/or small sample sizes and possibly differing exposure protocols.

Significantly adverse changes in endpoints are not observed in many studies, which may be due to the lower exposure levels characteristic of this type of study.

Healthy adults were 3-fold less sensitive to the respiratory effects of NO<sub>2</sub> than other subpopulations. For instance, whereas overall healthy individuals were not affected at exposure levels up to 1 ppm NO<sub>2</sub>, mixed results with asthmatics exposed to approximately 0.3 ppm NO<sub>2</sub> in two of five studies suggested that asthmatics were more sensitive, as evidenced by a rapid onset of decreased lung function parameters (e.g. FEV<sub>1</sub>), particularly when challenged with increased ventilation rates.

In considering studies examining respiratory, inflammatory, and immunology endpoints in humans exposed to concentrations below 1 ppm NO<sub>2</sub>, few consistent effects were observed in healthy individuals. In some studies, there were some sensitive subgroups (“responders”) identified at 0.6 ppm (increased α2-macroglobulin in BALF in one study; reduced ability to inactivate influenza virus *in vitro* in one of two studies). Decreased hematocrit, hemoglobin (≤5% each), and lymphocytes (<25% in females without dose dependence), or increased natural killer cells in BALF were also observed at 0.6 ppm in single studies. Although some of these isolated effects are of equivocal clinical significance in themselves, such inflammatory and respiratory effects may be of concern in certain subpopulations (e.g. asthmatics). Therefore, 1 ppm may be considered to be the level below which adverse effects have not been observed in healthy individuals, with the acknowledgement that some healthy individuals may be more sensitive to some endpoints at 0.6 ppm or less.

Asthmatics with allergies, exposed to at least 0.26 ppm NO<sub>2</sub> and challenged with an allergen, demonstrated decreased lung function in some studies but increased inflammatory response (increased neutrophils and ECP levels) in the absence of affected lung function in other studies. The changes in lung function tests were observed both in studies in which direct agents (histamine, carbachol, methacholine) or cold challenges were administered to induce hyperresponsiveness and in studies where no additional challenge beyond exercise was included in the study protocol. Specific allergens were also used to challenge subjects with allergies, and although the data were mixed, there was some evidence that NO<sub>2</sub> could potentiate allergen-induced airway responses in asthmatics.

Following exposure to at least 0.26 ppm NO<sub>2</sub>, direct bronchial challenges could increase bronchial responsiveness. There were a few studies where asthmatics were exposed to lower doses. No effects on symptoms or bronchial reactivity were observed in asthmatics challenged with histamine below 0.26 ppm or with methacholine. In one of these studies, 2 out of 21 subjects had a concentration-related increase in AHR due to exposures ranging from 0.15 to 0.6 ppm NO<sub>2</sub> for 75 min followed by methacholine challenge, but the significance of this is unclear, as the extent was not stated; nor were any data shown. The authors considered this as similar to chance. There were also no effects on symptoms or bronchial reactivity in asthmatics with allergies challenged with HDMA below 0.26 ppm.

However, while three studies of asthmatics exposed to lower concentrations of NO<sub>2</sub> did not demonstrate overall effects on lung function or bronchial reactivity, there were individuals in those studies who were responsive to the potentiating effects of NO<sub>2</sub> on bronchoconstrictors. In one study, in a subgroup of asthmatics (13 out of 20), the lowest tested exposure of 0.1 ppm NO<sub>2</sub> with intermittent exercise produced a slight increase in SRaw without statistical significance. The subgroup also demonstrated increased carbachol-induced response (20% or less carbachol was required for a reaction (D<sub>100</sub>, the dose of carbachol causing a 100% increase of initial SRaw) after NO<sub>2</sub> exposure, relative to air) in comparison to air exposure.

In another study, asthmatics exposed to 0.2 ppm NO<sub>2</sub> for 2 h with intermittent exercise did not have increased symptoms or adverse changes in pulmonary measurements (FVC, FEV<sub>1</sub>, R<sub>t</sub>) when assessed as a whole group. However, 0.2 ppm NO<sub>2</sub> decreased the respiratory function (4.8% decreased FEV<sub>1</sub>, not statistically significant) in 20 of the 31 subjects, relative to air. When

exposure was followed by a methacholine challenge, 17 of the 31 subjects required 5% less methacholine to elicit a 10% decrease in FEV<sub>1</sub> (statistically significant relative to air); lung function was similar or more responsive to air in the other third of the subjects. In another study with 0.1 ppm NO<sub>2</sub> exposure for 1 h/d for 3 d, 3 out of 15 people were responsive to methacholine. Responders in these studies were not the ones with the most compromised airways, based on pre-exposure measurements. It is unclear whether they represent the individuals in the population most sensitive to respiratory AHR without a bronchoconstricting agent. On the other hand, another paradigm is that perhaps individuals responsive to the potentiating effects of NO<sub>2</sub> should be of higher concern. These individuals suggest that intraspecies variation may not be supported by using the results of the study groups as a whole.

Other subpopulations that may be particularly sensitive to NO<sub>2</sub> are older adults with COPD and asthmatic children. Adults with COPD intermittently exercising demonstrated decreased respiratory function (decreased FVC or FEV<sub>1</sub>) with a 1- to 4-h exposure to 0.3 ppm NO<sub>2</sub> in two studies, but not after a 2-h exposure to 0.4 ppm NO<sub>2</sub> in a third study. There were no effects of an exposure to 0.3 ppm NO<sub>2</sub> on symptoms, heart function, inflammation, or AHR in older COPD subjects. It is unclear how adults with COPD compare to those with asthma, in terms of relative responsiveness to NO<sub>2</sub>, due to the mixed database of both adults with asthma and those with COPD. Epidemiological data support an increased vulnerability to NO<sub>2</sub> among asthmatics and COPD subjects (subsections 8.2.2, 8.3.1 and 8.4.1). Moreover, there are no studies evaluating less than 0.26 ppm NO<sub>2</sub> exposure in adults with COPD or in asthmatic children. The two studies of asthmatic children did not clearly suggest sensitivity. At rest, moderately to severely asthmatic children were affected similarly to asthmatic adults, in that both were not affected in symptoms, lung function, or AHR to histamine at 0.3 ppm NO<sub>2</sub> exposure, but both had AHR at 0.6 ppm. Asthmatic children (classification not stated) demonstrated increased symptoms and transient decreased lung function with no AHR to cold air at 0.3 ppm when exercising, but association of effects with NO<sub>2</sub> exposure was confounded with similarly increased symptoms prior to any NO<sub>2</sub> exposure and adaptation to exercise. Epidemiological data suggest that asthmatic children are more sensitive on a population basis (subsections 8.3.1, 9.3.1.3, 9.3.1.5). Thus, although there was no evidence of age or gender sensitivity in the controlled human database, it is possible that individual asthmatic children or adults with COPD could be more sensitive to exposures below 0.26 ppm NO<sub>2</sub>.

For cardiovascular effects, a single recent study identified an increase in HDL cholesterol and a decrease in QT variability index following exposure to 0.5 ppm for 2 h, while no effects were identified on heart rate, blood cell counts, coagulation factors, blood pressure, oxygen saturation or cardiovascular symptoms at levels ≤0.5 ppm in a limited number of new controlled human exposure studies. Some measures of HRV were decreased in one study, though not in another. One study suggested that NO<sub>2</sub> exposures may stimulate soluble factors to be released into the systemic circulation, where they may activate endothelial cells to produce inflammatory markers. Results of the recent studies are fairly consistent with the older literature (<2008), which also did not identify effects on heart rate with exposures of up to 4 ppm NO<sub>2</sub>, although one study did show a slight increase in heart rate among COPD subjects. Additionally the older literature did not identify changes in cardiac output or changes in blood pressure after exposure to up to 0.6 ppm NO<sub>2</sub>. A decrease in blood pressure was observed at 4 ppm in a single study, while two others showed slight decreases in hematocrit and hemoglobin starting at 0.6 ppm, with a decrease in RBC counts also being observed in only one of them.



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## 8. Epidemiological Studies of Health Effects Associated with Short-Term Exposure to Ambient NO<sub>2</sub>

### 8.1 Introduction

In this chapter, epidemiology studies of the health effects associated with short-term exposure to ambient NO<sub>2</sub> are reviewed.

**Chapter organization:** This chapter covers mortality and morbidity related to short-term exposures (on the order of days to weeks) to ambient NO<sub>2</sub>. This includes premature mortality, hospital admissions and ERVs for CVD and respiratory diseases, and the endpoints reported in panel studies: lung function, AHR, biomarkers of airway inflammation, respiratory symptoms or medication use, lung host defence and immunity, cardiovascular function, and biomarkers of CVD risk. The individual sections summarize the findings of epidemiological studies of associations between exposure to NO<sub>2</sub> (most often as ambient NO<sub>2</sub> measured at central sites) and the various health outcomes for each category.

**Focus on Canadian and US studies:** Epidemiology studies of health effects associated with NO<sub>2</sub> air pollution have been conducted all over the world, encompassing a wide range of climates, pollutant levels and mixes, standards of living, and health care systems. As a result, the literature is large and continually growing, and there are hundreds of published studies. Given this relatively large literature base, this chapter focuses on those studies that are considered to be particularly relevant to the risks associated with exposure to ambient NO<sub>2</sub> in Canada.

Studies from Canada and the US are considered to be the most relevant, and they are generally presented in the greatest detail, given the considerable similarities in these two countries with respect to such features as standard of living and (in some regions) climate. As well, the automobile industry is integrated on a North American basis, and in the urban settings where most people live engine emissions are a major source of NO<sub>2</sub> and co-emitted pollutants. Moreover, large parts of Canada have a common airshed with the US and are impacted by transboundary air pollution from this country. As well, both countries tend to be more multicultural due to the immigrant proportion of the population being higher than in many areas of Europe and the rest of the world, although the underlying proportions of ethnic or racial populations would be different between Canada and the US. Studies of populations in other developed regions of the world that have standards of living and levels of industrialization similar to those in Canada, primarily Europe and Australia, are also considered relevant, though there are some potentially important differences between Canada and these countries. For example, the European vehicle fleet is more heavily weighted to diesel vehicles, an important source of NO<sub>2</sub> and related pollutants.

**Use of the 2008 US EPA ISA as a starting point:** As with the other epidemiological chapters in this report that describe studies of ambient NO<sub>2</sub>, this chapter uses the 2008 US Environmental Protection Agency *Integrated Science Assessment for Oxides of Nitrogen—Health Criteria* (US EPA ISA) (US EPA, 2008) as the basis for summarizing the earlier epidemiological literature. There is a substantial relevant literature covered by the 2008 US EPA ISA, and it is important to retain this information as part of the overall weight of evidence. Hence, the summary of the earlier literature derived from the 2008 US EPA ISA is generally

fairly detailed and includes individual reference to key papers published prior to 2008 where this is of utility in illustrating important issues.

**Standard exposure increments:** In the epidemiological studies of ambient NO<sub>2</sub>, the effect estimates for health outcomes are usually reported as a percentage change in the outcome relative to a baseline mortality or morbidity rate, based on an incremental change in exposure. To enhance comparability of the risk estimates between studies, these relative risks need to be presented by a uniform increment of exposure. However, different NO<sub>2</sub> exposure indices with different averaging times have been used in the existing epidemiological literature. Since concentrations are lower and less variable for longer averaging times, risks of health outcomes for a given concentration range are not directly comparable across exposure metrics, which complicates the determination of a standard increment.

The short-term NO<sub>2</sub> exposure indices most often employed are the daily 1-h max and the 24-h avg concentrations. In this report, when the results from a number of studies are directly compared (for example, in figures and tables), the risks associated with these short-term exposure indices are expressed for the following standard increments, which are the same as those used in the 2008 US EPA ISA:

#### Daily Exposure Index

<u>Exposure Increment</u>	<u>ppb</u>
1-h max NO <sub>2</sub>	30
24-h avg NO <sub>2</sub>	20

As described in the 2008 US EPA ISA, these increments were developed using nationwide distributional data for NO<sub>2</sub> monitors in US metropolitan statistical areas; they are approximately representative of a low-to-high change in NO<sub>2</sub> concentrations, on the basis of the annual mean to 95<sup>th</sup> percentile differences. A review of recent Canadian data from the NAPS network indicated that this is also applicable to Canada.

Standardizing the risk estimates in this manner ensures that they are comparable across the different metrics. The different increments for each NO<sub>2</sub> exposure period do not represent inconsistencies; instead, they are scaled to enable direct comparisons between the risk estimates from various studies that used different NO<sub>2</sub> exposure indices.

**Potential Confounding by Co-pollutants:** The epidemiological studies yield valuable information on potential relationships between exposure to ambient NO<sub>2</sub> and health effects. However, the associations between ambient NO<sub>2</sub> and health outcomes in these studies may be confounded by co-pollutants. (A confounder is related to both the exposure and the effect. For example, confounding can occur between correlated pollutants that are associated with the same effect.) One way to remove spurious association is by means of statistical control of potential confounding, referred to as “adjustment.” Co-pollutant regression models are the most widely used technique to control for potential co-pollutant confounding in air pollution health effects studies. In such models, the NO<sub>2</sub> effect estimate represents the risk associated with NO<sub>2</sub> while keeping the level of the other co-pollutant(s) or other covariate(s) constant. There are limitations to multivariable models; in particular, high correlations between NO<sub>2</sub> levels and potential confounders can affect the magnitude or precision of the effect estimate for NO<sub>2</sub> or the covariate and are a concern for models that include a traffic-related co-pollutant or that include three or more pollutants in the same model (these are generically referred to as multi-pollutant models in this report). Because much of the uncertainty in inferring causality may arise from potential confounding by co-pollutants, control for these other pollutants is an important consideration when evaluating individual studies. In summarizing epidemiology studies in this

assessment, the results of both single- and co-pollutant models were considered and the robustness of findings to adjustment for co-pollutants was examined.

**GAMs:** Generalized additive models (GAMs) have been widely used in epidemiological analyses of health effects associated with air pollution. However, a number of years ago it was reported that using the default convergence criterion and default variance estimate method in the S-plus software package for the GAM function can lead to upward bias in regression estimates and an underestimation of the standard error of the risk estimate, respectively (Dominici et al., 2002; Ramsay et al., 2003). The default convergence criterion was problematic when the estimated risks were small and when the GAM included two or more nonparametric smoothing curves. The magnitude and direction of the bias depend in part on the concurvity of the independent variables in the GAM and the magnitude of the risk estimate. Consequently, the results from studies reporting that they employed GAMs with the default convergence criterion and at least two nonparametric smoothing terms are generally not included in this chapter.

## 8.2 Studies of Mortality

This section on epidemiology studies of the association between ambient NO<sub>2</sub> and mortality from various causes is focused principally on multi-city studies. Such studies are considered most informative because their large size reduces the potential for spuriously negative findings as a result of limited statistical power, which is a problem in some single-city studies. In addition, such studies analyze data from multiple cities in a consistent fashion, which reduces the impact of publication bias that can result in single-city studies with negative findings going unreported. The risk estimates from multi-city studies are also generally reported for consistent lags, reducing potential bias as a result of selecting the lag that yields the greatest risk estimate.

The 2008 US EPA ISA reported that the results of several large US and European multi-city studies and a meta-analysis indicated positive associations between ambient NO<sub>2</sub> concentrations and risks of total non-accidental mortality that were generally robust to adjustment for other pollutants (US EPA, 2008). Both cardiovascular and respiratory mortality were associated with increased NO<sub>2</sub> concentrations, but similar associations were observed for other pollutants in these studies.

The results of a number of key multi-city studies conducted in Canada, the US, and other developed countries are summarized in this section, including several reviewed in the 2008 US EPA ISA that contribute significantly to the weight of evidence. The findings of meta-analyses are also reported; however, because the heterogeneity in risk estimates reported in these studies is partly the result of differences in analytical approaches among studies the focus is on the multi-city studies. As noted above, studies that were affected by the GAM with convergence criterion issue are generally not discussed.

### 8.2.1 Multi-city Studies

There have been several Canadian multi-city studies by the same group of researchers. The following discussion focuses on the four studies that are most extensive in terms of the coverage of cities and the duration for which mortality data were available (Burnett et al., 2004; Brook et al., 2007; Shin et al., 2008; Stieb et al., 2008).

Burnett et al. (2004; US EPA, 2008) investigated mortality from total non-accidental, cardiovascular, and respiratory causes in 12 Canadian cities from 1981 to 1999. For NO<sub>2</sub>, the strongest association with mortality was found for the average of 0-, 1-, and 2-d lags, whereas it was with lag 1 d for PM<sub>2.5</sub>, PM<sub>10-2.5</sub>, PM<sub>10</sub>, SO<sub>2</sub>, CO, and coefficient of haze, and with the 2-d moving avg for O<sub>3</sub>. Of the single-day lags for NO<sub>2</sub>, lag 1 d showed the strongest association.



The pooled non-accidental mortality risk estimate for NO<sub>2</sub> in a single-pollutant model was 2.25% (95% CI 1.26%, 3.24%) per 22.4 ppb increase in the 0–2 d avg. The risk estimates were similar for total, cardiovascular, and respiratory mortality, were greatest in (but not limited to) the warmest months, and were increased in older adults (≥65 years of age). The NO<sub>2</sub>–mortality association was not sensitive to adjustment for any of the other pollutants in two-pollutant models, and was actually increased in models with the various particle size fractions. However, Burnett et al. (2004) reported that in two-pollutant models with daily PM<sub>2.5</sub> (available for 1998 to 2000 only, in contrast to the every-sixth-day PM<sub>2.5</sub> in the main analysis), the risk estimate for NO<sub>2</sub> was reduced (from 0.85% per 10 ppb 24-h NO<sub>2</sub>, lag 2 d to 0.32%, no longer significant), though detailed results were not reported.

Stieb et al. (2008) conducted a subsequent analysis of essentially the same data as Burnett et al. (2004) in the course of developing an Air Quality Health Index (AQHI), except that mortality from all internal causes was related to the daily 3-h max pollutant concentrations. In single-pollutant models, mortality was significantly related to all pollutants considered, including NO<sub>2</sub>: risk was increased 2.08% (95% CI 0.98%, 3.28%) at the mean concentration of 33.6 ppb 3-h max NO<sub>2</sub>, lag 1 d, and was lower but still significant at 0- and 2-d lags. NO<sub>2</sub>-related mortality was slightly greater in 1991–2000 than in 1981–1990 and in the warm season than in the cool season, though each of these associations was still significant. In two-pollutant and multi-pollutant models, NO<sub>2</sub> was robust to adjustment for other pollutants in all but a five-pollutant model with PM<sub>10</sub>, in which it was borderline significant. A linear model of concentration–response provided a better fit than quadratic or cubic polynomials for most pollutants, including NO<sub>2</sub>.

Brook et al. (2007; US EPA, 2008) analyzed data on total non-accidental mortality in relation to particulate and gaseous pollutants collected between 1980/84 and 2000 from 10 Canadian cities, with a particular focus on NO<sub>2</sub> and other traffic-related pollutants. NO<sub>2</sub> was most strongly related to mortality among the pollutants examined, including NO, various PM size fractions, sulphate, and a number of particulate trace elements. Using data for all days, the mortality risk was increased, with an RR of 1.013 (95% CI 1.008, 1.018) per 10.29 ppb 24-h NO<sub>2</sub>, lag 1 d, compared with an RR of 1.018 (95% CI 1.007, 1.028) when the analysis was restricted to days with data for all pollutants. The NO<sub>2</sub> risk estimate was strongest at lag 1 d and was greater in the warm season, though it remained significant in all-year and cool-season analyses. The NO<sub>2</sub>–mortality association was robust to adjustment for any of the other single pollutants, whereas none of the other pollutants was significantly associated with mortality after adjustment for NO<sub>2</sub>. NO<sub>2</sub> was generally more strongly correlated than PM<sub>2.5</sub> to a range of pollutants that were indicators of motor vehicle exhaust, including NO, BTEX, acetylene, 1,3-butadiene, selected PAHs, and hopane.

Shin et al. (2008) investigated the association of short-term exposure to NO<sub>2</sub> and O<sub>3</sub> with all-cause mortality from internal causes in 24 of Canada's largest cities over the 17-year period from 1984 through 2000. To examine whether the short-term association changed over the years of study, they developed two weighted multi-year estimators that used current plus several previous years of data to estimate the short-term risk during each year. The pooled common risk across all cities was significant (regression coefficient (β) 0.0859% (SE 0.0166%) per ppb 2-d running avg 24-h NO<sub>2</sub>). The annual average concentrations of NO<sub>2</sub> decreased significantly (by roughly 20%) over the study period, and the associated short-term risk for mortality appeared to change little or to increase minimally over this time (depending on whether data from one or two outlier years were included). The authors noted that the risk per unit of air pollutant could vary over time for a variety of reasons, including that the measured pollutant was acting as a surrogate for the true toxic agent, the population or the monitoring sites changed over time and space, or the association between exposure and mortality was not linear. Shin et



al. (2009) extended this work to also examine trends in the percentage of attributable risk over time, calculated as the product of the annual air pollutant concentrations and annual risks for a given pollutant. The percentage of attributable risk did not change significantly over time, even though the annual pollutant concentrations declined over the study period.

Recently, Shin et al. (2012) expanded this work still further, by extending the time period for four more years to 2004, employing a hierarchical two-level model to estimate regional risks in addition to city-specific and national risks, and estimating risks by cause of death and by season. Over the entire period, the NO<sub>2</sub>-related risk for CP mortality was slightly lower but with a narrower credible interval (a Bayesian construct similar to a confidence interval) than that for non-CP mortality (0.00071; 95% credible interval 0.00019, 0.00123 per ppb 24-h NO<sub>2</sub>, lag 0–2 d avg vs. 0.00089; 95% credible interval 0.00016, 0.00155, respectively), mainly due to an unusually low CP mortality risk for a single year. The warm-season risk associated with NO<sub>2</sub> was greater than the cold-season risk for both CP and non-CP mortality. For both categories, there were no significant regional differences in NO<sub>2</sub>-related mortality for 21 years, and no strong evidence that NO<sub>2</sub>-related risks changed over time at either the national or regional levels.

In the US, some large time-series analyses of mortality have been conducted using the National Morbidity, Mortality, and Air Pollution Study (NMMAPS) dataset (Samet et al., 2000; Dominici et al., 2003; US EPA, 2008). They reported on all-cause mortality in relation to air pollution in the 90 largest US cities between 1987 and 1994. Only limited results were reported for NO<sub>2</sub>, since PM<sub>10</sub> was the principal focus of this study; NO<sub>2</sub> was only measured in 58 of the cities. The strongest association for NO<sub>2</sub> was at lag 1 d, with a statistically significant increase in mortality of roughly 0.25% per 10 ppb 24-h NO<sub>2</sub> in the 2003 report (graphical results only presented). In two- and three-pollutant models with PM and gaseous pollutants, the NO<sub>2</sub> risk estimates remained about the same or increased, though they were less precise and no longer significant. This study used more terms for weather effects than others in the literature: i.e. the model adjustment for potential confounders was more aggressive than in other studies.

Recently, Moolgavkar et al. (2013) reported the results of analyses of mortality in relation to air pollutants using the NMMAPS dataset, in which they employed a sub-sampling bootstrap approach rather than the hierarchical Bayesian approach used in previous analyses of these data. Mortality data from 1987 to 2000 were assessed, and NO<sub>2</sub> data were available for 72 cities. At lag 1 d, all the pollutants were significantly associated with non-accidental mortality; for NO<sub>2</sub>, the risk was increased by 1.03% (95% CI 0.91%, 1.18%) per 10 ppb 24-h NO<sub>2</sub>. In two- and three-pollutant models with SO<sub>2</sub> and CO or with PM<sub>10</sub>, the risk estimates for NO<sub>2</sub> were attenuated somewhat but remained significant. In analyses using flexible models to investigate the shape of the concentration–response relationship, there was a suggestion of a threshold around 20 ppb for ambient NO<sub>2</sub>, though the confidence bounds were wide and the authors concluded that the concentration–response relationship was consistent with linearity.

NO<sub>2</sub>-related mortality has also been investigated in a number of multi-city studies from other developed regions of the world (Biggeri et al., 2005; Simpson et al., 2005; Samoli et al., 2006; Bellini et al., 2007; Berglind et al., 2009; Chiusolo et al., 2011; Zauli Sajani et al., 2011; Faustini et al., 2013).

In the largest analysis, Samoli et al. (2006; US EPA, 2008) estimated NO<sub>2</sub>-associated mortality risks in the Air Pollution and Health: A European Approach (APHEA2) project among 30 APHEA2 cities in the period 1990–1997. Results using a distributed lag model showed multi-day increases in risks for each of total, cardiovascular and respiratory mortality. Increases of 0.30% (95% CI 0.22%, 0.38%), 0.40% (95% CI 0.29%, 0.52%) and 0.38% (95% CI 0.17%, 0.58%) were observed in the pooled estimates using random effects models for total, cardiovascular and respiratory mortality, respectively, per 10 µg/m<sup>3</sup> (5.32 ppb) increase in daily NO<sub>2</sub> levels (lag

0–1 d avg). The associations were generally strongest at lag 1 d and cumulative risks over lag 0–5 d were 22–45% greater than the average of 0–1 d. In two-pollutant models, estimated risks for total, cardiovascular and to a lesser extent respiratory mortality were robust to adjustment for black smoke (BS), PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub>. In analyses of effect modifiers, the associations of NO<sub>2</sub> mortality with total and cardiovascular mortality were greatest in northwestern cities, with cardiovascular mortality in cities with greater natural gas consumption, and with respiratory mortality in cities with high median PM<sub>10</sub> levels and a greater proportion of older adults (≥65 years of age).

Chiusolo et al. (2011) conducted a case-crossover study of mortality in relation to daily air pollution in 10 Italian cities between 2001 and 2005, conducting detailed analyses of lags, co-pollutants, and effect modifiers. There were statistically significant increases in mortality due to all natural (2.09% for lag 0–5 d avg; 95% CI 0.96%, 3.24%), cardiac (2.63% for lag 0–5 d avg; 95% CI 1.53%, 3.75%) and respiratory causes (3.48% for lag 1–5 d avg; 95% CI 0.75%, 6.29%) per 10 µg/m<sup>3</sup> (5.32 ppb) increase in ambient NO<sub>2</sub>. Cerebrovascular deaths were only significantly increased during the warm season (7.87% for lag 0–5 d avg; 95% CI 4.78%, 11.05%). Overall, associations were strongest for exposures lagged 0–5 d (in contrast to most other studies, for single-day lags only cerebrovascular mortality was greatest for a 1-d lag), were stronger in the warm season, and were independent of PM<sub>10</sub> and O<sub>3</sub> in two-pollutant models. With respect to other effect modifiers, the association for total natural mortality was strongest: for subjects >84 years of age; during the warm season; among subjects with at least one hospital admission 29 d–2 years before death (especially for those with pulmonary circulation disorders, heart conduction disorders, diabetes, heart failure or ischemic heart disease (IHD)); and for subjects with three or more chronic conditions.

Biggeri et al. (2005; US EPA, 2008)) analyzed mortality between 1995 and 1999 in six Italian cities. An increase of 10 µg/m<sup>3</sup> (5.32 ppb) in the daily ambient NO<sub>2</sub> level (lag 0–1 d avg) was significantly associated with similarly increased risks for total (0.93%; 95% CI 0.58%, 1.27%) and cardiovascular mortality (1.31%; 95% CI 0.76%, 1.85%) and slightly greater risks for respiratory mortality (1.42%; 95% CI 0.05%, 2.81%). However, SO<sub>2</sub>, CO, PM<sub>10</sub> and O<sub>3</sub> were also related to each category of mortality; no two-pollutant or multi-pollutant models were run; nor were the correlations among the pollutants reported.

In a subsequent study by this same research group (Bellini et al., 2007) the associations between ambient air pollutants and mortality and hospital admissions were investigated in 15 Italian cities from 1996 to 2002. In all-year analyses (avg of lag 0–1 d), the risk of all natural cause mortality (0.59%; 95% CI 0.26%, 0.94%) was increased in relation to an increase of 10 µg/m<sup>3</sup> (5.32 ppb) in the 24-h avg NO<sub>2</sub>, while the risks were smaller and not statistically significant for respiratory (0.38%; 95% CI -0.63%, 1.74%) and cardiovascular (0.40%; 95% CI -0.46%, 1.05%) deaths. When analyses were stratified by season, mortality was significantly increased only in the summer, during which associations with respiratory and to a lesser extent cardiovascular mortality were greater than with all natural causes. However, mortality was also significantly associated with PM<sub>10</sub> and CO in some analyses, and NO<sub>2</sub>-related risk estimates adjusted for other pollutants were not presented in this brief English report of the study.

Simpson et al. (2005; US EPA, 2008) reported the results of a short-term mortality study in four Australian cities, using similar methods to the APHEA2 studies. NO<sub>2</sub> (per ppb 1-h max) was most strongly associated with respiratory mortality (RR = 1.0038; 95% CI 1.0017, 1.0058; lag 1 d), though the relation with total (RR = 1.0012; 95% CI 1.0006, 1.0018; lag 1 d) and cardiovascular (RR = 1.0018; 95% CI 1.0008, 1.0027; lag 3 d) mortality was also significant. In two-pollutant models for total mortality, the NO<sub>2</sub> risk estimate was not sensitive to adjustment for PM (nephelometer readings), whereas the nephelometer risk was substantially reduced by the inclusion of NO<sub>2</sub> in the model.

Investigating a potentially susceptible subpopulation, Berglind et al. (2009) reported air-pollution-related mortality over 6–12 years of follow-up among a cohort of more than 25,000 first myocardial infarction (MI) survivors from five European cities. Daily non-trauma deaths were associated with the average of daily NO<sub>2</sub> lag 0–4 d (3.25% increase; 95% CI 0.19%, 6.39% per interquartile range (IQR) of 8 µg/m<sup>3</sup> (4.26 ppb) 24-h NO<sub>2</sub>), and non-significantly increased for each of the average of lag 0–1 d and of lag 0–14 d. The associations with most other pollutants were also significant, and those for particle mass and number were generally strongest and most consistent. No co-pollutant models were presented. Cardiovascular mortality risk was not generally greater than that for all-cause mortality for any pollutant.

In a recent time-series investigation of a range of respiratory effects in relation to air pollution in six Italian cities from 2001 to 2005, Faustini et al. (2013) observed that relatively high mean levels of ambient NO<sub>2</sub> were significantly associated with out-of-hospital respiratory deaths (6.92% increase; 95% CI 3.53%, 10.42% per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub>, lag 2–5 d), and that this was most pronounced in the warm season. However, findings were similar for PM<sub>10</sub>, and in two-pollutant models, the risk estimates for both pollutants were reduced and no longer significant, especially for NO<sub>2</sub>.

Zauli Sajani et al. (2011) reported the results of an Italian six-city case-crossover study in which they examined how air-pollution-related mortality from all non-injury causes combined varied as a function of the geographic scale over which ambient pollutants were averaged. Associations with most of the pollutants, including NO<sub>2</sub>, were observed but not significant, likely as a result of limited statistical power in this small regional study. Based on the NO<sub>2</sub> city averages, an increase of 0.23% (95% CI -0.68%, 1.14%) was observed in daily mortality per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> at lag 1 d.

Stieb et al. (2002, 2003; US EPA, 2008) reported the results of a meta-analysis of time-series studies in which they estimated the overall effects on mortality of several air pollutants. In the 2003 update they separated the estimates for those studies that were affected by the GAM convergence issue from those that were not. For non-GAM estimates, the combined estimate for total mortality was 0.8% (95% CI 0.2%, 1.5%) per 20 ppb increase in the 24-h avg NO<sub>2</sub> in single-pollutant models, and 0.4% (95% CI -0.2%, 1.1%) in two-, three-, four-, and five-pollutant models. It should be noted that the number of co-pollutant estimates was small (three), and also that the data extraction procedure for the two-pollutant and multi-pollutant models was to use the estimate from the model from each study that yielded the most reduced risk compared with that for single-pollutant models.

### 8.2.2 Selected Single-City Studies

The available multi-city studies have only reported NO<sub>2</sub>-related mortality for all-cause or for broad cause-specific categories of mortality. More specific causes of death were investigated in several single-city studies.

In a single-city study in Vienna, Austria (Neuberger et al., 2007), using APHEA2 methods and data from 2000 to 2004, a 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> (avg of lag 0–7 d) was significantly associated with increased risk of mortality from a variety of causes, including all non-trauma (2.1% increase; 95% CI 0.8%, 3.5%), CVD (3.1%; 95% CI 1.2%, 5.0%), IHD (2.8%; 95% CI 0.3%, 5.3%), other heart disease (4.6%; 95% CI 0.4%, 9.1%), respiratory (5.7%; 95% CI 1.1%, 10.6%), and COPD (9.9%; 95% CI 3.0%, 17.3%). A significant association was also found for cerebrovascular mortality (4.4%; 95% CI 0.8%, 8.2%) but with a longer lag period (avg of 0–14 d). Significant associations were restricted to all-age and >65 years of age analyses. NO<sub>2</sub>-related risks for categories of mortality were increased for up to 14 d using a distributed lag model; the risk estimate over this period was 2.4 to 11.5 times greater than that for lag 0–1 d,

depending on the cause of death. Similar findings were, however, reported for PM<sub>10</sub> and PM<sub>2.5</sub>, and only single-pollutant models were run.

Faustini et al. (2012) investigated mortality associated with short-term exposure between 2005 and 2009 to high levels of air pollution (e.g. annual avg NO<sub>2</sub> of 32.1 ppb) in a cohort of 147,541 COPD patients from Rome. More than ¾ of the cohort members were identified from repeated respiratory medication use, and the remainder from hospital discharge diagnoses. NO<sub>2</sub>-related risks for mortality from respiratory causes were increased almost 7-fold more in COPD patients (32.2% increase; 95% CI 5.5%, 65.5%, per 20 ppb 24-h NO<sub>2</sub>, lag 0–5 d) compared with the entire population of Rome, excluding those who met the study criteria for COPD (4.7% increase; 95% CI -14.1%, 27.6%). There were also significant associations between PM<sub>10</sub> and PM<sub>2.5</sub> and respiratory deaths, and between NO<sub>2</sub> and total natural-cause deaths, though the increase in COPD versus non-COPD patients was only roughly 2-fold.

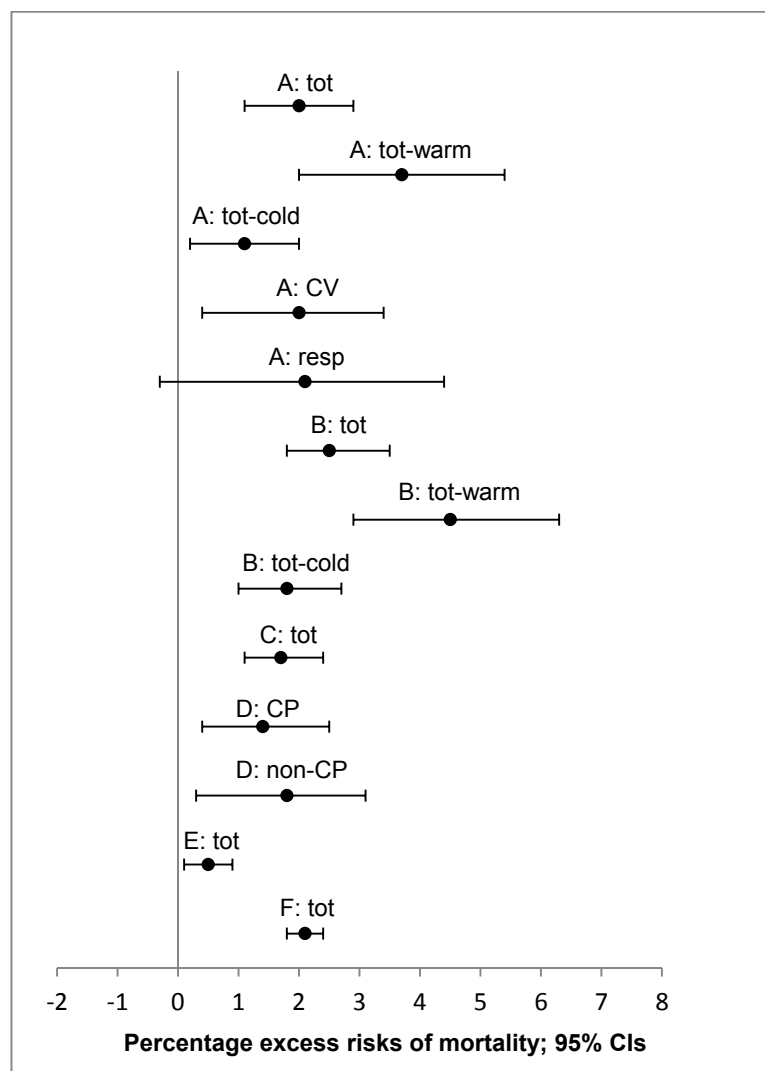
### 8.2.3 Summary and Considerations—Mortality Studies

In a number of multi-city studies from developed countries around the world, ambient NO<sub>2</sub> was consistently associated with increased risk of total mortality from non-trauma causes. These increases were most often greatest with short-term lags, especially for a 1-d lag. The risk estimates for short-term lags (in the range of 0–2 d singly or combined) in North American studies in all-season analyses mostly ranged from 1.7% to 3.5% per 20 ppb 24-h NO<sub>2</sub> (Figure 8.1). Cardiovascular and respiratory mortality, reported less frequently, exhibited similar or somewhat greater associations than for total mortality; however, the risk estimates for these categories were quite variable between studies. NO<sub>2</sub>-related mortality risks were generally robust to adjustment for other pollutants. This independence was evident in co-pollutant models with common air pollutants including PM<sub>10</sub>, O<sub>3</sub>, and SO<sub>2</sub>, but was also observed in models adjusted for traffic-related pollutants including CO, PM<sub>2.5</sub>, and (in a single study) NO and a wide range of particulate metals including copper, zinc and iron. The risks for NO<sub>2</sub>-related deaths were greater in the warmer months (Figure 8.1) as well as in older adults and subjects with pre-existing heart and lung conditions. In some European studies, cumulative risks over periods of roughly 1 to 2 weeks were increased several times over those for single- or few-day lags.

## 8.3 Studies of Hospitalizations and Emergency Room Visits

In the 2008 US EPA ISA, studies of hospital admissions and ERVs were discussed together by category of health effect (e.g. respiratory). Generally, individuals who require hospitalization represent a small but seriously ill subset of all individuals who may be affected by air pollution, whereas morbidities that result in ERVs are related but generally less severe. In addition, each is affected by factors aside from the severity of morbidity. For example, admissions are affected by the availability of beds and the criteria for admission (which will vary between individual physicians and between health care systems), while ERVs involve a greater element of subjectivity, since a visit is usually the result of an individual's or family's own decision that health has been seriously impaired and that medical attention should be sought immediately. As well, the relationship between hospital admissions and ERVs will be different for different categories of effects. For example, individuals presenting to the emergency room with cardiovascular complaints would be more likely to be admitted than those with respiratory conditions such as asthma.

**Figure 8.1: Percentage excess risks of mortality and 95% CIs per standardized increment (20 ppb for 24-h avg) in short-term NO<sub>2</sub> ambient concentration in single-pollutant models in Canadian and US studies\***



- A. 12 Canadian cities; Burnett et al. (2004); 0–2-d avg
- B. 10 Canadian cities; Brook et al. (2007); lag 1 d
- C. 24 Canadian cities; Shin et al. (2008); 2-d avg
- D. 24 Canadian cities; Shin et al. (2012); lag 0–2 d
- E. 90 US cities; Dominici et al. (2003); lag 1 d
- F. 72 US cities; Moolgavkar et al. (2013); lag 1 d

\*Tot = total mortality; CP = cardiopulmonary mortality; CV = cardiovascular mortality; resp = respiratory mortality; warm = warm season only; cold = cold season only.

The following sections summarize epidemiological studies of the association of hospital admissions or ERVs with short-term ambient NO<sub>2</sub>. Studies of hospital admissions or ERVs for respiratory, cardiovascular, and other causes are discussed separately. Within each of these categories of health effect, a summary of the assessment of the 2008 US EPA ISA is presented first, followed by separate sections describing the results of more recent studies of respiratory hospitalizations and ERVs. Finally, an overview of the entire evidence base is provided for each of hospital admissions and ERVs for each of respiratory, cardiovascular, and other causes.



### 8.3.1 Respiratory Hospitalizations and Emergency Room Visits

#### 8.3.1.1 Summary of the 2008 US EPA ISA

Temporal associations between hospital admissions and ERVs for respiratory causes had been investigated in a large number of earlier studies reviewed in the 2008 US EPA ISA.

For all respiratory causes combined, most of the studies reported positive associations between ambient NO<sub>2</sub> and hospitalizations and ERVs for these outcomes in analyses for participants of all ages (US EPA, 2008). (Total respiratory causes typically include asthma, bronchitis and emphysema (collectively referred to as COPD), upper and lower respiratory infections, and other minor categories.) Greater and more consistent associations were observed for children and older adults (≥65 years of age), with an IQR of 1–13% risk per 20 ppb increment in 24-h avg NO<sub>2</sub> or 30 ppb increase in 1-h max NO<sub>2</sub>. The strongest associations were mostly with short-term lags of between 0 and 3 d, though risks were not clearly greater for any specific single-day lag. In addition, risk estimates were often greater for those studies that considered combined exposure over several days, though the magnitude was also quite variable between studies. Findings were generally very similar in studies of different designs, including time-series, case-crossover, and multi-city studies. In two-pollutant models, the associations of admissions/ERVs with NO<sub>2</sub> were generally not very sensitive to adjustment for PM or other gaseous pollutants.

With respect to asthma hospital admissions and ERVs, the 2008 US EPA ISA considered that there was suggestive evidence of an association between these outcomes and ambient NO<sub>2</sub> levels. Risk estimates were most often positive, and they were generally greater for children than for adults and older adults (≥65 years of age), with an IQR of 1–25% excess risk estimated per 20 ppb 24-h avg NO<sub>2</sub> or 30 ppb 1-h max NO<sub>2</sub>. Those for adults as a whole and for older adults (aged ≥65) were generally positive, but few were statistically significant. In analyses for subjects of all ages combined, associations were overwhelmingly positive, especially in relation to daily NO<sub>2</sub>. The risk estimates with NO<sub>2</sub> were generally robust to adjustment for other gaseous and particulate pollutants in co-pollutant models.

As for the possible role of ambient NO<sub>2</sub> in hospitalizations or ERVs for other respiratory outcomes, the 2008 US EPA ISA reported that a limited number of studies had investigated COPD, and still fewer had examined upper respiratory tract infections (URTIs), pneumonia, bronchitis, allergic rhinitis, and lower respiratory disease. While some of these studies reported positive and statistically significant associations, others reported null or negative associations, and based on the limited available data the US EPA concluded that it was difficult to draw conclusions with respect to the effects of NO<sub>2</sub> on these other respiratory conditions.

#### 8.3.1.2 Recent Studies of Respiratory Hospital Admissions

In the more recent studies reviewed for this assessment, there continues to be evidence that ambient NO<sub>2</sub> is associated with increases in hospitalizations for respiratory causes, primarily for total respiratory and asthma admissions.

With respect to total respiratory causes, in a large Canadian time-series study in 10 Canadian cities between 1993 and 2000, Cakmak et al. (2006) observed that all-age admissions were significantly related to ambient NO<sub>2</sub>. In single-pollutant models, the increased risk was estimated at 2.5% (95% CI 0.2%, 4.8% per 21.4 ppb 24-h NO<sub>2</sub>; pooled mean concentration across cities, with an average lag of 1.4 d). The risk remained significant after adjustment for other gaseous pollutants, i.e. with O<sub>3</sub>, SO<sub>2</sub> and CO (1.9%; 95% CI 0.1%, 3.7%), and it was significantly related to lower mean incomes and education levels across communities.

Dales et al. (2006) investigated the risk of respiratory hospital admissions in neonates (from birth to 27 d) in 11 large Canadian cities between 1986 and 2000. In time-series analyses, ambient NO<sub>2</sub> was significantly related to increases in neonatal respiratory hospitalizations



(2.94%; 95% CI 1.93%, 3.95% per 10.0 ppb 24-h NO<sub>2</sub>, lag 1 d). Admissions were also significantly related to each of O<sub>3</sub>, SO<sub>2</sub>, and CO, but in four-pollutant models with all four gases, or in models with these gases and every-sixth-day PM<sub>10</sub>, the risk estimates for NO<sub>2</sub> and the other gases remained positive and significant.

There were also significant NO<sub>2</sub>-related increases in total respiratory hospitalizations in several European studies, though not all. Findings were positive in two multi-city studies and mixed in single-city studies; in both cases questions remain concerning the possible role of co-pollutants.

With respect to multi-city studies, Bellini et al. (2007) investigated the associations between ambient air pollutants and mortality and hospital admissions in 15 Italian cities from 1996 to 2002. The team reported significant NO<sub>2</sub>-related increased risks of admission for respiratory disease (0.77%; credible interval excludes 0) and cardiac disease (0.57%; credible interval excludes 0), but not for cerebrovascular conditions, per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h avg, lag 0–3 d NO<sub>2</sub>. However, risks were also significantly associated with PM<sub>10</sub> and CO in some analyses, and risk estimates from joint models including NO<sub>2</sub> and other pollutants were not presented in this brief English report of the study. In a more recent study of a suite of respiratory effects in adult residents of six Italian cities between 2001 and 2005, at mean ambient NO<sub>2</sub> levels greater than those observed in Canada, Faustini et al. (2013) reported significant NO<sub>2</sub>-related increases in hospital admissions for all respiratory diseases (1.19%; 95% CI 0.23%, 2.15% per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub> increase at lag 0–5 d), and for COPD (1.20%; 95% CI 0.17%, 2.23% at lag 0 d), along with non-significant increases in lower respiratory tract infections (LRTIs) in COPD patients (1.79%; 95% CI -1.16%, 4.83% at lag 0–5 d). Significant increases in out-of-hospital respiratory deaths were also found; these results are discussed in subsection 8.2.1. Risk estimates for NO<sub>2</sub> were greatest in the warm season. However, findings were broadly similar for PM<sub>10</sub>, and in two-pollutant models the risks were generally reduced and no longer significant.

As for single-city studies, in a time-series investigation from Copenhagen, Denmark, Andersen et al. (2007) found that respiratory admissions were significantly increased in adults ≥65 years of age in single-pollutant models (RR = 1.040; 95% CI 1.009, 1.072 per 7 ppb 24-h NO<sub>2</sub> lagged 0–5 d), though associations were reduced somewhat and no longer significant following adjustment for PM<sub>10</sub>. Namdeo et al. (2011) reported that hospital admissions were significantly increased (1.45%; 95% CI 0.36%, 2.37% per 18.02 µg/m<sup>3</sup> (9.6 ppb) increase in 24-h NO<sub>2</sub> at lag 0 d) in persons 0–59 years of age from a rural county in the UK, and they remained so in two-pollutant models with SO<sub>2</sub>. In a study from a tourist region in Greece, Kalantzi et al. (2011) reported that respiratory hospitalizations in adults >14 years of age were significantly increased in relation with 10 µg/m<sup>3</sup> (5.32 ppb) increases in same-day 24-h NO<sub>x</sub> levels (RR = 1.015; 95% CI 1.007, 1.023), though they were also associated with PM<sub>10</sub>, CO and O<sub>3</sub> at similar lags, and only single-pollutant models were run. By contrast, in time-series studies from Lisbon, Portugal (Alves et al., 2010) and from Madrid, Spain (Linares and Diaz, 2010) all-age respiratory hospitalizations were related to other pollutants but not to ambient NO<sub>2</sub>, though no quantitative results were reported for NO<sub>2</sub>. Finally, in a study that focused on children, Giovannini et al. (2010) reported that total respiratory hospital admissions in children ≤14 years of age from Milan, Italy (9.0%; 95% CI 1.2%, 16.8%), as well as admissions for asthma (2.1%; 95% CI 0.0%, 4.0%), upper respiratory diseases (3.3%; 95% CI 0.0%, 6.0%), and lower respiratory diseases (5.3%; 95% CI 1.05%, 10.0%), were associated with increases of 10 µg/m<sup>3</sup> (5.32 ppb) in the moving weekly average of ambient NO<sub>2</sub>, though the increased risks were marginal and sometimes rendered non-significant by adjustment for CO.

A number of recent North American studies have examined the relationship between ambient NO<sub>2</sub> and asthma hospitalizations. In a large Canadian time-series study (To et al., 2013) of all-age hospital admissions for asthma, including all 14 Local Health Networks in Ontario (a population greater than 12 million, of which 1.5 million were estimated to have asthma), there

were significant associations with same- or 1-d lagged ambient NO<sub>2</sub> adjusted for PM<sub>2.5</sub> and O<sub>3</sub> (RR = 1.025; 95% CI 1.017, 1.034 per 10 ppb 24-h NO<sub>2</sub> at lag 0 d, and roughly half this risk at lag 1 d). Only three-pollutant models were run, as this study focused on associations of asthma-related health service use and the three-pollutant AQHI, which was also significantly related to asthma hospitalizations. The authors reported that NO<sub>2</sub> was associated with higher risks of hospitalizations in the summer, and that in general risks were greater in younger age groups (data not shown).

Similar risks were reported by Grineski et al. (2011) in a smaller case-crossover study of all-age hospital admissions in El Paso, TX. In single-pollutant models, there were borderline NO<sub>2</sub>-related increases in hospitalizations for asthma (OR = 1.03; 95% CI 1.00, 1.06 per 10 ppb 24-h NO<sub>2</sub> lagged 2 or 3 d) and for acute bronchitis (OR = 1.03; 95% CI 0.98, 1.08 for the same increment and lags), while neither outcome was significantly related to PM<sub>2.5</sub>. The focus of this study was on associations of hospital admissions with dust storms or with inversions, for which the risk estimates were higher for some age groups. In a time-series study of children ≤14 years of age in Oklahoma City, OK, Magas et al. (2007) reported that admissions for asthma were significantly related to ambient NO<sub>2</sub> (increase of 6.21 cases (95% CI 0.68, 11.74 cases per ppb 1-h max NO<sub>2</sub>), whereas there was no association with PM<sub>2.5</sub> or O<sub>3</sub>. In a Phoenix, AZ, study that examined effect modification by medical insurance status and race, Grineski et al. (2010) reported that children without insurance status had a 1.4–1.9 times greater risk of NO<sub>2</sub>-related asthma hospitalizations than those with insurance, whether private or Medicaid.

Asthma hospitalizations in children were also related to ambient NO<sub>2</sub> in European studies reviewed for this assessment, though some results were sensitive to adjustment for co-pollutants and most studies were quite small. Ambient NO<sub>2</sub> was related to increased children's hospital admissions for asthma in time-series studies in Copenhagen (Andersen et al., 2007) (RR = 1.128; 95% CI 1.029, 1.235 per 7 ppb 24-h NO<sub>2</sub> lagged 0–5 d) and in Milan, Italy (Giovannini et al., 2010) (RR = 1.002; 95% CI 1.000, 1.004 per 1 µg/m<sup>3</sup> (.532 ppb) NO<sub>2</sub> lagged 0–6 d), but risk estimates were reduced somewhat and no longer statistically significant after adjustment for PM<sub>10</sub> and CO, respectively. Similarly, Samoli et al. (2011) reported that children's admissions for asthma in Athens, Greece, were non-significantly increased in relation to ambient NO<sub>2</sub> (1.10%; 95% CI -0.68%, 2.91%) per 10 µg/m<sup>3</sup> (5.32 ppb) 1-h max NO<sub>2</sub>, lag 0 d) and were reduced by adjustment for PM<sub>10</sub> and SO<sub>2</sub>, though a significant increase in males was observed (2.29%; 95% CI 0.13%, 4.50% per 10 µg/m<sup>3</sup> (5.32 ppb) 1-h max NO<sub>2</sub>, lag 0 d; only single-pollutant analyses were done by gender). Conversely, in a recent large case-crossover study of children ≤18 years of age in Copenhagen (Iskandar et al., 2012), risks for asthma hospitalizations were significantly related to both NO<sub>x</sub> and NO<sub>2</sub> (for NO<sub>2</sub>, OR = 1.10; 95% CI 1.04, 1.16 per 6.53 ppb 24-h NO<sub>2</sub>, lag 0–4 d), and were not materially affected by adjustment for PM<sub>10</sub>, PM<sub>2.5</sub> or UFPs. Risks were significantly increased with individual lags of 3 or 4 d, and there was no significant effect modification by age or gender. When these data were analyzed as a time-series using a GAM, results were almost identical.

In contrast to these generally positive findings in children, in a small time-series study from Lisbon (Alves et al., 2010) all-age asthma hospitalizations (predominantly adults) were related to other pollutants but not to ambient NO<sub>2</sub>; no quantitative results were reported for NO<sub>2</sub>.

Most studies of short-term air-pollution-related hospitalizations for asthma have examined associations over lags of zero to several days. However, Delamater et al. (2012) investigated the relationship between all-age admissions for asthma, ambient air pollution, and weather conditions in Los Angeles County, CA, using monthly time-series analysis. Using Bayesian linear regression with temporal random effects, asthma hospitalizations each month were significantly associated with NO<sub>2</sub> averaged over the month in single-pollutant models ( $\beta = 0.37$ ;

90% CI 0.22, 0.52), and with NO<sub>2</sub> and relative humidity or maximum temperature in multivariable models. Positive findings were also reported for CO and PM<sub>2.5</sub>.

Limited evidence is available with respect to NO<sub>2</sub>-related hospital admissions for other respiratory conditions, and the results are mixed and/or marginal. Hospitalizations of adults for COPD were increased in relation to NO<sub>2</sub> and other pollutants in a multi-city study in six Italian cities (Faustini et al., 2013) (1.20% increase; 95% CI 0.17%, 2.23% per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub> at lag 0 d) and in a rural study in the UK (Sauerzapf et al., 2009) (OR = 1.220; 95% CI 1.092, 1.362 per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub> lagged 1–8 d avg), but were not significantly associated with NO<sub>2</sub> in a small study in Lisbon (Alves et al., 2010); no quantitative results were reported for NO<sub>2</sub>. Giovannini et al. (2010) reported that ambient NO<sub>2</sub> in Milan was marginally associated with increases in admissions of children for upper respiratory disease (RR = 1.003; 95% CI 1.000, 1.006 per 1 µg/m<sup>3</sup> (0.53 ppb) 24-h NO<sub>2</sub> lagged 0–6 d avg) in a single-pollutant model and in two-pollutant models with CO. Similar borderline findings were reported for lower respiratory diseases, including bronchitis (RR = 1.005; 95% CI 1.001, 1.010 per 1 µg/m<sup>3</sup> (0.53 ppb) 24-h NO<sub>2</sub> lagged 0–6 d), in this study as well as in another of children from El Paso (Grineski et al., 2011) (OR for acute bronchitis = 1.03; 95% CI 0.98, 1.08 per 10 ppb 24-h NO<sub>2</sub> lagged 2 or 3 d).

### 8.3.1.3 Recent Studies of Respiratory Emergency Room Visits and Other Medical Visits

In the more recent studies reviewed for this assessment, there continues to be evidence that ambient NO<sub>2</sub> is associated with increases in ERVs and other medical visits for respiratory causes, primarily for total respiratory and asthma admissions.

Only a small number of studies have examined total ERVs for all respiratory causes in the recent literature. In a large case-crossover study of more than 1 million all-age respiratory ERVs in Atlanta, GA, between 1994 and 2004 (Darrow et al., 2011), respiratory ERVs were related to various ambient NO<sub>2</sub> metrics, including the 1-h max and 24-h avg, as well as commuting, daytime and nighttime averages. The focus of this study was on the use of alternative pollutant metrics in time-series studies of ambient air pollution; for NO<sub>2</sub> as for other pollutants, most metrics for a given pollutant were highly correlated both spatially and temporally. The strongest NO<sub>2</sub> risk estimate per 10 ppb was for the 24-h avg (RR = 1.009; 95% CI 1.005, 1.013 at lag 1 d). However, total respiratory ERVs were also associated with most metrics for CO and O<sub>3</sub>, and no two-pollutant or multi-pollutant models were run. Findings were reported to be generally similar in analyses with time-series generalized linear models (GLMs).

The association between ambient NO<sub>2</sub> and ERVs from all respiratory causes was also the subject of a small number of Italian studies reviewed for this assessment. In two time-series studies from areas with somewhat higher levels of ambient NO<sub>2</sub> than occur in Canada, there were significant increases in children's respiratory ERVs (Bedeschi et al., 2007) (11.0%; 95% CI 3.6%, 18.8% per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub> at lag 4 d); (Vigotti et al., 2007) (11.8%; 95% CI 1.4%, 23.3% per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub> at lag 2 d), and non-significant increases for older adults in the Vigotti et al. (2007) study (6.0%; 95% CI -3.3%, 16.2% at lag 2 d). However, the risks for ERVs from respiratory causes were also related to other pollutants in these studies, and no analyses adjusting the NO<sub>2</sub> risks for these other pollutants were conducted. Similarly, in a case-crossover study in Palermo, Italy (Tramuto et al., 2011), risks of adult respiratory ERVs were significantly associated with ambient NO<sub>2</sub>, an effect that was most pronounced in the warm season (OR = 1.043; 95% CI 1.021, 1.065 per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub> at lag 0 d). Again only single-pollutant models were run and respiratory ERVs were also related to other pollutants (PM<sub>10</sub>, SO<sub>2</sub>, CO).

The relationship between ambient NO<sub>2</sub> and ERVs for asthma was investigated in a number of studies reviewed for this assessment. Positive and significant associations have often been observed, very consistently when analyses were focused on children's asthma ERVs and restricted to the warm season.

For example, Villeneuve et al. (2007) reported the results of a 10-year case-crossover study of ambient air pollution and asthma ERVs in Edmonton, AB. Associations with air pollution were consistently found for asthma ERVs in the spring/summer season, and to a lesser extent in all-season analyses, while they were largely absent in fall/winter. For NO<sub>2</sub>, the estimated risks were greatest for the warm season in children 2–4 years old (OR = 1.50; 95% CI 1.31, 1.71 per 13.5 ppb 24-h NO<sub>2</sub>, lag 0–5 d avg) and in adults ≤75 years of age (OR = 1.37; 95% CI 1.02, 1.84, lag 0–5 d avg), though there were also significant but smaller NO<sub>2</sub>-related increases in children aged 5–14 and in adults aged 15–44 and 65–74. Findings were generally similar, though the ORs were lower, with other short-term lags. Asthma ERVs were also related to CO, which was highly correlated with NO<sub>2</sub>, but in models with both pollutants the findings for NO<sub>2</sub> were more robust than those for CO.

Szyszkowicz (2008a) analyzed the same dataset using GLMs. For children <10 years of age, the risk of asthma ERVs was related to ambient pollutants, including NO<sub>2</sub>, in all-year (5.3%; 95% CI 2.2%, 8.5% per 12.8 ppb 24-h NO<sub>2</sub>, lag 2 d) and April–September analyses (16.1%; 95% CI 9.5%, 23.0%), and was greater in boys (19.2%; 95% CI 11.4%, 27.6% in the warm season) than in girls (12.6%; 95% CI 2.8%, 23.3%). For patients aged 10 and older, the only significant finding was in females during April–September (6.2%; 95% CI 1.4%, 11.3%). However, asthma ERV risks were also related to several other pollutants, and only single-pollutant models were run.

Lavigne et al. (2012) conducted a time-stratified case-crossover study of asthma ERVs between 2002 and 2009 in Windsor, ON. Statistically significant increased ambient NO<sub>2</sub>-related risks for asthma ERVs were observed only in children aged 2–14 in the warm season (OR = 1.25, 95% CI 1.04, 1.50 per 9 ppb IQR 24-h NO<sub>2</sub>, lag 1 d). There was no association in all-season or cold-season-only analyses, nor for any other combination of time of year and age (all-ages, 15–39, 40–59 and ≥60). However, similarly increased risks in children 2–14 years of age in the warm season were also related to SO<sub>2</sub> and CO, and only single-pollutant models were reported.

Strickland et al. (2010) conducted a large case-crossover study of asthma ERVs in children aged 5–17 in Atlanta between 1993 and 2004. The population-weighted average of ambient NO<sub>2</sub> was related to ERVs for the entire year (RR = 1.036; 95% CI 1.018, 1.055 per 12.9 ppb 1-h max NO<sub>2</sub>, lag 0–2 d avg) and for the warm season (RR = 1.066; 95% CI 1.038, 1.095), but not in the cold season (RR = 1.016; 95% CI 0.992, 1.040). Individual day lag risks were greatest at lag 0 d, but were significant for each day up to d 7. There was an apparent dose–response relationship in risk increases across quintiles of NO<sub>2</sub> concentrations, and no apparent threshold down to roughly 10 ppb 3-d moving avg warm-season 1-h max NO<sub>2</sub>. In two-pollutant models with O<sub>3</sub>, the NO<sub>2</sub> risk was attenuated but remained significant, as was also the case for other markers of traffic. NO<sub>2</sub>-related risks also remained significant in alternative time-series and case-crossover analyses, but they disappeared when ERVs were related to the next day's pollution (i.e. lag -1 d); the association was only related to pollution preceding the medical visit. This exhibits temporality and supports the veracity of the findings.

Strickland et al. (2011) conducted further time-series analyses using the same warm-season data from Atlanta as Strickland et al. (2010). This article focused on the variation in risk estimates across different exposure metrics, including the concentration at a single central site monitor, the unweighted average concentration across monitors in the city, and the average concentration across monitors weighted by population. The risk estimates for NO<sub>2</sub> were all



significant, and very similar per IQR, with central estimates of RR of 1.051–1.053 for the different exposure metrics. By contrast, when expressed per standard unit of NO<sub>2</sub> (i.e. per 20 ppb 1-h max NO<sub>2</sub>, lag 0–2 d avg), the risk estimates increased from the central site monitor (RR = 1.052) to the unweighted average (RR = 1.079) to the population-weighted average (RR = 1.105). This was also the case for other spatially heterogeneous pollutants (EC, CO, SO<sub>2</sub>). The authors noted that these differences in risk estimates would be important for health benefit analyses, where epidemiology study estimates of health effects per unit change in concentration are used to predict the impact of a reduction in pollutant concentrations.

Li et al. (2011) studied asthma ERVs and hospitalizations combined (80.3% ERVs only) in relation to air pollution in Medicaid children aged 2–18 from Detroit, MI. NO<sub>2</sub> was related to asthma hospital events at a 5-d lag, but not at any other individual day or 3- or 5-d moving avg. In case-crossover analyses the NO<sub>2</sub> risk was estimated as RR = 1.039; 95% CI 1.010, 1.070 per 9.65 ppb 24-h NO<sub>2</sub>, which was very similar to that using GAM (RR = 1.038; 95% CI 1.005, 1.072). Asthma ERVs/hospitalizations were also related to PM<sub>2.5</sub>, SO<sub>2</sub> and CO; only single-pollutant analyses were run. In models to investigate thresholds for effects, there was no significant evidence of a threshold for NO<sub>2</sub>, SO<sub>2</sub> or CO, whereas there was an apparent threshold for PM<sub>2.5</sub>-related ERVs and hospital admissions.

In a time-series study of asthma ERVs in children aged 2–17 from St. Louis, MO, in which analyses were stratified by season, there were significantly increased risks for the all-ages group in spring (RR = 1.05; 95% CI 1.01; 1.09) and fall (RR = 1.03; 95% CI 1.00, 1.16) and non-significant increases in summer in relation to an increase of 10 ppb in 24-h ambient NO<sub>x</sub> after adjustment for other particulate and gaseous pollutants (EC, O<sub>3</sub>, SO<sub>2</sub>) (Mohr et al., 2008). However, the increases were marginal and only briefly reported in this study, which was focused on the possible role of EC as a determinant of asthma ERVs in children.

Jalaludin et al. (2008) conducted a case-crossover study of children's asthma ERVs in Sydney, Australia, a location with relatively low NO<sub>2</sub> levels. In children aged 1–4, risks were significantly increased (3.0%; 95% CI 1.8%, 4.2%) in relation to a 9.5 ppb increase in same-day 1-h max NO<sub>2</sub> avg, whereas there were no significant increases in children aged 5–9 (1.1%; 95% CI -0.7%, 2.9%) and 10–14 (1.2%; 95% CI -1.2%, 3.6%). The association for young children was significant using all-year data, was greater for the warm months but was not significant in the cold season, and was reduced but still significant in two-pollutant models with various particulate and gaseous pollutants. Similarly, in a case-crossover study from Perth, Australia (Pereira et al., 2010), an increase of 1 ppb in 1-d lagged 24-h avg NO<sub>2</sub> was associated with asthma ERVs only in children 0–4 years old (OR = 1.21, 95% CI 1.03, 1.43) and in males (OR = 1.16, 95% CI 1.01, 1.32), but not in older children (aged 5–9 and 10–19) or in females. However, only single-pollutant models were run in this study, and risks were also related to CO, which was highly correlated with NO<sub>2</sub>. Halonen et al. (2008) conducted a time-series study of asthma ERVs in all public hospitals in the Helsinki, Finland, metropolitan area. In children <15 years of age, an IQR increment of 7.6 ppb 24-h avg NO<sub>2</sub> was related to significantly increased risk of asthma ERVs at lags 3, 4, and 5 d (10.9%; 95% CI 6.38%, 15.5%, lag 4 d), which overall was independent of other pollutants (data not shown). However, there was no significant association with NO<sub>2</sub> for adults aged 15–64 or >65 with any individual day lag.

By contrast, ambient NO<sub>2</sub> was not significantly related to ERVs for asthma in two large Canadian studies, but in these studies the analyses were for all ages combined and used monitoring data from the entire year rather than stratifying by age category and season. Stieb et al. (2009) conducted a time-series study of respiratory ERVs based on nearly 400,000 visits to hospitals in 14 hospitals from seven Canadian cities during the 1990s and early 2000s. Ambient NO<sub>2</sub> was related to a number of cardiac conditions (subsection 8.3.2) but not to respiratory ERVs including asthma (0.0%; 95% CI -2.4%, 2.5% per 18.4 ppb 24-h NO<sub>2</sub>, lag 2 d). Similarly,

in a large time-series study that included all 14 Local Health Networks in Ontario (To et al., 2013), all-age asthma ERVs were not positively related to ambient NO<sub>2</sub> in analyses that adjusted for PM<sub>2.5</sub> and O<sub>3</sub>; in fact, they were significantly decreased (RR = 0.976; 95% CI 0.971, 0.980 per 10 ppb 24-h NO<sub>2</sub> at lag 0 d). No single-pollutant models were run, as the study focused on associations of asthma-related health service use and the three-pollutant AQHI.

The findings with respect to other medical visits for respiratory conditions, which encompass a range of medical settings and health outcomes, are somewhat mixed.

With respect to asthma outcomes, all-year ambient NO<sub>2</sub> was significantly associated with asthma outpatient medical visits in two studies in Ontario, including all-age physician visits in a large multicentre study after adjustment for PM<sub>2.5</sub> and O<sub>3</sub> (RR = 1.117; 95% CI 1.114, 1.120 per 10 ppb 24-h NO<sub>2</sub> at lag 0 d) (To et al., 2013), and ambulatory visits in children aged 1–17 and adults aged 18–64 (both males and females) for which risks were similar across income quintiles in a 10-year study in Toronto (Burra et al., 2009). Yet in another study, an increase of 19.9 ppb in 1-h max NO<sub>2</sub> (lag 3–5 d) over a 25-month period was not significantly related to urgent daily outpatient visits to Atlanta clinics for child asthma (RR = 1.049; 95% CI 0.982, 1.121), adult asthma (RR = 1.039; 95% CI 0.967, 1.116), URTI (RR = 1.022; 95% CI 0.999, 1.047) or LRTI (RR = 1.042; 95% CI 0.965, 1.126), though NO<sub>2</sub> exposure was only based on a single central monitoring station that was removed from the clinics and the bulk of the population (Sinclair et al., 2010). A similar lack of association was also noted over another (28-month) period, with the exception of a significant increase in visits for LRTI (RR = 1.062; 95% CI 1.005, 1.123 per 13.1 ppb 1-h NO<sub>2</sub> at lag 6–8 d). Doctors' house calls for asthma were not significantly associated with ambient NO<sub>2</sub> in two French studies: Chardon et al. (2007) (excess risk of -0.3%; 95% CI -3.3%, 2.7% per 10 µg/m<sup>3</sup> (5.32 ppb) increase in 24-h NO<sub>2</sub> lag 0–3 d avg); and Larrieu et al. (2009) (excess risk of 1.1%; 95% CI -3.0%, 5.2% per 10 µg/m<sup>3</sup> (5.32 ppb) increase in 24-h NO<sub>2</sub>, lag 0–3 d avg), whereas the risks for upper respiratory disease (0.8%; 95% CI -0.7%, 2.3%) and lower respiratory disease (2.6%; 95% CI 0.2%, 4.9%) with the average of lag 0–3 d were increased in one of these studies, particularly in the adults ≥65 years of age (Larrieu et al., 2009).

Concerning other respiratory outcomes, an increase of 18.4 ppb 24-h NO<sub>2</sub> (at lag 2 d) was not significantly related to all-age ERVs for COPD (0.1%; 95% CI -5.6%, 6.2% at lag 0 d) and for respiratory infections (0.7%; 95% CI -3.7%, 5.3% at lag 1 d) in a large Canadian multi-city study (Stieb et al., 2009). However, ambient NO<sub>2</sub> was associated with a significantly increased risk of COPD ERVs in patients >65 years of age (5.76%; 95% CI 1.49%, 10.2% per 7.6 ppb 24-h avg NO<sub>2</sub>), though not in children and other adults, in a time-series study in Helsinki (Halonen et al., 2008). There were non-significant increases in risks of ERVs for wheezing (2.8%; 95% CI -1.0%, 6.7% at lag 0–6 d avg per IQR of 22.2 ppb 24-h NO<sub>2</sub>) and gastroenteritis (2.9%; 95% CI -1.6%, 7.6% at 0–4 d avg) in children 0–2 years of age in a multi-city Italian study (Orazzo et al., 2009). Finally, in a longitudinal study of a birth cohort of children up to 4.5 years of age from two districts of the Czech Republic, ambient NO<sub>x</sub> was related to increased doctors' visits for respiratory infections (Ghosh et al., 2012). Risks were greatest in children <2 years of age (for bronchitis: RR = 1.31; 95% CI 1.07, 1.61 per approx. 19 ppb 24-h NO<sub>x</sub> averaged over the prior 30 d, and for upper airway inflammation: RR = 1.24, 95% CI 1.03, 1.37 for the same increment averaged over 14 d), but were generally also increased for children aged 2–4.5 and for averaging times between 3 and 45 d.

## 8.3.2 Cardiovascular Hospitalizations and Emergency Room Visits

### 8.3.2.1 Summary of the 2008 US EPA ISA

This assessment (US EPA, 2008) reviewed the results of a number of earlier epidemiology studies of short-term exposure to ambient NO<sub>2</sub> and hospitalizations or ERVs for CVD.



With only a few exceptions, studies reported a positive and statistically significant association between 24-h avg or 1-h max NO<sub>2</sub> levels and hospital admissions or ERVs for all CVDs combined in univariate models. Similarly, most investigators who distinguished cardiac disease from all CVDs reported significant positive associations in single-pollutant models. Among studies of the association between short-term ambient NO<sub>2</sub> and admissions or ERVs for more specific cardiovascular conditions, including IHD, MI, and congestive heart failure (CHF) in univariate models, the results were often positive but not statistically significant. However, most investigators reporting results from co-pollutant models observed diminished and non-significant effect estimates for NO<sub>2</sub> and hospitalizations or ERVs for these CVD categories or for CVDs as a whole, especially in analyses that adjusted for CO or PM.

Results from studies reviewed in the 2008 US EPA ISA that related all cerebrovascular disease admissions or ERVs to ambient NO<sub>2</sub> were generally inconsistent, and studies of more specific cerebrovascular diseases also provided little evidence for an effect of NO<sub>2</sub>.

### 8.3.2.2 Recent Studies of Cardiovascular Hospital Admissions

A small number of more recent studies reviewed for this assessment investigated the association between ambient NO<sub>2</sub> and hospitalizations for CVDs as a whole or for broad categories of CVD.

In a large US multi-site time-series study, Bell et al. (2009) investigated the association between ambient air pollutants and emergency hospital admissions for CVDs in >9.3 million Medicare enrollees aged 65 or over in 126 urban counties during 1999–2005. The study was focused on CO, but in online supplementary material it was reported that for the 92 counties with sufficiently complete data for NO<sub>2</sub>, there was a significant association between an IQR of 9.4 ppb same-day 24-h NO<sub>2</sub> and increased risk for all cardiovascular hospitalizations following adjustment for CO (1.34%; 95% CI 1.06%, 1.62%) or for CO and PM<sub>2.5</sub> (1.30%; 95% CI 0.87%, 1.73%). The corresponding estimates for CO and PM<sub>2.5</sub> on an IQR basis were lower and substantially attenuated by adjustment for NO<sub>2</sub>. On average across communities, NO<sub>2</sub> was only moderately correlated with both CO and PM<sub>2.5</sub>.

Ito et al. (2011) reported the findings of a time-series study of total emergency/urgent CVD hospitalizations in New York City, NY, from 2000 to 2006. Hospital admissions for CVD were associated with ambient NO<sub>2</sub> (at levels somewhat greater than those in Canada) on lag d 0 and were non-significantly increased on lag d 1, with similar risk estimates in all-year, warm-season, and cold-season analyses (risks presented in figure only, no quantitative data estimates given). However, the risks for CVD hospitalizations were also related to several other pollutants, including CO, and no analyses adjusting for these other pollutants were run.

Larrieu et al. (2007) conducted a large time-series study in eight French cities using APHEA2 methodology. The range of mean concentrations across cities overlapped those observed in Canada. An increase of 10 µg/m<sup>3</sup> (5.32 ppb) in 24-h ambient NO<sub>2</sub> was associated with hospital admissions for all CVDs (0.5%; 95% CI 0.1%, 1.0%), for cardiac diseases (1.0%; 95% CI 0.5%, 1.5%) and for IHD (1.7%; 95% CI 0.9%, 2.6%), but was not significantly related to those for stroke (-0.2%; 95% CI -1.1%, 0.7%). For each of the positive findings, risk estimates were higher for subjects >65 years of age compared with all ages. However, similar findings were reported for PM<sub>10</sub>, and no two-pollutant or multi-pollutant analyses were conducted.

In a multi-city study of mortality and hospital admissions in 15 Italian cities from 1996 to 2002, Bellini et al. (2007) reported significant NO<sub>2</sub>-related increases in cardiac admissions (an excess risk of 0.57% (credible interval excludes 0) per 10 µg/m<sup>3</sup> (5.32 ppb) in the 24-h avg, lag 0–3 d), but not in cerebrovascular admissions (0.77%; credible interval includes 0). However, risks were also significantly associated with PM<sub>10</sub> and CO in some analyses, and NO<sub>2</sub> risks adjusted for other pollutants were not presented in the brief English report of this study.

Ambient NO<sub>2</sub> was also positively associated with CVD admissions in some single-city European studies. Alves et al. (2010) reported the results of a time-series study in Lisbon, Portugal, where ambient NO<sub>2</sub> levels were similar to those at some near-road sites in Canada. NO<sub>2</sub> was significantly associated with hospital admissions for all circulatory and cardiac diseases after adjustment for other particulate and gaseous pollutants. For an increase of 10 µg/m<sup>3</sup> (5.32 ppb) in 1-h max NO<sub>2</sub> at a 3-d lag, the risk estimates for all circulatory diseases were 0.8% (95% CI 0.793%, 0.807%), 0.5% (95% CI 0.49%, 0.51%) and 2.2% (95% CI 2.19%, 2.21%), respectively, for the <15, 15–64, and >64 age groups. Similar risk estimates were obtained for hospital admissions due to cardiac diseases.

In most other European single-city studies reviewed, ambient NO<sub>2</sub> was related to CVD hospitalizations, but only single-pollutant models were run or adjustment for other pollutants attenuated the association with NO<sub>2</sub>. Andersen et al. (2007) reported that admissions for CVD in adults ≥65 years of age in Copenhagen, Denmark, were increased in relation to ambient NO<sub>2</sub> (RR = 1.013; 95% CI 0.993, 1.033 per 7 ppb 24-h NO<sub>2</sub> lagged 0–5 d avg), but the risk was non-significant and was reduced to zero by adjustment for PM<sub>10</sub> (RR = 1.000; 95% CI 0.975, 1.026). In a Greek study from a tourist region with relatively low NO<sub>2</sub> levels, cardiovascular hospital admissions in adults were associated with ambient NO<sub>x</sub> (RR = 1.017; 95% CI 1.009, 1.020 per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>x</sub>, lag 0 d) but also with PM<sub>10</sub>, CO and O<sub>3</sub>, and no analyses adjusting for these other pollutants were conducted (Kalantzi et al., 2011). Finally, in a time-series study in Madrid, Spain, all-age circulatory hospital admissions were associated with PM<sub>2.5</sub> but not with other pollutants, including NO<sub>2</sub> (no quantitative results reported) (Linares and Diaz, 2010).

Based on the results of a small number of studies reviewed for this assessment, there is only limited evidence of a relation between ambient NO<sub>2</sub> and hospitalizations for more specific cardiovascular causes, including MI, stroke and diabetes.

In a case-crossover study from New Jersey, USA, Rich et al. (2010) reported that a non-significant risk of hospitalizations for transmural MI in relation to ambient NO<sub>2</sub> was reduced to near-null (OR = 1.04; 95% CI 0.88, 1.22 per IQR of 16 ppb in the previous 24 h) by adjustment for PM<sub>2.5</sub>, the pollutant that was the focus of this study. In papers that examined hospital admissions for MI, ambient NO<sub>2</sub> was associated with borderline increased risks in case-crossover studies in six urban areas in Tuscany, Italy (Nuvolone et al., 2011) (OR = 1.028; 95% CI 1.000, 1.057 per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub>, lag 3 d), and in the cities of Copenhagen, Denmark (Wichmann et al., 2012) (no quantitative results were reported for NO<sub>2</sub>) and Kaunas, Lithuania (Vencloviene et al., 2012) (OR = 1.19; 95% CI 0.98, 1.45 per 19.05 µg/m<sup>3</sup> (10.1 ppb) 24-h NO<sub>2</sub>, lag 0–1 d avg). However, the findings either were not robust in two-pollutant models with PM<sub>10</sub> or CO (Nuvolone et al., 2011), or other pollutants were also related to admissions and no risk estimates for NO<sub>2</sub> adjusted for these other pollutants were presented (Wichmann et al., 2012), or other pollutants were not investigated (Vencloviene et al., 2012).

Bhaskaran et al. (2011) studied MI admissions in England and Wales in relation to very short-term fluctuations in air pollution. NO<sub>2</sub> (1.1%; 95% CI 0.3%, 1.8% per 10 µg/m<sup>3</sup> (5.32 ppb) and PM<sub>10</sub> levels were associated with increased risk of MI 1–6 h after exposure, but this was offset by a very similar negative risk between 7 and 12 h, and there was no net excess risk associated with any of the five pollutants studied over a 3-d period. In online supplementary material, these findings were restricted to patients with pre-existing CHD, which in conjunction with the temporal patterns suggested that they might simply reflect short-term displacement of risks by air pollution, rather than increased overall risk.

Ambient NO<sub>2</sub> was related to hospitalizations for ischemic stroke conditions in a small number of single-city US and European studies reviewed for this assessment, but either the findings were

not robust in two-pollutant models (Andersen et al., 2010) (OR = 1.02; 95% CI 0.85, 1.23 per 8.8 ppb in 24-h NO<sub>2</sub>, lag 4 d) or they were also related to other pollutants and only single-pollutant models were run (Vidale et al., 2010) (RR = 1.039; 95% CI 1.013, 1.066 at lag 2 d; no specific increment reported); (Wellenius et al., 2012) (OR = 1.12; 95% CI 1.03, 1.22 per 8.1 ppb in 24-h NO<sub>2</sub>, lag 1 d). In a case-crossover analysis from southern Sweden, hospital admissions for ischemic stroke and for hemorrhagic stroke were associated with ambient PM<sub>10</sub> but not with NO<sub>x</sub> levels modelled at each patient's residence, in both single- and three-pollutant models (Oudin et al., 2010).

### 8.3.2.3 Recent Studies of Cardiovascular Emergency Room Visits and Other Medical Visits

A number of studies have investigated the association between ambient NO<sub>2</sub> and all cardiovascular ERVs combined in Atlanta, GA, taking advantage of a rich dataset that exists for this city. In the following paragraphs, the findings from a particularly informative earlier report are summarized (Metzger et al., 2004), after which the results of a series of subsequent papers that have examined the influence of exposure measurement error on the risk estimates for cardiovascular ERVs in this dataset are discussed. This is followed by accounts of several studies from North American cities of ERVs for more specific cardiovascular outcomes.

Metzger et al. (2004) conducted a time-series study of the association between short-term exposure to ambient NO<sub>2</sub> and more than 102,000 ERVs for cardiovascular causes between 1993 and 2000. NO<sub>2</sub> was associated with significantly increased risk for cardiovascular ERVs, including for all CVDs (RR = 1.025; 95% CI 1.012, 1.039) per 20 ppb 1-h max NO<sub>2</sub>, lag 0–2 d avg. Risks were greater and significant for some more specific CVD categories, including IHD (RR = 1.029; 95% CI 1.005, 1.053) and peripheral and cerebrovascular disease ERVs (RR = 1.041; 95% CI 1.013, 1.069), but not for CHF (RR = 1.010; 95% CI 0.981, 1.040) or dysrhythmia (RR = 1.019; 95% CI 0.994, 1.044). Associations were also observed for other pollutants, including CO, PM<sub>2.5</sub>, OC, EC and oxygenated hydrocarbons, and they were generally robust to various model structures and specifications. In two-pollutant models using data for the entire period, the NO<sub>2</sub>-related risk for all CVDs was reduced but still significant, whereas the CO-related risk was reduced and non-significant. By contrast, using Aerosol Research and Inhalation Epidemiology Study (ARIES) data (encompassing a shorter period with more intensive sampling of a wider range of pollutants), CO-related risks were robust to adjustment for various pollutants, including NO<sub>2</sub>, while those for NO<sub>2</sub> were substantially reduced and no longer significant after adjustment for CO, PM<sub>2.5</sub>, total carbon or oxygenated hydrocarbons. (NO<sub>2</sub> and CO levels were highly correlated, making it difficult to disentangle any independent effect of each). The authors reported that associations for CVD visits by adults and older adults (≥65) were similar to those in all-age analyses, and that associations tended to be highest during colder months and lowest during warmer months, though no data were presented. This seasonal pattern, which is the opposite of that reported in a number of studies from other locations, may be the combined result of open windows (increasing infiltration) during the mild winters and prevalent use of air conditioning (decreasing infiltration) in the hot humid summers in Atlanta.

A number of more recent papers used this dataset to investigate the effect of exposure measurement error resulting from the use of ambient data from a central site monitor as a surrogate for exposure.

A study by Sarnat et al. (2010) focused on the effect of proximity to the monitoring station on the magnitude of risk estimates for ERVs for circulatory disease in this Atlanta dataset between 1998 and 2004. For the entire population, risk estimates for NO<sub>2</sub> were similar regardless of the monitor used (RRs = 1.017–1.020 per IQR 1-h max NO<sub>2</sub>, lag 0 d), except for the most distant rural sites, where risks were reduced and not significant (RRs = 0.996 and 1.007). For

subpopulations within 5 miles of a given monitor, risks were again similar using data from monitors within 20 miles, or when population-weighted averages were calculated, in spite of substantial differences in SES among the subpopulations. However, risks were reduced and non-significant using data from the more distant rural sites, which were at least 30 miles away. Findings were similar for other pollutants, except that for spatially homogeneous pollutants (PM and O<sub>3</sub>) the more distant rural sites yielded similar risks to the nearer urban/suburban stations.

Goldman et al. (2010) estimated the reductions in the risk of ERVs for CVD in Atlanta between 1999 and 2004 resulting from measurement error from instruments and from spatial heterogeneity for several pollutants. After conducting base-case time-series analyses of the association between various pollutants and CVD, simulations were then conducted with instrument and spatial heterogeneity measurement errors added. It was estimated that measurement error from these sources reduced the risk estimates for NO<sub>2</sub> by 4% and 67% (no longer significant) respectively. Pollutants largely of secondary origin had much less bias to the null (i.e. less than 16%), while those of mixed origin were intermediate.

In a subsequent simulation study of this dataset, Goldman et al. (2011) evaluated the effect of classical versus Berkson measurement error applied to instrument imprecision and spatial heterogeneity based on data for various pollutants. Measurement error resulted in lower p-values for all amounts and types of error. When modelled as classical-type error, risk estimates were attenuated towards the null, particularly for primary pollutants, as a result of their greater spatial heterogeneity (e.g. RRs were reduced by 92% per unit of NO<sub>2</sub>, and by 86% per IQR, and were no longer significant). When modelled as Berkson-type error, the RR per unit of NO<sub>2</sub> was biased away from the null by 31%, and attenuated toward the null by 34% per IQR; both associations were still significant.

Some Canadian studies reviewed for this assessment investigated the association of ambient NO<sub>2</sub> with ERVs for more specific cardiovascular causes.

Stieb et al. (2009) conducted a large time-series study of CVD ERVs in 14 hospitals in seven Canadian cities between 1992 and 2003. In single-pollutant models, all-age ERV risks were significantly increased for angina/MI (2.7%; 95% CI 0.2%, 5.3% per 18.4 ppb 24-h NO<sub>2</sub>, lag 1 d) and for heart failure (4.7%; 95% CI 1.2%, 8.4%), but not for dysrhythmia (0.3%; 95% CI -2.6%, 3.3%). Warm-season risks for NO<sub>2</sub> were greater for angina/MI (4.0%; 95% CI -0.5%, 8.8%) and for heart failure (7.2%; 95% CI 0.5%, 14.4%), while there was no consistent association for any pollutant or outcome during the winter. Ambient CO was also related to angina/MI ERVs; in two-pollutant models with NO<sub>2</sub> and CO, risks for both pollutants were reduced and non-significant (e.g. an excess risk of 1.18%; 95% CI -2.64%, 5.15% for NO<sub>2</sub>).

Szyszkowicz (2009) investigated the association between air pollution and ERVs for chest pain not otherwise diagnosed in a time-series study of data from 11 hospitals in six Canadian cities between 1992 and 2001–2003. Using GLMs, the risk for ERVs was significantly increased in relation to ambient NO<sub>2</sub> in the warm season (5.9%; 95% CI 3.3%, 8.6% per 20.1 ppb 24-h NO<sub>2</sub>, lag 0 d), and to a lesser extent in all-year (4.0%; 95% CI 2.6%, 5.5%) and cold-season (3.2%; 95% CI 1.5%, 5.0%) analyses. However, ERVs were also significantly associated with CO, O<sub>3</sub>, PM<sub>10</sub> and PM<sub>2.5</sub>, and only single-pollutant models were run. It was also reported that air pollutants including NO<sub>2</sub> were related to increased risks for angina/MI, heart failure and dysrhythmia, but little detail was given and only graphical results were provided in this brief article.

A significant NO<sub>2</sub>-related increase for IHD was also found in a GLM analysis of ERVs in Montreal, QC, from 1997 to 2002 (Szyszkowicz, 2007a). Using single-pollutant models, risks of IHD ERVs were increased in all-age analyses (5.9%; 95% CI 2.1%, 9.9% per 9.5 ppb 24-h NO<sub>2</sub>, lag 0 d). Risk estimates were similar for males and females in all-age analyses, but were



substantially higher in males >64 years of age (9.1%; 95% CI 2.8%, 15.7%). Ambient CO was also related to IHD ERVs in all ages and in males; no analyses adjusting for other pollutants were conducted.

Szyszkowicz (2008b) investigated the relation between ambient air pollution and ERVs for ischemic stroke, using data for 1992–2002 from five Edmonton-area hospitals. In time-series analyses using GLMs, there were significant increases in NO<sub>2</sub>-related risk for ischemic stroke ERVs in all-year analyses of 20- to 64-year-olds for both genders combined (6.3%; 95% CI 0.2%, 12.8% per 12.8 ppb 24-h NO<sub>2</sub>, lag 0 d), as well as in all-year analyses and cold-season analyses for females in this age group. There was also a significant increase in warm-season risk for both genders aged 65–100 (8.2%; 95% CI 0.4%, 16.7%). However, there were in addition a number of significant positive findings for CO, O<sub>3</sub> and SO<sub>2</sub>, and only single-pollutant models were run in this briefly reported study.

In a similar analysis of data from Edmonton, AB (Szyszkowicz et al., 2012), ambient NO<sub>2</sub> was significantly related to ERVs for hypertension (OR = 1.08; 95% CI 1.01, 1.15 per 12.8 ppb 24-h NO<sub>2</sub>, lag 2–4 d avg; also significant at lag 3 d). Risks for other lags and for males and females separately were positive but not significant. However, the risk for NO<sub>2</sub> was reduced and non-significant in two-pollutant models with PM<sub>10</sub>, and it disappeared entirely in three-pollutant models with PM<sub>10</sub> and SO<sub>2</sub>.

In four recent case-crossover studies, ambient NO<sub>2</sub> was not significantly associated with out-of-hospital cardiac arrest in New York City, NY (Silverman et al., 2010) (no quantitative results reported for NO<sub>2</sub>), Houston, TX (Ensor et al., 2013) (excess risk of 0.9%; 95% CI -3.0%, 5.0% per IQR of 6 ppb 24-h NO<sub>2</sub>), Melbourne, Australia (Dennekamp et al., 2010) (excess risk of 2.99%; 95% CI -2.57%, 8.86% per IQR of 6.64 ppb 24-h NO<sub>2</sub>; lag 0–1 d avg) and Helsinki, Finland (Rosenthal et al., 2013) (OR = 1.05; 95% CI 0.97, 1.14 per IQR of 20.0 µg/m<sup>3</sup> (10.6 ppb) in the same-day 24-h NO<sub>2</sub>; negative associations were observed for several other lags), whereas there were significant associations for PM<sub>2.5</sub> and often O<sub>3</sub> in each location.

### **8.3.3 Hospitalizations and Emergency Room Visits for Other Causes**

The 2008 US EPA ISA did not discuss hospital admissions or ERVs for causes other than respiratory or cardiovascular conditions. The following discussion briefly summarizes the results from the limited number of more recent epidemiology studies of hospitalizations/ERVs for non-cardiorespiratory outcomes.

With respect to hospital admissions, the literature reviewed subsequent to the 2008 US EPA ISA includes a small number of studies of diverse endpoints, all conducted by Canadian researchers. In a case-crossover study in Calgary, AB, Kaplan et al. (2009) found that hospitalizations for appendicitis were related to all the air pollutants examined. The estimated risks for NO<sub>2</sub> were significant in each season, strongest in the warm months, greater than risks for other pollutants, and remained similar in two-pollutant models with several other pollutants.

Headache risks were also related to ambient NO<sub>2</sub> in Canadian ERV studies in Montreal, QC (Szyszkowicz, 2008c) and Ottawa, ON (Szyszkowicz, 2008d). However, risks for ERVs for headaches were also significantly associated with various other pollutants in these studies, in which only single-pollutant models were run. There was no association between ambient NO<sub>2</sub> and ERVs for headache in a case-crossover study of patients seen at a single medical facility in Boston, MA (Mukamal et al., 2009).

ERVs for depression were significantly related to ambient NO<sub>2</sub> in studies from Edmonton, AB (Szyszkowicz, 2007b; Szyszkowicz and Tremblay, 2011), and in another study from six Canadian cities (Szyszkowicz et al., 2009). Estimated risks were increased using various analytical methods (GLMs with hierarchical clusters, case-crossover) and were most

pronounced in the warm season. However, ERVs for depression were also associated with several other air pollutants, and only single-pollutant models were run in most of these studies (Szyszkowicz, 2007b; Szyszkowicz et al., 2009), or risks were no longer statistically significant after adjustment for some other pollutants (Szyszkowicz and Tremblay, 2011).

Ambient NO<sub>2</sub> was associated with ERVs for suicide attempts/suicidal thoughts using various analytical approaches in a study from Vancouver, BC (Szyszkowicz et al., 2010). The estimated risk of suicide ERVs was most pronounced in the cold season, and was associated with other pollutants, especially CO and PM. No analyses were conducted to adjust the NO<sub>2</sub>-related risks for these co-pollutants.

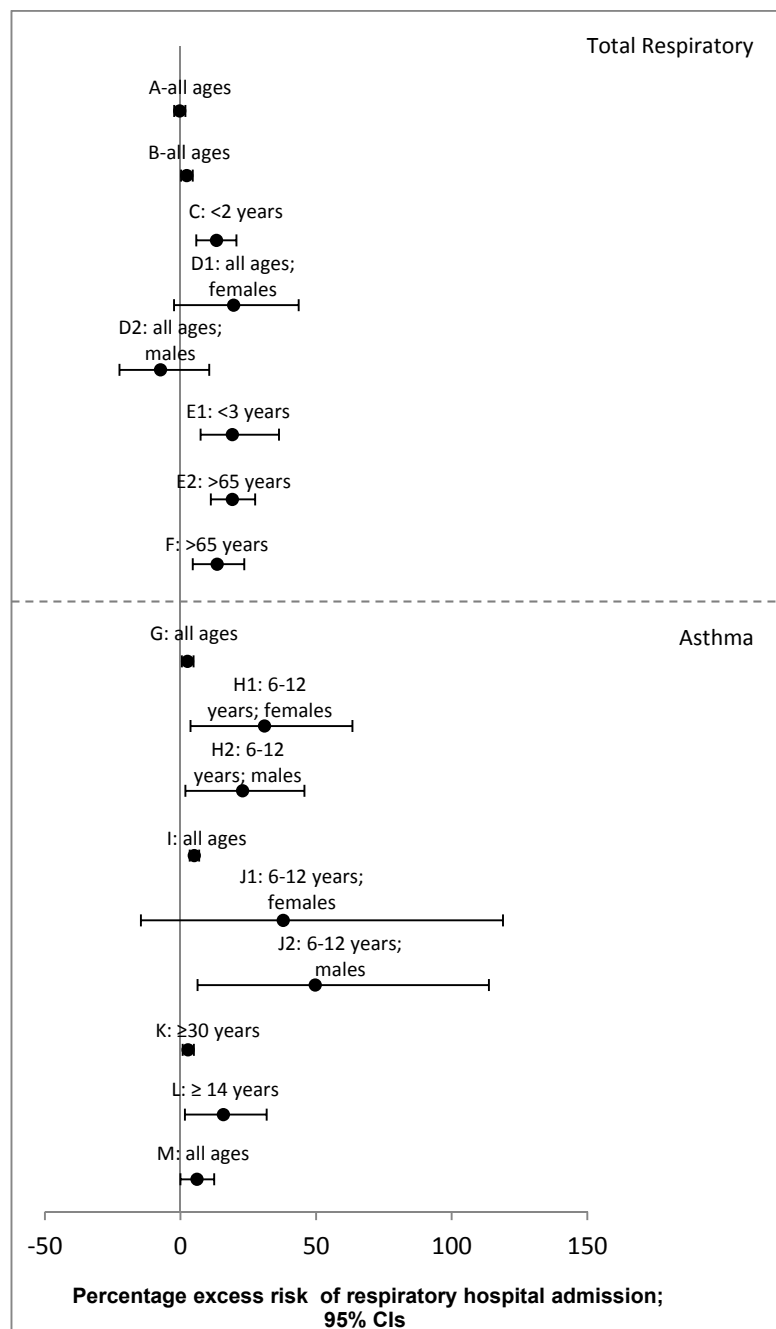
Finally, in a large study from Edmonton, ERVs for otitis media were significantly associated with ambient NO<sub>2</sub> and other pollutants (Zemek et al., 2010) in warm-, cold- and all-season analyses. Findings were similar for CO and PM<sub>10</sub>, and there were some associations with O<sub>3</sub>. NO<sub>2</sub>-related risks were not sensitive to adjustment for PM<sub>2.5-10</sub> or O<sub>3</sub> but were reversed following adjustment for CO.

### **8.3.4 Summary and Considerations—Studies of Medical Visits**

**Respiratory hospital admissions/ERVs/other medical visits:** For hospital admissions for all respiratory causes combined and for asthma, most of the studies reviewed in this assessment and in the 2008 US EPA ISA (US EPA, 2008) reported positive associations with short-term ambient NO<sub>2</sub>. As illustrated in Figure 8.2, which summarizes the results of Canadian and US studies, these associations were often observed in analyses for participants of all ages, but were greater and more consistent for total respiratory hospitalizations of children and older adults, and for asthma hospitalizations in children. The strongest single-day associations were mostly for short-term lags of between 0 and 3 d, though they were not consistently greater for any particular single-day lag (Figure 8.2). Findings were generally not highly sensitive to study design, and they were robust to adjustment for PM or other gaseous pollutants (including various traffic-related pollutants such as CO, PM<sub>2.5</sub>, and UFPs) in co-pollutant models in a number of studies, though not in all. Other respiratory hospital admissions have not been extensively studied, though there are mixed and often marginal results for COPD, URD and LRD.



**Figure 8.2: Percentage excess risk for respiratory hospital admissions and 95% CIs per standardized increment (20 ppb for 24-h avg and 30 ppb for daily 1-h max) in short-term NO<sub>2</sub> ambient concentration from all seasons in single-pollutant models (\*unless otherwise noted) in Canadian and US studies**



- A. 16 Canadian cities; Burnett et al. (1997b); lag 0 d; \*three-pollutant model
- B. 10 Canadian cities; Cakmak et al. (2006); lag 1–4 d
- C. Toronto, ON; Burnett et al. (2001); summer; 2-d avg
- D. Windsor, ON; Luginaah et al. (2005); lag 2 d
- E. Vancouver, BC; Yang et al. (2003); lag 1 d
- F. Vancouver, BC; Fung et al. (2006); 3-d avg
- G. Toronto, ON; Burnett et al. (1999); lag 0 d
- H. Toronto, ON; Lin et al. (2003); 6-d avg
- I. Ontario, Canada; To et al. (2013); lag 0 d; \*three-pollutant model
- J. Vancouver, BC; Lin et al. (2004); children w/ low SES; 4-d avg
- K. Los Angeles, CA; Linn et al. (2000); ≥30 years of age; lag 0 d
- L. Oklahoma City, OK; Magas et al. (2007); lag not reported
- M. El Paso, TX; Grineski et al. (2011); lag 2 or 3 d

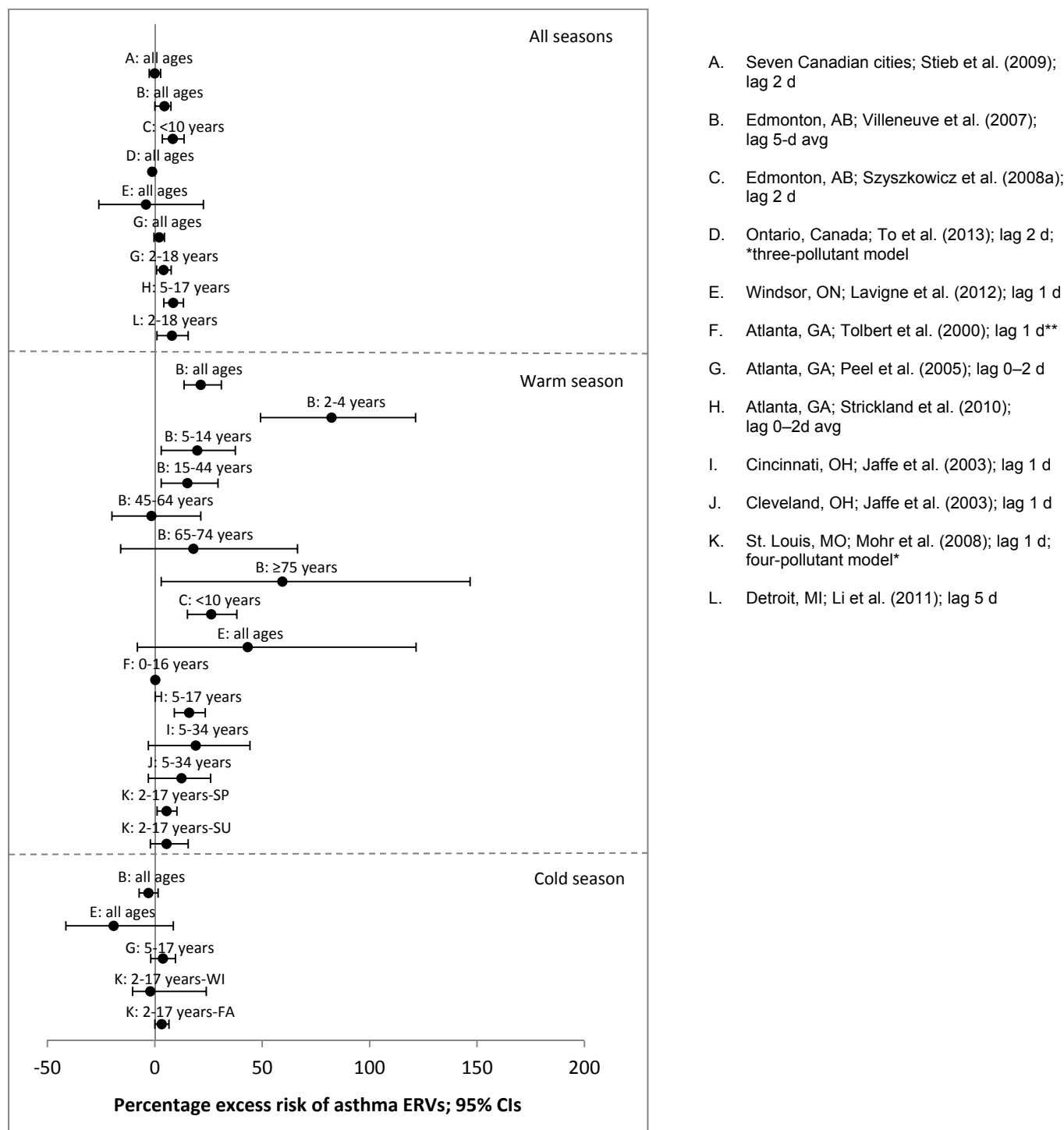
ERVs for all respiratory causes in children or adults have been consistently associated with short-term ambient NO<sub>2</sub> in the studies reviewed in this assessment, though ERVs were also related to other pollutants, and few co-pollutant analyses were conducted. For asthma ERVs, Figure 8.3 below summarizes the results of earlier and recent Canadian and US studies, most of which reported positive risk estimates. These were greatest and most consistent in warm-season analyses but were also increased in all-season analyses, whereas there was little evidence of an association during the cold season (Figure 8.3). Increased risks of asthma ERVs in children in particular were observed in a number of studies; however, significant associations were also seen in all-age analyses (Figure 8.3). In a large Canadian study in Edmonton in which analyses included a detailed age breakdown, risks were greatest for very young children, though they were also significantly increased in older children, in adults aged 15–44, and even more in older adults (Figure 8.3). While single-day associations were generally greatest with lags of 0–2 d, the risk estimates were often greater for those studies that combined ambient levels over several days. Significant associations were observed using various study designs, and they most often remained significant following adjustment for co-pollutants including certain traffic-related co-pollutants (CO, PM<sub>2.5</sub> and UFPs) in some studies. In two studies, there was no evidence of a threshold for the association of short-term ambient NO<sub>2</sub> with asthma ERVs in children. Findings with respect to ERVs for other respiratory conditions or for other medical visits for respiratory conditions are too limited to draw firm conclusions, though short-term ambient NO<sub>2</sub> was associated with asthma outpatient medical visits in two large studies in Ontario.

**Cardiovascular hospital admissions/ERVs:** Most studies reviewed in the 2008 US EPA ISA and in this assessment observed a positive and statistically significant association between CVD, cardiac or circulatory hospitalizations in single-pollutant models. With respect to more specific causes, positive associations were reported with admissions for MI in several studies, and also for IHD or ischemic stroke in some, though these were sometimes borderline. In those studies that stratified by age, risk estimates were generally greatest in older adults. However, in many of these studies these categories of hospital admissions were also related to other pollutants, most often PM and/or CO, and either only single-pollutant models were run or the results of two-pollutant and multi-pollutant analyses were mixed.

Findings in studies of cardiovascular ERVs in relation to ambient NO<sub>2</sub> are generally consistent with those for hospital admissions. In large studies in Atlanta, GA, and in seven Canadian cities, NO<sub>2</sub> was associated with increased risks for all CVD and/or cardiovascular categories, including IHD and angina/MI, though results were mixed for heart failure and negative for dysrhythmia. Increased risks for IHD ERVs with NO<sub>2</sub> were also reported in single-city studies. However, these outcomes were also related to other pollutants, particularly CO, and risk estimates for both of these substances were often sensitive to adjustment for the other, likely because they were highly correlated. Interestingly, associations with air pollutants were most pronounced in the warm season in Canada and in the cold season in Atlanta, perhaps as a result of prevalent use of air conditioning (and consequent reduced infiltration of ambient NO<sub>2</sub>) during the hot humid summers in the latter city.

**Hospitalizations/ERVs for other causes:** A small number of studies, most conducted in Canada, have investigated the relationship between ambient NO<sub>2</sub> and a variety of non-respiratory and non-cardiovascular conditions. In these studies, there were significant associations of NO<sub>2</sub> with increased hospital admissions for appendicitis and headache, and with increased ERVs for headache, depression, suicide attempts/suicidal thoughts, and otitis media. However, each of these outcomes was only examined in a very small number of studies, and they were also significantly related to most other particulate and gaseous pollutants.

**Figure 8.3: Percentage excess risk for asthma ERVs and 95% CIs per standardized increment (20 ppb for 24-h avg and 30 ppb for daily 1-h max) in short-term NO<sub>2</sub> ambient concentration in single-pollutant models (\*unless otherwise noted) in Canadian and US studies**



\*\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>.  
SP = spring; SU = summer; FA = fall; WI = winter.

**Studies of exposure measurement error:** Several studies have investigated the bias in estimates of risk for CVD ERVs in the Atlanta dataset as a result of using fixed area monitors as a measure of exposure. Simulation studies indicated that spatial heterogeneity of air pollutants was a much greater source of measurement error than instrument imprecision. The combined error could bias estimates either towards or away from the null, depending on whether the error was classical-type or Berkson-type. For NO<sub>2</sub>, most results suggested that this exposure measurement error markedly attenuated the risk estimates, even resulting in a loss of statistical significance.

## 8.4 Panel and Related Epidemiology Studies of Health Effects

In addition to the population-based studies of the relation between short-term exposure to ambient NO<sub>2</sub> and mortality, hospital admissions, ERVs and other medical visits, many epidemiology studies have investigated less severe health outcomes in smaller groups of subjects. Most of these are panel studies of the association between changes in air pollution and adverse health effects in groups of people (panels) engaged in normal activities in the environment. Exposures are usually to the ambient mix of pollutants, and pollutant levels can be closely monitored. A particular strength of this design is that panel studies allow investigators to make repeated measurements of physiological and biochemical changes in the respiratory, cardiovascular or hematological systems in individuals in “real world” settings. Subjects act as their own controls over time. Even the most sensitive populations can be observed in these studies to evaluate the relationship between exposure and health effects. Each subject’s medical history, lifestyle, activity patterns and episodes of illness can be closely followed.

Numerous panel and other epidemiological studies investigating the short-term effects of ambient NO<sub>2</sub> on various health endpoints have been published over the last few years. The results of these studies are presented in the following subsections, first for respiratory effects and then for those related to the cardiovascular system.

### 8.4.1 Respiratory Effects

#### 8.4.1.1 Lung Function

The 2008 US EPA ISA (US EPA, 2008) summarized the results of a number of epidemiological studies of lung function of children in relation to short-term ambient NO<sub>2</sub> air pollution. The report noted that measuring lung function in children is challenging and that spirometry, which requires special equipment and trained examiners, produces the most accurate results. Of the studies reviewed in the 2008 US EPA ISA that used spirometry, all conducted repeated measurements of lung function in schoolchildren. All these studies reported significant associations between ambient NO<sub>2</sub> and small decrements in one or more measures of lung function. These included: reductions in PEF (but not FVC, FEV<sub>1</sub>, or maximal mid-expiratory flow (MMEF)) among 1079 Netherlands children (Hoek and Brunekreef, 1994); decrements in morning FVC and morning-to-evening changes in FEV<sub>1</sub> in 269 Los Angeles, CA, schoolchildren (Linn et al., 1996); reduced baseline FVC and FEV<sub>1</sub> at lag 2 d in 33 Finnish children with chronic respiratory symptoms (Timonen et al., 2002); and decrements in FEV<sub>1</sub>, FVC, FEV<sub>0.5</sub>, MEF<sub>50</sub> and MEF<sub>25</sub> in 163 healthy Austrian schoolchildren related to overnight 8-h ambient NO<sub>2</sub> in both single and two-pollutant models with PM<sub>2.5</sub> (Moshhammer et al., 2006).

With respect to studies using peak flow devices, the 2008 US EPA ISA reported that those with supervised lung function measurements in schoolchildren did not show a consistent association between NO<sub>2</sub> and measurements of peak flow, while none of the several studies of children in

which home-use peak flow meters were employed reported significant associations with ambient NO<sub>2</sub>.

In those studies in adults in which spirometry was used (US EPA, 2008), there were significant NO<sub>2</sub>-related reductions in measures of lung function, including FVC, FEV<sub>1</sub> and FEF<sub>25–75%</sub> in a large Swiss cross-sectional study, and FEV<sub>1</sub> in a small number of US and European studies of subjects with COPD and/or asthma. In the studies of adults that used portable peak flow meters to measure their own lung function, no significant associations with ambient NO<sub>2</sub> were reported.

A number of more recent Canadian and US studies have examined associations between NO<sub>2</sub> exposure and lung function in asthmatic children.

Delfino et al. (2008a) reported the results of a repeated measures panel study of lung function in relation to personal and/or ambient exposure to relatively high levels of NO<sub>2</sub>, PM<sub>2.5</sub>, EC, OC and O<sub>3</sub> in 53 children aged 9–18 with persistent asthma from Riverside and Whittier, CA. Subjects self-administered spirometry at home 3 times/d for 10 d, and data quality was supported by detailed training and quality assurance protocols and validation analyses (Thompson et al., 2006). Personal NO<sub>2</sub> was related to significant decrements in FEV<sub>1</sub> (-1.217%; 95% CI -1.958%, -0.476% per 16.8 ppb 24-h personal NO<sub>2</sub>, lag 0 d). Central site NO<sub>2</sub> was also associated with reduced FEV<sub>1</sub> (-0.408%; 95% CI -0.768%, -0.047% per 6.3 ppb 24-h ambient NO<sub>2</sub>, lag 0 d). Decrement in FEV<sub>1</sub> were only significant in subjects not taking preventative controller medications. Gas stoves affected personal exposures to NO<sub>2</sub> but not the association between NO<sub>2</sub> and FEV<sub>1</sub>. Reduced lung function was also associated with personal PM<sub>2.5</sub>, though the risk estimate per IQR increment was greater for NO<sub>2</sub>; in two-pollutant models with personal PM<sub>2.5</sub>, decrements in FEV<sub>1</sub> with personal NO<sub>2</sub> were modestly reduced and still significant.

O'Connor et al. (2008) studied lung function (measured by self-administered spirometry), asthma symptoms and school absences in relation to air pollutants in 861 inner city children with persistent asthma from seven US cities for 2 weeks every 6 months for 2 years. The 5-d avg of ambient NO<sub>2</sub> (but not the 1-d avg) was significantly associated with reductions in FEV<sub>1</sub> (-1.36% of predicted FEV<sub>1</sub>; 95% CI -1.92%, -0.80% per 20.4 ppb 5-d avg NO<sub>2</sub>) and the PEF rate (-1.66%; 95% CI -2.24%, -1.08%). The risk of experiencing a lung function measure more than 10% lower than personal best was significantly related to NO<sub>2</sub> for both FEV<sub>1</sub> (OR = 1.17; 95% CI 1.01, 1.37 per increment from 10<sup>th</sup> to 90<sup>th</sup> percentile of 5-d avg NO<sub>2</sub>) and PEF rate (OR = 1.23; 95% CI 1.05, 1.44). PM<sub>2.5</sub> and SO<sub>2</sub> were also related to significant decrements in these parameters. In three-pollutant models with PM<sub>2.5</sub> and O<sub>3</sub>, NO<sub>2</sub> remained significantly associated with decrements in percentage FEV<sub>1</sub> and percentage PEF rate, as did PM<sub>2.5</sub> or O<sub>3</sub>. In five-pollutant models that also included SO<sub>2</sub> and CO, NO<sub>2</sub> remained a significant predictor of FEV<sub>1</sub> and NO<sub>2</sub> and O<sub>3</sub> remained significant predictors of PEF rate (data not shown).

Liu et al. (2009) conducted a panel study of 182 children 9–14 years old with a history of asthma from non-smoking homes in Windsor, ON. The children were followed for approximately a month in the fall of 2005, with weekly spirometry testing by a technician and the measurement of markers of airway inflammation and oxidative stress. The authors found that FEV<sub>1</sub> and FEF<sub>25–75%</sub> were consistently negatively associated with all pollutants, although this was only significant for the latter with NO<sub>2</sub> (FEV<sub>1</sub>: -0.8%; 95% CI -1.9%, 0.3%, per 6.8 ppb 24-h NO<sub>2</sub>, 3-d avg; FEF<sub>25–75%</sub>: -2.8%; 95% CI -5.0%, -0.5%) and with PM<sub>2.5</sub>. Risk estimates for several pollutants including NO<sub>2</sub> were most pronounced in children who did not use inhaled corticosteroids. However, the risk for NO<sub>2</sub> disappeared in two-pollutant models with PM<sub>2.5</sub>, whereas that for PM<sub>2.5</sub> was little changed.

Dales et al. (2009) reported the results of twice-daily self-administered PEF testing over 28 d in the same children studied by Liu et al. (2009). Ambient NO<sub>2</sub> was non-significantly related to decreases in bedtime percentage predicted FEV<sub>1</sub> (-0.24%; 95% CI -0.79%, 0.30% per 9.8 ppb

NO<sub>2</sub> averaged over the 24 h prior to lung function testing), whereas the association with PM<sub>2.5</sub> was significant and remained so in two-pollutant models with other pollutants. None of the pollutants were associated with decreased morning percentage predicted FEV<sub>1</sub>; in fact, most of the point estimates were positive. However, diurnal declines in FEV<sub>1</sub> during the daytime were inversely related to daytime NO<sub>2</sub> (-0.34%; CI -0.64%, -0.04% per IQR NO<sub>2</sub> in the previous 12 h [value not reported]), though they were also related to PM<sub>2.5</sub> and SO<sub>2</sub>. In two-pollutant models, the risks for each of these pollutants were attenuated and non-significant except in models with O<sub>3</sub>, which was not itself related to daytime declines in FEV<sub>1</sub>.

In a panel study of 40 fifth-grade children with asthma at four Bronx, NY, elementary schools that differed with respect to proximity to highways, Spira-Cohen et al. (2011) investigated lung function and respiratory symptoms in relation to exposure to air pollutants. Spirometry was self-administered twice daily over 1 month. None of the pollutants were significantly associated with decreased lung function, though decrements in PEF and FEV<sub>1</sub> were non-significantly associated with personal EC and PM<sub>2.5</sub>, and to a lesser extent with school site EC and PM<sub>2.5</sub>. Risk estimates per 60 ppb 6-h avg NO<sub>2</sub> were essentially null for FEV<sub>1</sub> (0.01 L; 95% CI -0.07 L, 0.09 L) and non-significantly positive for PEF (5.97 L/min; 95% CI -6.53 L/min, 18.46 L/min).

The association of ambient NO<sub>2</sub> with lung function in children has also been examined in a small number of recent epidemiological studies from Mexico and from Europe. The results of these studies have been somewhat mixed. While most observed NO<sub>2</sub>-related decrements in lung function, these were not always statistically significant. In addition, in studies of asthmatics, possible effect modification as a result of the use of asthma medications has not been extensively investigated in most studies through either the study design or data analysis.

There was some slight indication of NO<sub>2</sub>-related reductions in lung function in two studies of Mexican children exposed to somewhat higher levels of NO<sub>2</sub> than those in Canada. Ambient NO<sub>2</sub> was associated with non-significant decrements in FEV<sub>1</sub>, FVC, and (in one study) FEF<sub>25-75%</sub> in other panels that included both asthmatic and non-asthmatic children from Ciudad Juarez (Holguin et al., 2007) (risks presented in figure only, no quantitative results reported) and from Mexico City (Barraza-Villarreal et al., 2008) (FEF<sub>25-75%</sub> in asthmatics = -5.04 mL; 95% CI -22.6 mL, 12.5 mL per IQR of 13.4 ppb 8-h moving avg, lag 0 d; FEF<sub>25-75%</sub> in non-asthmatics = -12.1 mL; 95% CI -47.0 mL, 22.7 mL). However, in the latter study only, there were significant associations with PM<sub>2.5</sub>; only single-pollutant models were run in these studies.

In a small study of asthmatic children from Viseu, Portugal, with a history of wheezing who were exposed to similar ambient NO<sub>2</sub> concentrations to those in Canada, Martins et al. (2012) reported NO<sub>2</sub>-related changes in lung function. A 5-d avg exposure of each child to air pollutants was estimated using an inadequately described combination of time-activity data for each child and indoor and outdoor concentrations in microenvironments based on active and passive monitoring and air quality modelling. Asthma control medication was discontinued for virtually all the children for 3 weeks prior to each clinical visit. For NO<sub>2</sub>, the 5-d avg exposure was associated with significant decrements in FEV<sub>1</sub> (-6.31%; 95% CI -11.84%, -0.76% per increase of 10 µg/m<sup>3</sup> (5.32 ppb) in means of NO<sub>2</sub> exposure over a week) and also in FEF<sub>25-75%</sub> (-10.20%; 95% CI -18.80%, -1.59%), a non-significant decrement in FEV<sub>1</sub>/FVC (-2.79%; 95% CI -5.71%, 0.14%), and a significant increase in FEV<sub>1</sub> as a percentage of initial value after bronchodilator use (4.72%; 95% CI 0.91%, 8.53%). NO<sub>2</sub> had the greatest risk per 10 µg/m<sup>3</sup> (5.32 ppb) weekly increase for any of the pollutants. In two-pollutant models, decrements for NO<sub>2</sub> with FEV<sub>1</sub> were greater in models with PM<sub>10</sub> and attenuated with benzene and ethylbenzene, though all of these associations were non-significant.

Ofstedal et al. (2008) reported the associations between air pollutants and lung function of 2107 9- to 10-year-old lifetime residents of Oslo from the longitudinal Oslo Birth Cohort. Outdoor



residential concentrations of air pollutants were estimated using dispersion modelling. There were negative associations between lifetime exposures and first-year exposures to NO<sub>2</sub>, PM<sub>10</sub> and PM<sub>2.5</sub> and expiratory flow variables (especially in girls), but not forced volumes. With shorter time lags, similar but weaker associations were observed with NO<sub>2</sub> only. For the previous week and lag 1–3 d, only PEF in all children combined was significantly decreased with NO<sub>2</sub>, while FEF<sub>25%</sub> and FEF<sub>50%</sub> were non-significantly decreased; all three measures were found to a slightly greater extent in girls (risks presented in figure only, no quantitative results reported for short-term exposures to NO<sub>2</sub>). The authors noted that forced volumes provide information primarily on central airways, whereas expiratory flow variables represent peripheral airways.

Weinmayr et al. (2010) conducted a systematic global literature review and meta-analysis of studies of lung function and respiratory symptoms in asthmatic children in relation to NO<sub>2</sub> and PM<sub>10</sub> air pollution. The search spanned 1990 to 2008, excluded indoor and laboratory studies, and was restricted to studies of asthmatic or symptomatic children that reported quantitative effects and controlled for temperature and day of the week. There were a total of 24 studies of NO<sub>2</sub>, spanning a range of mean 24-h NO<sub>2</sub> levels between 4.3 and 41.0 ppb. In combined estimates using random effects models, ambient NO<sub>2</sub> was not related to decrements in PEF in analyses with all studies combined (0.180 L/min; 95% CI -0.184 L/min, 0.544 L/min per 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub>) or excluding the multi-centre Pollution Effects on Asthmatic Children in Europe (PEACE) studies (0.170 L/min; 95% CI -0.590 L/min, 0.929 L/min). However, there were fairly clear decrements in PEF in relation to PM<sub>10</sub> in analyses from all studies combined and excluding the PEACE studies. There was significant between-study heterogeneity for both pollutants. The authors noted that the evaluation of the effect on PEF is difficult because of large inter-individual differences in this measure, which is likely to be strongly influenced by medication use among diseased subjects, something that was not considered in the analysis. In addition, they discussed concerns and presented limited evidence indicating that the entire PEACE study series (largely null) may have been influenced by an influenza epidemic early in the study period and by increased respiratory infections in general during the study period, given each study's short duration and their conduct solely in winter.

In most recent studies of adults that were reviewed in this assessment (including healthy subjects, asthmatics, and COPD patients), lung function was not related to ambient NO<sub>2</sub> or was not robust to adjustment for other pollutants.

In healthy adult cyclists, there were no significant NO<sub>2</sub>-related decrements in ΔFEV<sub>1</sub>, ΔFVC or ΔFEF<sub>25–75%</sub> measured by a technician using spirometry up to 4 h after exposure to ambient NO<sub>2</sub> measured at a central site during an hour of cycling in Ottawa, ON. Instead, most of the risk estimates were positive, including a significant association between an IQR increase of 4 ppb in ambient NO<sub>2</sub> and ΔFEV<sub>1</sub> both 2 h (β = 121 mL; 95% CI 27 mL, 216 mL) and 3 h (β = 129 mL; 95% CI 23 mL, 234 mL) after cycling (Weichenthal et al., 2011). In a large study of healthy adults from Tel Aviv, Israel (Steinvil et al., 2009), an IQR of 12.8 ppb in ambient 24-h NO<sub>2</sub> was associated with reductions in FEV<sub>1</sub> with various lags (-31 mL; 95% CI -61 mL, -1 mL, lag 3 d; -35 mL; 95% CI -66 mL, -3 mL, and lag 5 d; -62 mL; 95% CI -116 mL, -9 mL, avg of lag 0–7 d) and FVC (-45 mL; 95% CI -83 mL, -7 mL, lag 5 d), but these lung function measures were more consistently and strongly related to SO<sub>2</sub>. In two-pollutant models risks for SO<sub>2</sub> were robust, while NO<sub>2</sub> and CO were attenuated and non-significant, and O<sub>3</sub> was significant but in the opposite direction of expectation.

In contrast to these generally negative findings, Strak et al. (2012a, b) reported significant and robust NO<sub>2</sub>-related decrements in lung function measured by technicians using spirometry in a panel study of healthy adults following short-term exposure to a wide range of air pollutants at various sites in Utrecht, the Netherlands. The sites, selected to provide exposure contrasts and reduced correlations among PM constituents, included an underground train station, sites near

moving traffic and near stop-and-go traffic, a farm, and an urban background site. Consistent associations of NO<sub>2</sub> with FVC were found immediately after exposure, 2 h after, and the next morning, whether in all sites combined or at outdoor-only sites. For all sites combined, the adjusted associations between an IQR of 10.54 ppb in ambient 24-h NO<sub>2</sub> and the percentage change (post/pre) in FVC were, respectively, -1.82%; 95% CI -3.16%, -0.44%; -1.54%; 95% CI -2.82%, -0.26%; and -1.88%; 95% CI -3.17%, 0.58% immediately after exposure, 2 h afterward and the next morning after exposure. Similar risk estimates were observed with the outdoor sites only. There were also associations with FEV<sub>1</sub>, though weaker and only after 2 h and the next morning. There were similar findings for NO<sub>x</sub>. In two-pollutant models, findings for NO<sub>2</sub> and NO<sub>x</sub> were robust to adjustment for virtually all of the 25 pollutants, except for NO<sub>x</sub> and NO<sub>2</sub>, respectively, (the latter findings probably because they were very highly correlated ( $r = 0.91$ ) with each other). There were no associations of any pollutant with FEF<sub>25–75%</sub> or with PEF.

In persistent asthmatics, Qian et al. (2008) investigated the relationship between air pollutants and lung function testing in the US multi-city Salmeterol Off Corticosteroids Study, in which subjects initially treated with inhaled corticosteroids (ICSs) and as-needed rescue medications were randomized into groups receiving inhaled triamcinolone (an ICS), inhaled salmeterol (a long-acting  $\beta_2$ -adrenergic agonist (BAA), and a placebo; they measured their own morning PEF each day. Ambient NO<sub>2</sub> was associated with a decrement in PEF (-1.53 L/min; 95% CI -2.93 L/min, -0.14 L/min per 10 ppb 24-h NO<sub>2</sub>, lag 0 d), which was more pronounced in those on salmeterol (-2.52 L/min; 95% CI -4.93 L/min, -0.11 L/min). PM<sub>10</sub> was negatively associated with PEF only in the placebo group and SO<sub>2</sub> in the triamcinolone group. In two-pollutant models, the NO<sub>2</sub> association was robust in models with O<sub>3</sub> (which was not itself related to reduced PEF), but it was non-significant with PM<sub>10</sub> and SO<sub>2</sub>. In another study, Canova et al. (2010) reported that there was no association between NO<sub>2</sub> and self-administered peak flow measurements of morning or evening PEF or FEV<sub>1</sub> in moderate-to-severe asthmatics from Padua, Italy (risks presented in figure only, no quantitative results reported for NO<sub>2</sub>). Instead, lung function was significantly associated with CO and remained so in two- and four-pollutant models.

Peacock et al. (2011) reported a study of air-pollutant-related peak flow measurements of lung function in moderate-to-severe COPD outpatients at a clinic in London, England. There were significant associations with some pollutants in 1996, including a significant decrease in FVC per 1 ppb increase in daily 1-h max ambient NO<sub>2</sub> during the summer period (-0.528 mL; 95% CI -0.032 mL, -1.024 mL); these were, however, not replicated in 1997 (0.606 mL; 95% CI -0.083 mL, 1.295 mL). When the 2 years' data combined were analyzed, most regression coefficients were positive rather than negative, including a significant association between NO<sub>2</sub> and PEF (0.013 L/min; 95% CI 0.002 L/min, 0.024 L/min, lag 1 d); the coefficients were positive and near-null for FEV<sub>1</sub> (0.005 mL; 95% CI -0.106 mL, 0.116 mL, lag 0 d) and FVC (0.071 mL; 95% CI -0.189 mL, 0.332 mL, lag 1 d). NO<sub>2</sub> was not associated with increased risks of large PEF decrements or COPD exacerbations, and it was marginally associated with a symptomatic fall in PEF (defined *a priori* as a fall in PEF of  $\geq 10$  L/min for  $\geq 2$  d accompanied by a reported increase in shortness of breath). In two- and three-pollutant models, the risk for PM<sub>10</sub> was robust, whereas there was no evidence of any adverse effect of NO<sub>2</sub> or BS after adjusting for PM<sub>10</sub>.

#### 8.4.1.2 Airway Hyperresponsiveness

Findings from experimental studies in animals and humans indicate that exposure to NO<sub>2</sub> causes increased AHR to bronchial challenges (Sections 6.1, 7.2 and 7.3). These experimental results support those from a recent cross-sectional analysis of data from a cohort's initial baseline evaluation, in which associations between increases in ambient NO<sub>2</sub> and in AHR were observed among 85 asthmatic children (aged 7–12) from Mexico City (Hernandez-Cadena et al., 2009). In this study, high levels of ambient NO<sub>2</sub> were associated with a 15% decrease in FEV<sub>1</sub> response to short-acting  $\beta$ -agonists (SABA) (95% CI -29%, -0.5%) per 10 ppb daily 1-h

max NO<sub>2</sub>, lag 0 d (after adjustment for covariates), with similar decreases for lags of 1, 2, or 3 d. These findings were limited to children not currently using ICSs, and to children with mild intermittent asthma rather than mild persistent or moderate asthma (it is likely that those with mild intermittent asthma would generally not be using ICSs). A reduced FEV<sub>1</sub> response to SABA was also associated with O<sub>3</sub> (r with NO<sub>2</sub> = 0.35), but only at lag 5 d, whereas there was no association with PM<sub>2.5</sub> (r = 0.59) at any of the lags. None of the pollutants was significantly related to the FVC percentage response to SABA. Hence, in this study, exposure to ambient NO<sub>2</sub> was associated with reduced bronchodilating response to SABA in asthmatic children, indicating increased AHR.

#### 8.4.1.3 Biomarkers of Airway Inflammation

A number of studies have been published examining associations between NO<sub>2</sub> exposure and changes in biomarkers of airway inflammation. In these studies, the following were measured: fractional exhaled nitric oxide (eNO), a marker of airway inflammation; TBARS, a marker of lipid peroxidation and oxidative stress; 8-isoprostane, a marker of oxidative stress; pH, a marker of oxidative stress and inflammation; and IL-8, a pro-inflammatory mediator. In the 2008 US EPA ISA, it was concluded that the few epidemiological studies available at that time indicated an association between ambient NO<sub>2</sub> exposure and markers of airway inflammation in children (US EPA, 2008). More specifically and in terms of panel studies, Delfino et al. (2006) found a relationship between personal and ambient NO<sub>2</sub> levels and eNO (1.6 ppb; 95% CI 0.43 ppb, 2.83 ppb per 17 ppb personal NO<sub>2</sub>, lag 0–1 d, and 1.36 ppb; 95% CI 0.39 ppb, 2.33 ppb per IQR of 12 ppb 24-h central NO<sub>2</sub>, lag 0–1 d) among 45 asthmatic schoolchildren in Southern California. In two-pollutant models these effects were largely independent of PM<sub>2.5</sub>. These results were consistent with observations from two studies from a research group in the Netherlands in which there were associations between ambient NO<sub>2</sub> and levels of eNO in schoolchildren (Steerenberg et al., 2001, 2003). However, among the panel studies involving adult populations, the majority of the studies found no significant association between NO<sub>2</sub> and markers of airway inflammation and damage (US EPA, 2008).

Since the 2008 US EPA ISA, several studies from Canada and the US have been published investigating the relationship between ambient NO<sub>2</sub> and increases in respiratory biomarkers related to oxidative stress and inflammation. All except one of these studies involved groups of schoolchildren, both asthmatic and non-asthmatic.

In a Canadian study conducted in Windsor, ON, the association between ambient air pollutants including NO<sub>2</sub> and fractional eNO, TBARS and 8-isoprostane in exhaled breath condensate (EBC) measured in 182 asthmatic children (aged 9–14) once a week for 4 weeks was examined (Liu et al. 2009). A significant increase in TBARS (32.9%; 95% CI 7.2%, 64.6% per 9.8 ppb increase in 24-h NO<sub>2</sub>, 3-d avg) was related to NO<sub>2</sub>, while 8-isoprostane was non-significantly decreased (-4.0%; 95% CI -16.3%, 10.2%, 3-d avg) and fractional eNO (0.5%; 95% CI -12.1, 14.9%, 3-d avg) was not markedly changed. Similar associations were observed for SO<sub>2</sub> and PM<sub>2.5</sub>, and in two-pollutant models with PM<sub>2.5</sub> and SO<sub>2</sub>, the NO<sub>2</sub>-related risk for TBARS was attenuated somewhat and was no longer significant.

Patel et al. (2013) found an increase in 8-isoprostane levels (0.59 unit; 95% CI 0.25, 0.94 (log-transformed) per 3.8 ppb increase in 24-h NO<sub>2</sub>, 4-d avg), but no significant association for pH in EBC (-0.06; 95% CI -0.22, 0.09, 2-d avg) among 18 asthmatic and 19 non-asthmatic adolescents in New York City, NY. Black carbon (BC) was similarly associated with both of these markers, while O<sub>3</sub> and fine PM were not consistently associated. No two-pollutant or multi-pollutant analyses were conducted with NO<sub>2</sub>. Associations did not differ between asthmatics and non-asthmatics, though no data were shown and the number of subjects from each group was small.

One US study (Berhane et al., 2011) identified a non-significant increase in eNO in relation to NO<sub>2</sub> levels among 2,240 schoolchildren (aged 7–9) from 13 Southern Californian communities in the Children's Health Study (CHS) (5.13%; 95% CI -2.34%, 13.17% per 10.62 ppb increase in 24-h NO<sub>2</sub>, 1–6-d avg) Multi-day averages for each of PM<sub>10</sub>, PM<sub>2.5</sub> and O<sub>3</sub> were related to eNO levels, and the associations remained significant with adjustment for O<sub>3</sub>.

Another panel study involved 119 asthmatic adults and adolescents (aged 12–65) in six US cities (Boston, MA; New York, NY; Denver, CO; Philadelphia, PA; San Francisco, CA; and Madison, WI) (Qian et al., 2009). NO<sub>2</sub> was related to an increase in eNO of 0.13 ppb (95% CI 0.06 ppb, 0.19 ppb per 10 ppb increase in 24-h NO<sub>2</sub>, lag 0 d), with stronger associations among those being treated with the asthma control medications triamcinolone or salmeterol. A similar association was observed for PM<sub>10</sub>, but in two-pollutant models involving these pollutants, the results for both pollutants were attenuated and no longer significant. In contrast to the findings for NO<sub>2</sub> and PM<sub>10</sub>, O<sub>3</sub> was inversely associated with eNO, a finding that remained significant in two-pollutant analyses.

Some studies from Mexico and Europe (including one cross-border US–Mexico panel study) also examined the relationship between NO<sub>2</sub> and eNO (and in some of these studies, also pH in EBC and IL-8 in nasal lavage fluid).

In a study of 58 asthmatic children (mean age 8.7 years) from two schools in El Paso, TX, and two in Ciudad Juarez, Mexico, NO<sub>2</sub> levels measured outside the schools but not at a central site were associated with an increase in the fraction of exhaled nitrogen oxide (FeNO) (outdoor school = 3.8%; 95% CI 1.5%, 6.1% per 12.3 ppb increase 96-h NO<sub>2</sub>; central site = 0.8%; 95% CI -0.5%, 2.1% per 9.6 ppb increase 96-h NO<sub>2</sub>) (Sarnat et al., 2012). FeNO was also positively, though marginally, related to indoor school NO<sub>2</sub>, but in a two-pollutant model with outdoor school NO<sub>2</sub>, neither finding remained significant (further details not discussed). FeNO was consistently increased in relation to various PM metrics, and the authors concluded that traffic-related and non-traffic-related particles were more robust predictors of FeNO than NO<sub>2</sub>, as the associations for NO<sub>2</sub> were highly sensitive to model specification.

Barraza-Villarreal et al. (2008) examined the relationship between NO<sub>2</sub> and the markers FeNO, IL-8 levels in nasal lavage fluid, and pH of EBC from 158 asthmatic and 50 non-asthmatic children (aged 7–11) in Mexico City, Mexico. FeNO was significantly associated with an IQR increase of 13.4 ppb 8-h moving avg NO<sub>2</sub> levels (lag 0 d) in both groups (1.05 ppb; 95% CI 0.98 ppb, 1.12 ppb in asthmatics and 1.10 ppb; 95% CI 0.99 ppb, 1.23 ppb in non-asthmatics). IL-8 was also increased in both asthmatics (1.03 pg/mL; 95% CI 0.94 pg/mL, 1.12 pg/mL) and non-asthmatics (1.15 pg/mL; 95% CI 1.01 pg/mL, 1.32 pg/mL). (The authors reported some of these findings as non-significant, but the CIs do not include zero.) No relation was found between NO<sub>2</sub> exposure and pH in either group. O<sub>3</sub> was significantly associated with all three markers examined, and was robust in two-pollutant models including PM<sub>2.5</sub>. Furthermore, in a study conducted in Ciudad Juarez involving 200 children (aged 6–12) with or without physician-diagnosed asthma, NO<sub>2</sub> was not significantly associated with an increase in FeNO (Holguin et al., 2007; risks presented in figure only, no quantitative results reported for NO<sub>2</sub>). This was also the case for PM<sub>2.5</sub> and EC, whereas road density near the child's residence was significantly associated with FeNO.

NO<sub>2</sub> was also not associated with increased FeNO in three European studies reviewed for this assessment. Flamant-Hulin et al. (2010) found no significant association between FeNO and NO<sub>2</sub> (risks presented in figure only, no quantitative results reported), although this biomarker was related to PM<sub>2.5</sub>, formaldehyde, and acetaldehyde, in a cohort of 104 asthmatic and non-asthmatic children from 14 schools in France. Martins et al. (2012) also reported that there was no significant relationship between weekly personal exposure to NO<sub>2</sub> (estimated using active



and passive sampling outdoors and indoors, air quality modelling, and time-activity data) and FeNO in a panel of 51 children with wheeze in Viseu, Portugal, though there was a negative association between NO<sub>2</sub> and pH in EBC (-0.69; 95% CI -1.04, -0.35 per increase of 10 µg/m<sup>3</sup> (5.32 ppb) in means of NO<sub>2</sub> exposure over a week). The pH of EBC was also inversely related to PM<sub>10</sub>, benzene, and ethylbenzene, whereas no pollutants examined were associated with FeNO. In a Dutch intervention study, which involved exposure of 31 participants to a wide range of particulate and gaseous pollutants at different locations (an underground train station, two traffic sites, a farm, and an urban background site) for 5 h with intermittent exercise, NO<sub>x</sub> and NO<sub>2</sub> were mostly associated with non-significant increases in FeNO in single-pollutant models or in two-pollutant models with a wide range of gaseous and particulate pollutants. For all sites combined, the adjusted association between an IQR of 10.54 ppb in ambient 24-h NO<sub>2</sub> and the percentage change (post/pre) in FeNO was 8.45% (95% CI 1.37%, 15.53%) 2 h after exposure. However, in two-pollutant models this was not robust to adjustment for particle number concentration and related particle metrics. Instead, particle number concentration was consistently related to FeNO in both single- and two-pollutant models after adjustment for all other pollutants examined (Strak et al., 2012a, 2012b).

In a unique panel study from Erfurt, Germany, involving 38 males with COPD, NO was associated with an increase in blood levels of monocytes and lymphocytes, while NO<sub>2</sub> was not related to any white blood cell counts (Bruske et al., 2010; risks presented in figure only, no quantitative results reported for NO<sub>2</sub>). The authors noted that most gaseous and particulate pollutants were associated with multiple changes in the differential white blood cell count.

#### 8.4.1.4 Respiratory Symptoms and Medication Use

The 2008 US EPA ISA (US EPA, 2008) reported that epidemiology studies using community ambient monitors found associations between ambient NO<sub>2</sub> and respiratory symptoms. Some of the strongest evidence was provided by three large longitudinal multi-city studies of children in the continental US and Southern Ontario (Schwartz et al., 1994; Mortimer et al., 2002; Schildcrout et al., 2006).

Schwartz et al. (1994; US EPA, 2008) reported findings from a study of a representative sample of 1884 schoolchildren from the Harvard Six Cities study followed for 1 year, for which symptoms (in 13 categories, analyzed as cough, lower or upper respiratory symptoms) were recorded daily. There was a significant association between a 4-d mean of ambient NO<sub>2</sub> and incidence of cough among all children in single-pollutant models (standardized to a 20 ppb increase in 24-h NO<sub>2</sub>, OR = 1.61; 95% CI 1.08, 2.43), but not with NO<sub>2</sub> on the previous day. The association with 4-d mean NO<sub>2</sub> was robust in two-pollutant models that included O<sub>3</sub>, but was attenuated somewhat and no longer significant in models with PM<sub>10</sub> or SO<sub>2</sub>. Lower respiratory symptoms were non-significantly related to the previous 24-h ambient NO<sub>2</sub>, and this was reduced in two-pollutant models with PM, while no pollutants were associated with upper respiratory symptoms.

In Mortimer et al. (2002; US EPA, 2008), 864 asthmatic children with a high prevalence of severe asthma/atopy from eight US National Cooperative Inner-City Asthma Study (NCICAS) cities were monitored daily for morning and evening asthma symptoms (none versus any) and peak flow for four 2-week periods over the course of 9 months. The greatest association between the pollutants studied and morning asthma symptoms was for a 6-d moving avg of ambient NO<sub>2</sub>; the OR for any asthma symptoms (cough, chest tightness, wheeze) among the study children was 1.48 (95% CI 1.02, 2.16 for a 20 ppb increase in 4-h NO<sub>2</sub>). The risk estimate for NO<sub>2</sub> was generally similar, though no longer statistically significant, using two-, three-, or four-pollutant models with O<sub>3</sub>; with O<sub>3</sub> and SO<sub>2</sub>; or with O<sub>3</sub>, SO<sub>2</sub>, and PM<sub>10</sub>.

Schildcrout et al. (2006; US EPA, 2008) reported pollutant-related daily asthma symptom (none versus any) and medication use for roughly 2 months in 990 mild–moderate asthmatic children who were participants in the Childhood Asthma Management Program (CAMP) study in eight North American cities (including Toronto). Ambient NO<sub>2</sub> was associated with increased risk of cough (OR = 1.09; 95% CI 1.03, 1.15 per 20 ppb 24-h NO<sub>2</sub>, lag 2 d) and increased use of rescue medication (OR = 1.05; 95% CI 1.01, 1.09). Risks were positive with most lags, though they were generally strongest for a 2-d lag. Findings were similar for CO, which was highly correlated with NO<sub>2</sub>, while risks were weaker and less significant for SO<sub>2</sub>, and still less for PM<sub>10</sub> and O<sub>3</sub>. No results from conventional two-pollutant or multi-pollutant models were reported.

The 2008 US EPA ISA also reviewed a number of single-city studies of NO<sub>2</sub>-related respiratory symptoms in children. The results of these studies generally confirmed the association between ambient NO<sub>2</sub> and asthma-related symptoms, though symptoms were often also related to other pollutants and few of the studies conducted co-pollutant analyses. In contrast to the generally positive findings in children, studies of respiratory symptoms or inhaler use in adults that were summarized by the US EPA (2008) yielded inconsistent results.

The more recent Inner-City Asthma Study of asthmatic inner-city children from seven US cities (O'Connor et al., 2008) largely confirms the findings of the earlier multi-city studies described above. In this later study, data on spirometry and asthma symptoms and asthma school absences were collected periodically over 2 years for 861 inner-city children (average age 7.7 years) with persistent asthma and atopy. Using generalized estimating equation (GEE) models to relate asthma-related symptoms and school absences to air pollutants, a 20.4 ppb 19-d avg NO<sub>2</sub> (six cities) was associated with the caregiver-reported 2-week frequencies of wheeze/cough (RR = 1.17; 95% CI 0.99, 1.39), nighttime asthma (RR = 1.37; 95% CI 1.08, 1.73), slow play days (RR = 1.26; 95% CI 1.04, 1.54), and asthma-related school absences (OR = 1.67; 95% CI 1.18, 2.36). In three-pollutant models with NO<sub>2</sub>, O<sub>3</sub>, and PM<sub>2.5</sub>, associations for NO<sub>2</sub> remained significant or nearly so with each of the symptoms, though asthma-related school absences were attenuated and not significant. In five-pollutant symptom models including SO<sub>2</sub> and CO, the associations with NO<sub>2</sub> were reported to be slightly attenuated and no longer statistically significant (results not shown). The authors reported that indoor levels of NO<sub>2</sub> in the homes of study subjects were significantly correlated with outdoor levels measured at central monitoring sites (data not shown).

Findings in recent Canadian and American single-city panel studies of NO<sub>2</sub>-related respiratory symptoms in asthmatic children are mixed, perhaps reflecting the more limited number of subjects and/or duration of follow-up in these studies, and the relatively low air pollutant exposures in some studies.

Dales et al. (2009) reported results for respiratory symptoms and lung function testing in a panel of 182 children aged 9–14 with a history of asthma from non-smoking homes in Windsor, ON. Respiratory symptom data collected in daily diary reports over 28 d (including difficulty breathing, cough, wheeze, and chest tightness) were not significantly related to ambient NO<sub>2</sub> in mixed effects regression models adjusting for confounding variables (data not shown). The findings for other pollutants were also largely negative except for SO<sub>2</sub>, where a significant association was found with chest tightness.

Mann et al. (2010) conducted a similar longitudinal study in 315 asthmatic children from Fresno, CA, exposed to comparable levels of ambient NO<sub>2</sub> to those in the study by Dales et al. (2009). Subjects underwent skin prick testing, followed by one or more 14-d sessions of twice-daily symptom reporting. Most NO<sub>2</sub> lags of 1–7 d and moving averages of 3–12 d were associated with increased odds of wheeze (greatest single-day risk for 2 d, OR = 1.10; 95% CI 1.02, 1.20 per 8.7 ppb 24-h NO<sub>2</sub>). Wheeze was also associated with PM<sub>10–2.5</sub>, but less so and not



significantly with  $\text{NO}_3$ ,  $\text{PM}_{2.5}$ , EC, or  $\text{O}_3$ .  $\text{NO}_2$  was also significantly and more strongly associated (as were all pollutants but  $\text{O}_3$ ) with wheeze in children who were allergic to cat (OR = 1.27; 95% CI 1.06, 1.51), allergic to common fungi (OR = 1.23; 95% CI 1.10, 1.39), and especially among boys with mild intermittent asthma (OR = 1.51; 95% CI 1.23, 1.85) (all lag 2 d). In two-pollutant models with  $\text{PM}_{10-2.5}$ , the OR for  $\text{NO}_2$  for all subjects decreased by 39% and was no longer significant, but was robust for the atopy subgroups and for boys with mild intermittent asthma.

Patel et al. (2010) conducted a longitudinal panel study of 249 adolescents at four high schools in or near New York City, NY, with different degrees of exposure to traffic pollutants. Daily symptom data were collected for 4–6 weeks from students, almost 23% of whom were asthmatics, and related to pollutants using multilevel GLMs. Multiple daily lags of central site  $\text{NO}_2$  were associated with wheeze and shortness of breath, and ORs increased with the number of days over which concentrations were averaged (for wheeze, OR = 1.70; 95% CI 1.36, 2.13 per 16 ppb 24-h  $\text{NO}_2$ , lag 5-d avg; for shortness of breath, OR = 1.35; 95% CI 1.09, 1.67, lag 5-d avg). Risks were positive but not significant for chest tightness (OR = 1.08; 95% CI 0.97, 1.20, lag 0 d), asthma medication use (OR = 1.14; 95% CI 0.74, 1.76, lag 4-d avg), and cough (OR = 1.05; 95% CI 0.87, 1.26, lag 5-d avg). In interaction analyses,  $\text{NO}_2$  was associated with shortness of breath in urban but not suburban schools, and with wheeze and with chest tightness in asthmatics but not in non-asthmatics. Wheeze symptoms was also associated with levels of BC (also shortness of breath) and  $\text{PM}_{2.5}$  measured at the schools, both of which were moderately to strongly correlated with  $\text{NO}_2$ ; no two-pollutant or multi-pollutant analyses were conducted.

Spira-Cohen et al. (2011) reported the results of a repeated measures panel study of the personal/outdoors-at-school 1-month exposure to air pollutants of 40 inner-city asthmatic children aged 10–12 at four New York City schools. Daily symptom severity index (reported 3 times daily) and lung function testing were analyzed in relation to air pollutants using mixed models. The 6-h avg school site  $\text{NO}_2$  was non-significantly associated with an increased risk of severity of cough, wheeze, shortness of breath, or total symptoms using the same-day concentrations (risk estimates only reported in figure). Personal and school site EC were robustly associated with symptoms for various short-term lags; in a three-pollutant model with  $\text{NO}_2$  and EC, the authors reported that EC “remained the significant exposure factor” (data not shown).

Sarnat et al. (2012) conducted a 16-week repeated measures panel study of daily respiratory symptoms and eNO in 58 asthmatic children aged 6–12 from two schools differing with respect to exposure to traffic-related pollution in each of El Paso, TX, and Ciudad Juarez, Mexico. There was no significant association between levels of any pollutant including  $\text{NO}_2$  over the school week at the central site, outdoors at school, or indoors at school with the risk of respiratory symptoms, including cough, wheeze, difficulty breathing, missed school, or SABA use (data not shown). This contrasts with the generally positive findings for eNO (subsection 8.4.1.3). However,  $\text{NO}_2$  exposures were measured for 4 d combined each week and therefore were not highly variable at outdoor/ambient sites, and daily symptom data were reported weekly by each child’s guardian, both of which factors may have limited the study’s power to detect an  $\text{NO}_2$ -related effect.

Andersen et al. (2008) made a prospective study of wheezing in a birth cohort of 205 children with asthmatic mothers from Copenhagen, Denmark, where they were exposed to ambient levels of  $\text{NO}_2$  measured at a central background monitoring station similar to those in Canada. Using logistic regression by GEE,  $\text{NO}_2$  was associated with incident wheezing symptoms (reported daily over the first 3 years of life, a total of 174,259 person-days) at lags of 3 d, 4 d and 2–4 d avg in infants 0–1 year of age (OR = 1.45; 95% CI 1.08, 1.95 per 6.5 ppb 24-h  $\text{NO}_2$ , lag 2–4 d avg). The OR was less but still significant for children aged 0–3, and non-significantly

increased in children 1–2 and 2–3 years of age. In children living within 5 km of the central site monitor at which monitoring data were collected, risk estimates were greater (for NO<sub>2</sub>, OR = 1.84; 95% CI 1.07, 3.17 for infants up to 1 year old). Findings were very similar for NO<sub>x</sub>, which was highly correlated with NO<sub>2</sub> ( $r = 0.98$ ), and generally similar for other pollutants, though ORs were smaller and sometimes non-significantly increased. In two-pollutant models with PM<sub>10</sub> or with UFPs, risks for both NO<sub>2</sub> and the other pollutant were still elevated, though somewhat attenuated and non-significant. There was no significant effect modification by gender, paternal asthma history, and ICS medication use.

Several small- to moderate-sized studies of NO<sub>2</sub>-related respiratory symptoms from other countries reviewed for this assessment provide mixed results. In a longitudinal study of 158 asthmatic and 50 non-asthmatic schoolchildren aged 6–14 from Mexico City exposed to mean levels of ambient NO<sub>2</sub> greater than those in Canada, respiratory symptoms, spirometry and inflammatory biomarkers were reported every 15 d, an average of 11 times per subject (Barraza-Villareal et al., 2008; Escamilla-Nunez et al., 2008). For respiratory symptoms, an increase in NO<sub>2</sub> of 34 ppb (1-h max) was associated with increased risk of cough (OR = 1.10; 95% CI 1.04, 1.16, lag 0 d) and of wheezing (OR = 1.10; 95% CI 1.03, 1.18, lag 0 d) in asthmatics. Escamilla-Nunez et al. (2008) reported that these effects were stronger when the cumulative exposure from the previous 2–5 d was considered, and noted similar effects on bronchodilator use. The associations with cough and bronchodilator use were robust to adjustment for O<sub>3</sub> and PM<sub>2.5</sub>, whereas that with wheeze was attenuated and no longer significant. Among non-asthmatic children, cough was significantly increased in relation to ambient NO<sub>2</sub> (OR = 1.22; 95% CI 1.03, 1.45 for an increase of 24.5 ppb in 2-d cumulative exposure), and was not associated with PM<sub>2.5</sub> or O<sub>3</sub>.

Holguin et al. (2007) reported a longitudinal study of respiratory symptoms, lung function and eNO in almost 200 asthmatic and non-asthmatic children aged 6–12 from Ciudad Juarez, Mexico. Mean outdoor levels of NO<sub>2</sub> at the children's schools were similar to ambient levels in some Canadian urban centres. There was no significant association of NO<sub>2</sub>, PM<sub>2.5</sub> or EC with cough, wheeze, or phlegm reported daily over 4 months (data not shown), though road density near the subjects' homes was associated with increased respiratory symptoms in asthmatics. The authors suggested that the study was somewhat underpowered, and noted that most of the asthmatics had a mild form of the disease. Rodriguez et al. (2007) studied respiratory symptoms reported in daily diaries for a birth cohort of 263 children from Perth, Australia, who had at least one parent with doctor-diagnosed clinical atopic disease. Relatively low levels of ambient NO<sub>2</sub> were associated with increased risks for cough, especially in same-day analyses (OR = 1.028; 95% CI 1.002, 1.055 per ppm 24-h avg NO<sub>2</sub>, and with non-significant risks for wheeze/chest rattle and increased body temperature. However, each of these symptoms was also related to other air pollutants, and only single-pollutant models were run. Finally, Martins et al. (2012) reported a small panel study from Viseu, Portugal, of respiratory effects (symptoms, lung function, eNO and pH of EBC) in schoolchildren with a history of wheezing. Respiratory symptoms during the previous 6 months were reported by parents at each clinic visit, while NO<sub>2</sub> was estimated using an inadequately described combination of active and passive monitoring, air quality modelling, and time-activity information. The risk of wheezing was significantly decreased in relation to increases of 10 µg/m<sup>3</sup> (5.32 ppb) in weekly personal exposure to NO<sub>2</sub> (-2.08%; 95% CI -3.59%, -0.58%) while risks for use of rescue medication (-0.01%; 95% CI -1.44%, 1.46%) and for respiratory ERVs (-0.02%; 95% CI -2.10%, 2.05%) were near null. These results are in apparent contrast to the study's findings of significant adverse effects on lung function (subsection 8.4.1.1).

Weinmayr et al. (2010) conducted a systematic global literature review and meta-analysis of studies of lung function and respiratory symptoms in asthmatic children in relation to NO<sub>2</sub> and

PM<sub>10</sub> air pollution (methods described in subsection 8.4.1.3). There were a total of 24 studies of NO<sub>2</sub>, spanning a range of mean 24-h NO<sub>2</sub> levels between 4.3 and 41.0 ppb. In combined estimates using random effects models, for an increase of 10 µg/m<sup>3</sup> (5.32 ppb) 24-h NO<sub>2</sub> for all possible lags, there was a significant increase in asthma symptoms across all studies (OR = 1.031; 95% CI 1.001, 1.062), in studies excluding the multi-centre PEACE studies (OR = 1.039; 95% CI 1.018, 1.061), and for trim and fill estimates accounting for publication bias (OR = 1.032; 95% CI 1.008, 1.057). However, there was no significant association with asthma symptoms in a sensitivity analysis restricted to lag 0–1 d (for all studies, OR = 0.997; 95% CI 0.984, 1.010). For the same increment of ambient NO<sub>2</sub>, there was no significant increase in cough (for all studies OR = 0.987; 95% CI 0.960, 1.014), except if the PEACE studies were excluded (OR = 1.031; 95% CI 1.005, 1.057). Conversely, there were fairly clear PM<sub>10</sub>-related increases in asthma symptoms and in cough in analyses from all studies combined and excluding the PEACE studies. In stratified analyses with lag 0–1 d, the risk of asthma symptoms was significantly increased in relation to NO<sub>2</sub> solely in summer-only studies (OR = 1.122; 95% CI 1.026, 1.227), and in studies of diagnosed asthmatics (OR = 1.073; 95% CI 0.999, 1.152), whereas risks were essentially null in studies conducted in other seasons or in children who just reported respiratory symptoms. There was significant between-study heterogeneity for both pollutants. The authors discussed concerns and presented limited evidence indicating that the entire PEACE study series (largely null) may have been influenced by an influenza epidemic early in the study period and by increased respiratory infections in general during the study period, given their short duration and their conduct solely in winter.

In recent studies that included adults, ambient NO<sub>2</sub> was not associated with respiratory symptoms in subjects with various pre-existing diseases, but was related to increased sales of asthma medications in two studies.

In a small daily diary study of 31 CHF patients in Montreal, QC, over 8 weeks, ambient NO<sub>2</sub> and other pollutants were not significantly related to self-reported shortness of breath at night (adjusted mean difference of 0.177; 95% CI -0.689, 1.044 per IQR of 16 µg/m<sup>3</sup> (8.5 ppb) in 24-h ambient NO<sub>2</sub>) (Goldberg et al., 2009). Similarly, in a panel study of 94 COPD patients from London, England, a 1 ppb increase of the 1-h max ambient NO<sub>2</sub> levels the previous day was not significantly associated with increases in sputum change (OR = 1.006; 95% CI 0.999, 1.013), dyspnea (OR = 1.003; 95% CI 0.997, 1.008), nasal discharge or congestion (OR = 0.999; 95% CI 0.991, 1.006), wheeze or tight chest (OR = 1.002; 95% CI 0.996, 1.009), or upper respiratory symptoms (OR = 0.999; 95% CI 0.990, 1.008), whereas PM<sub>10</sub> was related to some of these symptoms. In two-pollutant models, PM<sub>10</sub> relationships were robust while those of NO<sub>2</sub> were reduced to near null (Peacock et al., 2011). Finally, in a study of 137 patients with mild-to-moderate allergy to grass and/or olive pollen from two Spanish cities, the risk of asthma was not significantly related to ambient NO<sub>2</sub> (2.5%; 95% CI -3.1%, 8.4% in Ciudad Real) (2.4%; 95% CI -7.7%, 3.3% in Puertollano), but was related to PM<sub>10</sub>, SO<sub>2</sub> and O<sub>3</sub>, especially in the latter, more industrialized city (Feo Brito et al., 2007).

In a case-crossover study of subjects <40 years old in Strasbourg, France, OR for SABA sales to children, teens and young adults were significantly increased in relation to modelled daily NO<sub>2</sub> in census blocks at individual lags of 5–10 d (Laurent et al., 2009). An increase of 8.4% in SABA sales (95% CI 5%, 11.9%) was observed per 10 µg/m<sup>3</sup> (5.32 ppb) increase in 24-h NO<sub>2</sub>, lag 4–10 d avg (results were similar for PM<sub>10</sub> but not O<sub>3</sub>). Similarly, Carlsen et al. (2012) reported a time-series study of adults aged ≥18 in Reykjavik, Iceland, in which both 24-h and daily 1-h max NO<sub>2</sub> were associated with increased dispensing of any asthma drug and of adrenergic inhalants with lags of several days or more. Excess risks of 3.1% (95% CI 1.5%, 4.8%) and 1.6% (95% CI 1.0%, 2.2%) in dispensing of any asthma drug were found, respectively, with an increase of 10 µg/m<sup>3</sup> (5.32 ppb) in 24-h mean and 1-h peak NO<sub>2</sub> with the average of lag 12–14 d. Similar

results were calculated for adrenergic drugs. In both of these studies, however, increased asthma medication sales were also related to other pollutants, and only single-pollutant models were run (although in the study by Carlsen et al. (2012) the positive findings with other pollutants were generally for shorter lags than for NO<sub>2</sub>).

#### 8.4.1.5 Lung Host Defence and Immunity

The results of a small number of epidemiology studies have suggested that increases in exposure to ambient NO<sub>2</sub> can impair host defences in children, by showing associations with viral infections. Most of these are time-series studies of hospital admissions and ERVs (subsections 8.3.1.2 and 8.3.1.3). However, an earlier panel study of 114 asthmatic children aged 7–12 in England investigated whether personal exposure to NO<sub>2</sub> prior to proven respiratory viral infection increased the severity of asthma exacerbation (Chauhan et al., 2003). Viral infections, which are a major cause of asthma exacerbations in young children, were confirmed by analysis of nasal aspirates for a variety of respiratory-illness-causing viruses. Personal NO<sub>2</sub> exposure in the highest tertile (>7.4 ppb) the week before infection increased lower respiratory tract symptom scores for all virus types together (score increase 0.6; 95% CI 0.01, 1.18) and for respiratory syncytial virus alone (2.1; 95% CI 0.52, 3.81) compared with the lowest tertile (<4.0 ppb). The severity of PEF reduction was also more pronounced in the corresponding comparison for picornavirus, another common respiratory virus in children, with a reduction of 12 L/min (95% CI -23.6 L/min, -0.80 L/min). There was no significant change in these health measures with personal exposure to NO<sub>2</sub> in the week after infection. Hence, in this prospective study, personal exposure to NO<sub>2</sub> appeared to modify the risk of asthma exacerbation as a result of respiratory viral infection.

There is some support for these findings in the results of an earlier panel study of asthmatic children in Southern California (Delfino et al., 2002). In this small study, asthma symptom scores were only associated with ambient NO<sub>2</sub> in children not taking anti-inflammatory medication (OR = 1.91; 95% CI 1.07, 3.39 per 20 ppb 8-h max NO<sub>2</sub>, lag 0 d). However, the risk was increased several-fold during self-reported respiratory infections (OR = 6.72; 95% CI 1.73, 26.1). Pollutant exposures were not associated with risk of respiratory infections in this study, suggesting that the risk of infection onset was independent of the exposures, but the probability of an asthmatic response to respiratory infection was enhanced on high-pollution days.

### 8.4.2 Cardiovascular Effects

#### 8.4.2.1 Cardiovascular Function

In the 2008 US EPA ISA (US EPA, 2008), it was concluded that overall the evidence from epidemiological studies of NO<sub>2</sub>-related effects on functional cardiovascular endpoints (including HRV, arrhythmias, and repolarization changes) was inconsistent. Approximately six studies were identified examining the relation between NO<sub>2</sub> exposure and HRV, of which three reported an adverse effect, while the others reported no significant change. In addition, in two of the three studies reporting a significant change in HRV, the effect of PM was similar to that observed for NO<sub>2</sub>. In terms of ventricular arrhythmias, the studies described in the 2008 US EPA ISA also reported inconsistent results, and the strongest associations observed were for PM rather than for NO<sub>2</sub> (although their concentrations tended to be highly correlated). Only one study was identified involving an examination of the effect of NO<sub>2</sub> on repolarization abnormalities, in which no association was observed (US EPA, 2008).

One Canadian and four American panel studies of the relationship between NO<sub>2</sub> exposure and changes in HRV have been published since the 2008 US EPA ISA.

In a study conducted in Ottawa, ON, involving 42 healthy non-smoking adults (Weichenthal et al., 2011), the effect on HRV of cycling either in traffic (high- or low-traffic routes) or indoors for



1-h intervals was assessed by electrocardiogram (ECG). Levels of NO<sub>2</sub> at a central site monitor during the outdoor cycling periods were significantly associated with the ratio of low-frequency to high-frequency power ( $\beta$  for LF:HF = 1.4; 95% CI 0.35, 2.5 per 9 ppb 1-h post), which was stable with adjustment for other pollutants; this ratio is considered a reflection of the balance of sympathetic and parasympathetic modulation. In addition, NO<sub>2</sub> was also inversely associated with the standard deviation of normal-to-normal intervals ( $\beta$  for SDNN [a reflection of total power] = -10 msec; 95% CI -20 msec, -0.34 msec), and the association was reportedly not sensitive to adjustment for other pollutants. UFPs and O<sub>3</sub> were also inversely and robustly related to HRV, though to different measures.

Suh and Zanobetti (2010) observed a decrease in pNN50 (a measure of parasympathetic function) related to 24-h personal exposure to NO<sub>2</sub> (-11.03%; 95% CI -19.29%, -1.93%) per 6.83 ppb increase, lag 0 d) among a group of 30 non-smoking adults, with either a recent MI or COPD, in Atlanta, GA. The association was greatest in those with MI, and similar to what was observed for EC but weaker and less consistent. A significant association was also found with decreased HF, but only among subjects with a recent MI (data not shown). Interestingly, for NO<sub>2</sub> and EC there was an inverse association with the percentage change in rMSSD that was strongest for personal exposures, less for ambient concentrations, and near null for indoor and outdoor levels measured at subjects' residences. Percentage change in overall SDNN and the ratio of LF:HF were also not significantly related to either ambient or personal NO<sub>2</sub> levels. Only single-pollutant models were run.

In another study from Boston, MA, involving 46 patients with coronary artery disease (CAD), HF (an HRV measure of vagal tone) was significantly decreased in relation to NO<sub>2</sub> exposures for all averaging times measured (30 min–5 d) (e.g. -9.4%; 95% CI -14.1%, -4.4% per 0.006 ppm (6 ppb) increase, 5-d avg) (Zanobetti et al., 2010). This association remained robust to adjustment for PM<sub>2.5</sub>, but not BC, in two-pollutant models.

Conversely, while Laumbach et al. (2010) found an association between riding in the back seat of a vehicle for 90–110 min during rush hour in New Jersey and HRV (but not with heart rate or blood pressure) changes, this was not significant in relation to NO<sub>2</sub> (percentage change from pre-exposure to next day: HF, -9%; 95% CI -79%, 61%; LF:HF ratio, -81%; 95% CI -225%, 63%), or to any other pollutant alone. The authors noted that the study was a proof of concept undertaking and underpowered to adequately evaluate the associations between the individual pollutants and the outcomes measured, as it only involved single exposure sessions of 21 non-smoking adults with diabetes (mean age 61). In a study involving 5,465 subjects from six US communities participating in the Multi-Ethnic Study of Atherosclerosis cohort (Park et al., 2010), no significant relationship was observed between NO<sub>2</sub> exposure and unspecified HRV measures (data not shown), though the authors noted the ambient measures for gaseous pollutants used in this study may not be appropriate for the situation being evaluated. Significant associations were observed for PM<sub>2.5</sub> exposure, which became stronger with adjustment for NO<sub>2</sub> and CO.

One Mexican and one European panel study investigating air-pollution-related effects on cardiovascular function including HRV have been recently published. In a study conducted in Mexico City involving 16 researchers in a mobile laboratory van chasing diesel buses for approximately 9.5 h over 8 d, SDNN was significantly decreased in relation to NO<sub>2</sub> exposure ( $\beta$  = -3.90%;  $p$  = 0.0007 per IQR of 31 ppb in NO<sub>2</sub> for the 60-min window; significant decrements were also noted for the 5-min, 30-min and 90-min window periods), while the other functions measured (HF, LF, LF:HF) were non-significantly decreased. SDNN was also significantly related to O<sub>3</sub>, CO, CO<sub>2</sub>, and formaldehyde (the latter three were strongly correlated with NO<sub>2</sub>). PM<sub>2.5</sub> was also significantly associated with a number of HRV parameters measured (SDNN, HF, and LF) though in the opposite direction (i.e. an increase) of NO<sub>2</sub> (Shields et al., 2013). In a



study conducted in Aberdeen, Scotland, involving 132 CHF patients, personal NO<sub>2</sub> exposure was significantly related to a decrease in normalized LF, another measure of HRV ( $\beta = -2.452$ ; 95% CI -4.392, -0.511 per ppb, lag 0–2-d avg), but was not significantly associated with other HRV outcomes, heart rate, or various arrhythmias, and none of the endpoints was related to NO<sub>2</sub> at a central site (Barclay et al., 2009). Different particle metrics, most often particle number, were associated with changes in heart rate and in HRV measures, including a significant decrease in normalized LF.

Two Canadian and five additional American studies examined the effect of NO<sub>2</sub> exposure on cardiovascular function endpoints other than HRV.

In a study conducted in Montreal, QC, involving 31 subjects (aged 50–85) with CHF, oxygen saturation and pulse rate were measured each morning over a 2-month period (Goldberg et al., 2008). Non-significant associations between hourly measures of NO<sub>2</sub> and oxygen saturation (mean difference -0.021; 95% CI -0.137, 0.095 per 16  $\mu\text{g}/\text{m}^3$  (8.51 ppb), 3-d avg) were found but were largely eliminated when adjusted for personal and weather co-variables. Pulse rate was significantly increased in relation to NO<sub>2</sub> (0.375; 95% CI 0.008, 0.742, 1-d lag), though when personal and weather variables were incorporated, the CI widened to include zero. PM and especially SO<sub>2</sub> were more strongly related to both outcomes.

In an additional Canadian study, Dales et al. (2007) assessed the impact that sitting for 2-h intervals at two different Ottawa bus stops had on flow-mediated vasodilation (FMD), heart rate, and blood pressure among 39 healthy non-smoking adults aged 18–50. NO<sub>2</sub> concentration at the bus stop was related to an increase in diastolic blood pressure ( $\beta = 0.44$  mm Hg; 95% CI 0.15 mm Hg, 0.73 mm Hg per ppb 2-h NO<sub>2</sub>), but was not associated with a change in any of the other cardiovascular endpoints measured. An association between PM<sub>2.5</sub> and FMD was also observed.

In a study conducted in Detroit, MI, an increase in brachial artery diameter (0.0041 mm; 95% CI 0.0004 mm, 0.0078 mm per ppb personal 24-h NO<sub>2</sub>, lag 1 d) and FMD (0.072 mm; 95% CI 0.008 mm, 0.136 mm per ppb personal 24-h NO<sub>2</sub>, lag 1 d) was observed among 65 adults in relation to personal exposure to NO<sub>2</sub> (Williams et al., 2012). Same-day ambient NO<sub>2</sub> concentrations were associated with a significant decrease in heart rate (-0.0982 bpm;  $p = 0.0023$ ) per ppb 24-h NO<sub>2</sub>. Neither personal nor ambient NO<sub>2</sub> was related to blood pressure. However, at lag 2 d BAD was significantly associated with personal NO<sub>2</sub> in the opposite direction, and each of these endpoints was also significantly related to one or more PM<sub>2.5</sub> components.

Baja et al. (2010) found increases in ECG measurements of QTc (a marker of ventricular repolarization) in relation to NO<sub>2</sub> exposure among a cohort of 580 older men (mean age 74.8 years) in Boston (change in mean QTc = 2.56 msec; 95% CI -0.88 msec, 6.00 msec), though this was marginally non-significant. A similar though significant association was found for BC (none was found for PM<sub>2.5</sub>, SO<sub>2</sub> or O<sub>3</sub>). These relationships were increased for those with diabetes, the obese and never-smokers, though for NO<sub>2</sub>, none of these associations reached the statistically significant level. There was also evidence that an individual's genetic susceptibility to oxidative stress also increased the likelihood of an increased QTc response to air pollution.

In two additional US studies, the relationship between NO<sub>2</sub> exposure and cardiac ischemia was examined through measurement of ST-segment changes. In a study conducted in Boston involving 48 patients (aged 43–75) with CAD, ambient NO<sub>2</sub> was significantly associated with ST-segment depression, a non-specific marker of MI (RR = 1.51; 95% CI 1.23, 1.85 per 6.92 ppb increase in 24-h NO<sub>2</sub>) (Chuang et al., 2008). These results remained significant in a two-pollutant model with PM<sub>2.5</sub>. Associations were similar for BC. Delfino et al. (2011) also found a

relationship between NO<sub>2</sub> exposure and ST-segment depression in a study conducted in Los Angeles, CA. Among 38 older subjects (aged ≥65) with CAD, ambient NO<sub>2</sub> concentrations were significantly associated with ST-segment depression of ≥1 mm (OR = 3.83; 95% CI 1.20, 12.16 per 17.4 ppb hourly NO<sub>2</sub>, lag 3-d avg). Associations were also found with other markers of traffic exposure (BC, OC, PM<sub>2.5</sub> and CO), with the strongest relationship being measured for primary (but not secondary) OC.

Finally, Wellenius et al. (2007) observed no significant association between circulating levels of B-type natriuretic peptide (BNP) (levels associated with symptom severity in patients with heart failure and cardiac hemodynamics) and any air pollutant measured (including NO<sub>2</sub>) among 28 patients with chronic stable heart failure and impaired systolic function in Boston (data in graph). However, the authors concluded that due to considerable within-person variability, BNP measurements are unlikely to be useful in the study of air-pollution-related health effects.

In a unique study in Atlanta of 4,277 high-risk infants (<6 months of age) prescribed cardiorespiratory monitors, the relationship between NO<sub>2</sub> and apnea and bradycardia was assessed (Peel et al., 2011). NO<sub>2</sub> exposure was borderline significantly associated with bradycardia (OR = 1.025; 95% CI 1.000, 1.050 per 20 ppb daily 1-h max NO<sub>2</sub>, lag 0–1 d), but not apnea (OR = 1.011; 95% CI 0.972, 1.052) in this cohort. A similar result was observed with O<sub>3</sub>, although only the association with O<sub>3</sub> remained significant in a two-pollutant model with NO<sub>2</sub>.

With respect to European studies, Briet et al. (2007) found an increase in brachial artery FMD in relation to NO ( $\beta$  = -0.83; 95% CI -1.42, -0.24) per 10 µg/m<sup>3</sup> (8.15 ppb) increase in 24-h NO, 5-d mean), but not with NO<sub>2</sub> exposure ( $\beta$  = 0.64; 95% CI -0.26, 1.55) per 3 µg/m<sup>3</sup> (1.6 ppb) increase in 24-h NO<sub>2</sub>, 5-d mean), while NO<sub>2</sub> exposure was related to an increase in small artery reactive hyperemia ( $\beta$  = 13.8; 95% CI 3.6, 24 per 8 µg/m<sup>3</sup> (4.3 ppb) increase in 24-h NO<sub>2</sub>, 5-d mean) in 40 healthy non-smokers in Paris, France. SO<sub>2</sub> and CO were also associated with brachial artery FMD, while PM<sub>2.5</sub> and PM<sub>10</sub> were also associated with small artery reactive hyperemia; only single-pollutant models were run. Pekkanen et al. (2002) reported that ambient NO<sub>2</sub> was related to increased risk of exercise-induced ST-segment depression >0.1 mV (a marker of ischemia) among adults with stable CHD in Helsinki, Finland (OR = 2.02; 95% CI 1.34, 3.04 per 10 µg/m<sup>3</sup> (5.3 ppb) 24-h NO<sub>2</sub>, lag 2 d). However, this outcome was also related to a wide range of PM size fractions and to CO, and no analyses were conducted with NO<sub>2</sub> adjusted for other pollutants. Finally, Henneberger et al. (2005) found an association between QTc and NO<sub>2</sub> exposure among 56 males with IHD (4.47%; 95% CI 1.02%, 7.93% per IQR of 17.2 µg/m<sup>3</sup> (9.15 ppb) in NO<sub>2</sub> during the 6–11 h before the recording) in Erfurt, Germany. However, the risk estimates for an IQR change in OC and EC were greater than those for gaseous pollutants, including NO<sub>2</sub>.

#### 8.4.2.2 Biomarkers of Cardiovascular Disease Risk

In the 2008 US EPA ISA, epidemiological studies of the relationship between NO<sub>2</sub> exposure and markers of inflammation, cell adhesion, coagulation, and thrombosis were reviewed (US EPA, 2008). However, there were few studies and the dataset exhibited a significant level of inconsistency and somewhat conflicting results. For example, a study by Pekkanen et al. (2000) reported a significant increase in fibrinogen related to NO<sub>2</sub> exposure, while Steinvil et al. (2007) observed a significant decrease in the same measure. Moreover, while Schwartz (2001) reported an increase in fibrinogen associated with NO<sub>2</sub> in single-pollutant models, in two-pollutant models with PM<sub>10</sub> the opposite outcome was observed. Overall, the US EPA concluded that the associations between NO<sub>2</sub> exposure and the cardiovascular biomarkers measured appear to be significantly confounded by other traffic-related air pollutants (in particular, PM), with the majority of these studies providing stronger evidence for an effect of PM rather than for NO<sub>2</sub> (US EPA, 2008).

Since the 2008 US EPA ISA, three US studies have been published examining the association between NO<sub>2</sub> exposure and changes in biomarkers of CVD risk.

A panel study involving weekly measurements of 13 biomarkers of systemic inflammation over 12 weeks in 29 older adults (aged ≥65) with a history of CAD was conducted in Los Angeles, CA (Delfino et al., 2008b). Both interleukin-6 (IL-6) and tumour necrosis factor- $\alpha$  soluble receptor-II (TNF-RII) were significantly increased in relation to ambient NO<sub>2</sub> ( $\beta$  = 0.61 pg/mL; 95% CI 0.22 pg/mL, 1.01 pg/mL at lag 0 d and 288 pg/mL; 95% CI 9.7 pg/mL, 566 pg/mL at 9-d avg, respectively, per 13.87 ppb increase in hourly NO<sub>2</sub>), with C-reactive protein (CRP) being non-significantly increased. PM exposure was associated with changes in a greater number of the biomarkers assessed, and the authors concluded that the association with NO<sub>2</sub> was a result of particulate traffic emissions, specifically OC and quasi-UFPs.

In a follow-up study by the same group involving 60 older subjects (aged ≥65) from Los Angeles also with CAD, weekly measurements of biomarkers of inflammation and erythrocyte antioxidant activity were analyzed in relation to ambient levels of NO<sub>2</sub> and NO<sub>x</sub> over two 6-week periods (Delfino et al., 2009). Although no significant associations were found for NO<sub>2</sub> (data not shown), NO<sub>x</sub> exposure was related to significant increases in levels of the inflammatory markers IL-6 ( $\beta$  = 0.56 pg/mL; 95% CI 0.25 pg/mL, 0.87 pg/mL, 7-d avg per 41.6 ppb increase in hourly NO<sub>x</sub>), P-selectin (6.85 ng/mL; 95% CI 2.92 ng/mL, 10.78 ng/mL, 9-d avg), TNF-RII (251 pg/mL; 95% CI 62 pg/mL, 441 pg/mL, 9-d avg), and CRP (1081 ng/mL; 95% CI 371 ng/mL, 1791 ng/mL, 7-d avg). Other traffic-related pollutants, such as EC and CO, were also associated with an increase in these biomarkers of inflammation, as well as with decreased erythrocyte antioxidant enzyme activity.

In a panel study conducted by Bind et al. (2012) in Boston of 704 older men (mean age of 73.2 years), the relationship between NO<sub>2</sub> exposure and markers of inflammation, thrombosis, and endothelial dysfunction was assessed. In addition, the epigene–environment impact of the DNA methylation status of specific genes and intergenic regions on the NO<sub>2</sub> exposure effect was considered. NO<sub>2</sub>, particle number, BC, and CO were related to an increase in fibrinogen (procoagulant and pro-inflammatory) levels (4.5%; 95% CI 2.6%, 6.4% per 11 ppb increase in NO<sub>2</sub>, 3-d avg), as well as higher ICAM-1 and VCAM-1 (transmembrane proteins involved in endothelial transmigration of white blood cells in the inflammatory response) levels after intermediate-term (24-h) exposure (risks presented in figure only, no quantitative results reported). In terms of epigenetic effects, high DNA methylation of the *Alu* element and low methylation of the *F3* tissue factor were related to a greater increase in fibrinogen associated with NO<sub>2</sub> exposure, while low TLR-2 methylation was related to greater CRP levels, indicating that epigenetic states may affect susceptibility to air pollution.

An examination of the level of urinary 8-hydroxy-2'-deoxyguanosine ((8-OHdG), a marker of oxidative DNA damage) in relation to air pollutants among 320 older men (mean age of 76.7 years) in Boston, MA, was conducted by Ren et al. (2011). The amount of 8-OHdG excreted in urine was significantly increased for NO<sub>2</sub> moving averages up to 28 d, with a peak in the relationship for the IQR of the 21-d moving avg (32.2% increase in urinary 8-OHdG level; 95% CI 7.4%, 56.9%); similar associations were observed for O<sub>3</sub>, SO<sub>4</sub><sup>2-</sup>, and OC exposure (characterized as secondary pollutants) but not for CO, EC, and BC (characterized as primary pollutants).

A number of European studies also investigated the relationship between NO<sub>2</sub> exposure and levels of biomarkers related to inflammation and coagulation, specifically in regards to the cardiovascular system.

In a study conducted in the Lombardia region of Italy, NO<sub>2</sub>-related effects on blood coagulation were assessed in 1,218 healthy subjects (Baccarelli et al., 2007a). Prothrombin time (a measure

of time to the formation of the fibrin clot by interaction between tissue factor and activated coagulation factor VII) was significantly decreased ( $\beta = -0.08$ ; 95% CI -0.5, 0.00), in relation to ambient NO<sub>2</sub> levels averaged over the 30 d (and shorter averages) prior to the study (increment not given), whereas it was not associated with levels of activated partial thromboplastin time ( $\beta = 0.03$ ; 95% CI -0.04, 0.11), natural anticoagulants ( $\beta$  for protein C = -0.04; 95% CI -0.12, 0.03, for protein S = -0.08; 95% CI -0.14, 0.06, and for fibrinogen = -0.01, 95% CI -0.08, 0.005). PM<sub>10</sub> and CO were also related to a decrease in prothrombin time. The authors hypothesized that inflammation can induce tissue factor expression, generating a tendency for hypercoagulation. In an additional publication by the same group using the same cohort, no association was found between NO<sub>2</sub> exposure and total plasma homocysteine (0.0%; 95% CI -2.4%, 2.4% per an IQR increase of 20.1 ppb in 24-h NO<sub>2</sub>) (an increase of which is an indicator of inflammation, and an independent risk factor for vascular disease) (Baccarelli et al., 2007b). In a panel study conducted in Erfurt involving 38 male patients with COPD, no significant association was found between NO<sub>2</sub> and levels of fibrinogen or prothrombin fragment 1+2, but a decrease in von Willebrand factor in relation to NO<sub>2</sub> was observed (no quantitative results reported for NO<sub>2</sub>) (Hildebrandt et al., 2009). By contrast, particulate pollutant fractions were also associated with an increase in fibrinogen and E-selectin, and a decrease in prothrombin 1+2 and von Willebrand factor in this study.

In addition to cardiovascular function (subsection 8.4.2), Barclay et al. (2009) examined the association between ambient NO<sub>2</sub> and blood markers of endothelial activation, inflammation, and coagulation in a panel of 132 patients with CHF in Aberdeen. Exposure to 24-h ambient NO<sub>2</sub> was associated with an increase in IL-6 levels ( $\beta = 6.267$ ; 95% CI 0.594, 11.940 per ppb, lag 0 d), while 72-h personal NO<sub>2</sub> exposure was associated with a decrease in fibrinogen levels ( $\beta = -2.357$ ; 95% CI -3.900, -0.815 per ppb, lag 0–2 d avg); reduced fibrinogen was also associated with PM<sub>2.5</sub>. In a similar study, Rudez et al. (2009) found associations between ambient NO<sub>2</sub> exposure and an increase in platelet aggregation (5.6%; 95% CI 1.5%, 9.7% per IQR increase (not given) in NO<sub>2</sub> levels within 48–72 h before blood sampling) and thrombin generation (8.0%; 95% CI 2.4%, 13.6% per IQR increase (not given) in NO<sub>2</sub> levels within 24–48 h before blood sampling), but not with levels of fibrinogen or CRP, among a panel of 40 healthy volunteers in Rotterdam, the Netherlands. Findings for PM, CO, and NO exposure were similar, and the authors concluded that air pollution appeared to increase prothrombotic activity, with the potential to contribute to adverse cardiovascular events, though it had no clear effect on systemic inflammation.

In another study attempting to determine underlying mechanisms between air pollution exposure and inflammation, Bruske et al. (2011) found a significant association between increases in plasma-lipoprotein-associated phospholipase A2 (Lp-PLA2) concentrations and NO<sub>2</sub> at lags 4 d (2.62%; 95% CI 1.08%, 4.16% per IQR increase of 13.8 µg/m<sup>3</sup> (7.34 ppb) and 5 d (risk presented in figure only, no quantitative result reported for this lag) (with an inverse association at shorter time lags) in 200 patients with a previous MI. Oxidized low-density lipoprotein (LDL) is converted by Lp-PLA2 to oxidized free fatty acids and lysophosphatidylcholine, which triggers inflammation. Similar associations were also observed for PM<sub>10</sub>, PM<sub>2.5</sub>, particle number concentration, SO<sub>2</sub>, and O<sub>3</sub>.

Finally, Ljungman et al. (2009) examined the association between ambient NO<sub>2</sub> and plasma IL-6 levels, as well gene–environment interactions due to single nucleotide polymorphisms in IL-6 and fibrinogen genes, among a cohort of 955 MI survivors in six European cities. NO<sub>2</sub> was related to an increase in IL-6 levels (1.7%; 95% CI 0.2%, 3.4% per IQR of 15.9 µg/m<sup>3</sup> (8.5 ppb) in 24-h NO<sub>2</sub>, lag 0–1-d avg), although no significant gene–environment interactions were found for this pollutant. CO was also associated to a lesser degree overall; however, the association of



this pro-inflammatory biomarker with ambient CO was significantly modified by specific polymorphisms in both the IL-6 and fibrinogen genes.

### 8.4.3 Summary and Considerations—Panel and Related Studies

**Lung function:** In several earlier and more recent Canadian or American studies with some strong design features, there were small but significant NO<sub>2</sub>-related decrements in one or more measures of lung function in schoolchildren tested by spirometry: most often FEV<sub>1</sub> but also including such measures as FVC, PEF rate and FEF<sub>25–75%</sub> (US EPA, 2008; Figure 8.4). A number of these studies examined asthmatics, but NO<sub>2</sub>-related reductions in lung function were also reported in groups of subjects drawn from the general population. In asthmatic children, associations were often most pronounced with lags of a few days, though they were seen within a few hours in some studies, and were primarily limited to those not taking asthma control medications in those studies that stratified analyses on this basis (Figure 8.4). In a study of Californian schoolchildren with asthma, personal NO<sub>2</sub> was related to decrements in FEV<sub>1</sub> with a similar risk to that for central site NO<sub>2</sub> (Figure 8.4). However, lung function decrements were also associated with other pollutants in most of these studies, and the results of co-pollutant modelling (conducted in a limited subset of studies) were mixed. In some studies ambient NO<sub>2</sub> was independently associated with reductions in lung function, whereas in others the relation with NO<sub>2</sub> was attenuated and rendered non-significant by adjustment for other pollutants.

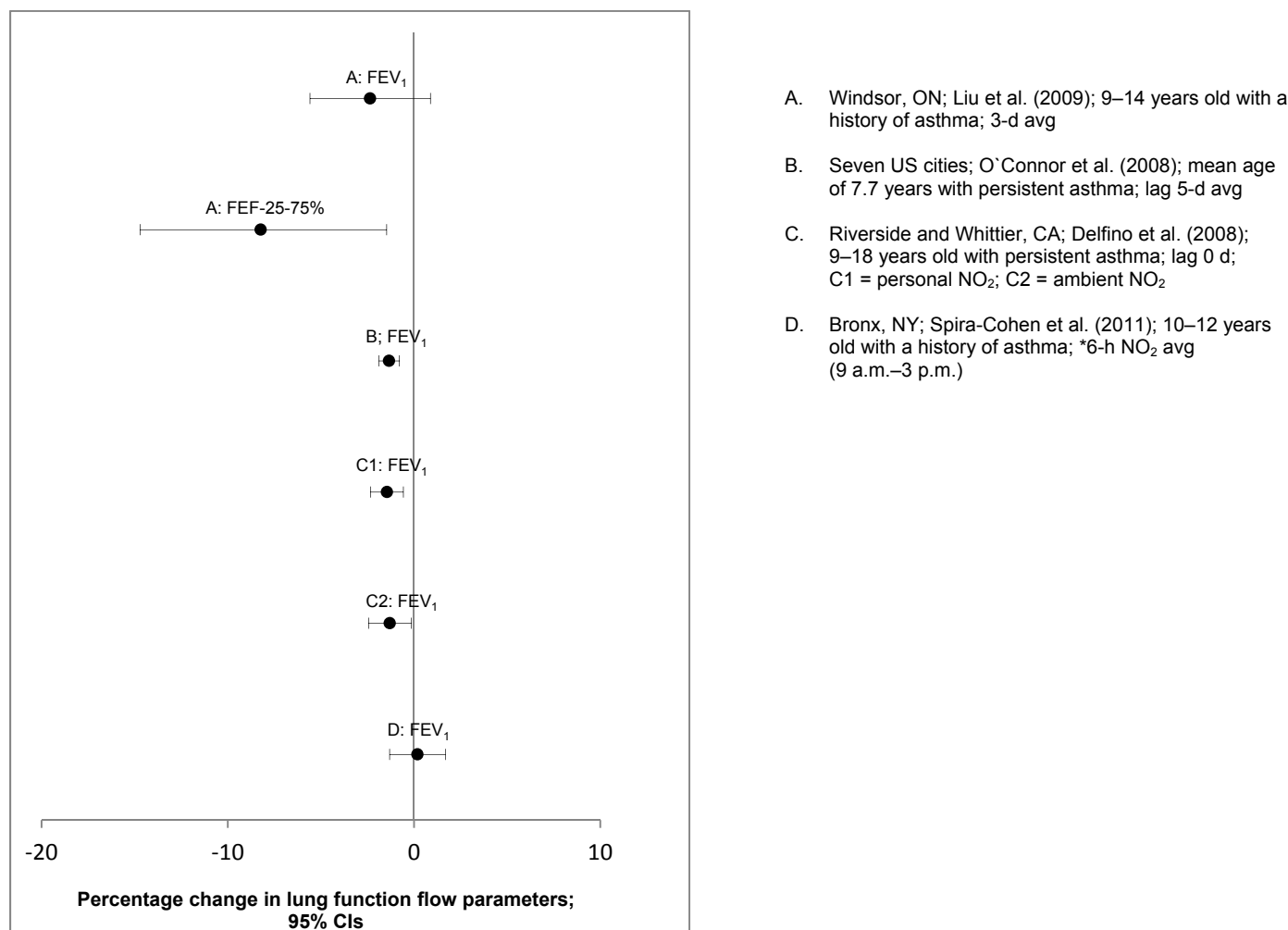
In most studies in adults, lung function was not related to ambient NO<sub>2</sub> or was not robust to adjustment for other pollutants, although there were positive findings in healthy adults in an earlier Swiss cross-sectional study and in a more recent Dutch scripted study, and in subjects with COPD and/or asthma in a small number of earlier US and European studies.

**AHR:** The results of experimental studies in animals and humans demonstrate that short-term exposure to NO<sub>2</sub> causes increased AHR in response to bronchial challenge. There has been little epidemiological study of this endpoint, though there is support for these findings in a recent investigation in which ambient NO<sub>2</sub> was related to increases in AHR in asthmatic children from Mexico City. The associations were limited to children not currently using ICSs and to those with mild intermittent asthma, and other pollutants were related to AHR with a different lag than for NO<sub>2</sub>, or not at all.

**Biomarkers of airway inflammation:** Following earlier reports of associations between personal and ambient NO<sub>2</sub> and increases in eNO, a number of recent studies have examined air-pollution-related effects on this and other biomarkers of airway inflammation and oxidative stress. Most of these have studied schoolchildren, including both asthmatic and non-asthmatic children. A number of these studies have reported NO<sub>2</sub>-related changes in these biomarkers, and while consistency is lacking in the findings for FeNO, there have been positive findings for other inflammatory biomarkers, including TBARS, isoprostane, IL-8, and pH of EBC. Associations are also apparent with other pollutants (most often PM) in most of these studies, and these were often more consistent than those with NO<sub>2</sub>, though few studies employed co-pollutant models. Findings were generally similar for asthmatics and non-asthmatics in these studies.

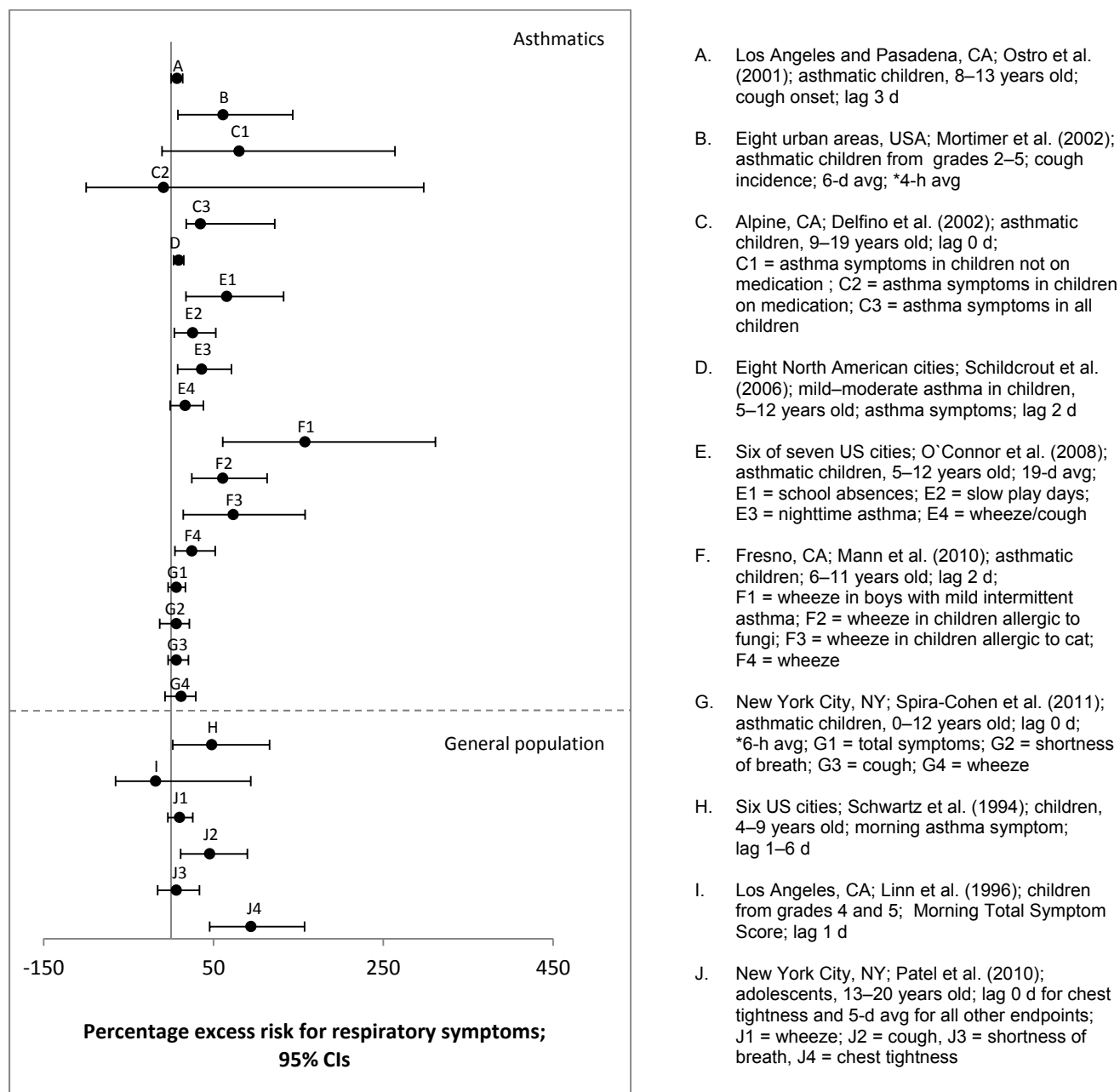


**Figure 8.4: Percentage change in lung function flow parameters in children and 95% CIs per standardized increment (20 ppb for 24-h avg) in single-pollutant models (\*unless otherwise noted) in Canadian and US studies**



**Respiratory symptoms and asthma medication use:** A number of epidemiological studies have investigated whether ambient NO<sub>2</sub> is related to risks for respiratory symptoms or asthma medication use, most often in panels of asthmatic schoolchildren. In most earlier and more recent Canadian and American studies, there were consistent and mostly significant associations between NO<sub>2</sub> concentrations and increases in asthma-related respiratory symptoms, including one or more of wheeze, cough, chest tightness, total asthma symptoms, slow play days, asthma-related school absences, and rescue medication use (Figure 8.5). In most of those studies that used co-pollutant models, the risks for NO<sub>2</sub> were not highly sensitive to adjustment for other pollutants, including traffic-related pollutants such as PM<sub>2.5</sub>, CO and UFPs in the small number of cases where these were investigated. In studies of panels of schoolchildren drawn from the general population, findings again highlighted effects in asthmatics, with increased risks between NO<sub>2</sub> and such asthma-related symptoms as cough, wheeze, shortness of breath, chest tightness and asthma medication use (Figure 8.5). In one study, NO<sub>2</sub>-related effects on wheeze and chest tightness were observed in asthmatic adolescents but not in non-asthmatics.

**Figure 8.5: Percentage excess in respiratory symptoms in children and 95% CIs per standardized increment (20 ppb for 24-h avg and 30 ppb for daily 1-h max, \*unless otherwise noted) in short-term NO<sub>2</sub> ambient concentration in single-pollutant models in Canadian and US studies**



In studies of both asthmatic and general population schoolchildren, respiratory symptoms were not generally associated with same-day NO<sub>2</sub>, but instead were most strongly related to averages of several days and/or with individual lags of a few days. In one study of asthmatic children from Fresno, CA, increased odds for wheeze were most pronounced in children allergic to cat or allergic to fungi, or in boys with mild intermittent asthma. These findings for allergic children may reflect a role for NO<sub>2</sub> in exacerbating allergic asthma. The interaction with mild intermittent asthma is likely because asthma control medications are not commonly used by patients with such a mild form of the condition; most studies that have stratified analyses by medication use have indicated that control medications provide protection against NO<sub>2</sub>-related symptoms.

With respect to studies in adults, the 2008 US EPA ISA noted that associations between NO<sub>2</sub> and respiratory symptoms or inhaler use were found in a number of studies, but not in all. In more recent studies reviewed for this assessment, ambient NO<sub>2</sub> was not associated with respiratory symptoms in subjects with various pre-existing diseases, but it was related to increased sales of asthma medications in both studies in which this endpoint was examined.

**Cardiovascular effects:** A number of panel studies investigated the association between NO<sub>2</sub> exposure and changes in cardiovascular function. Several studies reported NO<sub>2</sub>-related effects on decreases in HRV, although others observed no significant associations, and even among the positive findings the specific HRV measures affected varied between studies. In addition to HRV, there is some indication in the available studies that NO<sub>2</sub> exposure is associated with blood pressure changes, vasodilation, and ST-segment depression in adults, as well as with bradycardia in high-risk infants, though the dataset is small in size and the results are also somewhat inconsistent. In virtually all these studies the cardiac function endpoints were also related to other air pollutants, most often particulates or selected components thereof. In those studies that conducted co-pollutant models, the association with NO<sub>2</sub> was not robust to adjustment for other pollutants, with the exception of HRV changes in a Canadian study of urban cyclists, and ST-segment depression in a US study of CAD patients. Hence, although there is substantive evidence of an effect of air pollution on some aspects of cardiac function, to date there is little indication of an independent effect of NO<sub>2</sub>.

A number of panel studies assessed the impact of NO<sub>2</sub> exposure on biomarkers of CVD risk, specifically those related to inflammation and coagulation. In general, studies reported an association between ambient or personal NO<sub>2</sub> and increases in inflammatory (e.g. IL-6, TNF-RII, CRP, 8-OHdG) and coagulation (e.g. fibrinogen) biomarkers. In one study, the DNA methylation status of specific genes also resulted in an epigene–environment interaction affecting these associations. However, in virtually all these studies the potential confounding of NO<sub>2</sub>-related effects by other traffic-related pollutants was also apparent; these pollutants, most often PM and CO, were also associated with effects on biomarkers of CVD risk, and co-pollutant models were not reported in any of the studies.

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# 9. Epidemiological Studies of Health Effects Associated with Long-Term Exposure to Ambient NO<sub>2</sub>

## 9.1 Introduction

In this chapter, mortality and morbidity epidemiological studies related to long-term human exposure to ambient NO<sub>2</sub> are reviewed.

The effects of long-term exposure to ambient NO<sub>2</sub> have been mostly examined with prospective cohort studies. However, in contrast to the large number of studies on mortality and morbidity endpoints associated with daily variations in air pollutants, there have been relatively few that examine the health effects of long-term exposure to air pollutants. This is in large measure because of the technical difficulty in conducting methodologically sound, long-term epidemiological studies.

**Focus on North American studies:** As with the other categories of epidemiological data, mortality and morbidity studies associated with long-term NO<sub>2</sub> air pollution have been conducted all over the world. This chapter focuses on those studies that are considered to be particularly relevant to the risks associated with exposure to ambient NO<sub>2</sub> in Canada; therefore, for reasons discussed in detail in the introduction to Chapter 8, the focus will remain on studies conducted in North America. However, given the limited number of long-term epidemiological studies conducted in North America and the existence of a number of well-designed cohort studies performed in other OECD countries, particularly in Europe, this research is also taken into consideration. Some caution is necessary when interpreting the European studies, given the differences between some aspects of the European and North American sources (e.g. on-road fleets). Nonetheless, the European studies provide an important proportion of the database on long-term effects and illustrate some issues that are not highlighted in the North American database.

**2008 US EPA ISA as a starting point:** Consistent with other epidemiological chapters of this report that describe studies of ambient NO<sub>2</sub>, the US Environmental Protection Agency *Integrated Science Assessment for Oxides of Nitrogen—Health Criteria* (US EPA ISA) (US EPA, 2008) is used as the basis for summarizing the earlier literature. However, key individual references published prior to 2008 are cited where this is of utility in illustrating important issues, in order to retain this information as part of the overall weight of evidence.

**Standard exposure increments:** The long-term NO<sub>2</sub> exposure index most often employed is the annual mean concentration. In this report, consistent with the approach taken in the 2008 US EPA ISA, the mortality and morbidity risks associated with long-term exposure to NO<sub>2</sub> have been converted to the same exposure increment, in figures, for comparison purposes. A standard increment of 10 ppb, which approximates the annual average of NO<sub>2</sub> levels observed in Canada, was selected. The original risk estimates have been reported throughout the document.

## 9.2 Studies of Mortality

### 9.2.1 Summary of the 2008 US EPA ISA

Some epidemiological studies investigating the relationship between long-term exposure to NO<sub>2</sub> and mortality (all-cause, CP, respiratory, lung cancer) published since the release of the 1993 *Air Quality Criteria for Oxides of Nitrogen* document (AQCD) were discussed in the 2008 US EPA ISA. The relationship between mortality and long-term exposure to NO<sub>2</sub> had been studied in a limited number of US and European-based cohort studies, and results from these studies had generally not been consistent. US studies used ecologic exposure estimates, i.e. community-level exposure estimates, while European studies used more spatially resolved exposure estimates at the individual level. More accurate exposure estimates may have been obtained with the European approach, but additional sources of heterogeneity might also have been introduced, given different exposure assessment methods or the use of traffic variables or emission estimates incorporated in air dispersion models. While a weak or no association was reported in some studies, others found positive and significant associations. For comparison purposes, the US EPA only presented the converted risk estimates based on a standardized increment of 10 ppb; these are the ones reported in the next few paragraphs.

In the US cohort studies, which primarily focused on PM, NO<sub>2</sub> showed mostly weak or no associations with the mortality outcomes in both the American Cancer Society (ACS) study (Krewski et al., 2000; Pope et al., 2002) and the Washington University–Electric Power Research Institute (EPRI) Veterans' Cohort Mortality Study (Lipfert et al., 2000, 2003, 2006a, 2006b). In the reanalyzed Harvard Six Cities Study (Krewski et al., 2000) significant associations with NO<sub>2</sub> were, however, reported for both all-cause mortality (RR = 1.15; 95% CI 1.04, 1.27) and CP mortality (RR = 1.17; 95% CI 1.02, 1.34); a positive but non-significant association was reported with lung cancer (RR = 1.09; 95% CI 0.76, 1.57). Similar risk estimates were also reported for PM<sub>2.5</sub>, which was highly correlated with NO<sub>2</sub> (r = 0.78). No association between long-term exposures to NO<sub>2</sub> and all-cause, CP or respiratory mortality was observed in the Adventist Health and Smog study (AHSMOG) Californian cohort (Abbey et al., 1999) for either gender. By contrast, a significant association was observed for lung cancer in females (RR = 1.69; 95% CI 1.07, 2.65), but this association became negative in two-pollutant models with SO<sub>2</sub>.

In European studies, NO<sub>2</sub> was a significant predictor of both total mortality (RR = 1.21; 95% CI 1.03, 1.42) and CP mortality (RR = 1.72; 95% CI 1.28, 2.29) in the women's cohort living in North Rhine-Westphalia, Germany (Gehring et al., 2006). NO<sub>2</sub> and PM<sub>10</sub> were strongly correlated and PM<sub>10</sub> was also found to be associated with an increased mortality due to CP causes. A similar risk estimate (RR = 1.70; 95% CI 1.02, 2.81) was found between living within (versus beyond) a 50-m radius of a major road and cardiovascular mortality, while a positive but non-significant association (RR = 1.29; 95% CI 0.93, 1.78) was observed for total mortality. In the Netherlands Cohort Study (NLCS) on Diet and Cancer in subjects aged 55–69 (Hoek et al., 2002), a higher and significant risk estimate was found with the indicator of living near a major road (RR = 1.41; 95% CI 0.94, 2.12) as compared with NO<sub>2</sub> exposure (RR = 1.15; 95% CI 0.60, 2.23). A significant association between total mortality (RR = 1.16; 95% CI 1.12, 1.22) and increased estimated long-term exposure to NO<sub>x</sub>, based on a 10 ppb increase, was observed in a Norwegian male cohort (Nafstad et al., 2004) followed from 1972/1973 through 1998. Significant associations were also measured with respiratory (RR = 1.32; 95% CI 1.12, 1.54) and IHD mortality (RR = 1.16; 95% CI 1.06, 1.24) as well as with lung cancer mortality (RR = 1.22; 95% CI 1.06, 1.39). NO<sub>x</sub> and SO<sub>2</sub> levels were moderately correlated, and no associations were found with yearly SO<sub>2</sub> concentrations. Similar findings were also observed in a different cohort in Oslo, Norway (Naess et al., 2007), but the effect estimate was only significant in the

women aged 51–70 (HR = 1.23; 95% CI 1.10, 1.38), while a positive but non-significant association was found in the men's group (HR = 1.07; 95% CI 0.97, 1.18). Based on the quartiles of exposure, the effects appeared to increase at daily NO<sub>2</sub> levels above 21 ppb in the youngest men (aged 51–70); a linear dose–response relationship was observed for the oldest men (aged 71–90) for NO<sub>2</sub> daily levels between 10.6 and 32 ppb. The high correlation between NO<sub>2</sub> and the PM indices made the interpretation of the independent contribution of NO<sub>2</sub> difficult to determine. The study of seven French cities (Filleul et al., 2005), which used a design similar to the Harvard Six Cities and ACS studies, found no associations between NO<sub>2</sub> air pollution and mortality when monitors from all the 24 areas were included in the analysis. However, after exclusion of monitoring stations directly influenced by local traffic, significant associations were found with total (RR = 1.28; 95% CI 1.07, 1.55), CP (RR = 1.58; 95% CI 1.07, 2.33) and lung cancer (RR = 2.12; 95% CI 1.11, 4.03) mortality per 10 ppb increase in mean NO<sub>2</sub>. The authors suggested that the first approach, i.e. the inclusion of the air monitoring data from stations directly influenced by local traffic, might have overestimated the mean population exposure and biased the results. Long-term exposures to total suspended particles (TSP) were also associated with increased risks of both total and cardiovascular mortality, while SO<sub>2</sub>, TSP, BS, and NO were associated with lung cancer mortality.

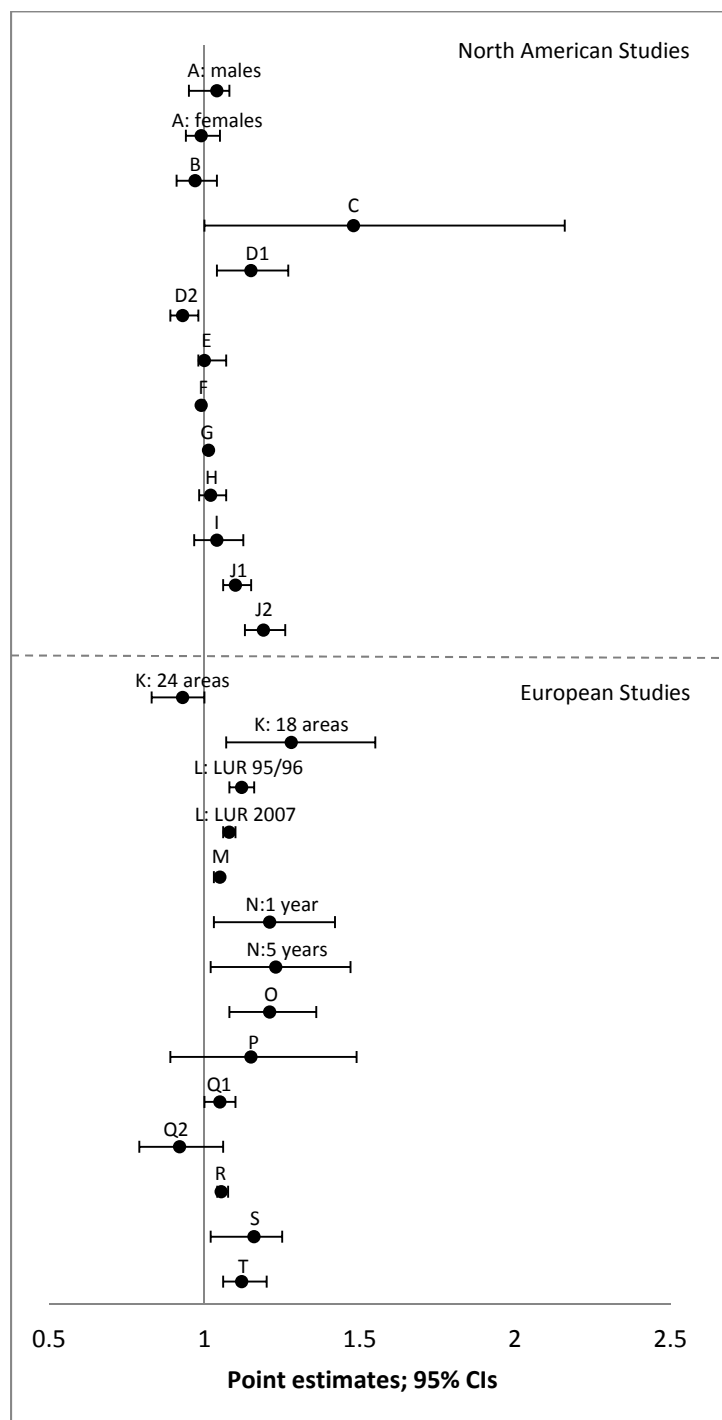
The weight of evidence from long-term exposure mortality studies available at the time of the 2008 US EPA ISA was difficult to assess because of fewer studies being available, uncertainty associated with high correlations between NO<sub>2</sub> and other air pollutants (mainly PM indicators), and uncertainties associated with exposure measurement error. The US EPA concluded at that time that the health database was inadequate to infer the presence or absence of a causal relationship between total mortality and long-term exposure to NO<sub>2</sub>.

### 9.2.2 Recent Mortality Studies

A total of 25 new chronic mortality studies published between 2008 and April 2013 have been reviewed in this assessment. Of these, 9 studies were conducted in North America, 14 were performed in different locations throughout Europe and 1 was undertaken in Australia. The studies were carried out under various climate conditions and approaches. All cohort and case-control studies except one (Rosenlund et al., 2009a) used Cox proportional hazard models for their statistical analysis. Most studies only used single-pollutant models, while a few (Jerrett et al., 2009; Lipfert et al., 2009; Gan et al., 2011; Hart et al., 2011; Raaschou-Nielsen et al., 2012; Cesaroni et al., 2013) incorporated some measures of traffic exposure/traffic air pollutants into co-pollutant models. A fully adjusted model, including PM<sub>2.5</sub> as a covariate, was also performed in England by Tonne and Wilkinson (2013). NO<sub>2</sub> was used in all studies except for two (Lipfert et al., 2009; Haining et al., 2010), where NO<sub>x</sub> was the pollutant used; another North American study (Lipsett et al., 2011) provided results for both NO<sub>x</sub> and NO<sub>2</sub> concentrations. A systematic review conducted by Chen et al. (2008a) was also published; major results from this study are presented in this section as well.

Relative risk estimates, based on 10 ppb annual NO<sub>2</sub> concentrations, for all-cause, respiratory and cardiovascular mortality from North American and European cohort studies are presented in Figures 9.1 to 9.3. Figure 9.4 presents lung cancer mortality RR estimates associated with long-term exposure to NO<sub>2</sub>.

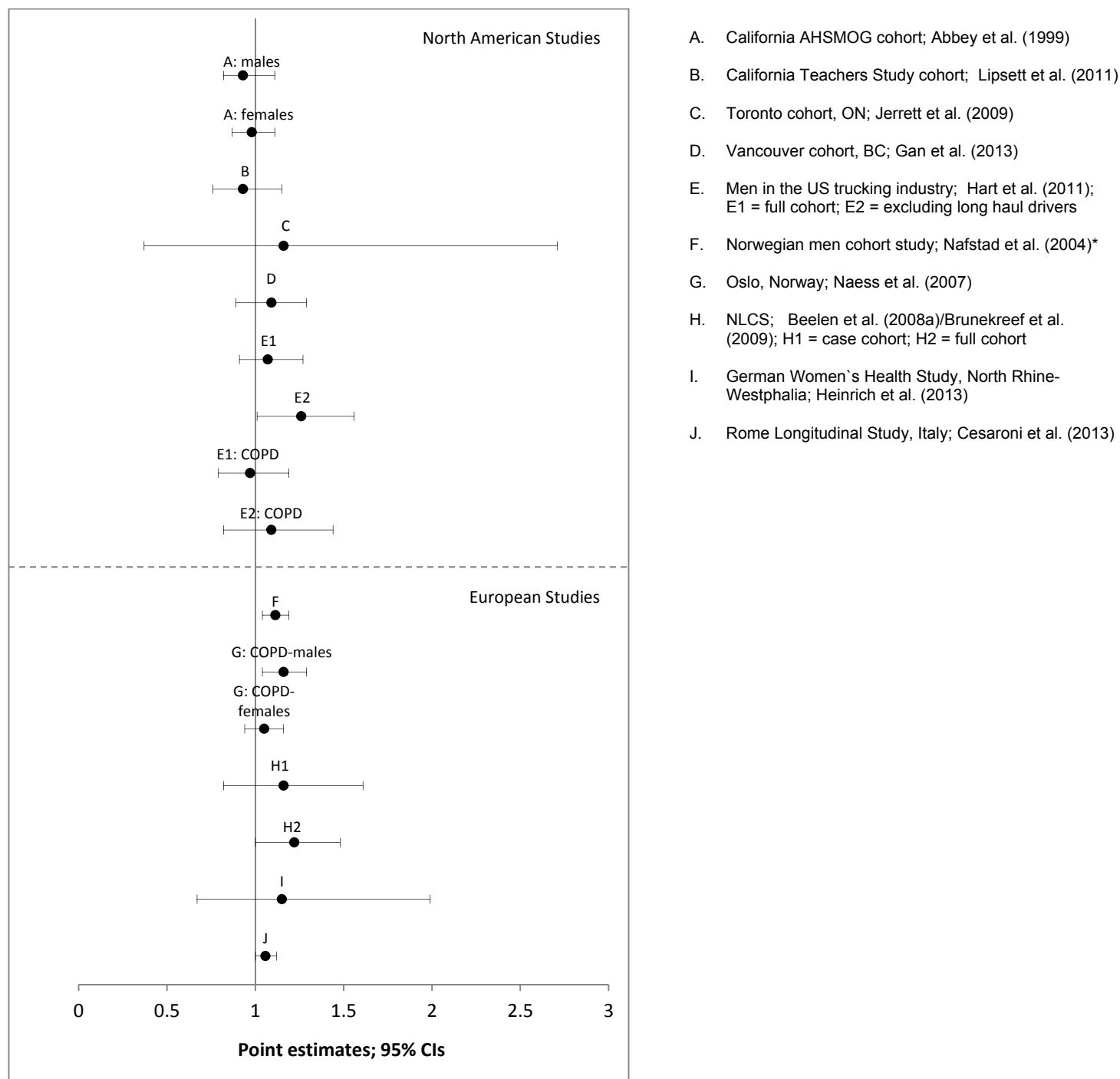
**Figure 9.1: Point estimates (RR or HR) and 95% CIs from cohort studies for risks of mortality from all causes per standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration in single-pollutant models**



- A. California AHSMOG cohort; Abbey et al. (1999)
- B. California Teachers Study cohort; Lipsett et al. (2011)
- C. Toronto cohort, ON; Jerrett et al. (2009)
- D. Harvard Six Cities and ACS study cohorts, USA: Krewski et al. (2000); D1 = Harvard Six Cities Cohort; D2 = ACS Cohort
- E. ACS cohort reanalysis, USA; Pope et al. (2002)
- F. ACS cohort extended reanalysis, USA; Kreswki et al. (2009)
- G. Military veterans' cohort, USA; Lipfert et al. (2009)\*
- H. Military veterans' cohort, USA; Lipfert et al. (2006a)
- I. Military veterans' cohort, USA; Lipfert et al. (2006b)
- J. Men in the US trucking industry, USA; Hart et al. (2011); J1 = full cohort; J2 = excluding long haul drivers
- K. Seven French cities, France; Filleul et al. (2005)
- L. Rome Longitudinal Study, Italy; Cesaroni et al. (2012)
- M. Rome Longitudinal Study, Italy; Cesaroni et al. (2013)
- N. German Women's Health Study, North Rhine-Westphalia; Gehring et al. (2006)
- O. German Women's Health Study, North Rhine-Westphalia; Heinrich et al. (2013)
- P. NLCS; Hoek et al. (2002)
- Q. NLCS; Beelen et al. (2008a)/Brunekreef et al. (2009); Q1 = full cohort; Q2 = case cohort
- R. Norwegian men cohort study; Nafstad et al. (2004)\*
- S. Danish cohort study; Raaschou-Nielsen et al. (2012)
- T. England and Wales; Tonne and Wilkinson (2013)

\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>. The risk ratios reported by Krewski et al. (2009), Lipsett et al. (2011) and Heinrich et al. (2013) were HRs; all others were RRs.

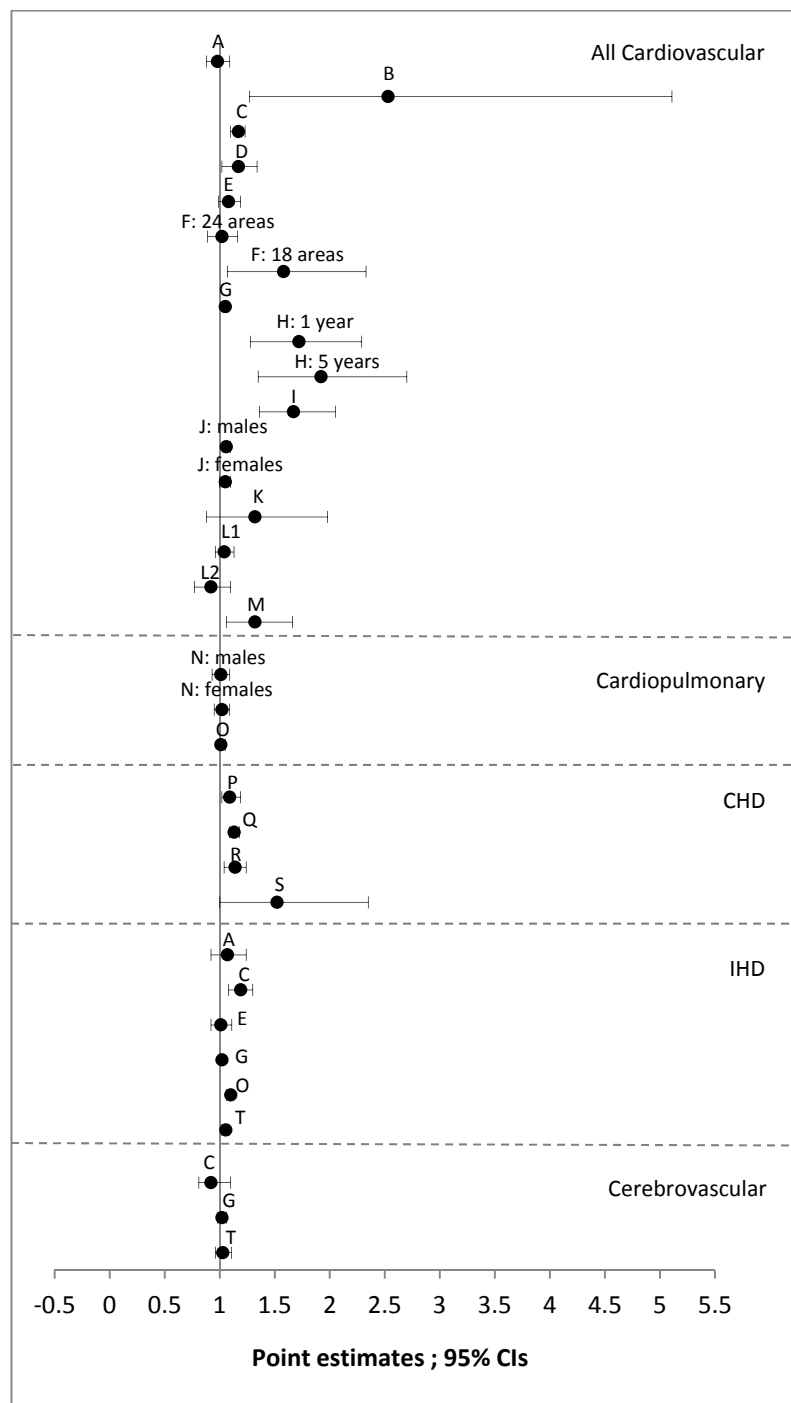
**Figure 9.2: Point estimates (RR or HR) and 95% CIs from cohort studies for risks of respiratory mortality (all respiratory diseases unless otherwise noted) per standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration in single-pollutant models**



\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>. The risk ratios reported by Lipsett et al. (2011) and Heinrich et al. (2013) were HRs; all others were RRs.



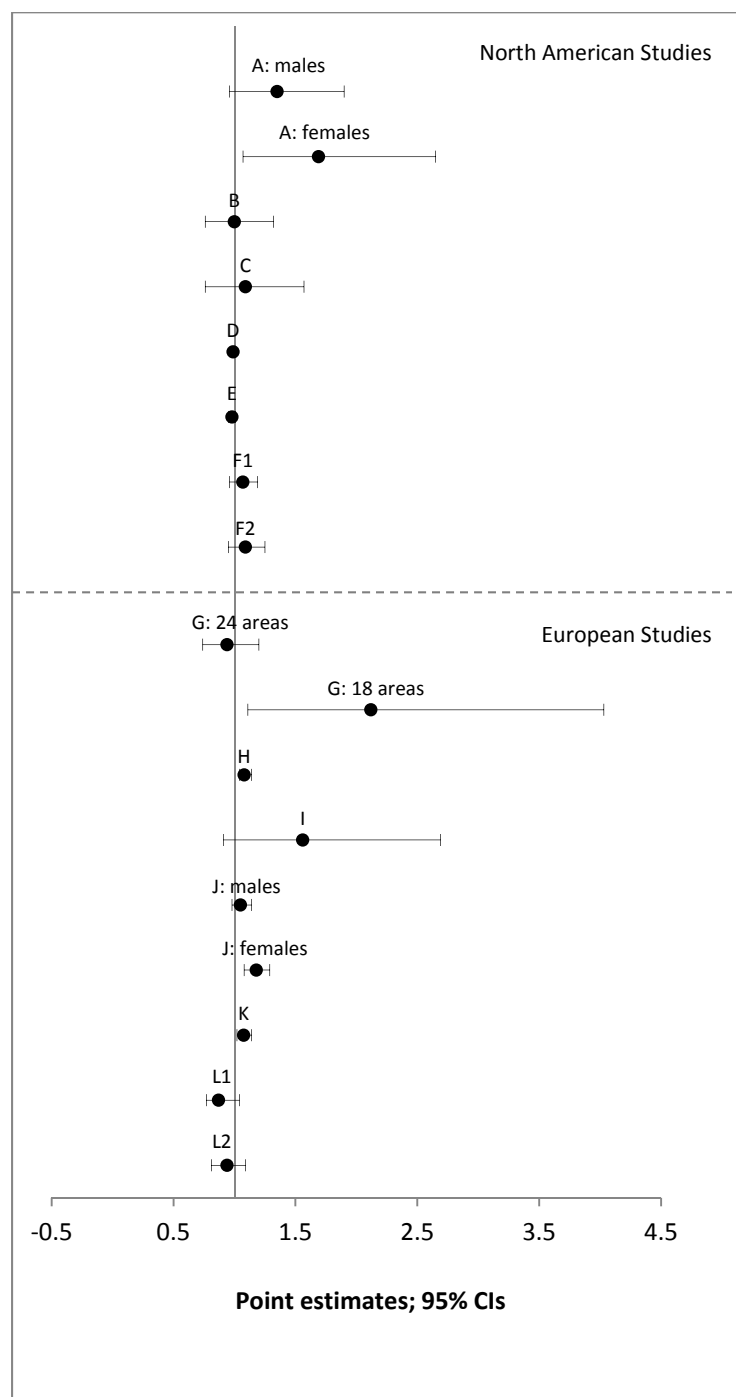
**Figure 9.3: Point estimates (RR or HR) and 95% CIs from cohort studies for risks of cardiovascular mortality per standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration in single-pollutant models**



- A. California Teachers Study cohort; Lipsett et al. (2011)
- B. Toronto cohort, ON; Jerrett et al. (2009)
- C. Toronto, Hamilton, Windsor, ON; Chen et al. (2013)
- D. Harvard Six Cities Study cohort, USA; Krewski et al. (2000)
- E. Men in the US trucking industry, USA; full cohort; Hart et al. (2011)
- F. Seven French cities, France; Filleul et al. (2005)
- G. Rome Longitudinal Study, Italy; Cesaroni et al. (2013)
- H. German Women's Health Study, North Rhine-Westphalia; Gehring et al. (2006)
- I. German Women's Health Study, North Rhine-Westphalia; Heinrich et al. (2013)
- J. Oslo, Norway; Naess et al. (2007)
- K. NLCS; Hoek et al. (2002)
- L. NLCS; Beelen et al. (2008a)/Brunekreef et al. (2009); L1 = full cohort; L2 = case cohort
- M. Danish cohort study; Raaschou-Nielsen et al. (2012)
- N. California AHSMOG cohort; Abbey et al. (1999)
- O. ACS cohort extended reanalysis, USA; Krewski et al. (2009)
- P. Vancouver cohort, BC; Gan et al. (2011); (mortality due to CHD)
- Q. Stockholm cohort, Sweden; Rosenlund et al. (2009a); (fatal MI)
- R. Rome, Italy; Rosenlund et al. (2008); (coronary mortality among survivors of first coronary event)
- S. Danish cohort study; Andersen et al. (2012) (fatal stroke (both ischemic and hemorrhagic heart diseases))
- T. Norwegian men cohort study; Nafstad et al. (2004)\*

\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>. The risk ratios reported by Krewski et al. (2009) and Lipsett et al. (2011) were HRs; all others were RRs.

**Figure 9.4: Point estimates (RR or HR) and 95% CIs from cohort studies for risks of lung cancer mortality per standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration in single-pollutant models**



- A. California AHSMOG cohort; Abbey et al. (1999)
- B. California Teachers Study cohort; Lipsett et al. (2011)
- C. Harvard Six Cities Study cohort, USA; Krewski et al. (2000)
- D. ACS cohort extended reanalysis, USA; Krewski et al. (2009)
- E. ACS cohort; USA; Pope et al. (2002)
- F. Men in the US trucking industry, USA; Hart et al. (2011); F1 = full cohort; F2 = excluding long haul drivers
- G. Seven French cities, France; Filleul et al. (2005)
- H. Rome Longitudinal Study, Italy; Cesaroni et al. (2013)
- I. German Women's Health Study, North Rhine-Westphalia; Heinrich et al. (2013)
- J. Oslo, Norway; Naess et al. (2007)
- K. Norwegian men cohort study; Nafstad et al. (2004)\*
- L. NLCS; Beelen et al. (2008b)/Brunekreef et al. (2009); L1 = case cohort; L2 = full cohort

\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>. The risk ratios reported by Lipsett et al. (2011) and Heinrich et al. (2013) were HRs; all others were RRs.

Chen et al. (2008a) provides a systematic review of all cohort and case-control studies published between 1950 and 2007 investigating the relationship between long-term exposure to ambient air pollution and the risks in adults for premature mortality. This review provides only a moderate indication of an effect of long-term NO<sub>2</sub> exposure on mortality. For NO<sub>2</sub> levels, based on an IQR increase of 8 ppb, a pooled RR of 1.00 (95% CI 0.99, 1.02) was found for all-cause mortality based on six studies (three from North America; three from Europe). Based on four studies (two from North America; two from Europe), positive but non-significant pooled RRs of 1.01 (95% CI 0.94, 1.09) and 1.02 (95% CI 0.98, 1.07) were found, respectively, for lung cancer and CP mortality. For respiratory mortality, a pooled RR of 1.01 (95% CI 0.94, 1.09) was found for NO<sub>2</sub>, based on only two European studies. Living close to traffic (distance to the nearest major road/local traffic density) was also associated with elevated, but non-significant, risks for all-cause, CP and lung cancer mortality. Long-term exposure to PM<sub>2.5</sub> was significantly related to all-cause, cardiovascular and lung cancer mortality, while long-term exposure to SO<sub>2</sub> was positively associated with lung cancer mortality. For many of the outcomes and air pollutants the review concluded that the information was insufficient to reach robust conclusions.

#### 9.2.2.1 Canadian and American Studies

Since 2008 only four Canadian studies (Jerrett et al., 2009; Gan et al., 2011, 2013; Chen et al., 2013) have examined the effects of long-term exposure to NO<sub>2</sub> on mortality outcomes. In Toronto, ON, long-term exposure to NO<sub>2</sub> levels obtained from LUR models ( $R^2 = 0.69$ ) was associated with mortality in 2,360 patients from a respiratory disease clinic (Jerrett et al., 2009). Positive and significant associations were observed between long-term exposure to NO<sub>2</sub> with both all-cause (RR = 1.17; 95% CI 1.00, 1.36) and circulatory mortality (RR = 1.45; 95% CI 1.10, 1.92) in single-pollutant models, based on a 4 ppb increase. After inclusion of traffic proximity in the model the RR was robust for circulatory mortality with an RR of 1.39 (95% CI 1.05, 1.85) but became non-significant for all-cause mortality (RR = 1.13; 95% CI 0.97, 1.32). The effect for traffic was attenuated but remained significant following adjustment for NO<sub>2</sub>. In sensitivity analyses, similar results were observed after adjustment for pre-existing conditions (diagnoses of COPD, IHD and diabetes). Non-significant associations were observed between long-term exposures to NO<sub>2</sub> and respiratory mortality, while results for lung cancer (data not shown) were inconclusive, and the authors noted that this might be due to the low number of cases ( $n = 35$ ). NO<sub>2</sub> was the principal focus of this study and no other air pollutants were analyzed.

A population-based cohort study of the relationship between long-term exposure to traffic-related air pollution and the risk of CHD mortality of all residents aged 45–85 (without previous diagnosis of CHD) who resided in Metropolitan Vancouver ( $n = 418,826$ ) was conducted by Gan et al. (2011). NO<sub>2</sub> annual average levels and other traffic-related air pollutants (TRAPs)—BC, PM<sub>2.5</sub> and NO—were estimated at residences of participants using LUR models ( $R^2 = 0.56$  for NO<sub>2</sub>). Long-term NO<sub>2</sub> concentrations, based on an IQR increase of 8.4 µg/m<sup>3</sup> (4.47 ppb), were significantly associated with CHD mortality in single-pollutant models (RR = 1.04; 95% CI 1.01, 1.08); the association was attenuated and became non-significant (RR = 1.03; 95% CI 0.99, 1.07) after the inclusion of PM<sub>2.5</sub> and BC in the models. A significant association was also observed with BC in a single-pollutant model; it remained significant in three-pollutant models including both PM<sub>2.5</sub> and NO<sub>2</sub>, while positive but non-significant associations were found for PM<sub>2.5</sub>. Moderate correlation coefficients were found between NO<sub>2</sub> and BC ( $r = 0.39$ ) and PM<sub>2.5</sub> ( $r = 0.47$ ). BC was strongly associated with increased risk of CHD mortality with a linear exposure–response relationship. An increased risk was also found between long-term exposure to NO<sub>2</sub> levels, obtained through LUR models, and mortality due to COPD (Gan et al., 2013), but only in the unadjusted single-pollutant models. An RR of 1.24 (95% CI 1.15, 1.33) was found per 8.4 µg/m<sup>3</sup> (4.47 ppb) increase in NO<sub>2</sub> levels. This association was attenuated (RR = 1.04;

95% CI 0.95, 1.12) and no longer significant following adjustment for covariates (age, sex and SES); only the association with BC remained significant.

The Ontario tax cohort is a retrospective study of approximately 600,000 residents of that Canadian province. Using single-pollutant LUR models for NO<sub>2</sub>, Chen et al. (2013) evaluated the relationship of mortality from CVD (1982–2004) in Toronto, Hamilton and Windsor within the cohort (n = 204,440 adults, aged 35–85). Positive and significant associations were found between the relatively low levels of NO<sub>2</sub> in these cities and cardiovascular mortality among adults living in the three cities between 1982 and 1986. Based on a 5 ppb increase, annual NO<sub>2</sub> levels, estimated by LUR models ( $R^2$  = 0.69, 0.76, and 0.77 for Toronto, Hamilton, and Windsor, respectively) were associated with all cardiovascular mortality (pooled RR of 1.08; 95% CI 1.05, 1.11) and IHD mortality (pooled RR of 1.09; 95% CI 1.04, 1.14), respectively. Stronger associations were found using long-term cumulative exposure estimates, although exposure at baseline provided nearly as good a proxy for long-term exposure. No associations were found with cerebrovascular mortality (RR = 0.96; 95% CI 0.90, 1.05). No other air pollutants were analyzed in the current study.

Five American studies (Krewski et al., 2009; Lipfert et al., 2009; McKean-Cowdin et al., 2009; Hart et al., 2011; Lipsett et al., 2011) using prospective cohort designs have also examined the relationship between long-term exposure to NO<sub>2</sub> and mortality.

The phase III reanalysis of the ACS cohort (Kreswski et al., 2009) extends the follow-up period for the ACS cohort from 1982 through 2000. This analysis used advancements in statistical methods and incorporated sophisticated controls for confounders and known sources of bias. Annual average NO<sub>2</sub> concentrations gathered from central monitoring stations were not found to be strongly associated with any cause-of-death category; IHD was the only outcome significantly associated with NO<sub>2</sub> exposure. Hazard ratios (HRs) for a 10 ppb incremental change in annual NO<sub>2</sub> concentration were, respectively, 0.99 (95% CI 0.99, 1.00), 1.01 (95% CI 1.00, 1.05), 1.02 (95% CI 1.00, 1.03) and 0.99 (95% CI 0.97, 1.01) for all-cause mortality, CP mortality, IHD and lung cancer. Using the random effect Cox model, which includes added control for ecological covariates, an increased HR of 1.035 (95% CI 1.017, 1.053) was found between NO<sub>2</sub> and IHD. PM<sub>2.5</sub> was strongly associated with several causes of mortality, including all-cause, cardiovascular, IHD and lung cancer; these results mainly corroborate earlier findings from this cohort.

In single-pollutant models, Lipfert et al. (2009) observed significant associations (RR = 1.076; 95% CI 1.061, 1.092) between all-cause mortality and increases of 19.5 ppb in NO<sub>x</sub> levels in the Washington University–EPRI veterans' cohort; a nationwide prospective cohort of 70,000 male US veterans recruited in 1975 for a hypertension study. Exposure estimates were developed using emissions inventories and atmospheric dispersion models. NO<sub>x</sub> was the metric used as a surrogate for NO<sub>2</sub> and was highly correlated ( $r$  = 0.84) with EC and to a lesser extent with traffic density ( $r$  = 0.56). A higher RR (1.18; 95% CI 1.16, 1.20) was observed for subjects living in the high traffic density counties; the risk was lower but still statistically significant for subjects living in low traffic density counties (RR = 1.03; 95% CI 1.01, 1.05). The association between NO<sub>x</sub> and all-cause mortality in the all-subjects group was reduced but remained significant (RR = 1.067;  $p$  < 0.05) when traffic density was included in the models; this observation was also true for several other air pollutants. The highest mortality risks were found with benzene, and strong associations were also found with diesel particulate matter (DPM). The authors concluded that selected TRAPs, including benzene, formaldehyde, DPM, NO<sub>x</sub> and EC, were better predictors of mortality than traffic density per se.

McKean-Crowdin et al. (2009) analyzed the association between brain cancer mortality and long-term exposures to ambient air pollution (PM<sub>10</sub>, PM<sub>2.5</sub>, TSP, SO<sub>2</sub>, NO<sub>2</sub>, CO and O<sub>3</sub>) for

participants in the ACS Cancer Prevention Study II, a large cohort of adult men and women from the US and Puerto Rico, in which a total of 1,284 brain cancer deaths were identified from 1982 to 2000. NO<sub>2</sub> levels in the area of each participant's residence, compiled from various central monitoring stations, were not associated with increased risk of mortality due to brain cancer; the RR was 0.88 (95% CI 0.81, 0.96) for a 10 ppb increase in NO<sub>2</sub>. No significant associations were found with any other air pollutants.

Significant associations were observed between NO<sub>2</sub> levels, estimated at residential addresses using models combining spatial smoothing of ambient monitors and geographic covariates (e.g. distance to roads, population density), and all-cause mortality in the American cohort of trucking industry employees (Hart et al., 2011). In single-pollutant models, based on an IQR increase of 8 ppb in NO<sub>2</sub> annual levels, an 8.2% increased risk (95% CI 4.5%, 12.1%) was found for all-cause mortality; this association was attenuated but remained robust (7.4%; 95% CI 2.4%, 12.5%) in three-pollutant models with PM<sub>10</sub> and SO<sub>2</sub>. A significant increased risk of 6.9% (95% CI 0.6%, 13.6%) was also found for cardiovascular mortality in single-pollutant models, but this association was no longer significant in the three-pollutant models. It should be noted that all three pollutants were highly correlated ( $r_s$  ranged from 0.64 to 0.98). Positive but non-significant associations were observed between NO<sub>2</sub> and increased risks for mortality from both lung cancer (5.5%; 95% CI -3.4%, 15.3%) and respiratory diseases (5.9%; 95% CI -7.4%, 21.1%) in single-pollutant models. Larger associations (and ones significant for respiratory causes) were found when long-haul drivers who, each week, spend one to three nights away from their home were excluded from the analysis. In this study, increased risks for all-cause mortality were also found for both PM<sub>2.5</sub> and PM<sub>10</sub>. Positive associations with cause-specific mortality (lung cancer, CVD and respiratory disease) were also observed for PM<sub>2.5</sub> and SO<sub>2</sub> but not PM<sub>10</sub>. In three-pollutant models, the most dramatic attenuations were observed for the associations with PM<sub>10</sub>, which were no longer significant after adjustment for NO<sub>2</sub> and SO<sub>2</sub>.

Another major prospective cohort study in the US is the California Teachers Study cohort initiated in the fall of 1985, which is made up of current and former public school professionals. Lipsett et al. (2011) examined air pollution–mortality relationships for women in this cohort (n = 124,614), where monthly average concentrations for PM<sub>10</sub>, PM<sub>2.5</sub>, NO<sub>2</sub>, NO<sub>x</sub>, CO and SO<sub>2</sub> were estimated from levels at fixed-site monitoring stations using IDW interpolation linked with geocoded residential addresses. Based on a 10.29 ppb increase, a positive association was found between exposure to NO<sub>2</sub> and IHD mortality (HR = 1.07; 95% CI 0.92, 1.25) while no association (HR = 1.00; 95% CI 0.75, 1.33) was noted for lung cancer. No associations were observed with all-cause mortality and mortality due to cerebrovascular disease. Some positive associations were also reported with both IHD mortality (HR = 1.25; 95% CI 1.00, 1.55) and all cardiovascular mortality (HR = 1.13; 95% CI 0.98, 1.31) based on NO<sub>x</sub> increases (49.31 ppb). The authors also reported an elevated and significant association between PM<sub>2.5</sub> and IHD mortality, as well as incident stroke, which combined fatal and nonfatal events. Overall it was concluded that PM<sub>2.5</sub>, PM<sub>10</sub> and NO<sub>x</sub> were associated with elevated risks for IHD mortality.

#### 9.2.2.2 European and Australian Studies

Fourteen studies of the effects of long-term exposure to NO<sub>2</sub> reviewed in this assessment were conducted in Europe. Several methodological approaches have been used, including population-based case-control studies (Rosenlund et al., 2008, 2009a); cohort studies (Beelen et al., 2008a/Brunekreef et al., 2009; Andersen et al., 2012a; Cesaroni et al., 2012, 2013; Raaschou-Nielsen et al., 2012; Heinrich et al., 2013; Tonne and Wilkinson, 2013), ecological studies (Janke et al., 2009; Haining et al., 2010; Maheswaran et al., 2012) and survival analysis (Maheswaran et al., 2010).



The German Women's Health Study cohort was used to investigate the effects of long-term exposure to air pollution on mortality. In an initial follow-up period (Gehring et al., 2006), discussed in the 2008 US EPA ISA, NO<sub>2</sub> was strongly associated with both all-cause and CP mortality. Results from the second follow-up period (2004–2008) (Heinrich et al., 2013) confirm the earlier findings; an increase of 16 µg/m<sup>3</sup> (8.51 ppb) in NO<sub>2</sub> levels, obtained from the air-monitoring station nearest to the subjects' residences, was significantly associated with increased risks in both all-cause (RR = 1.18; 95% CI 1.07, 1.30) and CP (RR = 1.55; 95% CI 1.30, 1.84) mortality. Similar results were observed with PM<sub>10</sub>. The risks for all-cause and CP mortality for both PM<sub>10</sub> and NO<sub>2</sub> were lower in the second follow-up period, during which lower PM<sub>10</sub> levels (but not NO<sub>2</sub> concentrations) were measured. With the extension period the impact of long-term exposure on more specific causes of mortality was examined; positive but non-significant relationships were found between NO<sub>2</sub> (HR = 1.46; 95% CI 0.92, 2.32) and lung cancer, while significant associations were noted with PM<sub>10</sub>. No associations were observed with respiratory mortality for either air pollutant. The authors also found a significant inverse relationship between distance of the home address to a major road (≤50 m) and both all-cause and CP mortality. Overall, the study concluded that each of long-term exposure to PM<sub>10</sub> and NO<sub>2</sub>, as well as living in close proximity to major roads, was associated with increased mortality risks.

Tonne and Wilkinson (2013) examined the relationship between long-term exposure to air pollution and premature all-cause mortality in England and Wales. Annual average background levels of NO<sub>x</sub> and NO<sub>2</sub>, estimated by dispersion modelling, were not associated with all-cause mortality in a cohort of survivors of hospital admissions for acute coronary syndrome after inclusion of PM<sub>2.5</sub>; following adjustment for fine particles, HRs of 1.01 (95% CI 0.98, 1.04) and 1.00 (95% CI 0.99, 1.02) were found, respectively, per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> and NO<sub>x</sub> levels, which were both highly correlated with PM<sub>2.5</sub> (r = 0.65 for each). However, PM<sub>2.5</sub> demonstrated significant associations with all-cause mortality.

The Rome Longitudinal Study is a closed prospective cohort of residents of this city enrolled in 2001. Cesaroni et al. (2012) selected 684,000 adults from this cohort for which NO<sub>2</sub> levels at residential address were estimated from LUR models for 1995/96 and 2007 (R<sup>2</sup> = 0.737 and 0.704, respectively). The adult residents were followed from 2001 to 2006 to study the association between all-cause mortality and concentrations of NO<sub>2</sub>, which was the only air pollutant studied. The RRs for all-cause mortality were 1.06 (95% CI 1.04, 1.08) in 1995/96 and 1.04 (95% CI 1.03, 1.05) in 2007 per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub>. In general the study found similar increased mortality risks in a large population with the use of LUR-based NO<sub>2</sub> exposures developed employing independent measurement data collected 12 years apart.

Recently, the same researchers (Cesaroni et al., 2013) used data from the Rome Longitudinal Study with 9 years of follow-up (October 2001–December 2010) in order to assess the roles of NO<sub>2</sub> and PM<sub>2.5</sub> with respect to cause-specific mortality. A total of 1,265,058 residents aged ≥30 who had resided in Rome for at least 5 years were included. Annual NO<sub>2</sub> concentrations were estimated for each residence with an LUR model (R<sup>2</sup> = 0.704) based on levels measured in 2007. Based on a 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub>, statistically significant associations were observed in single-pollutant models for several mortality outcomes: all-cause (RR = 1.03; 95% CI 1.02, 1.03), cardiovascular (RR = 1.03; 95% CI 1.02, 1.04), IHD (RR = 1.05; 95% CI 1.03, 1.06), respiratory (RR = 1.03; 95% CI 1.00, 1.06) and lung cancer (RR = 1.04; 95% CI 1.02, 1.07) mortality. A positive but non-significant association was found for mortality due to cerebrovascular disease, with an RR of 1.01 (95% CI 0.99, 1.03). Similar, but slightly stronger, associations were observed for PM<sub>2.5</sub>. Despite the high correlation between NO<sub>2</sub> and PM<sub>2.5</sub> (r = 0.79), using two-pollutant models the risk estimate between NO<sub>2</sub> and all-cause mortality was reduced but still statistically significant (RR = 1.02; 95% CI 1.01, 1.03), while that for PM<sub>2.5</sub> was

no longer significant. Two-pollutant models were not performed for the other mortality categories. The authors reported that no changes were observed in the risk estimates for NO<sub>2</sub> and PM<sub>2.5</sub> following the inclusion of proximity to high-traffic roads or traffic intensity in the model (data not shown).

Cesaroni et al. (2013) also examined the form of the concentration–response function for all-cause mortality and cause-specific mortality based on quintiles of exposure (Q1 = ≤36.5 µg/m<sup>3</sup> (≤19.41 ppb), Q2 = 36.5–42.7 µg/m<sup>3</sup> (19.41–22.71 ppb), Q3 = 42.7–46.2 µg/m<sup>3</sup> (22.71–24.57 ppb), Q4 = 46.2–50.4 µg/m<sup>3</sup> (24.57–26.81 ppb) and Q5 = >50.4 µg/m<sup>3</sup> (>26.81 ppb). Risk estimates increased across the quintile NO<sub>2</sub> distributions for several mortality outcomes: all-cause (*P* trend <0.001), cardiovascular (*P* trend <0.001), IHD (*P* trend <0.001) and lung cancer (*P* trend = 0.002) mortality. A non-significant *P* trend was found for mortality due to cerebrovascular or respiratory diseases. Statistically significant dose–response relationships were also observed between PM<sub>2.5</sub> and all-cause, respiratory, cardiovascular, IHD and cerebrovascular mortality. Based on a 20% random sample of the study population the concentration–response curves showed no evidence of deviation from linearity with the exception of the association between NO<sub>2</sub> and IHD mortality.

The effect of long-term exposure to air pollution on coronary mortality among survivors of a first coronary event was previously studied in Rome by Rosenlund et al. (2008) by following a cohort of survivors aged 35–84. NO<sub>2</sub> levels were estimated in the participant's census block residence by an LUR model (*R*<sup>2</sup> = 0.69). No other air pollutants were analyzed. After adjustment for age, sex and SES, an RR of 1.07 (95% CI 1.02, 1.12) was observed between a fatal coronary event within 28 d (*n* = 4,654 cases) and NO<sub>2</sub> levels, based on a 10 µg/m<sup>3</sup> (5.32 ppb) increase. RRs of 1.08 (95% CI 1.02, 1.13) and 1.05 (95% CI 0.97, 1.15) were found with out-of-hospital deaths and fatal hospitalizations, respectively.

Additional studies have analyzed the impact of long-term exposure to air pollution on cause-specific mortality. A case-control study performed by Rosenlund et al. (2009a) investigated the association between long-term air pollution exposure and fatal MI in individuals living in Stockholm County, Sweden, from 1985 to 1996 and aged 15–79 with a first MI. Air pollution levels were assessed by dispersion modelling at home addresses for both cases (*n* = 24,347) and controls (*n* = 276,926); a correlation coefficient of 0.96 was found between modelled and measured annual NO<sub>2</sub> levels in 1995. The relationship was analyzed with logistic regression and controlled for age, gender and calendar year. An adjusted OR of 1.23 (95% CI 1.15, 1.32) was found between the incidence of fatal MI and an increase of 31.6 µg/m<sup>3</sup> (16.81 ppb) in the NO<sub>2</sub> levels. Risk was much greater when the analysis was restricted to subjects who did not move between population censuses (OR = 2.54; 95% CI 1.96, 3.29). In addition, the OR for out-of-hospital death was significantly elevated (OR = 1.34; 95% CI 1.23, 1.46) as compared to in-hospital death (OR = 1.08; 95% CI 0.96, 1.20). Both CO and PM<sub>10</sub> were also strongly associated with out-of-hospital death.

Recently, the members (*n* = 52,061; aged 50–64) of a Danish cohort living in the Copenhagen and Aarhus, Denmark, areas who were enrolled between 1993 and 1997 were followed through 2009 to investigate the impact of traffic-related air pollution on stroke mortality (Andersen et al., 2012a). NO<sub>2</sub> levels were estimated at the residence of each cohort participant since 1971 using dispersion modelling; NO<sub>2</sub> was the only air pollutant included in the current study. A significant association (RR = 1.22; 95% CI 1.00, 1.50) between NO<sub>2</sub> and fatal stroke (stroke hospitalization followed by death within 30 d) was observed based on an 8.9 µg/m<sup>3</sup> (4.73 ppb) increase in NO<sub>2</sub> levels. The exposure–response relationship between NO<sub>2</sub> and fatal stroke was judged to be linear. Stronger associations were observed for non-specified and ischemic strokes, while no association was reported for hemorrhagic strokes. Several sensitivity analyses were also conducted and larger risk estimates were found in current smokers, in people ≥56 years old, in

people with <8 years of education and in people suffering from asthma. After adjustment for potential confounders including traffic noise, Raaschou-Nielsen et al. (2012) reported significant associations between long-term exposure to NO<sub>2</sub> and mortality in the same Danish cohort. Long-term exposure to NO<sub>2</sub> based on a 10 µg/m<sup>3</sup> (5.32 ppb) increase (without log-transformation of NO<sub>2</sub> data) was linked with both all-cause (RR = 1.08; 95% CI 1.01, 1.14) and cardiovascular (RR = 1.16; 95% CI 1.03, 1.31) mortality after adjustment for road traffic noise at the baseline address using state-of-the art exposure models. NO<sub>2</sub> was highly correlated with traffic noise (r = 0.64). Positive but non-significant associations were found with IHD and cerebrovascular mortality. Sensitivity analyses revealed stronger associations with both all-cause and cardiovascular mortality in people with a low consumption of fruits and vegetables. No other air pollutants were analyzed.

Using participants (n = 117,528 subjects) from the ongoing NLCS, Brunekreef et al (Brunekreef et al., 2009/Beelen et al., 2008a) investigated the impact of traffic-related air pollutants (BS, NO<sub>2</sub>, SO<sub>2</sub> and PM<sub>2.5</sub>) and traffic variables on mortality. The authors expanded the work of the pilot study conducted in the Netherlands a few years previously (Hoek et al., 2002), which used only a sub-cohort of 5000 individuals randomly selected from the large cohort of older men and women. The current study used all the participants of the NLCS cohort as well as improved exposure assessment methods. Long-term exposure to ambient air pollution at the 1986 residential address was estimated as the sum of regional (IDW interpolation of levels), urban (regression models) and local traffic contributions (traffic variables estimated with a geographic information system (GIS)). The correlations among the different air pollutants were high (all > 0.8, except for SO<sub>2</sub> (>0.6)). In the full cohort analyses, an increase of 30 µg/m<sup>3</sup> (15.96 ppb) in NO<sub>2</sub> concentrations was significantly associated with mortality from both all natural causes (RR = 1.07; 95% CI 1.00, 1.16) and respiratory causes (RR = 1.37; 95% CI 1.00, 1.87). Positive but non-significant associations were found with cardiovascular mortality (RR = 1.07; 95% CI 0.94, 1.21), while a negative association was found for lung cancer (RR = 0.91; 95% CI 0.72, 1.15). Traffic intensity on nearest road was also significantly and independently associated with natural mortality after inclusion of background concentrations of BS in the model, while only positive associations were found with PM<sub>2.5</sub>. No significant associations were found between air pollution and mortality in case-cohort analyses; the authors argued that the results from the full cohort, which provided much more statistical power, were likely less biased than those from the case-cohort.

Some ecological studies (Janke et al., 2009; Haining et al., 2010; Maheswaran et al., 2012) that were conducted in the UK found inconsistent results between long-term exposure to modelled NO<sub>2</sub>/NO<sub>x</sub> levels and mortality. In England, PM<sub>10</sub> and O<sub>3</sub> were mainly associated with higher mortality rates; a significant association was, however, observed between all-cause mortality and NO<sub>2</sub> in the older adult group (aged >75) (Janke et al., 2009). In Sheffield a trend of increasing stroke mortality was observed with increasing quintile of NO<sub>x</sub> levels (Haining et al., 2010) while in London, Maheswaran et al. (2012) found no significant associations between NO<sub>2</sub> concentrations and fatal hemorrhagic or ischemic strokes. A positive but non-significant rate ratio (RR = 1.17; 95% CI 0.51, 2.70) was, however, noted for ischemic fatal stroke per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> levels; positive associations were also observed with PM<sub>10</sub>. A survival analysis also performed in London by Maheswaran et al. (2010) found that survival after stroke was lower among patients living in areas with higher ambient air pollution.

An Australian study (Wang et al., 2009) found no significant association (RR = 0.99; 95% CI 0.97, 1.02) between a 1 ppb increase in annual NO<sub>2</sub> levels, estimated by IDW method, and cardiorespiratory mortality to residents of Brisbane in both single and three-pollutant models with GEE models. The increased risk posed by SO<sub>2</sub> was higher than those reported for NO<sub>2</sub> or O<sub>3</sub> and remained significant in three-pollutant models with the other gaseous pollutants.

### 9.2.3 Summary and Considerations—Mortality Studies

While studies of the health effects of long-term exposure to air pollution are generally more complex to conduct than studies on daily variations in air pollutants, there is an increasing database that examines the consequences of long-term exposure to NO<sub>2</sub> and other air pollutants. It is noteworthy that a number of authors used NO<sub>2</sub>, NO<sub>x</sub> and/or NO as markers of the traffic air pollution mixture, not specifically attributing the effects observed to NO<sub>2</sub> per se. The independent relation of NO<sub>2</sub> to mortality has not been widely characterized in these epidemiological studies, given the high collinearity among the various air pollutants, and uncertainty remains with respect to possible confounding by co-pollutants. Most studies utilized single-pollutant models. In studies that included co-pollutant analyses (with traffic indicators, PM indices and/or BC) the results were somewhat inconsistent, though the effects of NO<sub>2</sub>, which were mostly attenuated, often remained significant or at least presented some evidence of association with adverse outcomes.

At the time of the 2008 US EPA ISA, the US and European cohort studies investigating the association between long-term exposure to NO<sub>2</sub> and mortality showed generally inconsistent results; when associations were observed, these results were difficult to interpret given the high correlation with other pollutants, especially measures of PM. In the North American dataset, neither the extended ACS study nor the AHSMOG study conducted in California found associations between ambient measured NO<sub>2</sub> and all-cause mortality, while a significant association was reported in the reanalyzed Harvard Six Cities Study. Cohort studies from Europe generally observed associations between NO<sub>2</sub> and mortality.

Since 2008, only five updated or new cohort studies have examined the effect of long-term exposure to NO<sub>2</sub> on all-cause mortality in North America (Figure 9.1). These newer findings, similar to previous results, provide inconsistent evidence of a role for this air pollutant. Generally null associations were observed between NO<sub>2</sub> and all-cause mortality in the extended analyses from the ACS cohort and the CTS cohort, while the extended and most recent analyses from the Washington University–EPRI veterans' cohort demonstrated an association between NO<sub>x</sub> and all-cause mortality, with stronger associations being observed for subjects living in counties with higher traffic density. A new American cohort of trucking industry employees also demonstrated significant associations between long-term exposure to NO<sub>2</sub> and premature mortality, though this association was attenuated but remained significant in three-pollutant models with PM<sub>10</sub> and SO<sub>2</sub>. Positive associations were also observed in the Toronto cohort, although these effects did not persist after the inclusion of traffic proximity in the model; this traffic indicator is, however, an important determinant of NO<sub>2</sub> exposure, and hence will potentially confound the findings.

A larger number of cohort studies have been performed in Europe and have mostly found positive and significant associations between long-term exposure to NO<sub>2</sub> and all-cause mortality (Figure 9.1). Moreover, several studies have observed similar risk estimates (Gehring et al., 2006; Beelen et al., 2008a/Bruneekreef et al., 2009; Cesaroni et al., 2012, 2013). Annual ambient concentrations of NO<sub>2</sub> (8.99–24.15 ppb) observed in the European studies reporting significant associations were relevant to those in Canada. Most of the European cohort studies estimated exposure based on more spatially resolved exposure estimates at the individual level, whereas community-level air pollution estimates are typically used in the US studies. More accurate exposure estimates may have been obtained with the European approach, but additional sources of heterogeneity might have also been introduced, given different exposure assessment methods or the use of traffic variables or emission estimates incorporated in air dispersion models (Section 4.6). An important and large prospective cohort study, the Rome Longitudinal Study, also found independent effects of NO<sub>2</sub> with respect to all-cause mortality; despite the high correlation between NO<sub>2</sub> and PM<sub>2.5</sub> the risk estimate between NO<sub>2</sub> and all-cause mortality was reduced but still statistically significant while no longer significant for PM<sub>2.5</sub>.



A small number of studies, including the Toronto cohort and some analyses of American and European cohorts, have explored the association between long-term exposure to NO<sub>2</sub> air pollution and mortality due to respiratory diseases. These studies generally found weak and inconsistent associations, as demonstrated in Figure 9.2. There was evidence of an effect of NO<sub>2</sub>/NO<sub>x</sub> on respiratory disease mortality in some European studies (Nafstad et al., 2004; Beelen et al., 2008a/Brunekreef et al., 2009; Cesaroni et al., 2013), though correlations among the different air pollutants were often high, complicating the interpretation of these results.

Cardiovascular mortality has also been studied in the reviewed literature, with a number of more specific cardiovascular outcomes being examined. Positive, and for the most part significant, associations have been observed between long-term exposure to ambient NO<sub>2</sub> and mortality due to all CVDs. As illustrated in Figure 9.3 risk estimates were, however, quite variable between studies. Strong NO<sub>2</sub>-related cardiovascular mortality risks have been observed in several cohorts from Canada, where NO<sub>2</sub> levels are low compared with other parts of North America and Europe. In Vancouver, where moderate correlations were observed between NO<sub>2</sub> and both PM<sub>2.5</sub> and BC, the association between NO<sub>2</sub> and CHD mortality was no longer significant following inclusion of these pollutants in three-pollutant models. It should be noted that the phase III reanalysis of the ACS cohort, which extended the follow-up period and used advancements in statistical methods, found significant associations between annual average NO<sub>2</sub> concentrations gathered from central monitoring stations and both CP and IHD mortality. Significant associations have also been found in several European cohorts where new published studies confirmed earlier findings.

Several cohort studies conducted in North America and in Europe showed positive associations between long-term NO<sub>2</sub> exposure and increased mortality due to cancer, but most of these associations were not significant (Figure 9.4). The only significant associations observed were in the AHSMOG females group, where robust associations were found only with ambient SO<sub>2</sub> concentrations in two-pollutant models, and in the Norwegian men cohort study where NO<sub>x</sub>, instead of NO<sub>2</sub>, was the exposure metric and where the authors concluded that this result might represent the combined effects of traffic-related air pollutants. A significant association was observed in adult participants in the Pollution atmosphérique et Affections respiratoires chroniques (PAARC) survey, who resided in seven French cities, after exclusion of monitoring stations directly influenced by local traffic. The phase III reanalysis of the ACS cohort found no association between long-term exposure to NO<sub>2</sub> and lung cancer; instead, long-term exposure to PM<sub>2.5</sub> ambient levels was found to be an important risk factor for death from lung cancer. Similarly, no associations were reported between ambient NO<sub>2</sub> and lung cancer in Europe with the large NLCS cohort. Overall, inconsistent associations were observed between NO<sub>2</sub> and lung cancer mortality among a limited number of studies.

Two recent studies have investigated the shape of the concentration–response relationship between long-term exposures to NO<sub>2</sub> and mortality outcomes. Statistically significant dose–response relationships were observed between NO<sub>2</sub> concentrations and several outcomes, including all-cause, cardiovascular, IHD and lung cancer mortality in the Rome Longitudinal Study, a population-based cohort with more than 1 million subjects. There was no evidence of deviation from linearity of the effects of either NO<sub>2</sub> or PM<sub>2.5</sub> on mortality, with the only exception being the association between NO<sub>2</sub> exposure and IHD mortality, which was initially near-linear but levelled off at higher concentrations. The shape of the C–R relationship for all-cause mortality and cause-specific mortality (cardiovascular, COPD and lung cancer) also appeared to be linear with NO<sub>2</sub>, PM<sub>2.5</sub> and PM<sub>10</sub> in a study population of men from Oslo, Norway (Naess et al., 2007).

Overall, the database on mortality from long-term exposure to NO<sub>2</sub>/NO<sub>x</sub> air pollution has been greatly enhanced over the past few years, with new analyses and reanalyses of existing cohort



data indicating potential impacts on public health. PM continues to be an important air pollutant, however, explaining the long-term effects of air pollution on premature mortality in several of these studies. Given the multicollinearity between air pollutants, results from co-pollutant models are difficult to interpret. Furthermore, no mortality studies performed co-pollutant models adjusting for key traffic-related air pollutants such as EC or UFPs. The role of NO<sub>2</sub> as a surrogate for traffic and traffic-sourced air pollution has not been fully explored and may explain the positive but inconsistent associations seen in this dataset.

## 9.3 Studies of Morbidity Outcomes

This summary of epidemiology studies of the association between long-term exposure to ambient NO<sub>2</sub> and morbidity outcomes focuses principally on respiratory effects, including lung function/lung function growth, respiratory symptoms, asthma-related outcomes and hospitalization due to asthma or other chronic respiratory disease. Some additional studies investigating the impact of long-term exposures to ambient NO<sub>x</sub>/NO<sub>2</sub> on various non-respiratory morbidity outcomes are also reviewed as part of this assessment.

Studies of various epidemiological designs are reviewed in this section, including cross-sectional, case-control and cohort or longitudinal studies. For asthma-related outcomes, several prospective cohort studies investigated the development of new-onset asthma (i.e. incidence of asthma) while prevalence of asthma and/or asthma-related symptoms was investigated with cross-sectional studies. Some of these cross-sectional studies also used subsets of subject data from larger prospective cohort studies. A major limitation of cross-sectional studies is that this type of design does not provide information on the time sequence of exposure and outcome. However, results from cross-sectional studies/analysis are also discussed, as they contribute to the overall evidence, in addition to providing information on issues such as genetic susceptibility.

Each subsection discusses results presented in the 2008 US EPA ISA, followed by brief accounts of the more recent studies.

### 9.3.1 Respiratory Effects

A number of epidemiological studies investigating the relationship between long-term exposures to NO<sub>2</sub> and respiratory endpoints (lung function, asthma prevalence/incidence, respiratory symptoms) published since the release of the 1993 NO<sub>x</sub> AQCD were discussed in the 2008 US EPA ISA. While most were performed in Europe, several were performed in the US, largely based on data from the CHS.

#### 9.3.1.1 Lung Function/Lung Function Growth

Decrements in lung function and deficits in lung function growth have been associated with long-term exposures to NO<sub>2</sub> in a number of epidemiologic studies previously discussed in the 2008 US EPA ISA (US EPA, 2008). In California, decrements in lung function and deficits in lung function growth related to long-term exposures to NO<sub>2</sub> were similar for boys and girls, as well as for children without a history of asthma (Peters et al., 1999b; Avol et al., 2001; Gauderman et al., 2004). Children were followed over several years, and the observed deficits in growth of lung function resulted in clinically significant changes in FEV<sub>1</sub> attained at the age of 18 (Gauderman et al., 2004, 2007). Significant reductions in lung function associated with NO<sub>2</sub> exposures were also measured in Germany (Moseler et al., 1994) but only in asthmatic children; no associations were detected in children without asthma. Associations between long-term NO<sub>2</sub> exposures and deficits in lung function growth have also been found in Mexican (Rojas-Martinez et al., 2007) and Norwegian children (Ofstedal et al., 2008); only the long-term effect remained significant in the Norwegian cohort following simultaneous adjustment for short-term NO<sub>2</sub>

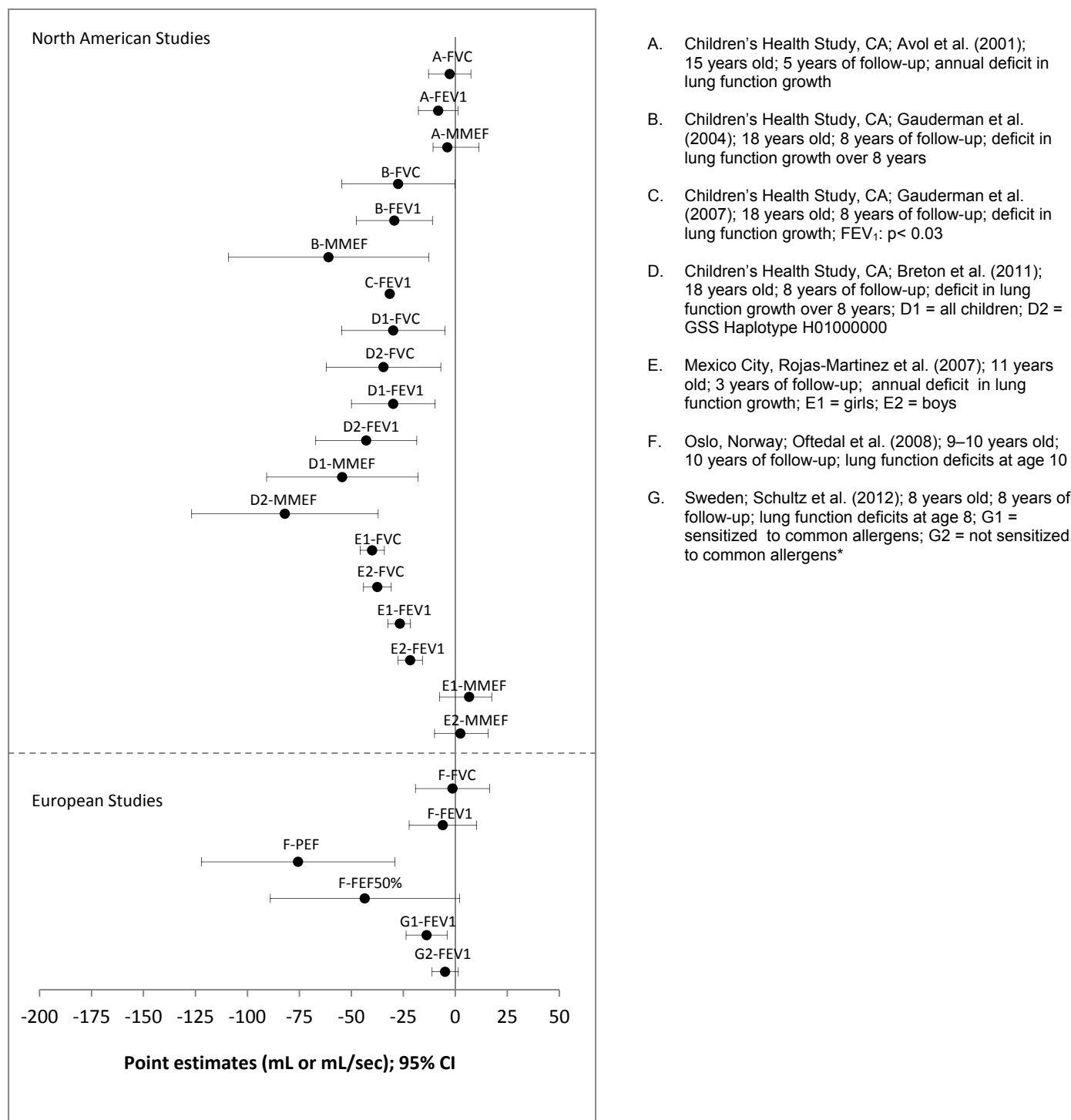
exposures. Significant associations between annual average NO<sub>2</sub> exposures and lung function decrements were also found in adults living in Switzerland (Ackermann-Lieblich et al., 1997). Given strong correlations between most air pollutants, very few co-pollutant models have been performed. The associations between NO<sub>2</sub> and deficits in lung function growth observed in Mexican children (Rojas-Martinez et al., 2007) remained significant in three-pollutant models including PM<sub>10</sub> and O<sub>3</sub> which were both not highly correlated with NO<sub>2</sub> ( $r = 0.17$  with O<sub>3</sub>;  $r = 0.25$  with PM<sub>10</sub>). Significant and robust associations were, however, also observed with both PM<sub>10</sub> and O<sub>3</sub> in these three-pollutant models. In Californian children, associations remained significant in two-pollutant models between deficits in lung function growth and any pair of pollutants, though it should be noted that there was high collinearity ( $r_s$  ranged from 0.64 to 0.94) among them (Gauderman et al., 2004). Both long-term NO<sub>2</sub> levels and distance from a freeway have also been independently associated with deficits in lung function growth in Californian children (Gauderman et al., 2007; correlation coefficients among the different air pollutants were not reported by the authors). It should be noted that similar associations were also found for several air pollutants (acid vapour and PM<sub>10</sub>) as well as for traffic-related pollutants (PM<sub>2.5</sub> and EC) and proximity to traffic (<500 m). Overall, previous epidemiological studies indicated positive associations between long-term exposure to low NO<sub>2</sub> levels and both decrements in lung function measurements and partially irreversible deficits in lung function growth. It should, however, be noted that it has been difficult to distinguish the independent effects of NO<sub>2</sub>, due to the high correlations with the other air pollutants for which similar risk estimates have been found.

This review includes 10 new studies that investigated the effects of long-term exposure to NO/NO<sub>x</sub>/NO<sub>2</sub> on lung function. In total, four studies were conducted in North America, including one in Canada, while the others were performed in Europe. The majority of these studies conducted only single-pollutant analyses due to the multicollinearity among the measured pollutants; one study included both single- and two-pollutant models with O<sub>3</sub>. Lung function indices in children from North America and European cohort studies per 10 ppb increase in NO<sub>2</sub> concentrations are presented in Figure 9.5.

Dales et al. (2008) conducted a study in Windsor, ON, using different health indicators to evaluate the health effects on children ( $n = 2,350$ ; ages 9–11) living near roadways. NO<sub>2</sub> levels at each child's residence were estimated using LUR models ( $R^2 = 0.77$ ) and roadway density around the homes. Pulmonary function volumes (FEV<sub>1</sub> and FVC) were about 40 mL less in the highest tertile than in the lowest tertile for NO<sub>2</sub>, SO<sub>2</sub> and coarse PM; none of these differences was statistically significant. From the highest (>14.44 ppb) to the lowest tertiles (<12.12 ppb) in annual NO<sub>2</sub> concentrations FEV<sub>1</sub> measured ranged from 2.15 L (standard error (SE) = 0.01) to 2.19 L (SE = 0.01) and FVC ranged from 2.49 L (SE = 0.02) to 2.53 L (SE = 0.02) for FVC. When the air pollution estimates were expressed as continuous measures no relationships between exposure and effects were observed.

Two recent studies, Breton et al. (2011) and Islam et al. (2011), are follow-up analyses using data from the CHS, a large 10-year prospective cohort in Southern California following 5,500 children from 12 communities with different combinations of high and low levels of various air pollutants, using ambient data gathered from central monitoring stations. Associations between declines in lung function and/or lung function growth with several air pollutants, including NO<sub>2</sub>, have been previously demonstrated in several CHS studies (Peters et al., 1999b; Avol et al., 2001; Gauderman et al., 2004, 2007).

**Figure 9.5: Lung function indices in children and 95% CIs from cohort studies per standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration in single-pollutant models**



\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>. FVC = forced vital capacity (mL); FEV<sub>1</sub> = forced expiratory volume in 1 sec (mL); FEF<sub>50%</sub> = flow rate at the 50% point of forced expiration (mL/sec); MMEF = maximal mid-expiratory flow rate (mL/sec); PEF = peak expiratory flow (mL/sec).

Glutathione (GSH) is an antioxidant playing an important role in detoxification pathways. One of the main objectives of a study by Breton et al. (2011) was to determine if sequence variation in genes involved in GSH synthesis pathways can modify the susceptibility to adverse effects of air pollution on lung function. About 2,106 children included in the CHS had their complete genetic information analyzed, based on genotyping assays performed on collected buccal cells. Results of this study demonstrated that variation in the glutathione synthetase gene (GSS locus) can affect an individual's lung function growth responses to air pollution. Significant associations were observed between NO<sub>2</sub> exposures and decrements in FEV<sub>1</sub> (-145 mL; 95% CI -227 mL, -63 mL), FVC (-117 mL; 95% CI -210 mL, -24 mL) and MMEF (-278 mL/sec; 95% CI -430 mL/sec, -126 mL/sec) per increase of 33.9 ppb NO<sub>2</sub> for the haplotype h0100000, the most common haplotype of GSS occurring in 48% of the children. Similar decrements were observed with other air pollutants, including PM<sub>10</sub>, PM<sub>2.5</sub>, EC, OC and O<sub>3</sub>. No significant decrements in lung function were found for the other haplotypes that were grouped together. Results remained significant in two-pollutant models with inclusion of O<sub>3</sub> in the model, which was negatively correlated with NO<sub>2</sub> ( $r = -0.11$ ), suggesting an independent effect of NO<sub>2</sub>. No other two-pollutant or multi-pollutant models were carried out.

Also using data from the CHS, Islam et al. (2011) assessed whether psychosocial stress could modify the adverse effects of traffic exposure on lung function. Results of this study suggested that children living in a high-stress home environment (parental perceived stress >4) were more susceptible to the detrimental effects of air pollution on lung function. Long-term exposures to traffic-NO<sub>x</sub>/NO/NO<sub>2</sub> levels were predicted from models using measurements of these air pollutants taken at homes and schools of study participants and incorporating population density, elevation, land-use, and several indicators of traffic ( $R^2 = 0.71, 0.61$  and  $0.68$  for NO<sub>x</sub>, NO and NO<sub>2</sub> levels). Among children with high parental stress, decrements in FVC of 4.73% (95% CI -6.7%, -2.7%), 4.84% (95% CI -6.9%, -2.8%), and 4.55% (95% CI -6.5%, -2.6%) were measured, respectively, for each 21.8 ppb increase in residential and school NO<sub>x</sub>, NO and NO<sub>2</sub> levels. No significant associations were measured in low-stress households. Similar decrements were obtained in FEV<sub>1</sub> while no statistically significant associations were found with MMEF. Sensitivity analyses among children without asthma were also performed; significant decrements in both FVC and FEV<sub>1</sub> were also related to indoor NO<sub>2</sub> exposure in the high-stress households.

Several studies have been conducted in Europe as well. Rosenlund et al. (2009b) also observed significant decrements in lung function of children aged 9–14 living in Rome who were participants in the Italian part of the International Study of Asthma and Allergies in Childhood (ISAAC) phase 2 cross-sectional survey. For a 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> estimated by an LUR model ( $R^2 = 0.69$ ) in the census block of residence, decreases of 13 mL (95% CI -31 mL, -5 mL) for FEV<sub>1</sub>, 85 mL/sec (95% CI -135 mL/sec, -35 mL/sec) for PEF and 62 mL/sec (95% CI -102 mL/sec, -21 mL/sec) for MMEF were observed, while a decrease of 0.62% (95% CI -1.05%, -0.19%) was found for the FEV<sub>1</sub>/FVC ratio. Based on sensitivity analyses, associations were usually stronger in girls, older children (aged 11–14, as compared with those aged 9–10), children with high SES and those exposed to parental ETS.

Long-term exposure to traffic-NO<sub>x</sub> air pollution was found to be associated with reduced lung function in 8-year-old children from the Swedish BAMSE (Children, Allergy, Milieu, Stockholm, Epidemiology) birth cohort study (Schultz et al., 2012). Lifetime residential, day care, and school addresses were geocoded, and time-weighted average outdoor levels were calculated using emission inventories and air dispersion models. Exposure to an increase of 47 µg/m<sup>3</sup> (25 ppb) in traffic-NO<sub>x</sub> during the first year of life was associated with a deficit of 34.9 mL (95% CI -80.1 mL, -10.4 mL) in FEV<sub>1</sub>. Stronger decrements (-98.9 mL; 95% CI -169.4 mL, -28.4 mL) were found among children sensitized to common allergens. Similar associations were also found for PM<sub>10</sub>

in single-pollutant models. No clear associations were found between lung function and air pollution exposure after the infancy period, suggesting that early life exposure is a critical period that can lead to long-term respiratory consequences. Nordling et al. (2008), analyzing the same cohort, found a non-significant decrease in PEF (-3.08 L/min; 95% CI -6.84 L/min, 0.68 L/min) at age 4 in relation to traffic-NO<sub>x</sub> exposure during the first year of life (per 44 µg/m<sup>3</sup> (23.40 ppb) increase). Significant decrements for this parameter were reported for traffic-PM<sub>10</sub>.

The European Community Respiratory Health Survey (ECRHS) is a comprehensive multi-centre cohort study performed in adults from 21 European cities. The first survey, ECRHS I, was performed in 1991–1993 and a second, ECRHS II, was conducted in 1999–2001. Decrements in lung function among participants in the ECRHS were not found to be associated with exposure to annual levels of NO<sub>2</sub>, SO<sub>2</sub>, PM<sub>2.5</sub> and light absorbance measured at central monitoring stations in each centre (Götschi et al., 2008). Results from a cross-sectional study performed in England by Forbes et al. (2009a) suggested that long-term exposure to NO<sub>2</sub> estimated with air dispersion modelling might also be associated with reduced FEV<sub>1</sub> in adults. Decrements in FEV<sub>1</sub>, based on 10 µg/m<sup>3</sup> (5.32 ppb) increases in NO<sub>2</sub> were, respectively, 22 mL (95% CI -31 mL, -14 mL) in all subjects, 30 mL (95% CI -42 mL, -17 mL) in men, and 16 mL (95% CI -24 mL, -7 mL) in women. However, as in all previous studies, high correlations were observed between PM<sub>10</sub> and NO<sub>2</sub>, which makes it difficult to discern the individual effect of NO<sub>2</sub>. Stronger effects were also observed in older adults and ex-smokers, while no significant associations were reported for the FEV<sub>1</sub>/FVC ratio. Exposure to modelled ambient air pollution in the UK has also been related to declines in FEV<sub>1</sub> in subjects with α-1-antitrypsin deficiency, which is a genetic disorder that predisposes people to the development of COPD (Wood et al., 2009). Only PM<sub>10</sub> and O<sub>3</sub> were significantly associated with declines in lung function; long-term exposure to NO<sub>2</sub> was not a significant predictor. No significant cross-sectional and longitudinal associations between modelled NO<sub>2</sub> and declines in FEV<sub>1</sub> were observed in a population-based study of adults aged 18–70 living in Nottingham, England (Pujades-Rodriguez et al., 2009). Significant decrements in lung function were found in mail carriers from Athens, Greece (Karakatsani et al., 2010) exposed to higher personal long-term exposure measurements of NO<sub>2</sub> (>44.41 µg/m<sup>3</sup> (>23.62 ppb)) following adjustment for several covariates.

### 9.3.1.2 Respiratory Symptoms

The 2008 US EPA ISA (US EPA, 2008) identified several studies investigating the relationship of ambient NO<sub>2</sub> exposure with respiratory symptoms. The results of these studies were somewhat inconsistent, with most reporting some positive associations but all reporting a large number of negative results. The 2008 US EPA ISA also noted that only one of these studies (Peters et al., 1999a) reported an association between long-term exposures to ambient NO<sub>2</sub> and wheeze (a respiratory symptom studied in a large number of studies) in children from California. Significant associations between increased bronchitis symptoms and long-term exposure to NO<sub>2</sub> were also found in these children; stronger associations were observed with yearly variations of NO<sub>2</sub> within-communities (McConnell et al., 2003), and some results indicated that dog ownership may worsen this relationship (McConnell et al., 2006). The effects estimates for NO<sub>2</sub> remained robust in two-pollutant models with several pollutants, including PM<sub>10</sub>, PM<sub>10-2.5</sub>, EC, inorganic and organic acid (*r<sub>s</sub>* varied from -0.22 to 0.65), but not with OC (*r* = 0.67). The effects of all other pollutants were reduced after adjustment for OC or NO<sub>2</sub>. Several additional studies reviewed by the US EPA were performed in Europe and found inconsistent results between long-term exposure to NO<sub>2</sub> and several respiratory symptoms.

More recently, there have been several studies published that deal with respiratory symptoms; one study was performed in Canada (Karr et al., 2009a) and three in the US (Karr et al., 2007, 2009b; Ebisu et al., 2011) while all the others have been conducted in Europe. Point estimates



(ORs) from cohort studies for associations between a 10 ppb increase in NO<sub>2</sub> concentration and respiratory symptoms in children (bronchitis or wheeze) are illustrated in Figure 9.6.

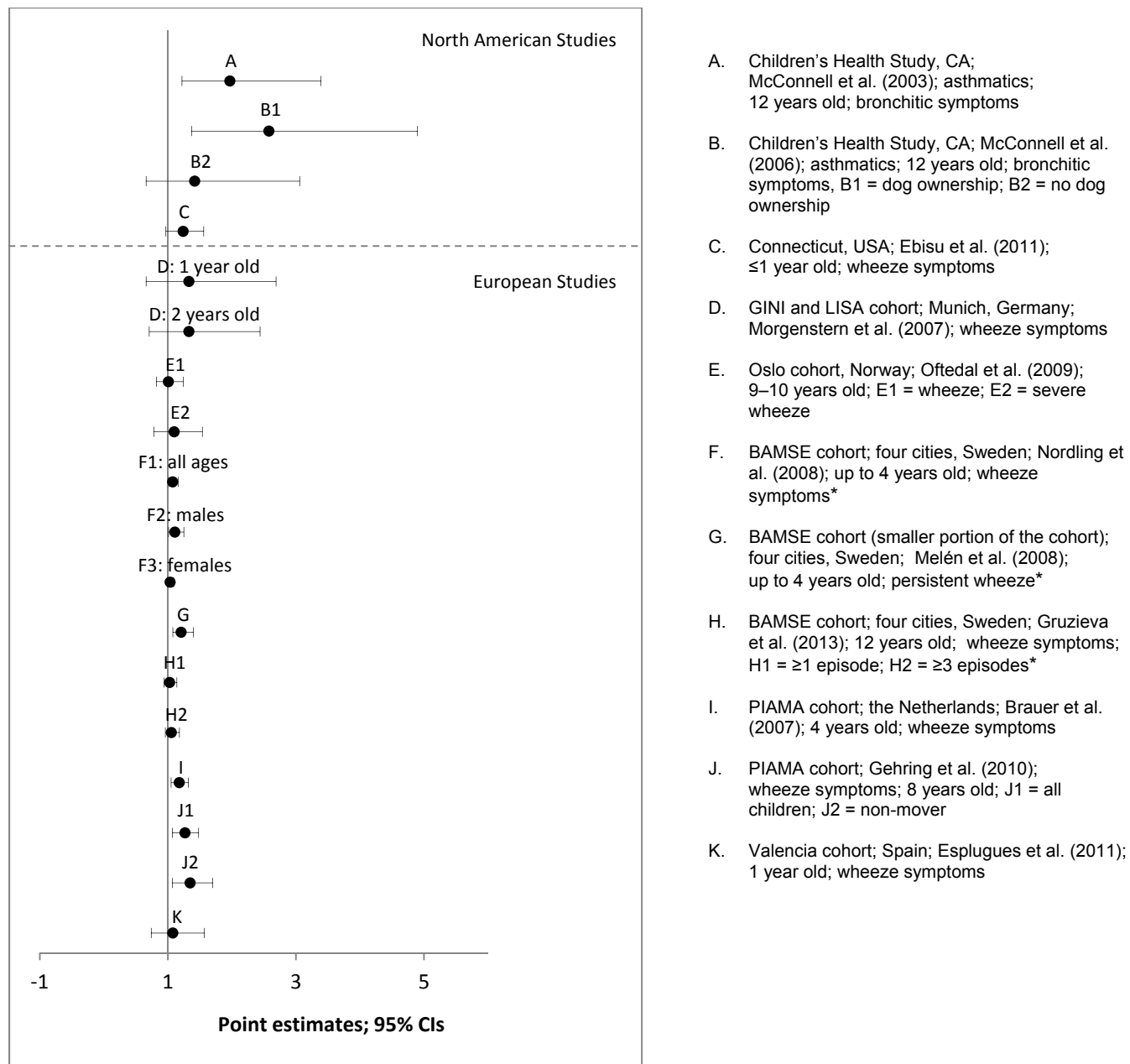
Bronchiolitis, an inflammation of the bronchioles, is an important cause of hospitalization in the first year of life. Some investigators have analyzed the influence of traffic-related air pollutant exposure, including NO/NO<sub>2</sub>, on infant hospitalization due to bronchiolitis. A nested case-control study (Karr et al., 2009a) was conducted in the Georgia Air Basin of British Columbia, Canada, an area where NO/NO<sub>2</sub> is mainly derived from vehicular emissions. The team used a dataset developed from a population-based cohort of all singleton infants (cases = 1,465) born from 1999 through 2002. Based on an IQR of 11.8 ppb in NO<sub>2</sub> lifetime exposure estimated with the proximal monitor within 10 km of the residence, an OR of 1.04 (95% CI 1.02, 1.07) was measured for bronchiolitis hospitalization. A similar and statistically significant association was also found with the NO proximal monitor (IQR = 19.6 ppb): an OR of 1.08 (95% CI 1.04, 1.12). The use of LUR models ( $R^2 = 0.56$ ) yielded similar but smaller effect estimates. Risk estimates based on quartile lifetime exposure were also computed, and higher risks were obtained with the highest NO<sub>2</sub> quartile than with the lowest one. For lifetime NO exposure the quartile-based assessment also suggested a dose–response relationship ( $P$  trend = 0.01).

In Los Angeles, CA, children's hospitalization due to bronchiolitis was associated with lifetime average exposure to PM<sub>2.5</sub> and not with NO and NO<sub>2</sub> in a matched case-control study (Karr et al., 2007). An OR of 1.03 (95% CI 0.99, 1.07) was found for each 16 ppb increase in long-term exposure to NO<sub>2</sub>, estimated based on the most relevant monitoring station. A matched case-control study performed by the same team (Karr et al., 2009b) in the Puget Sound region of Washington State found only modest and non-significant increases in children's hospitalizations due to bronchiolitis with both NO<sub>2</sub> (estimated based on an LUR model;  $R^2 = 0.72$ ) and PM<sub>2.5</sub>. Overall, these American studies did not observe any clear associations between long-term exposure to ambient NO/NO<sub>2</sub> and infant bronchiolitis.

In California, the Air Pollution and Absence Study is a population-based prospective cohort study conducted as part of the CHS; it included 1,935 fourth-grade schoolchildren where data on air pollutants were gathered from central site monitors. Wenten et al. (2009) found an increased risk of respiratory-illness absences with functional variants of the catalase (CAT) and myeloperoxidase (MPO) genes, which are involved in the oxidative stress defence pathway, in children with the combination of CAT (G/G) and MPO (G/A or A/A) genes. The risk was reduced for children with the CAT (G/A or A/A) and the MPO (G/A or A/A) genes. The epistatic effect of the CAT/MPO variants was most apparent in children living in communities with higher NO<sub>2</sub> air pollution ( $P$ -interaction = 0.002). The protective effect of the joint-effect analyses CAT GA or AA plus MPO GA or AA genotype was limited to children living in communities with high ambient NO<sub>2</sub> levels (RR = 0.63; 95% CI 0.37, 1.10), but stronger and significant associations were observed in high-O<sub>3</sub> exposure groups.

All but one of the new studies investigating the relationship between long-term exposures to ambient NO<sub>x</sub>/NO<sub>2</sub> and wheeze symptoms in children have been performed in Europe (Figure 9.6); most of these studies have found positive but non-statistically significant increases (Morgenstern et al., 2007; Oftedal et al., 2009; Esplugues et al., 2011; Gruzieva et al., 2013). Some significant associations were, however, observed in the Swedish (Nordling et al., 2008; Melén et al., 2008) and Dutch (Brauer et al., 2007; Gehring et al., 2010) cohorts.

**Figure 9.6: Point estimates (ORs) and 95% CIs from cohort studies for associations between a standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration and respiratory symptoms (bronchitis or wheeze) in children; single-pollutant models only**



\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>.

An OR of 1.22 (95% CI 0.98, 1.51 per IQR increment (9.21 ppb) of traffic-outdoor NO<sub>2</sub>), estimated using a GIS-integrated traffic exposure model, was found for wheeze symptoms in children living in Connecticut in a longitudinal cohort study focused on the impact of urban land-use (i.e. the fraction of urban land-use in a person's neighbourhood) on children's health (Ebisu et al., 2011). This association was reduced following the introduction of urban land-use in the two-pollutant models (OR = 1.10; 95% CI 0.86, 1.40); the association of urban land-use was slightly attenuated but no longer significant following adjustment for NO<sub>2</sub>. The correlation between the land-use categories and estimated NO<sub>2</sub> levels varied from 0.51 to 0.64. Positive but non-significant associations between outdoor NO<sub>2</sub> exposure and wheeze symptoms were also found by Morgenstern et al. (2007) in German children of 1 year (OR = 1.09; 95% CI 0.88, 1.35) and 2 years in age (OR = 1.09; 95% CI 0.90, 1.31) per IQR increase of 5.7 µg/m<sup>3</sup> (3.03 ppb) in annual concentrations, estimated using GIS-based exposure models. A moderate correlation ( $r = 0.59$ ) was found between NO<sub>2</sub> and PM<sub>2.5</sub> absorbance (a marker of diesel exhaust particles) in the Munich metropolitan area, which prevented the authors from disentangling the effects of individual air pollutants. This study, however, found a borderline significant association between NO<sub>2</sub> in the first year of life and dry cough at night (OR = 1.34; 95% CI 1.00, 1.81) and a significant association (OR = 1.30; 1.03, 1.66) with spastic/obstructive bronchitis symptoms, but only in 1-year-old children. In a cross-sectional survey performed in Rome, Italy, Rosenlund et al. (2009b) observed no associations between residential NO<sub>2</sub> levels estimated by LUR models ( $R^2 = 0.69$ ) and any of the self-reported respiratory symptoms in children aged 9–14, except for persistent wheeze, where a small positive but non-significant association was found (estimates only presented in a figure). Positive but still non-significant associations were observed in children aged 9–10 from the follow-up of the Oslo Birth Cohort study (Ofteidal et al., 2009) between NO<sub>2</sub> levels (estimated with air dispersion modelling) in the previous year (18 µg/m<sup>3</sup> (9.57 ppb)) and wheeze (OR = 1.01; 95% CI 0.83, 1.23), severe wheeze (OR = 1.10; 95% CI 0.79, 1.51) and dry cough (OR = 1.01; 95% CI 0.84, 1.21). In a birth cohort study conducted in Valencia, Spain (Esplugues et al., 2011), exposure to ambient NO<sub>2</sub> in children during their first year of life, estimated by LUR models ( $R^2 = 0.73$ ), was not found to be associated with wheeze symptoms; only a positive but non-significant association was measured (OR = 1.04; 95% CI 0.85, 1.27) per 10 µg/m<sup>3</sup> (5.32 ppb) increase in postnatal NO<sub>2</sub>. A significant association was found with persistent cough (OR = 1.40; 95% CI 1.02, 1.92).

In Stockholm, Sweden, lifetime residential, day care, and school addresses were geocoded, and time-weighted average outdoor levels were calculated using emission inventories and air dispersion models. Exposure to traffic-NO<sub>x</sub> (44 µg/m<sup>3</sup> (23.40 ppb) corresponding to the difference between the 5<sup>th</sup> and 95<sup>th</sup> percentile range) during the first year of life was associated with an excess risk (OR = 1.60; 95% CI 1.09, 2.36) of persistent wheeze in 4-year-old children from the Swedish BAMSE birth cohort study (Nordling et al., 2008). A stronger risk was found for girls (OR = 1.94; 95% CI 1.07, 3.50) than for boys (OR = 1.55; 95% CI 0.92, 2.63). A similar excess risk was also found with traffic-PM<sub>10</sub>, but the association was not statistically significant. No associations were found between transient and late onset wheezing with any of the air pollutants, and no clear dose–response relationship was observed in the quartile-based analyses. A significant association (OR = 3.0; 95% CI 1.6, 5.9) between exposure to NO<sub>x</sub> levels during the first year of life (44 µg/m<sup>3</sup> (23.40 ppb)) and persistent wheeze was found by Melén et al. (2008) using a small sub-cohort of the BAMSE cohort study, which mainly focused on the genetic interactions between exposure to traffic-related air pollution for development of childhood allergic diseases. In a follow-up study (Gruzieva et al., 2013) positive but non-significant associations were, however, reported between traffic-NO<sub>x</sub> levels (46.8 µg/m<sup>3</sup> (24.89 ppb)) and wheeze: ≥1 episode (OR = 1.12; 95% CI 0.84, 1.49) or ≥3 episodes (OR = 1.10; 95% CI 0.73, 1.66) during the children's first 12 years of life.

In the Netherlands (Gehring et al., 2010), a significant association was found in children at the age of 8 years between wheeze (OR = 1.14; 95% CI 1.04, 1.24) and an increase in NO<sub>2</sub> levels of 10.4 µg/m<sup>3</sup> (5.53 ppb) estimated by an LUR model (R<sup>2</sup> = 0.85). A stronger association was reported for children who did not change residences during the study (OR = 1.18; 95% CI 1.04, 1.34). Another interesting study is one performed by van Roosbroeck et al. (2008), where they explored the impact of adjustment for exposure measurement error on respiratory health effects of schoolchildren in the Netherlands using personal NO<sub>2</sub> data. For an increase of 17.6 µg/m<sup>3</sup> (9.36 ppb) in outdoor NO<sub>2</sub> levels measured at school, the adjusted and unadjusted prevalence ratios were 2.94 (95% CI 0.85, 10.18) and 1.58 (95% CI 0.99, 2.51) for wheeze and 3.82 (95% CI 1.03, 14.21) and 1.76 (95% CI 1.10, 2.81) for phlegm, respectively. Prevalence ratios of 1.74 (95% CI 0.99, 3.05) and 1.72 (95% CI 0.82, 3.62) were reported for wheeze and phlegm, respectively, in the original study performed by Janssen et al. (2003) based on exposure measurements outside the schools. Overall, the results of this study suggest that the effects from ambient air pollution may be substantially attenuated when based on measurements outside the school instead of personal exposure. The authors argued, however, that the unadjusted risk estimates were underestimated but also that the adjustments for the NO<sub>2</sub> measurement error were likely overestimated.

Additional single-city studies have investigated the potential impact of long-term exposures to NO<sub>2</sub> on other respiratory symptoms in adults. In a cross-sectional study performed in Bordeaux, France, positive but non-significant associations were found in older subjects (≥65 years of age) for cough (OR = 1.01; 95% CI 0.99, 1.04) and phlegm (OR = 1.02; 95% CI 0.98, 1.04) per 1 µg/m<sup>3</sup> (0.53 ppb) increase in NO<sub>2</sub> levels, estimated at the individuals' addresses with a dispersion model (Bentayeb et al., 2010). More pronounced and significant effects were, however, found with both SO<sub>2</sub> and PM<sub>10</sub>. Based on cross-sectional surveys assessing adult residents of Rome who had resided in the same place for at least 3 years, no association was found between any of the exposure indices of traffic-related air pollution (estimated with LUR models; R<sup>2</sup> = 0.69) and chronic bronchitis (Cesaroni et al., 2008), while significant associations were reported in Sweden (Lindgren et al., 2009a) for adults aged 18–77 following exposure to the highest modelled NO<sub>x</sub> category (>19 µg/m<sup>3</sup> (>10.11 ppb)). Based on a cross-sectional analysis, there was no relationship between increased quintiles of modelled NO<sub>2</sub> concentrations at home and increased risk of wheeze or COPD in adult participants in an existing cohort in Nottingham, England (Pujades-Rodriguez et al., 2009). Positive but non-significant associations between personal long-term exposure measurements of NO<sub>2</sub> concentrations and respiratory symptoms (bronchitis, cough, phlegm and bronchial asthma) were found in mail carriers through a cross-sectional study performed in Athens, Greece (Karakatsani et al., 2010).

### 9.3.1.3 Asthma-related Outcomes

Epidemiological studies have investigated both the incidence and prevalence of asthma in relation to long-term ambient NO<sub>2</sub>. Incidence is a measure of the risk to develop a specific disease in a population within a specified period (i.e. number of newly diagnosed cases of asthma or asthma onset) and prevalence is the proportion of cases in the population at a single point in time. The prevalence of asthma in a population at a given time or over a short period can be measured with cross-sectional studies or surveys, while cohort studies can be used to determine new-onset asthma cases.

In the 2008 US EPA ISA, several studies were identified that investigated the relationship between NO<sub>2</sub> exposure and either asthma incidence or asthma prevalence (US EPA, 2008). Most of these studies reported inconsistent findings, except for two major prospective cohort studies where positive and significant associations were observed. In a small group of children about 10 years old from the CHS, 4-wk avg NO<sub>2</sub> measured at homes, based on an IQR increase of 5.7 ppb, was strongly associated (OR = 1.83; 95% CI 1.04, 3.21) with a lifetime history of

asthma diagnosis (Gauderman et al., 2005). A birth cohort study performed in the Netherlands (Brauer et al., 2007) also reported a significant association with physician-diagnosed asthma in 4-year-old children (OR = 1.28; 95% CI 1.04, 1.56) based on 10.6  $\mu\text{g}/\text{m}^3$  (5.63 ppb) increases in  $\text{NO}_2$  levels estimated at each subject's residence, which were calculated by a model combining air pollution measurements with GIS. At that time, the US EPA concluded that the associations observed between long-term exposure to ambient  $\text{NO}_2$  and both asthma incidence and/or prevalence were inconsistent.

**Development of asthma:** Six recently published North American studies examined the association between new-onset asthma and long-term exposure to air pollutants including  $\text{NO}_x/\text{NO}_2$ . In addition, six studies from Europe were also identified. Point estimates (OR, HR or RR) from cohort studies for associations between a 10 ppb increase in  $\text{NO}_2$  concentration and incidence of asthma in children or adults are illustrated in Figure 9.7.

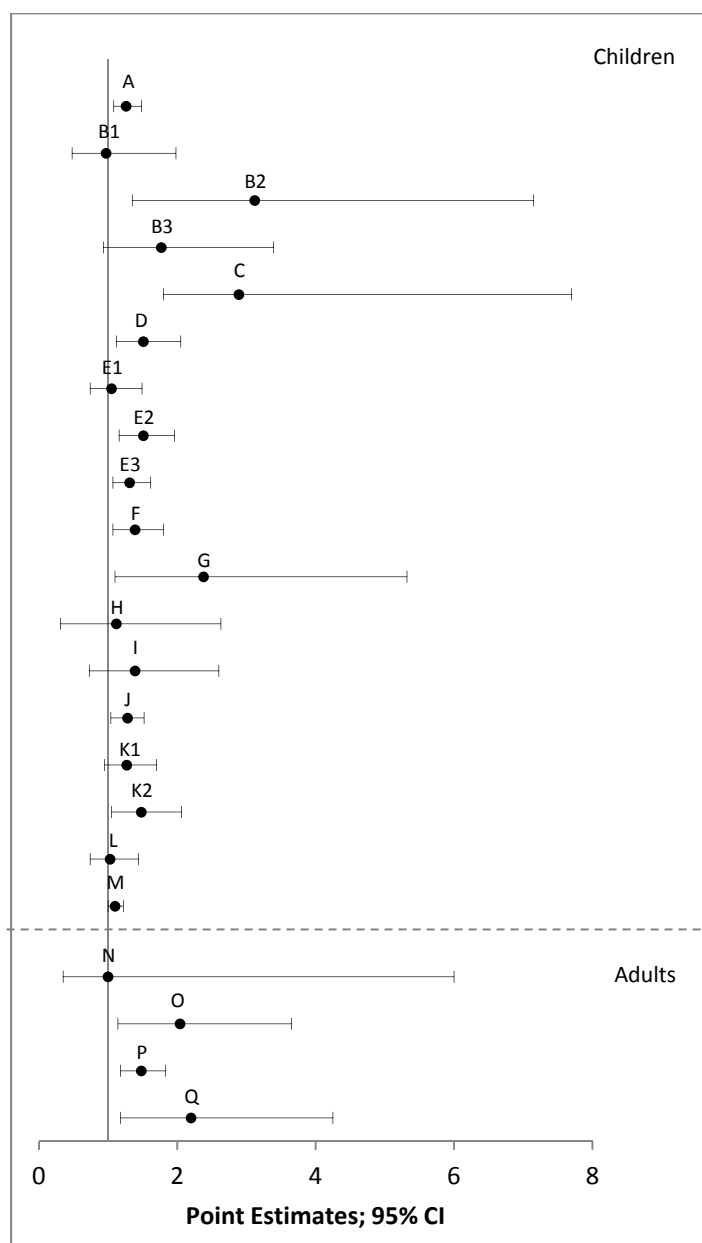
Using a nested case-control design, Clark et al. (2010) found an increased risk of asthma diagnosis in children up to 3 and 4 years of age who were born in southwestern British Columbia in 1999–2000. Based on the IDW summation, ORs of 1.08 (95% CI 1.04, 1.12) and 1.13 (95% CI 1.04, 1.23) were found with a 10  $\mu\text{g}/\text{m}^3$  (8.15 ppb) increase in levels of NO and  $\text{NO}_2$  (5.32 ppb) in the first year of life. Similar risks were observed using LUR models ( $R^2 = 0.56$ ). In quartile-based analyses elevated ORs were usually noted for the highest quartile, but the trend across quartiles was not consistently monotonic. Statistically significant increased risks of asthma diagnosis were also observed with CO,  $\text{PM}_{10}$ ,  $\text{SO}_2$ , BC, and proximity to point sources.

In a high-risk birth cohort study performed in Vancouver (Carlsten et al., 2011a), where asthma diagnosis was performed by an allergist, positive and borderline significant associations were found between the incidence of asthma in 7-year-old children and exposure to NO (OR = 1.2; 95% CI 0.9, 1.7 per 12.7  $\mu\text{g}/\text{m}^3$  (10.36 ppb) increase) and  $\text{NO}_2$  (OR = 1.5; 95% CI 0.9, 2.5 per 7.2  $\mu\text{g}/\text{m}^3$  (3.83 ppb) increase) in the year of birth. All air pollutants were estimated with LUR models ( $R^2 = 0.56$ ). Higher risk estimates were found in children with both allergist-diagnosed asthma and bronchial hyperreactivity ( $\text{PC}_{20} \leq 2 \text{ mg/mL}$ ): an OR of 1.8 (95% CI 1.1, 2.9) per 12.7  $\mu\text{g}/\text{m}^3$  (10.36 ppb) increase in NO levels and an OR of 2.3 (95% CI 1.0, 5.1) per 7.2  $\mu\text{g}/\text{m}^3$  (3.83 ppb) increase in  $\text{NO}_2$  levels in the year of birth. Significant associations were also observed with  $\text{PM}_{2.5}$ , which was moderately correlated with  $\text{NO}_x$ -air pollutants ( $\text{NO}_2$ - $\text{PM}_{2.5}$ ,  $r = 0.7$  and  $\text{NO}$ - $\text{PM}_{2.5}$ ,  $r = 0.5$ ). The risk of asthma increased monotonically with increasing exposure quartiles of both NO and  $\text{PM}_{2.5}$ , while no clear dose–response trend was found for  $\text{NO}_2$ . Higher risk estimates were observed for both the second and higher  $\text{NO}_2$  quartiles as compared to the first quartile. In an associated letter (Carlsten et al., 2011b) GSTP1 polymorphisms were found to modify the risk for incident asthma; the GSTP1 polymorphism rs1799811 interacted on the  $\text{NO}_2$  asthma association ( $p = 0.04$ ), with the variant polymorphism conferring decreased risk. Overall, these results support the hypothesis that early childhood exposure to air pollutants may play an important role in the development of asthma.

Some new studies investigating the relationship between long-term exposure to  $\text{NO}_2$  and new-onset asthma have also been performed in the US.



**Figure 9.7: Point estimates (OR, HR or RR) and 95% CIs from cohort studies for associations between a standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration and incidence of asthma in single-pollutant models**



- A. Vancouver cohort, BC; Clark et al. (2010); 3–4 years old; incidence of doctor-diagnosed asthma
- B. East Boston, MA; Clougherty et al. (2007); 4–11 years old; B1 = below median ETV; B2 = above median ETV; B3 = all children; parental reports of child's asthma diagnosis
- C. Children's Health Study, CA; Gauderman et al. (2005); 10 years old; lifetime history of doctor-diagnosed asthma
- D. Children's Health Study, CA; Jerrett et al. (2008); 10–18 years old; doctor-diagnosed asthma
- E. Children's Health Study, CA; Shankardass et al., 2009; 5–9 years old, doctor-diagnosed asthma; E1 = low parental stress; E2 = high parental stress; E3 = all children
- F. Children's Health Study, CA; McConnell et al. (2010); 4–9 years old; doctor-diagnosed asthma
- G. GINI and LISA cohorts; Munich, Germany; Morgenstern et al. (2007); 1 year old; doctor-diagnosed asthmatic/spastic/obstructive bronchitis
- H. GINI and LISA cohorts; Munich, Germany; Morgenstern et al. (2008); 6 years old; doctor-diagnosed asthmatic/spastic/obstructive bronchitis
- I. GINI and LISA cohorts; Wesel, Germany; Krämer et al. (2009); 6 years old; doctor-diagnosed asthmatic/spastic/obstructive bronchitis
- J. PIAMA cohort; the Netherlands; Brauer et al. (2007); 4 years old; incidence of doctor-diagnosed asthma
- K. PIAMA cohort; Gehring et al. (2010); 8 years old; K1 = movers; K2 = non-movers; risk of incident asthma
- L. Oslo cohort, Norway; Oftedal et al. (2009); 9–10 years old; incidence of doctor-diagnosed asthma (late onset (≥4 years old))
- M. BAMSE cohort, four cities, Sweden; Gruzieva et al. (2013); 12 years old; asthma incidence (≥4 episodes of wheeze or ≥1 episode in combination with prescription of inhaled corticosteroids, which would be provided following a doctor-diagnosed asthma)\*
- N. Luleå, Sweden; Modig et al. (2006); 20–60 years old; incidence of doctor-diagnosed asthma
- O. RHINE cohort, three cities, Sweden; Modig et al. (2009); 20–44 years old; new onset of doctor-diagnosed asthma
- P. ECRHS cohort, 17 European cities; Jacquemin et al. (2009a); 25–44 years old; new onset of self-reported asthma
- Q. ECRHS cohort, 13 European cities; Castro-Giner et al. (2009); 25–44 years old; new onset asthma (attack of asthma in preceding 12 months or current medication for asthma)

\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>. The risk ratios reported by Jerrett et al. (2008); Shankardass et al. (2009) and McConnell et al. (2010) were HRs; those reported by Oftedal et al. (2009) were RRs while all the others were ORs. ETV = exposure to violence

Three studies (Jerrett et al., 2008; Shankardass et al., 2009; McConnell et al., 2010) have been performed in California using data from the CHS. Jerrett et al. (2008) assessed the association between traffic-related air pollution and asthma onset in a selected sample ( $n = 217$ ) of children in 11 CHS communities during an 8-year follow-up.  $\text{NO}_2$  exposure estimates were based on outdoor measurements at homes of participants. The average within-community IQR of 6.2 ppb in annual residential  $\text{NO}_2$  was significantly associated with incident asthma (HR = 1.29; 95% CI 1.07, 1.56). Similar associations were observed in the summer and fall-winter season. For yearly averages and the fall-winter season, the within- and between-community  $\text{NO}_2$  pollution resulted in similarly increased risks, suggesting that both regional and local sources contributed to the asthma associations. In a 3-year follow-up of a Californian cohort of 2,497 children aged 5 to 9 years, Shankardass et al. (2009) analyzed whether low SES (based on parental education) or high parental stress could modify the association between  $\text{NO}_x$  levels, an indicator of the near-source mixture of traffic-related pollution, and childhood asthma incidence. Based on an IQR increase of 21 ppb  $\text{NO}_x$ , estimated with air dispersion models, an HR of 1.31 (95% CI 1.07, 1.61) was found with incident asthma in this population. The risk associated with  $\text{NO}_x$  was greater in subjects with parental stress above the median (HR = 1.51; 95% CI 1.16, 1.96) than in subjects with parental stress below the median (HR = 1.05; 95% CI 0.74, 1.49). Similar results were found with SES; an increased risk, based on  $\text{NO}_x$  levels, was observed in subjects of low SES (HR = 1.55; 95% CI 1.09, 2.19) compared with those of high SES (HR = 1.20; 95% CI 0.93, 1.55). The authors concluded that the results of this study suggest that children living in stressful households are more susceptible to the effects of traffic-related air pollution. They also noted that because of the high correlation between measures of  $\text{NO}_x$  and other pollutants ( $\text{CO}$ ,  $\text{NO}_2$ , EC and OC) and PM indices ( $\text{PM}_{10}$  and  $\text{PM}_{2.5}$ ), measures of  $\text{NO}_x$  represented not only primary local  $\text{NO}_x$  from vehicular traffic but a mixture of other pollutants related to near-source traffic exposure. McConnell et al. (2010) investigated the impact of exposure to traffic-related air pollution at home and school on new-onset asthma cases identified in a 3-year follow-up of a cohort of 2,497 kindergarten and first-grade children. Based on 23.6 ppb increases in  $\text{NO}_2$  levels, which were measured at a central monitoring site in each community, an HR of 2.18 (95% CI 1.18, 4.01) was observed in single-pollutant models. This association was attenuated and became non-significant (HR = 1.37; 95% CI 0.69, 2.71) in two-pollutant models with the inclusion of modelled traffic exposure. However, associations between new-onset asthma and traffic exposure modelled at school or at home remained robust in two-pollutant models including  $\text{NO}_2$ ,  $\text{PM}_{2.5}$ ,  $\text{PM}_{10}$  or  $\text{O}_3$ ; correlations among the different air pollutants were not reported by the authors.

In a retrospective community-based pregnancy cohort in East Boston, MA (Clougherty et al., 2007), a diagnosis of asthma was significantly associated with long-term exposure to  $\text{NO}_2$  (4.3 ppb increase), obtained by an LUR model ( $R^2 = 0.83$ ), but only in children exposed to above-median violence (OR = 1.65; 95% CI 1.16, 2.34); a positive but non-significant association was observed in the full cohort. Results of this study are suggestive of a synergistic effect between asthma etiology and social/physical variables.

Several European cohort studies (Morgenstern et al., 2007, 2008; Krämer et al., 2009; Oftedal et al., 2009; Gehring et al., 2010; Gruzieva et al., 2013) that evaluated the impact of long-term exposure to  $\text{NO}_2$  on the development of asthma in children have also been identified. In contrast to the American studies, a study conducted in Oslo, Norway (Oftedal et al., 2009) found no associations (RR = 0.82; 95% CI 0.67, 1.02) between long-term exposure to modelled  $\text{NO}_2$  levels (IQR =  $27.9 \mu\text{g}/\text{m}^3$  (14.84 ppb) increase) and onset of doctor-diagnosed asthma in schoolchildren aged 9–10. The team also investigated early asthma onset (from birth to 3 years of age) and late onset (from 4 years of age) in separate analyses. No association (RR = 0.78; 95% CI 0.62, 0.98) was found between  $\text{NO}_2$  and early asthma onset, while a positive but non-statistically significant association (RR = 1.05; 95% CI 0.64, 1.72) was found for late onset.

Some studies (Morgenstern et al., 2007, 2008; Krämer et al., 2009) have investigated the impact of traffic-related air pollution using data from two major prospective cohort studies, the GINIplus (German Infant Study on the influence of Nutrition Intervention Plus environmental and genetic influences on allergy development) and LISAplus (Influence of Lifestyle factors on the development of the Immune System and Allergies in East and West Germany Plus the influence of traffic emissions and genetics) study groups. Air-pollutant-exposure estimates were derived with GIS-based models and outdoor measurements at participants' homes. Morgenstern et al. (2007) found a positive and significant association of NO<sub>2</sub> levels with asthma/spastic/obstructive bronchitis in 1-year-old German children (OR = 1.30; 95% CI 1.03, 1.66) based on 5.7 µg/m<sup>3</sup> (3.03 ppb) NO<sub>2</sub> increases, while no association (OR = 0.82; 95% CI 0.33, 2.08) was observed in children at the age of 2. In a follow-up study, Morgenstern et al. (2008) found a positive, but still non-significant association between a diagnosis of asthma in the same cohort of young children at the age of 6 and NO<sub>2</sub> levels in urban areas of Munich, Germany. Based on 6.4 µg/m<sup>3</sup> (3.40 ppb) increases in NO<sub>2</sub> levels an OR of 1.04 (95% CI 0.67, 1.39) was found for doctor-diagnosed asthma. PM<sub>2.5</sub> was strongly and significantly associated with doctor-diagnosed asthma. Krämer et al. (2009) used subjects of these two cohorts living in rural, small-town areas, Wesel and northwest Germany, where NO<sub>2</sub> concentrations were considerably lower than in Munich, and found a positive but non-significant association per 9 µg/m<sup>3</sup> (4.79 ppb) increase in NO<sub>2</sub> concentrations estimated by LUR models (R<sup>2</sup> = 0.81) with the incidence of doctor-diagnosed asthma (RR = 1.17; 95% CI 0.86, 1.58) in 6-year-old children. Similar results were found with other traffic-related exposure metrics (soot and distance (<50 m between child's address and the nearest major road)).

Air pollution exposure in the Netherlands, where exposure estimates were obtained with LUR models (R<sup>2</sup> = 0.85), was shown to be a potential contributor to the development of asthma. Gehring et al. (2010) followed children during their first 8 years of life and found a positive and significant association between incident asthma and NO<sub>2</sub> levels over the entire period, with an OR of 1.19 (95% CI 1.05, 1.34) per 10.4 µg/m<sup>3</sup> (5.53 ppb) NO<sub>2</sub> increase. A slightly stronger association was reported for children who did not change residences (OR = 1.24; 95% CI 1.03, 1.49). Similar results were also found for both PM<sub>2.5</sub> and soot, which were both highly correlated with NO<sub>2</sub> (PM<sub>2.5</sub>: r = 0.93; soot: r = 0.96), with the result that it was impossible to disentangle the effects of individual air pollutants. In single-pollutant models, an increase of 46.8 µg/m<sup>3</sup> (24.89 ppb) in traffic-related NO<sub>x</sub> levels in the first year of life of the BAMSE cohort, corresponding to the 5<sup>th</sup>–95<sup>th</sup> percentile difference, was associated with incidence of asthma (OR = 1.87; 95% CI 1.01, 3.44) in children aged 12 (Gruziova et al., 2013). Lifetime residential, day care, and school addresses were geocoded, and time-weighted average outdoor levels were calculated using emission inventories and air dispersion models. Similar associations were found with PM<sub>10</sub> levels, and given the high correlation (r = 0.96) observed between NO<sub>x</sub> and PM<sub>10</sub> exposure levels, no two-pollutant models were conducted. Stronger associations were found between non-allergic asthma and oldest children. Several time aspects of long-term exposure were investigated, and the authors concluded that either early-life periods are critical windows of exposure or there is less measurement error before children start daycare or school.

Fewer studies have investigated the relationship between long-term exposure to air pollutants and asthma in adults. Three important multi-city studies (Jacquemin et al., 2009a, 2009b; Castro-Giner et al., 2009) have been performed in Europe using participants in the ECRHS. Jacquemin et al. (2009a) used a random sample of adults from 17 European centres (located in Sweden, the UK, Belgium, Germany, France, Italy and Spain) who participated in the first survey. New cases of asthma were defined as cases that occurred between ECRHS I and ECRHS II. Based on a 10 µg/m<sup>3</sup> (5.32 ppb) increase in the outdoor NO<sub>2</sub> levels modelled at the residence, an OR of 1.43 (95% CI 1.02, 2.01) was observed with new-onset asthma.

The asthma score, based on five different respiratory symptoms, was also found to be significantly associated with new-onset asthma in adults of ECRHS I and II aged 25–44 (Jacquemin et al., 2009b). Home addresses were geocoded and linked to modelled outdoor NO<sub>2</sub> estimates, as a marker of local traffic-related pollution. Results were expressed as the ratio of the mean asthma scores (RMS). Based on a 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> levels, which corresponded to the 5<sup>th</sup>–95<sup>th</sup> percentile difference in the city with the lowest NO<sub>2</sub> levels, an RMS of 1.23 (95% CI 1.09, 1.38) was found with asthma scores. The association remained significant (RMS = 1.25; 95% CI 1.05, 1.50) after exclusion of participants with asthma and symptoms at baseline. Another interesting study is the one conducted by Castro-Giner et al. (2009), who selected adults from 13 cities of the ECRHS II with available DNA test results and outdoor NO<sub>2</sub> estimates to investigate the role that polymorphisms in genes involved in oxidative stress pathways might play in modifying the relationship between asthma and traffic-related air pollution. All estimates were based on 10 µg/m<sup>3</sup> (5.32 ppb) increases in NO<sub>2</sub> levels. A significant association was found between NO<sub>2</sub> levels and new-onset asthma during the follow-up period (OR = 1.52; 95% CI 1.09, 2.16) in subjects with the most common genotype of NQO1 rs2917666. A higher risk (OR = 2.02; 95% CI 1.16, 3.73) was observed for carriers of the NQO1 rs2917666 C allele. The role of genetic polymorphism in influencing asthma prevalence was also analyzed; results are presented in the next subsection.

Positive and significant associations with the development of asthma have also been observed by Modig et al. (2009) in young adults from three Swedish cities, some of whom were included in the ECRHS. New cases of asthma were defined as those that occurred between ECRHS I (1991–1993) and ECRHS II (1999–2001) and two distinct definitions were used: asthma onset (year of onset unknown) and incident asthma (year of onset known). Incident asthma consisted of true cases of asthma development between the survey cycles, while onset could include subjects that were asymptomatic and not taking medications at the time of the first survey. Per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> levels estimated from air dispersion models, ORs of 1.46 (95% CI 1.07, 1.99) and 1.54 (95% CI 1.00, 2.36) were found, respectively, for asthma onset and incident asthma. The relationship between NO<sub>2</sub> and asthma was not modified by gender, hay fever or wheeze. With the NO<sub>2</sub> tertile-based exposure analyses a dose–response pattern was observed, with higher, but non-significant, risk estimates for the third tertile of exposure (3<sup>rd</sup> > 2<sup>nd</sup> < 1<sup>st</sup>). The risk of developing asthma was also strongly related to living close to a major road; overall, the team concluded that results from this study showed an increased risk of developing asthma with elevated levels of vehicle exhaust outside the home.

Recently, a meta-analysis of 17 cohorts (8 birth cohorts and 9 child/adult cohorts) examined the association between long-term exposure to air pollution and the incidence of asthma (Anderson et al., 2013a). The inclusion criteria were that the study be an original peer-reviewed paper published in English (up to July 2010), be based on a population sample, and report a numerical exposure–response relationship for incidence adjusted for confounders and accompanied by an estimate of precision. Asthma incidence was defined as the incidence of diagnosed asthma or of new wheeze symptom between two assessments or, in birth cohorts followed up to age 10, a lifetime prevalence estimate of asthma or wheeze symptoms. A pooled random-effect OR of 1.07 (95% CI 1.02, 1.13) per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> was found based on 13 studies. A positive but non-significant pooled estimate was observed with PM<sub>2.5</sub> based on five studies only; there were too few estimates to perform a meta-analysis of O<sub>3</sub> or SO<sub>2</sub>. In analyses adjusted for publication bias, the pooled estimates and the statistical significance were reduced but the estimates remained positive. The results of this meta-analysis support the findings from individual studies that long-term NO<sub>2</sub> exposure is associated with asthma incidence.

**Prevalence/symptoms of asthma:** Several new studies have investigated the relationship between long-term exposure to air pollutants and prevalence of asthma and/or asthma-related



symptoms. In a cross-sectional study performed in Hamilton, ON (Sahsuvaroglu et al., 2009), where several exposure methods were used to estimate NO<sub>2</sub> exposure, no associations were found between any of the exposure estimates and the prevalence of asthma in the whole childhood population. Strong associations were observed in the subgroup of children without hay fever, where an OR of 1.137 (95% CI 1.012, 1.278) was observed among girls per 1 ppb increase in NO<sub>2</sub> exposure (estimates obtained from LURs; R<sup>2</sup> = 0.76). This effect remained significant in two-pollutant models including either SO<sub>2</sub> (r = 0.38) or O<sub>3</sub> (r = 0.44) but was no longer significant when PM<sub>10</sub> (R = 0.72) was included in the models, except for the girls aged 13–14 (OR = 1.287; 95% CI 1.008, 1.643). The NO<sub>2</sub>-LUR associations were attenuated and no longer significant in four-pollutant models including SO<sub>2</sub>, O<sub>3</sub> and PM<sub>10</sub>. A cross-sectional study (Akinbami et al., 2010) conducted among children residing in US metropolitan areas showed that current asthma and asthma attacks were related to long-term exposure to other pollutants, mainly O<sub>3</sub> and PM<sub>2.5</sub>, and not to ambient NO<sub>2</sub> gathered from central monitoring stations. Similar results were found with the quartile-based exposure assessment; a positive but non-significant association (OR = 1.02; 95% CI 0.71, 1.47) was, however, observed with the highest NO<sub>2</sub> quartile (30.8–40.2 ppb).

NO<sub>2</sub>-related asthma outcomes in children have also been investigated in a number of studies from other developed regions of the world. A cross-sectional study performed in six French cities (Pénard-Morand et al., 2010), where major urban air pollutants were estimated at school addresses using air pollution models, found significant associations between NO<sub>x</sub> (per 52.1 µg/m<sup>3</sup> (27.71 ppb)) increase and both exercised-induced asthma (OR = 1.25; 95% CI 1.11, 1.46), and asthma symptoms in the last year (OR = 1.32; 95% CI 1.00, 1.89) in children aged 9–11 who had resided at their current address for the past 3 years. Positive but non-significant associations were measured with NO<sub>2</sub> levels, while stronger associations were found with PM<sub>10</sub> and benzene.

Asthma symptoms in subjects 6 years old living in rural, small-town areas (Wesel and northwest Germany) were also not found to be related to NO<sub>2</sub> levels (per 9 µg/m<sup>3</sup> (4.79 ppb) increase) estimated with LUR models (R<sup>2</sup> = 0.81); OR = 0.64 (95% CI 0.40, 1.03) (Krämer et al., 2009).

In another cross-sectional study (Rosenlund et al., 2009b) no significant adverse associations were found between long-term exposure to NO<sub>2</sub> estimated by LUR models (R<sup>2</sup> = 0.69) or any of the traffic-related exposure metrics used (traffic intensity and heavy traffic) and asthma symptoms in children aged 9–14 living in Rome, Italy.

The association between long-term exposure to ambient NO<sub>2</sub> levels and asthma-related symptoms has also been studied in adult populations. In the US, a cross-sectional study performed in Los Angeles and San Diego counties, CA (Meng et al., 2007) using data from the 2001 California Health Interview Survey investigated the impact of air pollution levels on adults with poorly controlled asthma by using the annual average concentration measured at the nearest station within 5 miles of the residential cross-street intersection. No association was reported between NO<sub>2</sub> and poorly controlled asthma, which was defined as having in the past 12 months either daily or weekly asthma symptoms or at least one ERV or hospitalization because of asthma (quantitative results for NO<sub>2</sub> not reported in the paper). O<sub>3</sub> and PM<sub>10</sub> exposures were strongly associated with poorly controlled asthma.

Several European studies also investigated the relationship between long-term exposure to NO<sub>2</sub> and asthma prevalence/asthma-related symptoms in adults. Using two different exposure methods, a cross-sectional study (Rage et al., 2009a) performed in five French cities (Paris, Lyon, Marseille, Montpellier, and Grenoble) found no association between asthma severity over the past 12 months in adult asthmatics and NO<sub>2</sub> exposure estimates obtained with two different approaches (level from the closest monitoring station and spatial models using geostatistical



interpolations linked with air pollutants to the geocoded residences). Long-term O<sub>3</sub> exposure was found to be strongly associated with this outcome. In a follow-up study (Jacquemin et al., 2012) no associations were observed between long-term exposure to NO<sub>2</sub> and asthma control; instead, long-term exposures to O<sub>3</sub> and PM<sub>10</sub> were related to decreased asthma control in these adults. The study by Castro-Giner et al. (2009) discussed in the previous subsection found a positive but non-significant association between long-term exposure to NO<sub>2</sub> levels and asthma prevalence. This team observed, however, that the association between NO<sub>2</sub> levels and asthma prevalence was significant for carriers of the most common genotypes of the genes NQO1 and TNF-α. The NQO1 gene encodes for the enzyme NAD(P)H dehydrogenase [quinine] 1, involved in oxidative stress pathways, while TNF-α gene encodes for TNF-α, which is implicated in inflammatory responses. A test for interaction between NO<sub>2</sub> and polymorphisms of these genes in relation to asthma prevalence only showed a significant association with NQO1 rs2917666.

Some additional cross-sectional studies (Modig and Forsberg, 2007; Lindgren et al., 2009a, 2009b) have been performed in Sweden. Using a random sample (n = 2,800) of adult residents from three different cities, Modig and Forsberg (2007) found that the odds of reporting asthmatic symptoms in the past 12 months rose with increasing levels of modelled NO<sub>2</sub> (OR of 1.04; 95% CI 1.01, 1.07 per 1 µg/m<sup>3</sup> (0.53 ppb) increment). Based on a quintiles of exposure analysis, ambient modelled NO<sub>x</sub> was associated with asthma symptoms (OR = 1.21; 95% CI 0.99, 1.46; *P* trend = 0.026) in adult residents of southern Sweden aged 18–77 in the highest quintile only (>19 µg/m<sup>3</sup> (>10.11 ppb)) (Lindgren et al., 2009a). Based on geographical stratification the effects of NO<sub>x</sub> at the highest exposure quintile were only found in the city of Malmö and not outside this area. Stronger associations were also observed with residential traffic intensity. In a follow-up study, Lindgren et al. (2009b) found that a higher prevalence of allergic asthma (asthma triggered by pollen/furred animals) was associated with traffic intensity, but not asthma triggered by non-allergic factors; neither was associated with modelled levels of NO<sub>x</sub>.

Some cross-sectional studies that mainly focused on respiratory symptoms (subsection 9.3.1.2) also found no associations between long-term exposure to NO<sub>2</sub> and prevalence of asthma in residents of Rome (Cesaroni et al., 2008) and adult residents (aged 18–70) of Nottingham, England (Pujades-Rodriguez et al., 2009) or with asthma attacks in older subjects living in Bordeaux, France (Bentayeb et al., 2010).

Recently, Anderson et al. (2013b) completed a meta-analysis of multi-community studies to examine the association between long-term exposure to air pollution and asthma prevalence. The meta-analysis included peer-reviewed cross-sectional studies (up to November 2009) that were conducted in at least five communities and reported quantitative associations between air pollution and the prevalence of wheeze symptoms or asthma diagnosis while controlling for confounding factors at the individual level. Pooled random-effects risk estimates of 1.00 (95% CI 0.95, 1.06) and 1.00 (95% CI 0.99, 1.01) per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> were found for period of prevalence of wheeze symptoms and asthma diagnosis and for lifetime prevalence, respectively. Positive and non-significant associations were also noted for PM<sub>10</sub>, O<sub>3</sub> and SO<sub>2</sub>. Based on the results obtained for the various air pollutants, the authors concluded that there is no evidence of an association between long-term exposure to community levels of air pollution and asthma prevalence.

**Pulmonary inflammation in children:** A limited number of studies have been published examining associations between NO<sub>2</sub> exposure and changes in biomarkers of airway inflammation. Chen et al. (2008b) observed an interaction between NO<sub>2</sub> air pollution and some asthma-related biological/clinical inflammatory markers in a small group of asthmatic children from Vancouver, BC, exposed to higher chronic stress. High-resolution NO<sub>2</sub> exposure estimates were obtained by an LUR model (*R*<sup>2</sup> = 0.56) and NO<sub>2</sub> stress interactions were found for IL-5, IgE, eosinophil counts, daily diary symptoms and parent-reported symptoms, as well as with

PEF rate. No significant associations emerged for IL-4 and IL-13. Results from this study support the hypothesis of an interaction between the social and physical environments; the interactive effects on respiratory health of combined air pollution and stress were stronger than either factor alone.

Exhaled NO, which has been shown to be a useful non-invasive marker of airway inflammation in the large Californian cohort of public-school students (Linn et al., 2009), has recently been investigated in relation to various measures of traffic-related pollution in some members of this cohort (Eckel et al., 2011). This biomarker was not related to predicted annual average NO/NO<sub>x</sub>/NO<sub>2</sub> levels obtained with a model using measurements of these air pollutants taken at homes and schools of study participants and incorporating population density, elevation, land-use, and several indicators of traffic ( $R^2 = 0.75$  for NO and NO<sub>x</sub> and 0.67 for NO<sub>2</sub>). Based on an increase of 10 ppb in predicted NO<sub>2</sub> levels a small and non-significant negative association was observed with eNO (-1.2%; 95% CI -8.6%, 6.9%). The only traffic-related measure that displayed significant positive associations with eNO in this study was the total length of roads within circular buffers; this finding was observed only in asthmatic children, and was stronger with smaller buffers. Similarly, eNO was not related to NO<sub>2</sub> in another study of children aged 9–11 living in Windsor, ON (Dales et al., 2008), where positive but not statistically significant associations (4% difference; 95% CI -10.2%, 20.6% per 10 ppb NO<sub>2</sub> increase) were found between NO<sub>2</sub> and eNO, while significant associations were found with PM<sub>2.5</sub> and roadway density (length of both local and all roadways within 200 m of the home).

**Asthma hospitalizations/ERVs:** The relationships between NO<sub>2</sub> and hospital admissions or ERVs due to asthma have been assessed mostly through time-series studies of short-term exposure (subsection 8.3.1). However, some new studies (Delfino et al., 2009; Meng et al., 2010; Andersen et al., 2012b) have investigated the relationship between asthma hospital admissions/ERVs and long-term NO<sub>2</sub> exposure.

In Orange County, CA, Delfino et al. (2009) conducted a longitudinal study and found that children (aged 0–18) faced a significant increased risk of repeated hospital encounters with a primary diagnosis of asthma with increases in NO<sub>x</sub> levels. Residential addresses were geocoded and linked with air pollution levels estimated from air dispersion models. An HR of 1.097 (95% CI 1.034, 1.164) per 4 ppb increase in NO<sub>x</sub> levels was found, while a positive but non-significant association (HR = 1.042; 0.987, 1.101) was related to NO<sub>2</sub> per 2.68 ppb increase. This study also showed that girls (HR = 1.136; 95% CI 1.075, 1.238) and infants (HR = 1.197; 95% CI 1.075, 1.333) were at higher risk following exposure to NO<sub>x</sub>. Similar associations were also found with CO, another traffic-related air pollutant. In a cross-sectional study performed in the San Joaquin Valley, CA (Meng et al., 2010), annual levels of NO<sub>2</sub>, estimated based on the nearest air monitoring station, were not associated with asthma-related ERVs and/or hospitalization in asthmatic subjects aged 1–65 years old. Significant associations were, however, observed with O<sub>3</sub>, while positive but non-significant associations were found with both PM<sub>10</sub> and PM<sub>2.5</sub> that were not correlated with NO<sub>2</sub>. No quantitative results for NO<sub>2</sub> were included in the paper.

Using participants in the Danish Diet, Cancer and Health cohort study (n = 57,053; subjects aged 50–65 at baseline (1993–1997)), Andersen et al. (2012b) observed that NO<sub>2</sub> levels in Copenhagen and Aarhus were associated with increased risk for asthma hospitalizations. Based on an increase of 5.8 µg/m<sup>3</sup> (3.09 ppb) in modelled NO<sub>2</sub> levels, an OR of 1.12 (95% CI 1.04, 1.22) was found in the full cohort, while an OR of 1.10 (95% CI 1.01, 1.20) was found for first-ever admissions. Higher risks were also found in subjects with a history of asthma or COPD. Based on quartiles of exposure, a dose–response relationship was observed for NO<sub>2</sub>, with higher risks for people living in areas with higher air pollution levels.

#### 9.3.1.4 Development of Other Chronic Respiratory Diseases

The potential impact of exposure to long-term ambient NO<sub>2</sub> levels on the risk of hospitalization for pneumonia (Neupane et al., 2010) and COPD (Andersen et al., 2011; Gan et al., 2013) has also been recently investigated.

Significant associations were observed between long-term exposure to NO<sub>2</sub> and PM<sub>2.5</sub> and hospitalization for community-acquired pneumonia in a population-based case-control study conducted in Hamilton, ON (Neupane et al., 2010) in patients at least 65 years of age. Exposure estimates for NO<sub>2</sub> were estimated with various methods; risk estimates were calculated based on the 5<sup>th</sup>–95<sup>th</sup> percentile range increment, which corresponded to 11.59, 7.16 and 8.04 ppb of NO<sub>2</sub> estimated by bicubic spline, IDW and an LUR model ( $R^2 = 0.76$ ), respectively. For NO<sub>2</sub> levels estimated by IDW, an OR of 2.30 (95% CI 1.25, 4.21) was reported for pneumonia hospitalization. Similar risks were observed based on NO<sub>2</sub> levels estimated by the bicubic spline method (OR = 2.19; 95% CI 1.25, 3.83) and by the LUR method (OR = 1.70; 95% CI 1.00, 2.89).

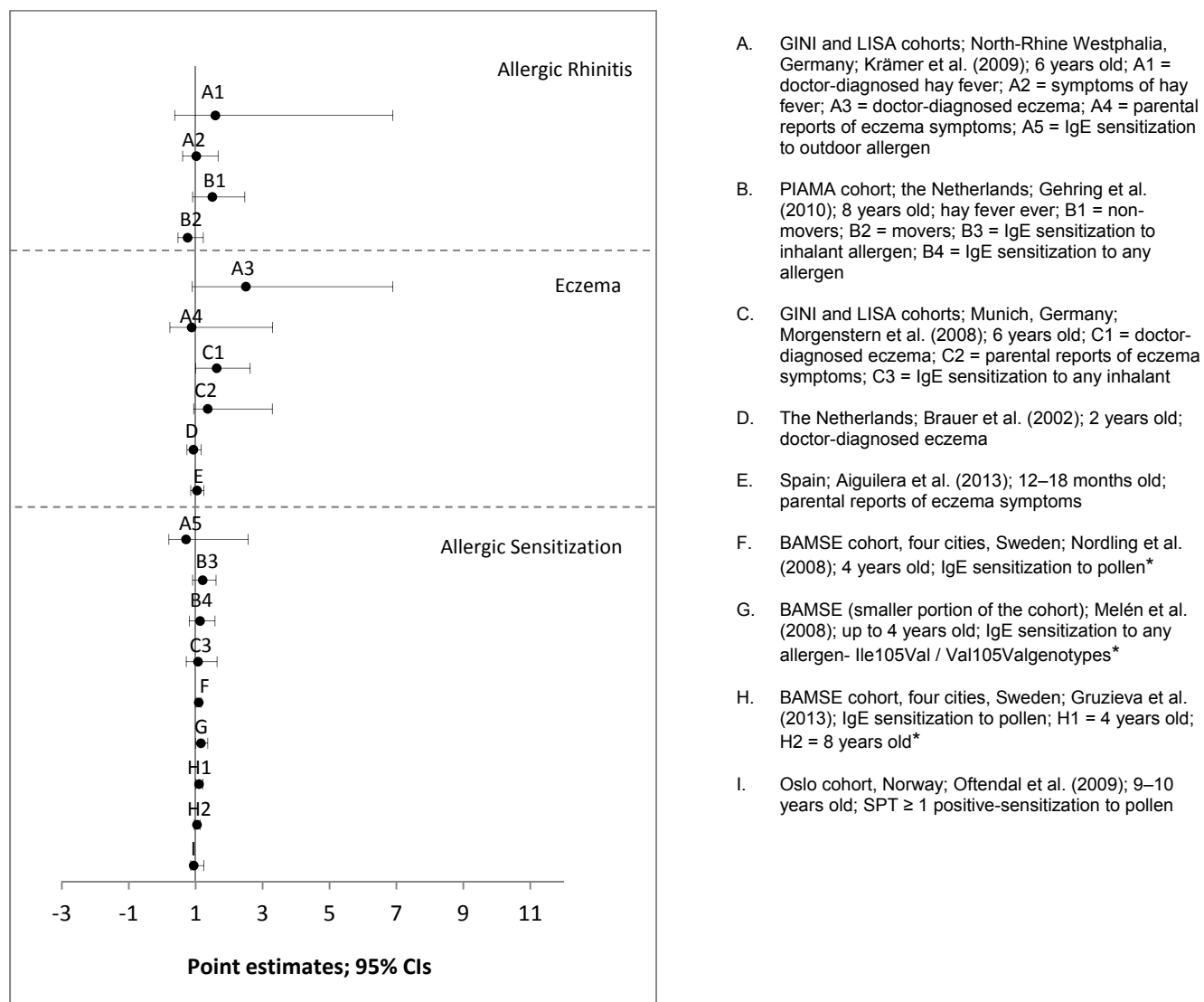
A significant association between long-term exposure to NO<sub>2</sub> and COPD hospitalization in residents aged 45–85 was found in a population-based study performed in Vancouver, BC (Gan et al., 2013), but only in the unadjusted single-pollutant models. An RR of 1.17 (95% CI 1.12, 1.21) was found per 8.4 µg/m<sup>3</sup> (4.47 ppb) increase in NO<sub>2</sub> levels; following adjustment for covariates this association was, however, attenuated and no longer significant. Long-term exposures to higher levels of BC as well as wood smoke air pollution were found to be associated with an increased risk of hospitalization due to COPD.

In Europe, with participants in the Danish Diet, Cancer and Health cohort study, a significant association was also found between first-ever hospital admissions for COPD and the 35-year mean NO<sub>2</sub> and NO<sub>x</sub> levels (1971–2006) estimated (with dispersion models) at each subject's residential address at the time of recruitment (Andersen et al., 2011). An adjusted HR of 1.08 (95% CI 1.02, 1.14) was estimated based on an IQR of 5.8 µg/m<sup>3</sup> (3.09 ppb) in NO<sub>2</sub> levels. Similar risk estimates were observed with the estimated 35-year NO<sub>x</sub> levels and shorter NO<sub>2</sub> exposure periods (25-year and 15-year NO<sub>2</sub> means). Sensitivity analyses were also performed between NO<sub>2</sub> (35-year mean) and COPD hospitalizations, and larger associations were observed in subjects with diabetes (HR = 1.29; 95% CI 1.05, 1.50) and asthma (HR = 1.19; 95% CI 1.03, 1.38).

#### 9.3.1.5 Allergic Responses

An allergic reaction occurs when a person's immune system reacts to normally harmless substances. An inflammatory response where IgE is produced is initiated following the exposure. Several epidemiological studies have reported increased risks of developing allergies in relation to ambient air pollution exposure. The studies reviewed in the 2008 US EPA ISA mainly investigated the effects of short-term exposure to NO<sub>2</sub> on allergic airway responses. Some recent studies have investigated the impact of long-term exposure to NO<sub>2</sub> levels in the development of allergic responses; all were conducted in Europe except for one US study. Point estimates (ORs), based on 10 ppb annual NO<sub>2</sub> concentrations, for allergic conditions (including allergic rhinitis, eczema and allergic sensitization to inhalant allergens) from North American and European cohort studies are presented in Figure 9.8.

**Figure 9.8: Point estimates (ORs) and 95% CIs from cohort studies for associations between a standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration and allergic responses in children in single-pollutant models**



\*Original risk estimates developed for NO<sub>x</sub>; a scaling factor of 0.35 was used for conversion to NO<sub>2</sub>. SPT = skin prick test

**Allergic rhinitis:** Allergic rhinitis (hay fever) is an allergic reaction that can lead to symptoms such as sneezing, nasal congestion and eye irritation. Several studies have examined the relationship between allergic rhinitis symptoms in children and air pollution.

Parker et al. (2009) examined the association between the reporting of respiratory allergy or hay fever in children who participated in the U.S. 1999–2005 National Health Interview Survey in a cross-sectional study and found no apparent relationship with NO<sub>2</sub> levels derived by spatial averages within 20 or 5 miles of a child's residence. Strong increased risks of respiratory allergies/hay fever were, however, found with both O<sub>3</sub> and PM<sub>2.5</sub>, and these effects remained

stable and significant in the five-pollutant models including several air pollutants ( $\text{O}_3$ ,  $\text{PM}_{2.5}$ ,  $\text{SO}_2$ ,  $\text{NO}_2$  and  $\text{PM}_{10-2.5}$ ).

In a cross-sectional study performed in six French cities (Pénard-Morand et al., 2010), positive and non-significant associations were observed with lifetime allergic rhinitis (OR = 1.14; 95% CI 0.92, 1.36) based on an IQR increase of  $18.5 \mu\text{g}/\text{m}^3$  (9.84 ppb) in  $\text{NO}_2$  in children aged 9–11 who had resided at their current address for the past 3 years. Similar results were found with exposure to  $\text{NO}_x$  levels, while more robust associations were found with  $\text{PM}_{10}$  and benzene. In this study, major urban air pollutants were estimated at school addresses using air pollution models.

In a cross-sectional study performed in Rome, Italy, a lack of association between  $\text{NO}_2$  levels (obtained with LUR models;  $R^2 = 0.69$ ) and allergic symptoms was also observed in children aged 9–14 (Rosenlund et al., 2009b; data only presented in figures). No significant associations were apparent between long-term exposure to  $\text{NO}_2$  (based on a  $9 \mu\text{g}/\text{m}^3$  (4.79 ppb) increase) estimated with an LUR model ( $R^2 = 0.81$ ) and prevalence of doctor-diagnosed hay fever (OR = 1.25; 95% CI 0.62, 2.52) and symptoms of hay fever (OR = 1.01; 95% CI 0.79, 1.28) in a cohort of children aged 6 living in rural areas of North-Rhine Westphalia, Germany (Krämer et al., 2009). In the Netherlands, where exposure estimates were obtained with LUR models ( $R^2 = 0.85$ ), Gehring et al. (2010) followed children during their first 8 years of life and found a positive association between  $\text{NO}_2$  exposures and hay fever, but only for those who did not change residence over the study period (OR = 1.25; 95% CI 0.95, 1.65 per  $10.4 \mu\text{g}/\text{m}^3$  (5.53 ppb) increase). Positive but non-significant associations were also found with symptoms of rhinitis in both movers and non-movers.

Some studies have also analyzed the impact on adults. In single-pollutant models, increased risks of rhinitis in adult residents of Rome (Cesaroni et al., 2008), who had been residents in the same place for at least 3 years, have been found to be associated with several exposure indices of traffic-related air pollution, including with  $\text{NO}_2$ , which was estimated using LUR models ( $R^2 = 0.69$ ). An OR of 1.13 (95% CI 1.04, 1.22) was observed for rhinitis per  $10 \mu\text{g}/\text{m}^3$  (5.32 ppb) increase in  $\text{NO}_2$ . Estimated  $\text{NO}_2$  and PM emissions were, however, highly correlated ( $r = 0.86$ ). Significant associations have also been observed in a randomly selected adult population (aged 18–77) in southern Sweden (Lindgren et al., 2009b) between modelled annual means of  $\text{NO}_x$  and both allergic rhinitis (rhinitis triggered by pollen, furred animals, house dust or mould), and non-allergic rhinitis. From quintiles of  $\text{NO}_x$  exposure the adjusted ORs based on highest ( $>19 \mu\text{g}/\text{m}^3$  ( $>10.11$  ppb)) vs. lowest quintile ( $0\text{--}8 \mu\text{g}/\text{m}^3$  ( $0\text{--}4.26$  ppb)) were, respectively, 1.33 (95% CI 1.13, 1.57) and 1.37 (95% CI 1.03, 1.81) for allergic and non-allergic rhinitis. For allergic rhinitis, significant associations were also observed with other traffic-exposure metrics, including self-reported traffic and GIS-measured traffic intensity (living within 100 m of a road with a traffic intensity of  $>10$  cars/min (24-h mean)). As similar results were found with allergic asthma, the team concluded that exposure to traffic was associated with higher prevalence of allergic asthma and allergic rhinitis.

In contrast to these results no significant cross-sectional associations (OR = 1.02; 95% CI 0.77, 1.37) were observed between hay fever and modelled  $\text{NO}_2$  levels based on the highest ( $>36.79 \mu\text{g}/\text{m}^3$  ( $>19.57$  ppb)) versus lowest quintile ( $<33.92 \mu\text{g}/\text{m}^3$  ( $<18.04$  ppb)) in adults aged 18–70 in the population-based study conducted in Nottingham, England (Pujades-Rodriguez et al., 2009). This study also found no evidence to suggest that living in close proximity to traffic is a major determinant of allergic diseases in adults.

**IgE /allergic sensitization:** The IgE response is a key trigger of allergic reactions, and several studies have investigated the impact of traffic-related air pollutants on the development of allergic sensitization in children.



In the Swedish BAMSE birth cohort study, exposure to air pollution from traffic (levels obtained with dispersion modelling) during the first year of life was associated with increased risk of allergic sensitization to inhalant allergens, measured as IgE, in 4-year-old children (Nordling et al., 2008). For pollen sensitization, an OR of 1.67 (95% CI 1.10, 2.53) per 44  $\mu\text{g}/\text{m}^3$  (23.40 ppb) increase in traffic- $\text{NO}_x$  was observed. The team also found a stronger association (OR = 2.02; 95% CI 1.20, 3.42) between sensitization to pollen and  $\text{NO}_x$  in children where parents reported an allergic disease. Similar results were found with traffic- $\text{PM}_{10}$ , a pollutant highly correlated with  $\text{NO}_x$  ( $r = 0.61$ ). No associations were found between air pollution and sensitization to furry pets. Using the same cohort, Melén et al. (2008) found that when exposed to high levels of traffic- $\text{NO}_x$ , children with Ile105Val/Val105Val genotypes (GSTP1 gene) were at increased risk of sensitization to any allergen (OR = 2.4; 95% CI 1.0, 5.3). The GSTP1- $\text{NO}_x$  interaction effect was more pronounced in children with the TNF-308 genotype (GA/AA). No interactions were observed between exposure to traffic- $\text{NO}_x$  and the variants in the gene ADRB2, the beta-2 adrenergic receptor gene.

The results of this study showed that genes involved in antioxidative and inflammatory pathways could play a role in the development of childhood allergy. A follow-up study of the Swedish population-based birth cohort (Gruzieva et al., 2012) found no relationship between long-term exposure to traffic-related modelled  $\text{NO}_x$  (46.7  $\mu\text{g}/\text{m}^3$  (24.84 ppb)) during the first 8 years of life and development of allergic sensitization in these children at age 8 (OR = 0.72; 95% CI 0.47, 1.11); a stronger association was, however, observed with exposure to  $\text{NO}_2$  (1-year avg) during the first year of life (OR = 1.30; 95% CI 0.76, 2.22). Similar results were observed for  $\text{PM}_{10}$  levels, which were highly correlated with  $\text{NO}_x$  levels during the study period. This study confirmed previous results from Nordling et al. (2008), where an increased risk of allergic sensitization to pollen at age 4 was associated with exposure to traffic-related  $\text{NO}_x$  during the first year of life (OR = 1.83; 95% CI 1.02, 3.28).

Significant associations between exposure to  $\text{NO}_2$  and elevated total IgE have also been observed in a subgroup of 881 schoolchildren in the Netherlands (van Roosbroeck et al., 2008). Based on an increase of 17.6  $\mu\text{g}/\text{m}^3$  (9.36 ppb) in outdoor  $\text{NO}_2$  levels measured at school, the prevalence ratio was 1.83 (95% CI 1.39, 2.42). A higher estimate was, however, observed following adjustment for measurement error using personal exposure data (PR = 4.20; 95% CI 1.54, 11.48).

In contrast to these studies, no association (RR = 0.94; 95% CI 0.76, 1.16) was observed with sensitization to any pollen allergen, based on an IQR of 19.5  $\mu\text{g}/\text{m}^3$  (10.37 ppb) in long-term  $\text{NO}_2$  air pollution, in schoolchildren aged 9–10 years old residing in Oslo, Norway (Ofstedal et al., 2007). Similar results were observed in children from two Munich birth cohorts, where an OR of 1.00 (95% CI 0.81, 1.23) for sensitization to outdoor inhalants was observed among 6-year-old children, based on 8.5  $\mu\text{g}/\text{m}^3$  (4.52 ppb) increases in  $\text{NO}_2$  levels (Morgenstern et al., 2008). In the German study, positive and significant associations were found with  $\text{PM}_{2.5}$ ,  $\text{PM}_{2.5}$  absorbance and distance to nearest main road (<50 m). In a cross-sectional study performed in Rome, a lack of association between  $\text{NO}_2$  levels and allergic sensitization was also observed in children aged 9–14 (Rosenlund et al., 2009b). In the Netherlands, Gehring et al. (2010), who followed children during their first 8 years of life, also found no significant associations between  $\text{NO}_2$  exposures and allergic sensitization. The cross-sectional study performed by Pénard-Morand et al. (2010) in six French cities also investigated the impact of long-term exposure to  $\text{NO}_2$  levels on sensitization to pollen. For allergic rhinitis, they observed no significant associations with sensitization to pollen (OR = 1.04; 95% CI 0.82, 1.36) based on an IQR increase of 18.5  $\mu\text{g}/\text{m}^3$  (9.84 ppb) in  $\text{NO}_2$  in children aged 9–11 who had resided at their current address for the past 3 years. Sensitization to pollens with  $\text{PM}_{10}$  and VOCs was particularly robust and reached borderline significance. No associations were noted between both

sensitization to indoor allergens (OR = 0.84; 95% CI 0.45, 1.60) and outdoor allergens (OR = 0.855; 95% CI 0.46, 1.57) in children age 6 living in rural areas of North-Rhine Westphalia, Germany (Krämer et al., 2009) based on 9  $\mu\text{g}/\text{m}^3$  (4.79 ppb) increases in  $\text{NO}_2$  levels estimated by LUR models ( $R^2 = 0.81$ ).

Some studies have also analyzed the impact on adults. In a large case-control study performed in five French centres (Paris, Lyon, Marseille, Montpellier and Grenoble) total IgE levels were not related to  $\text{NO}_2$  levels in 369 adult asthmatics in single-pollutant models (Rage et al., 2009b). Based on an IQR of 10  $\mu\text{g}/\text{m}^3$  (5.32 ppb) increase in  $\text{NO}_2$  levels, a percentage change in total IgE of -14% (95% CI -26.4%, -0.4%) was observed. The significant negative association between  $\text{NO}_2$  levels and total IgE levels was no longer significant when  $\text{O}_3$  was included in the model; the  $\text{NO}_2$  effect estimate decreased by 57%. No effects were found for either  $\text{SO}_2$  or  $\text{PM}_{10}$ , while a positive association was found with  $\text{O}_3$ . The authors concluded that the results from this study suggest that  $\text{O}_3$  may up-regulate total IgE levels among asthmatic adults. Similarly, no cross-sectional associations were found in adults aged 18–70 in a population-based study conducted in Nottingham (Pujades-Rodriguez et al., 2009) between long-term exposure to  $\text{NO}_2$  and total IgE (OR = 0.84; 95% CI 0.62, 1.15, based on the highest (>36.79  $\mu\text{g}/\text{m}^3$  (>19.57 ppb)) versus lowest quintile (<33.92  $\mu\text{g}/\text{m}^3$  (<18.04 ppb)). No significant cross-sectional associations were observed with home proximity to the roadside.

**Eczema:** This condition, which is also referred to as atopic dermatitis, is an inflammation of the outer layer of the skin. Several recent studies have investigated the impact of air pollution on eczema, especially in children. For the 4,907 children from the six French cities cross-sectional study (Pénard-Morand et al., 2010) who had resided at their current address for the past 3 years, lifetime eczema was significantly associated with long-term exposure to  $\text{NO}_2$  and  $\text{NO}_x$  levels. An OR of 1.23 (95% CI 1.04, 1.42) was found per increment of 18.5  $\mu\text{g}/\text{m}^3$  (9.84 ppb) in  $\text{NO}_2$  levels, corresponding to the annual IQR. Lifetime eczema was also significantly associated with both  $\text{PM}_{10}$  and CO. The authors concluded that these results support the emerging body of evidence that traffic-related air pollution can induce childhood asthma and allergic diseases.

Morgenstern et al. (2008), who used data from two major prospective cohort studies in Germany, found a positive and significant association between doctor-diagnosed eczema in 6-year-old German children in Munich and urban  $\text{NO}_2$  levels (OR = 1.18; 95% CI 1.00, 1.39) based on an annual IQR increase of 6.4  $\mu\text{g}/\text{m}^3$  (3.40 ppb), as well as a positive but non-significant association with parental reports of eczema symptoms (OR = 1.11; 95% CI 0.98, 1.50). Krämer et al. (2009), who used the subjects within these two cohorts living in rural, small-town areas, Wesel and northwest Germany, found in 6-year-old children a positive but non-significant association between long-term exposure to  $\text{NO}_2$  estimated by LUR models ( $R^2 = 0.81$ ), based on the inner 90% range of 9  $\mu\text{g}/\text{m}^3$  (4.79 ppb), and prevalence of doctor-diagnosed eczema (OR = 1.55; 95% CI 0.95, 2.52), but no association with symptoms of eczema (OR = 0.94; 95% CI 0.49, 1.77). By contrast, strong associations were observed with long-term exposure to soot (i.e. absorbance of particles less than 2.5  $\mu\text{m}$  diameter). Similar results were found also in the prospective birth cohort study performed in Spain (Aguilera et al., 2013), where a positive but non-significant association (OR = 1.02; 95% CI 0.92, 1.12) was found in children aged 12–18 months between a 10  $\mu\text{g}/\text{m}^3$  (5.32 ppb) increase in the annual mean  $\text{NO}_2$  level in their first year of life (estimated with LUR models;  $R^2 = 0.52$  to 0.75 for the different study areas) and eczema. Stronger associations were observed between postnatal exposure to benzene and eczema.

Only one study (Lindgren et al., 2009b) investigated the impact of long-term exposure to  $\text{NO}_2$  on eczema in adults; based on the  $\text{NO}_2$  quintiles of exposure no associations were found with self-reported allergic eczema, diagnosed hand-eczema and hand-eczema during the last 12 months.

Hand-eczema during the last 12 months was, however, significantly associated with living within 100 m of a road with >10 cars/min.

### 9.3.2 Cardiovascular Effects

The 2008 US EPA ISA (US EPA, 2008) did not provide insight into the role played by NO<sub>2</sub> in relation to cardiovascular effects. Only one study, the US Women's Health Initiative (Miller et al., 2007), was available at that time; it did not find any associations between long-term NO<sub>2</sub> exposure and fatal and nonfatal cardiovascular events, including first occurrence of coronary revascularization, MI, stroke or death from CHD. In this study, post-menopausal women (n = 65,893) from 36 US metropolitan areas without previous CVDs were followed from 1994 through 1998. In single-pollutant models, an HR of 0.98 (95% CI 0.89, 1.08) was found per 10 ppb increase in the annual NO<sub>2</sub> average for an overall risk of a first cardiovascular event. This association was attenuated (HR = 0.82; 95% CI 0.70, 0.95) in three-pollutant models including PM indices (PM<sub>2.5</sub> and PM<sub>10-2.5</sub>) and other gaseous pollutants (SO<sub>2</sub>, O<sub>3</sub> and CO). Among these postmenopausal women, only long-term exposure to PM<sub>2.5</sub> air pollution was independently associated with the incidence of CVD (correlation coefficients among the different pollutants were not reported by the authors).

Some new studies investigating the impact of long-term exposure to NO<sub>2</sub> on cardiovascular morbidity outcomes have been published recently. Most assessed the impact of traffic air pollutants on stroke incidence or hospitalization due to stroke; of these studies two have been performed in Canada (Johnson et al., 2010, 2013), and another in the US (Lipsett et al., 2011), while the rest have been conducted in Europe (Oudin et al., 2009, 2010; Andersen et al., 2012a; Maheswaran et al., 2012). Some additional studies investigating the impact of long-term exposure to ambient NO<sub>x</sub>/NO<sub>2</sub> on other cardiovascular outcomes, including incidence of CHD and MI events, HRV, chronic inflammation, and atherosclerosis, are also reviewed as part of this assessment.

#### 9.3.2.1 Hospitalizations and Emergency Room Visits

Stroke or cerebrovascular accident due to disturbance in the blood supply to the brain is a medical emergency that can cause permanent neurological damage or death. Johnson et al. (2010) performed an ecological analysis in Alberta to study the impact of long-term exposure to ambient air pollution on the incidence of stroke. Stroke data were extracted for patients who visited one of the 11 emergency department sites in the metropolitan area of Edmonton between 2003 and 2007. An increase of 7.14 ppb in NO<sub>2</sub> levels (avg 5-year ambient NO<sub>2</sub> concentrations/IQR) was positively and significantly associated with stroke incidence (RR = 1.08; 95% CI 1.05, 1.12) and more particularly with incidence of hemorrhagic stroke (RR = 1.14; 95% CI 1.06, 1.22). Higher risk estimates were observed with the fifth quintile (16.7–20.3 ppb) of NO<sub>2</sub> (RRs = 1.29; 95% CI 1.16, 1.43 for all stroke and 1.46; 95% CI 1.19, 1.80 for hemorrhagic stroke). Risk estimates were, however, strongly reduced and no longer statistically significant after adjustment for household income levels. Recently, the same team (Johnson et al., 2013) performed a case-control study in the Edmonton area to investigate the impact of NO<sub>2</sub> exposures (over the past year) on stroke incidence; cases were patients who visited one of the 11 emergency department sites in the metropolitan area of Edmonton between 2007 and 2009. Using highly resolved estimates of outdoor residential levels of NO<sub>2</sub> (obtained based on LUR models (R<sup>2</sup> = 0.81)) no significant associations between NO<sub>2</sub> and any stroke outcomes were found. ORs of 1.01 (95% CI 0.94, 1.08), 1.03 (95% CI 0.94, 1.13) and 1.07 (95% CI 0.92, 1.24) were, respectively, observed for all strokes, acute ischemic strokes and hemorrhagic strokes, based on increases of 5 ppb in NO<sub>2</sub> levels, while a negative OR of 0.95 (95% CI 0.86, 1.05) was found with transient ischemic attack.

In the female California Teachers Study cohort (Lipsett et al., 2011), long-term exposure to PM was found to be associated with increased risk of incident stroke, combining fatal and nonfatal (hospitalization) events. Details of this cohort and results of the mortality associations have been presented in subsection 9.2.2. Long-term exposure to NO<sub>2</sub> (10.3 ppb) was positively but not significantly associated with hospital admissions for stroke (HR = 1.02; 95% CI 0.90, 1.16) and MI (HR = 1.05; 95% CI 0.90, 1.24).

Weak positive and non-significant associations between exposure to NO<sub>2</sub> levels at residence (based on an IQR increase of 5.7 µg/m<sup>3</sup> (3.03 ppb)) and first-ever (incident) hospital admissions for any stroke (HR = 1.05; 95% CI 0.99, 1.11) and ischemic stroke (HR = 1.05; 0.95, 1.17) were also found in a Danish cohort (Andersen et al., 2012a) of 57,053 participants from Copenhagen or Aarhus aged 50–65 at recruitment. No associations were observed with hemorrhagic stroke (HR = 0.93; 95% CI 0.81, 1.07). Associations between NO<sub>2</sub> levels and fatal stroke, previously reported in subsection 9.2.2, were markedly stronger. Similar risks were also reported by Maheswaran et al. (2012), who performed a small-area level ecological study in the UK from 1995 to 2004 using data from the South London Stroke Register, which captures all incident cases of first-ever stroke among the population of a defined geographical area. For all ages, a positive but non-significant association was estimated for ischemic stroke (RR = 1.11; 95% CI 0.93, 1.32) per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> levels; higher risk estimates were observed among people aged 65–79 (RR = 1.23; 95% CI 0.99, 1.90), suggesting that this age group might be more susceptible to long-term exposure to NO<sub>2</sub>. As was found by Andersen et al. (2012a) there were no associations between long-term exposure to NO<sub>2</sub> and the incidence of hemorrhagic stroke (RR = 0.86; 95% CI 0.60, 1.24). Similar positive and non-significant associations were also observed with PM<sub>10</sub> levels.

No evident associations were found through registry-based case-control studies (Oudin et al., 2009, 2011) between long-term exposure to NO<sub>x</sub> and hospital admissions for ischemic stroke in Scania, southern Sweden, an area with relatively low levels of air pollution. Significant associations were, however, observed in diabetics for each NO<sub>x</sub> exposure category; ORs of 1.3 (95% CI 1.1, 1.6), 2.0 (95% CI 1.4, 2.7) and 2.0 (95% CI 1.2, 3.4) were, respectively, measured for NO<sub>x</sub> categories of <15 µg/m<sup>3</sup> (<7.98 ppb), 15–<25 µg/m<sup>3</sup> (7.98–<13.30 ppb) and ≥25 µg/m<sup>3</sup> (≥13.30 ppb).

NO<sub>2</sub>-related hospital admissions for cardiovascular conditions have also been studied in a large population-based cohort study (n = 466,727; aged 45–83) in metropolitan Vancouver, BC (Gan et al., 2011). No association was found between CHD hospitalizations and NO<sub>2</sub> (RR = 0.97; 95% CI 0.95, 0.99 per 8.4 µg/m<sup>3</sup> (4.47 ppb) increase); long-term exposure to fine PM, indicated by BC, was, however, related to CHD hospitalizations.

The relationship between long-term exposure to ambient NO<sub>2</sub> levels and the incidence of CHD and MI events has also been investigated. In Rome, Italy, using data from the regional hospital-discharge registry, which covers all public and private hospitals, Rosenlund et al. (2008) found a significant association between NO<sub>2</sub> (based on a 10 µg/m<sup>3</sup> (5.32 ppb) increase) and total incidence of CHD events (RR = 1.03; 95% CI 1.00, 1.07) in residents aged 35–84 for the 1998–2000 period. A positive but non-significant association was found with non-fatal hospitalizations (RR = 1.01; 95% CI 0.97, 1.05). Using cases of MI identified through registries of hospital discharges during 1985 to 1996, the same team (Rosenlund et al., 2009a) found no association between long-term exposure to the 5-year average NO<sub>2</sub> concentrations (obtained from reconstructed emission data, dispersion models, and GIS techniques) and incidence of nonfatal MI (OR = 0.94; 95% CI 0.89, 1.00 per 31 µg/m<sup>3</sup> (16.49 ppb) increase) in Stockholm County among individuals aged 15–79. As with the findings of Andersen et al. (2012a), stronger associations were observed for fatal coronary events and especially those occurring out-of-hospital. Significant associations between NO<sub>2</sub> levels (estimated at residential address from



dispersion models in 2002) and incident cases of heart failure were found in patients aged 40–89 who were registered with general practitioners in England (Atkinson et al., 2013). Cardiovascular events were identified through primary care records from national registers of hospital admissions and death certificates. With Cox proportional hazards models, an HR of 1.06 (95% CI 1.01, 1.11) was found for heart failure in single-pollutant models based on an IQR of  $10.7 \mu\text{g}/\text{m}^3$  (5.69 ppb) in annual  $\text{NO}_2$  concentrations. Other cardiovascular outcomes, including MI, stroke and arrhythmias, were not associated with  $\text{NO}_2$  levels. Similar associations were found with  $\text{PM}_{10}$  levels, which were highly correlated with  $\text{NO}_2$  ( $r = 0.86$ ), while  $\text{SO}_2$  was significantly associated with all cardiovascular outcomes.

### 9.3.2.2 Other Cardiovascular Outcomes

Some additional studies (Dietrich et al., 2008; Forbes et al., 2009b; Panasevich et al., 2009; Lenters et al., 2010) investigated the relationship of long-term exposure to ambient  $\text{NO}_2$  with other cardiovascular outcomes, including HRV, chronic inflammation, vascular damage, self-reported incidence of hypertension and both systolic and diastolic blood pressure. In the population-based Swiss cohort Study on Air Pollution and Lung Diseases in Adults (SAPALDIA) (Dietrich et al., 2008) significant decreases in HRV were observed in women, but not in men, with a  $10 \mu\text{g}/\text{m}^3$  (5.32 ppb) increase in 1-year averaged  $\text{NO}_2$  level. Reduced HRV is generally interpreted as a decreased ability to respond to changes in the environment and is associated with cardiac dysfunction and disease. Decrements of 3% (95% CI -4%, -1%), 6% (95% CI -11%, -1%) and 5% (95% CI -9%, 0%) were measured, respectively, in the SDNN, nighttime LF and nighttime LF:HF ratio. Stronger decrements were observed in women with self-reported cardiovascular problems.

Among adult participants in three cross-sectional studies performed in England (Forbes et al., 2009b) no associations were found between circulating levels of two markers of systemic chronic inflammation (fibrinogen or CRP concentrations) and concentrations of  $\text{NO}_2$ ,  $\text{SO}_2$ ,  $\text{O}_3$  or  $\text{PM}_{10}$ . Significant increases in serum levels of inflammatory markers (IL-6 and CRP) were reported following long-term exposure to traffic- $\text{NO}_2$  and heating- $\text{SO}_2$  in men and women of Stockholm, Sweden, aged 45–70 (Panasevich et al., 2009). No associations were, however, found between long-term exposure to traffic- $\text{NO}_2$  and TNF- $\alpha$  and blood coagulation markers (fibrinogen and PAI-1).

Exposure to ambient air pollution, particularly residential exposure to ambient  $\text{PM}_{2.5}$ , has been previously associated with carotid artery intima-media thickness (CIMT), a measure of atherosclerosis. Using data from the Atherosclerosis Risk in Young Adults Study (Lenters et al., 2010) no associations were found between any of the traffic air pollutants ( $\text{NO}_2$ ,  $\text{SO}_2$ , BS) and CIMT; risk estimates for  $\text{PM}_{2.5}$  were, however, in line with those from previous studies. Significant associations were measured between exposure to gaseous pollutants and arterial stiffness: a 4.1% change in pulse wave velocity (95% CI 0.1%, 8.0%) per  $25 \mu\text{g}/\text{m}^3$  (13.30 ppb) increase in  $\text{NO}_2$  levels. Long-term exposure to  $\text{NO}_2$  was also significantly associated with the augmentation index, a ratio calculated from the blood pressure waveform (37.58% change; 95% CI 2.23%, 72.93% per  $25 \mu\text{g}/\text{m}^3$  (13.29 ppb) increase). These associations remained stable and significantly positive in four-pollutant models including BS,  $\text{PM}_{2.5}$  and  $\text{SO}_2$ . Correlation coefficients were all  $>0.5$  for  $\text{NO}_2$ , BS and  $\text{PM}_{2.5}$ , while  $\text{SO}_2$  was poorly correlated with other pollutants ( $r = 0.1$ ). The percentage change in these vascular damage outcomes also tended to be higher in women and in subjects with less education, and the team concluded that low air pollution levels may cause early vascular damage.

Using the participants in the Danish Diet, Cancer and Health cohort study, Sørensen et al. (2012) found no clear associations between self-reported incidence of hypertension and long-term exposure to  $\text{NO}_x$  (RR = 1.01; 95% CI 0.95, 1.08 per doubling of  $\text{NO}_x$  exposure (specific increment not specified) in the 1-year period and RR = 1.01; 95% CI 0.94, 1.09 per doubling of



NO<sub>x</sub> exposure in the 5-year period). Long-term exposures to NO<sub>x</sub> were inversely associated with small changes in blood pressure. A doubling of NO<sub>x</sub> exposure during the 1-year period preceding enrollment was associated with decreases of 0.50 mmHg (95% CI -0.84 mmHg, -0.16 mmHg) and 0.24 mmHg (95% CI -0.42 mmHg, -0.07 mmHg), respectively, for systolic and diastolic blood pressure. Similar results were found based on a doubling of NO<sub>x</sub> exposure for the 5-year period. The relationship between NO<sub>x</sub> exposure and systolic blood pressure was stronger in women (-0.83 mmHg; 95% CI -1.23 mmHg, -0.43 mmHg).

### 9.3.3 Cancer Incidence

Only two epidemiologic studies conducted in Europe (Nyberg et al., 2000; Nafstad et al., 2003) were reviewed in the 2008 US EPA ISA. In Stockholm, Nyberg et al. (2000) conducted a population-based case-control study among men aged 40–75 and found that long-term exposure to NO<sub>2</sub> mapped at the residence addresses (10-year avg) was associated with lung cancer; ORs of 1.10 (95% CI 0.97, 1.23) and 1.44 (95% CI 1.05, 1.99) were found, respectively, for NO<sub>2</sub> increases of 10 µg/m<sup>3</sup> (5.32 ppb) and 29.26 µg/m<sup>3</sup> (15.56 ppb; 90<sup>th</sup> percentile). Similar incidence RRs were obtained in two-pollutant models with SO<sub>2</sub> ( $r = 0.64$ ), which remained robust for NO<sub>2</sub> while little association was observed for SO<sub>2</sub>. In a Norwegian cohort study among men aged 40–49 living in Oslo (Nafstad et al., 2003) an increase of 10 µg/m<sup>3</sup> (5.32 ppb) in NO<sub>2</sub> exposure levels (5-year avg) was associated with an OR of 1.08 (95% CI 1.02, 1.15) for lung cancer; the OR for NO<sub>2</sub> exposures ≥30 µg/m<sup>3</sup> (≥15.96 ppb) was 1.36 (95% CI 1.01, 1.83). The similarity in the estimates of effect in these two studies is striking. Overall, these studies showed an association between long-term NO<sub>2</sub> exposure, used as an indicator of traffic exposure, and increased risk of lung cancer. NO<sub>2</sub> may be acting as an indicator of traffic-related carcinogens (such as PAHs).

In recent years, additional studies have investigated the association between long-term exposures to NO<sub>2</sub> and incidence of cancer; most studies focused on the development of lung cancer while some investigated the relation to other types of cancer.

One new hospital-based case-control study by Crouse et al. (2010) performed in Montreal, QC, investigated the association between long-term exposure to traffic-NO<sub>2</sub> air pollution and incidence of post-menopausal breast cancer at 18 hospitals in the greater Montreal area. No other pollutants were analyzed. Ambient NO<sub>2</sub> was sampled in areas likely to experience high spatial variability in traffic-related pollution and population densities, and an LUR model was developed to predict the annual mean concentrations ( $R^2 = 0.80$ ). For each 5 ppb increase in NO<sub>2</sub> adjusted ORs of 1.16 (95% CI 0.94, 1.42), 1.31 (95% CI 1.00, 1.71) and 1.35 (95% CI 0.94, 1.94) were found, respectively, for NO<sub>2</sub> estimates of exposure for 1985, 1996 and 2006, with unconditional logistic regressions. Higher risks were found among subjects who lived at the same address for more than 10 years.

The relationship between predicted concentrations of NO<sub>2</sub> levels in 2006 (estimated by LUR at the current address;  $R^2 = 0.80$ ) and prostate cancer risk was analyzed in a case-control study in Montreal (Parent et al., 2013), where a significant association was observed (adjusted OR of 1.44; 95% CI 1.21, 1.73 based on a 5 ppb increase). Significant associations were also found using back-extrapolated estimates of NO<sub>2</sub> exposure 10 years earlier (1996). Associations were not always significant following several additional sensitivity analyses, including back-extrapolation of NO<sub>2</sub> estimates 20 years prior.

No cohort studies performed in the US were published in the review period; however, ecological studies assessing the correlation between trends in NO<sub>x</sub> emissions and regional variations in cancer incidence have been conducted. Age-adjusted incidence rates for lung adenocarcinoma (Chen et al., 2007) and female breast cancer (Wei et al., 2012) were evaluated for nine US

regions; they indicated that the increased trend in NO<sub>x</sub> emissions paralleled the increase in cancer incidence. Other environmental exposures could, however, also explain those findings.

A number of new studies investigating the relationship between long-term exposure to NO<sub>2</sub> and cancer incidence have been carried out in Europe.

Vineis et al. (2006) conducted a nested case-control study within the Epidemiology Prospective Investigation on Cancer and Nutrition (EPIC). The EPIC study was designed to investigate the relationship between the incidence of cancer and other chronic diseases and diet, nutritional status, lifestyle and environmental factors in 10 European countries. More than 500,000 healthy volunteers aged 35–75 were recruited in 1993–1998. Community-level exposure estimates were derived for NO<sub>2</sub> using the nearest background monitoring stations, based on the residence address at the time of enrollment. An increment of 10 µg/m<sup>3</sup> (5.32 ppb) in NO<sub>2</sub> levels was positively associated with an OR of 1.14 (95% CI 0.78, 1.67) for lung cancer. Stronger associations were observed for residences located in proximity to heavy-traffic roads, i.e. for exposure levels >30 µg/m<sup>3</sup> (>15.96 ppb) (OR = 1.56 (95% CI 1.13, 2.16)) and they remained significant following adjustments for occupational exposures. Multiple statistical analysis techniques, including profile regression, a powerful tool to examine the joint effect of multiple risk factors, were applied in a follow-up study (Papathomas et al., 2011) to the case-control study of lung cancer in non-smokers nested within the EPIC cohort. While the profile regression analysis indicated higher NO<sub>2</sub> and PM<sub>10</sub> exposures for the high-risk group, no associations were found between NO<sub>2</sub> and lung cancer incidence with the logistic regression model.

The relationship between long-term exposures to NO<sub>x</sub>/NO<sub>2</sub> and lung cancer has also been assessed in Europe using data from major cohorts: a Dutch cohort (Beelen et al., 2008b/Brunekreef et al., 2009) and a Danish cohort (Raaschou-Nielsen et al., 2010, 2011a). In the Dutch cohort, in which 2,183 lung cancer cases were identified among participants, no evidence of an association was found between NO<sub>2</sub> and lung cancer incidence, with ORs of 0.86 (95% CI 0.70, 1.07) and 0.86 (95% CI 0.57, 1.29), respectively, in the full and the case-cohort analyses, for an increase of 30 µg/m<sup>3</sup> (15.96 ppb) in NO<sub>2</sub> concentrations.

In Denmark, the AirGIS modelling system allows for the estimation of exposure at a high spatial resolution—at address level. Using a case-cohort design, Raaschou-Nielsen et al. (2010) observed an OR of 1.37 (95% CI 1.06, 1.76) between lung cancer incidence among members of three Danish cohorts and a 100 µg/m<sup>3</sup> (53.19 ppb) increment in NO<sub>x</sub> concentration. Incidence rate ratios for lung cancer were also computed for NO<sub>x</sub> exposure levels between 29.8 and 72.4 µg/m<sup>3</sup> (15.85–38.51 ppb) (RR = 1.30; 95% CI 1.07, 1.57) and for NO<sub>x</sub> levels ≥72.4 µg/m<sup>3</sup> (≥38.51 ppb) (RR = 1.45; 95% CI 1.12, 1.88). It should, however, be noted that a strong correlation between NO<sub>x</sub>, an indicator of traffic-related air pollution, and PM<sub>10</sub> (r = 0.70) was observed. Single-pollutant models for PM<sub>10</sub> were not performed.

Similar results were found by the same team in a follow-up study (Raaschou-Nielsen et al., 2011a) using only participants from the Danish Diet, Cancer and Health cohort study in which 592 lung cancer cases were identified. For each 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> concentrations (calculated at the residence by dispersion models) an OR of 1.08 (95% CI 0.94, 1.24) was observed with lung cancer incidence. Based on the quartiles of exposure, positive but non-significant associations were found with the second, third and fourth NO<sub>2</sub> quartiles. A significant risk estimate of 1.30 (95% CI 1.05, 1.61) was found with the highest quartile of NO<sub>x</sub> exposure (>29.7 µg/m<sup>3</sup> (>15.8 ppb)) compared with the lowest (<17.27 µg/m<sup>3</sup> (<9.15 ppb)). The associations throughout the second and third quartiles of exposure did not follow an increasing exposure–response pattern; a positive but non-significant association (OR = 1.09; 95% CI 0.84, 1.40) was observed in the second quartile, while a negative effect was noted in the third quartile. In sensitivity analyses, using the highest versus the lowest NO<sub>x</sub> exposure categories,

stronger associations were found among females (OR = 1.45; 95% CI 1.06, 1.99), non-smokers (OR = 1.91; 95% CI 1.10, 3.30), people with a longer school attendance (OR = 1.71; 95% CI 1.25, 2.33) and those with a relatively low fruit intake (OR = 1.55; 95% CI 1.12, 2.13). Positive associations were also observed with other markers of traffic exposure at the residence (e.g. traffic load within 200 m, major road within 50 m), and the authors concluded that the results of this study support the hypothesis of an association between lung cancer incidence and traffic-related air pollution.

Using data from the above cohort, Raaschou-Nielsen et al. (2011b), evaluated the association between long-term NO<sub>x</sub> exposures and 20 types of cancer. Increased risks for cervical (RR = 2.94; 95% CI 1.0, 5.93) and brain cancer (RR = 2.28; 95% CI 1.25, 4.19) were found per 100 µg/m<sup>3</sup> (53.19 ppb) increase in NO<sub>x</sub> at residential addresses. Positive but non-significant associations were also observed for several other types, including buccal cavity and pharynx, oesophagus, liver, uterus, kidney, bladder, and breast cancer and non-Hodgkin's lymphoma.

In a case-control study, intermediate (12.2–16.1 µg/m<sup>3</sup> (6.49–8.56 ppb)) and high (≥16.2 µg/m<sup>3</sup> (≥8.62 ppb)) traffic-NO<sub>2</sub> levels for mainland France (estimated at the place of residence by dispersion modelling) were significantly associated with acute leukemia incidence in 15-year-old children, with ORs of 1.3 (95% CI 1.06, 1.6) and 1.2 (95% CI 1.0, 1.5), respectively (Amigou et al., 2011). Significant associations were also found with indicators of proximity to or density of main roads. Stronger associations were found (data not shown) in children who had lived for at least 2 years at the same address.

### 9.3.4 Other Morbidity Effects

One area in which the recent literature has contributed new information is in the study of other potential adverse health effects related to long-term exposure to ambient NO<sub>2</sub>/NO<sub>x</sub>, including annoyance, diabetes, inflammatory bowel disease (IBD), neurological diseases/effects, otitis media, osteoporosis and rheumatoid arthritis.

#### 9.3.4.1 Annoyance

Some new studies have investigated the relationship between air pollution and annoyance. Exposure to NO<sub>x</sub> was found to be positively and significantly ( $p < 0.05$ ) associated with annoyance from traffic noise and exhaust fumes (Persson et al., 2007) in participants aged 18–80 from a population-based public health survey in Scania, Sweden. Modig and Forsberg (2007) also found that perceived annoyance to vehicle exhaust was significantly associated with NO<sub>2</sub> exposure in a random sample of persons aged 16–70 living in three different Swedish cities. Significant associations were also observed with reporting daily irritating and asthmatic symptoms. An association between self-reported annoyance and outdoor NO<sub>2</sub> levels in 20 European cities was also reported by Jacquemin et al. (2008). Different strengths were, however, observed from city to city, and the authors concluded that annoyance might not be a valid surrogate for ambient air pollution exposure but might be a measure of perceived ambient air quality that could serve as a complementary tool for health surveillance.

Based on quintiles of exposure, significant associations between increasing NO<sub>2</sub> levels and annoyance about air pollution problems among participants in the Oslo Health Study were also observed by Piro et al. (2009). In these participants, subjects with a chronic disease reported more air pollution problems than healthy people (even if they were exposed to similar air pollution levels), suggesting that health status might influence the reporting of air pollution problems. Finally, strong associations have also been reported more recently between odour annoyance and modelled NO<sub>2</sub> levels (estimated by LUR models;  $R^2 = 0.79$ ) in Sarnia, ON, a small but heavily industrialized area (Atari et al., 2009).

#### 9.3.4.2 Diabetes

Diabetes mellitus is a group of metabolic disorders characterized by high sugar levels in the blood due to an insufficient production of insulin or a lack of cell response to the insulin produced; it is a growing population health concern. Some recent studies (Brook et al., 2008; Krämer et al., 2010; Dijkema et al., 2011; Coogan et al., 2012; Andersen et al., 2012c) have investigated the relationship between air pollution and the prevalence and incidence of diabetes, especially Type 2 diabetes mellitus, which was formerly called non-insulin-dependent diabetes mellitus. Mortality from diabetes was also studied in a Danish cohort (Raaschou-Nielsen et al., 2013) while one another recent study (Malmqvist et al., 2013) performed in southern Sweden investigated the impact of air pollutants on gestational diabetes.

Brook et al. (2008) conducted a case-control study of diabetes in Southern Ontario. Predicted pollution exposures estimated with LUR models were assigned to patients recruited from two academic respiratory disease clinics located in Hamilton and Toronto ( $R^2 = 0.69$  and  $0.76$ , respectively). Logistic regression models were used to evaluate the associations between  $\text{NO}_2$  air pollution levels and diabetes while adjusting for age, body mass index, and neighborhood income. In Toronto, using logistic regression models, a borderline positive association was found in women (OR = 1.055; 95% CI 0.99, 1.11) per 1 ppb increase in  $\text{NO}_2$  levels; for the same increment, the risk estimate was about half the size in the Hamilton women's group (OR = 1.029; 95% CI 0.98, 1.08). There were no positive associations among men. In the random effects meta-analysis combining both cities a positive but non-significant association (OR = 1.015; 95% CI 0.98, 1.49) was found for both sexes combined, while a positive and significant association was found in women (OR = 1.04; 95% CI 1.00, 1.08). The authors suggested that the stronger effects seen in women could be explained by lower exposure measurement errors, as women usually tend to spend more time around the home.

An association between long-term exposure to  $\text{NO}_x$  and incidence of type 2 diabetes mellitus was also found in black women (aged 21–69) living in Los Angeles, CA (Coogan et al., 2012). Following adjustment for several potential confounders an incidence rate ratio of 1.25 (95% CI 1.07, 1.46) per 12.4 ppb increase in  $\text{NO}_x$  levels estimated at participants' residential addresses with LUR models ( $R^2 = 0.85$ ) was found in single-pollutant models. The association was robust in two-pollutant models with  $\text{PM}_{2.5}$  ( $r = 0.27$ ); positive, but non-significant, associations were observed with  $\text{PM}_{2.5}$  in both single and two-pollutant models.

Two cohort studies of diabetes have also been performed in Europe: one in Germany (Krämer et al., 2010) and one in Denmark (Andersen et al., 2012c). A relationship between traffic-related  $\text{NO}_2$  estimated by LUR models ( $R^2 = 0.81$ ) and incident Type 2 diabetes mellitus among older women (aged 54–55) was found in the German cohort study. The HRs associated with  $\text{NO}_2$  levels from monitoring stations, a traffic emission inventory and land-use regressions were, respectively, 1.34 (95% CI 1.02, 1.76) per  $24.9 \mu\text{g}/\text{m}^3$  (13.24 ppb), 1.15 (95% CI 1.04, 1.27) per  $19 \mu\text{g}/\text{m}^3$  (10.11 ppb) and 1.42 (95% CI 1.16, 1.73) per  $15 \mu\text{g}/\text{m}^3$  (7.98 ppb). This study also showed that women with high complement factor C3c (a marker for subclinical inflammation) in their serum at baseline were more susceptible to  $\text{NO}_2$ -related excess risk of diabetes than women with low C3c levels. Weak positive associations between exposure to  $\text{NO}_2$  levels at residence and incident diabetes (all cases) were found in the Danish study (Andersen et al., 2012c), which followed 57,053 participants from Copenhagen or Aarhus aged 50–65 at recruitment. The relationship became statistically significant when the analysis was restricted to confirmed cases of diabetes (stricter definition), with an HR of 1.04 (95% CI 1.00, 1.08) per IQR of  $4.9 \mu\text{g}/\text{m}^3$  (2.61 ppb) mean  $\text{NO}_2$  levels since 1971. Among the confirmed diabetes cases the risks were greater among women (HR = 1.07; 95% CI 1.01, 1.13), non-smokers (HR = 1.12; 95% CI 1.05, 1.20) and physically active people (HR = 1.10; 95% CI 1.03, 1.16).



In contrast to the other epidemiological studies, a cross-sectional screening study performed in the Netherlands (Dijkema et al., 2011), did not find consistent associations between Type 2 diabetes mellitus prevalence and predicted NO<sub>2</sub> concentrations from LUR models ( $R^2 = 0.82$ ) in the population studied.

Long-term exposure to NO<sub>2</sub>, estimated at participants' residential addresses ( $n = 52,061$ ) with a dispersion model, was associated with diabetes-related mortality in the 13-year prospective Danish Diet, Cancer and Health cohort (Raaschou-Nielsen et al., 2013). A mortality RR of 2.15 (95% CI 1.21, 3.83) was found for exposure above 19.4 µg/m<sup>3</sup> (10.32 ppb) compared with an RR of 1.31 (95% CI 0.98, 1.76) for the lowest exposure quartile (<13.6 µg/m<sup>3</sup> (<7.23 ppb)).

Recently, Malmqvist et al. (2013) observed an increased risk of gestational diabetes associated with modelled NO<sub>x</sub> exposure in a cohort of 81,110 pregnant women from 1999 to 2005 in southern Sweden. Across quartiles of exposure it was found that the risk for gestational diabetes increased monotonically with increased NO<sub>x</sub> exposure levels in the second trimester, with adjusted ORs of 1.19 (95% CI 0.99, 1.44), 1.52 (95% CI 1.28, 1.82) and 1.69 (95% CI 1.41 2.03), respectively, for the second (9.0–14.1 µg/m<sup>3</sup> (4.79–7.5 ppb)), third (14.2–22.6 µg/m<sup>3</sup> (7.55–12.02 ppb)) and fourth (>22.7 µg/m<sup>3</sup> (>12.07 ppb)) quartiles. Significant associations were also observed with all quartiles of NO<sub>x</sub> levels for mothers who did not change residence during pregnancy, as well as for Nordic-born mothers. In the fourth NO<sub>x</sub> quartile (>22.7 µg/m<sup>3</sup> (>12.07 ppb)), corresponding adjusted ORs from restricted analyses were 1.80 (95% CI 1.49, 2.19) for mothers who did not change residence during pregnancy and 1.56 (95% CI 1.24, 1.95) for Nordic-born mothers. Similar associations increasing monotonically with increased NO<sub>x</sub> exposure levels were also noted in the first trimester. Several sensitivity analyses were performed as well, and it was found that in Nordic-born women the prevalence of gestational diabetes was higher in women living in an urban area. In addition, the authors observed an increased risk of preeclampsia, which is discussed further in Section 10.3.

#### 9.3.4.3 Inflammatory Bowel Disease

In a nested case-control study undertaken in the UK, Kaplan et al. (2010) found that NO<sub>2</sub>, SO<sub>2</sub> and PM<sub>10</sub> were not associated with IBD. Some individuals (≤23 years of age) were more likely to be diagnosed with Crohn's disease (OR = 2.31; 95% CI 1.25, 4.28) if they resided in regions with NO<sub>2</sub> levels within the upper three quintiles (quintiles of concentrations not reported) following adjustments for potential confounders. A dose–response relationship ( $p = 0.02$ ) was also observed among these Crohn's disease patients, with the adjusted OR increasing linearly across the quintiles of concentrations of NO<sub>2</sub>.

#### 9.3.4.4 Neurological Diseases/Effects

Some recent cohort studies have been published that investigated the impact of long-term exposure to NO<sub>2</sub> on Parkinson's disease (Finkelstein and Jerrett, 2007), cognitive development in children (Freire et al., 2010; van Kempen et al., 2012; Clark et al., 2012) and autism (Volk et al., 2013).

The focus of the Canadian study (Finkelstein and Jerrett, 2007) was on the potential impact of industrial emissions of manganese on Parkinson's disease among subjects ( $n = 110,348$ ) attending primary care and respiratory clinics in Hamilton and Toronto, ON. A positive but non-significant risk (OR = 1.018; 95% CI 0.96, 1.08 per 1 ppb NO<sub>2</sub>) was found for having been diagnosed with Parkinson's disease or having been prescribed an L-Dopa-containing medication in relation to residential NO<sub>2</sub> levels (predicted using LUR models;  $R^2 = 0.76$  for Hamilton and 0.69 for Toronto).

In Spain, a population-based birth cohort (Freire et al., 2010) followed 4-year-old children for 1 year to study the impact of TRAPs on children's motor and cognitive abilities. A negative but non-significant association on the general cognitive score ( $\beta = -4.19$  points; 95% CI -14.02



points, 5.64 points) was found for exposure to higher NO<sub>2</sub> levels (>24.75 µg/m<sup>3</sup> (>13.16 ppb)) while a lesser estimate (β = -1.07 points; 95% CI -9.99 points, 7.85 points) was observed in relation to lower NO<sub>2</sub> exposure levels (15.40–24.75 µg/m<sup>3</sup> (8.19–13.16 ppb)).

Two recent studies (van Kempen et al., 2012; Clark et al., 2012) have used data that were part of the British and Dutch Road Traffic and Aircraft Noise Exposure and Children's Cognition and Health (RANCH) project to investigate the effect of traffic-related air pollution on cognitive performance of children. Exposure to NO<sub>2</sub> at school was found to be significantly associated with a decrement in the memory span length of Dutch children (aged 9–11); the link remained robust after inclusion of transportation noise in the model (van Kempen et al., 2012). Significant associations between road and traffic noise exposure at school were also found with other cognitive outcomes and were robust to inclusion of NO<sub>2</sub>. For long-term exposure to NO<sub>2</sub> at schools, no associations were found with any of the other outcomes (attention, locomotion and perceptual coding), while no associations with any cognitive outcomes were found from air pollution exposure at home.

Using data from a subsample of the British RANCH dataset, Clark et al. (2012) found no associations between exposure to NO<sub>2</sub> air pollution and a range of cognitive outcomes (reading comprehension, recognition memory, information recall, conceptual recall, working memory) in children aged 9–10 with and without adjustment for noise exposure. Exposure to aircraft noise at school was found to be associated with several cognitive outcomes, and the team found little evidence that air pollution moderated these associations.

Autism spectrum disorders are a range of complex neurodevelopmental disorders characterized mainly by pervasive impairment of social interaction and communication as well as by restricted and repetitive behaviour patterns. A population-based case-control study of preschool children performed in California by Volk et al. (2013) found that exposure to traffic-related air pollutants during the first year of life was significantly associated with increased risk of autism in children aged 2–5. An OR of 2.06 (95% CI 1.37, 3.09) was observed based on an increment of 14.1 ppb in NO<sub>2</sub> levels. The authors also observed an increased risk of autism with different exposure metrics during pregnancy; these results are discussed further in subsection 10.6.1. Similar associations were also found with PM<sub>2.5</sub> and PM<sub>10</sub>, while O<sub>3</sub> exposure was not related to autism.

#### 9.3.4.5 Ear Infections

Otitis media, an infection of the middle ear, is the most common reason young children visit a physician and receive antibiotics. Significant associations between long-term exposure to NO<sub>2</sub> and children's physician visits for otitis media during their first 2 years of life were found in southwestern British Columbia (MacIntyre et al., 2011), an area with relatively low levels of ambient air pollution. In single-pollutant models, RRs of 1.12 (95% CI 1.10, 1.15) per IQR of 14.0 µg/m<sup>3</sup> (7.45 ppb) and 1.09 (95% CI 1.07, 1.12) per IQR of 10 µg/m<sup>3</sup> (5.32 ppb) were found, respectively, with the predicted NO<sub>2</sub> levels in the 2 months prior to the visit (obtained from IDW and LUR models (R<sup>2</sup> = 0.56)). Consistent associations were also found between otitis media and several other air pollutants, including NO, PM<sub>2.5</sub> and wood smoke, while no associations were observed for PM<sub>10</sub> and BC. With further adjustment for otitis media season, however, only the RRs for NO remained significant. Estimates from two-pollutant models were also computed for NO including either wood smoke or PM<sub>2.5</sub>, and they showed that the risk for exposure to NO levels assessed by IDW was sensitive to the adjustment of these other pollutants, while the risk estimates from NO obtained by LUR models (R<sup>2</sup> = 0.62) remained significant with the inclusion of wood smoke (r = 0.19) but not PM<sub>2.5</sub> (r = 0.34). The authors mentioned that a possible explanation for these differences might be the temporal variability in the inverse-weighted NO data.

In a prospective birth cohort study performed in Spain, Aguilera et al. (2013) examined the effect of early-life exposure (prenatal and postnatal) to ambient air pollution on ear infections in 2,199 infants. A positive and significant association was found between NO<sub>2</sub> levels (estimated with LUR models; R<sup>2</sup> = 0.52 to 0.75 for the different study areas) and ear infections during the first 12–18 months of age. Based on the results from the random-effects meta-analysis across all four study areas, an adjusted OR of 1.15 (95% CI 1.01, 1.31) was reported per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub>. Positive but non-significant associations were observed for the different prenatal exposure periods (subsection 10.6.1). The authors mentioned, however, that given the high correlation between air pollution levels during the prenatal and postnatal periods they were unable to discern the relative importance of each period.

#### 9.3.4.6 Osteoporosis

Osteoporosis is a disease of bones that leads to an increased risk of fracture due to reduction in bone mineral density (BMD). Research on the effects of long-term exposure to NO<sub>2</sub> on BMD was not identified in previous US EPA assessments, but two studies (Alvaer et al., 2007, 2010) that were/are part of the Oslo Health Study in Norway have been identified in this review period. The first study (Alvaer et al., 2007) found that NO<sub>2</sub> levels (estimated at the residential address with a dispersion model) were positively but not significantly associated with reductions in BMD (OR = 1.24; 95% CI 0.97, 1.59 per 12.5 µg/m<sup>3</sup> (6.65 ppb) increase) in men aged 75–76, while significant associations were found for both PM<sub>2.5</sub> and PM<sub>10</sub>. Similar results were reported in a follow-up study (Alvaer et al., 2010) where no associations between long-term exposures to NO<sub>2</sub> air pollution (estimated based on fixed monitoring stations) and either self-reported forearm fracture or BMD were observed in groups of men and women, both aged 59–60 and 75–76. Significant associations were, however, noted between low BMD and forearm fractures and PM air pollution indicators in men aged 75–76.

#### 9.3.4.7 Rheumatoid Arthritis

Rheumatoid arthritis, an autoimmune disease, can lead to a chronic systemic inflammatory disorder that may affect many tissues and organs in adults. Hart et al. (2013) found no evidence of increased risk of rheumatoid arthritis with various ambient air pollutants, including NO<sub>2</sub>, SO<sub>2</sub>, PM<sub>2.5</sub> and PM<sub>10</sub>, using different time-varying exposures in participants of the Nurses' Health Study, a large American cohort of female nurses assessed prospectively every two years since 1976.

### 9.3.5 Summary and Considerations—Morbidity Studies

**Lung function/lung function growth:** In the past, associations between decrements in lung function and/or deficits in lung function growth with air pollutants including NO<sub>2</sub> have been demonstrated in several CHS studies as well as in Oslo, Norway and Mexico. New longitudinal cohort studies conducted in various locations have found significant decrements in lung function in children, which support results from previous cohorts. New analyses of the CHS also suggest that lung function deficits in children could be modified by psychosocial stress and by genetic variants for antioxidant enzymes involved in detoxification pathways. Results from an important European birth cohort study also suggest that early life exposure, i.e. exposure during infancy, is a critical period that can lead to long-term adverse respiratory consequences. Overall, findings were generally not highly sensitive to study design, but uncertainty remains about whether the effects related to NO<sub>2</sub> are independent of other pollutants. In a limited number of studies examining effects of NO<sub>2</sub> in co-pollutant models, robust associations were generally observed following adjustment for various air pollutants including PM indices, EC and/or gases (O<sub>3</sub> or SO<sub>2</sub>). In addition, results from these studies are coherent with associations found in children for asthma incidence and respiratory symptoms.

Some new studies have also investigated the impact of long-term NO<sub>2</sub> exposure on pulmonary function in adults, but results are inconsistent. Overall, the database is currently not adequate to determine the independent effect of NO<sub>2</sub> in adults.

**Respiratory symptoms:** The 2008 US EPA ISA identified several longitudinal and cross-sectional studies investigating the relationship of ambient NO<sub>2</sub> exposure to respiratory symptoms, with results from these studies being quite variable and mostly inconsistent. More recent studies reviewed in the current assessment also demonstrated inconsistent associations between ambient NO<sub>2</sub> exposure and respiratory symptoms. However, several studies found positive associations between long-term exposure to NO<sub>2</sub> and wheeze in children, and while most of them did not reach the level of statistical significance, they exhibited a consistent pattern of effect. While significant associations have been observed between long-term exposure to NO<sub>2</sub> and infant hospitalization due to bronchiolitis in a single Canadian study, no clear associations were found in two similar US studies. Analysis of the CHS showed an increased risk of respiratory-illness absences in children living in communities with higher NO<sub>2</sub> air pollution, though stronger associations were observed in high-O<sub>3</sub> exposure groups.

Weak or no associations have been found in a very small number of studies that investigated the impact of long-term exposure to NO<sub>x</sub>/NO<sub>2</sub> on respiratory symptoms in adults.

**Asthma-related outcomes:** Recent epidemiological studies using different designs and analysis and performed in multiple locations have consistently found positive and generally statistically significant associations between long-term exposure to ambient NO<sub>2</sub> and asthma incidence in children. The results of a recent meta-analysis which combined risk estimates from 17 cohorts also support the findings from individual studies that long-term NO<sub>2</sub> exposure is associated with asthma incidence. In California, where significant associations were found in single-pollutant models, these associations were attenuated and became non-significant following the inclusion of indicators of traffic exposure. Increases in markers of airway inflammation in relation to NO<sub>2</sub> exposure have also been measured in some participants in this cohort as well as in children living in Windsor, ON. In the Netherlands, NO<sub>x</sub>/NO<sub>2</sub> exposures were also associated with development of asthma in children. In these studies, significant associations were also found with PM<sub>2.5</sub> and/or some indicators of traffic-related exposures; as well, PM air pollution was shown to exhibit independent and significant associations with the development of asthma in children. Of note, more robust associations with NO<sub>2</sub> were observed with better exposure classification (children who did not change residences during the study) and in children with concurrent stress (exposure to violent environments). Long-term exposure to ambient NO<sub>2</sub> was also found to be associated with asthma-related symptoms in some cross-sectional studies.

While adults have also been the subject of some investigation, there are many fewer studies than for children. Some European studies, including meta-analyses, suggested that long-term exposure to NO<sub>2</sub> might be involved in new asthma onset in adults, and higher risks have been observed for carriers of specific genes implicated in oxidative stress pathways and inflammatory responses.

In the first studies investigating the impact of long-term NO<sub>x</sub>/NO<sub>2</sub> exposure on respiratory hospital admissions, some positive and significant associations have been observed for admissions due to asthma. In a longitudinal cohort study performed in Orange County, CA, higher risks for hospital admission were found for girls and infants. Positive but non-significant associations were observed in Denmark, where higher risks were found in adults with a history of asthma or COPD. In addition, higher risks were found for people living in areas within the highest quartile of NO<sub>2</sub> concentrations.

**Development of other chronic respiratory diseases:** A very small number of studies investigated the relationship between ambient NO<sub>2</sub> and the development of other chronic respiratory diseases in adults. In these studies, significant associations were found between long-term exposure to ambient NO<sub>2</sub> and increased hospital admissions for pneumonia and COPD. The effects of NO<sub>2</sub> exposure on COPD hospital admissions were stronger in subjects suffering from co-morbid conditions such as diabetes or asthma.

**Allergic responses:** Some new European epidemiological studies have investigated the effects of long-term exposure to ambient NO<sub>2</sub> levels on allergic responses, mostly in children. These studies have demonstrated some associations of NO<sub>2</sub> with allergic rhinitis, hay fever, allergic sensitization and eczema, though there are large between-study inconsistencies. Many of the studies demonstrating significant results found that specific characteristics of the cohort (e.g. children who had not moved residence, traffic proximity, oxidative pathway genetics) appeared to confer increased sensitivity to NO<sub>2</sub> effects, possibly explaining the considerable heterogeneity in overall results. Findings with respect to allergic responses in adults are too limited to draw conclusions. It should be noted that some of these studies, performed in children or in adults, reported significant associations between allergic responses and other air pollutants such as benzene, PM and O<sub>3</sub>. Overall, while there are important inconsistencies, there is an emerging body of evidence that traffic-related air pollution, and possibly NO<sub>2</sub>, can induce childhood allergic diseases

**Cardiovascular effects:** A small number of studies, including a few conducted in Canada, investigated the relationship between long-term exposure to ambient NO<sub>2</sub> and a variety of cardiovascular outcomes. Most of these new publications studied the impact of traffic air pollutants on stroke incidence or hospitalization due to stroke. Studies in Canada, the US and Europe find positive associations of stroke with NO<sub>2</sub>/NO<sub>x</sub>, though these results are generally not statistically significant. The database is similar for other outcomes such as CHD and MI hospitalization, though individual studies find positive and significant associations with heart failure and point to the possible involvement of co-morbid conditions (e.g. diabetes) in conferring sensitivity to NO<sub>2</sub>. Long-term exposure to PM was reported to be associated with hospital admissions due to cardiovascular events in some of these studies. A limited number of studies are investigating the effect of long-term exposure to ambient NO<sub>2</sub> on various preclinical cardiovascular outcomes. Such studies include examination of HRV, inflammation, vascular damage, self-reported incidence of hypertension and both systolic and diastolic blood pressure, with mixed results being observed. Greater consistency has been seen in results in females, with significant decrements in blood pressure and stronger decrements in HRV in women with self-reported cardiovascular problems.

Overall, the database is currently limited and provides inconsistent results on the relationship between long-term exposure to ambient NO<sub>2</sub> and cardiovascular morbidity. Moreover, most of these studies only reported single-pollutant models, and in several of these, associations were more strongly related to PM air pollution.

**Cancer incidence:** Most studies reviewed in the 2008 US EPA ISA and in this assessment suggested that long-term exposure to NO<sub>x</sub>/NO<sub>2</sub> could be associated with the incidence of certain types of cancer, especially lung cancer. A Canadian study suggested a possible association between long-term exposure to NO<sub>2</sub> levels and post-menopausal breast cancer incidence, while in France acute leukemia was found to be associated with traffic-NO<sub>2</sub> levels and other indicators of traffic. Additional studies are required, however, to confirm these observations on cancer incidence given the difficulty in disentangling any effect associated with NO<sub>2</sub> from those of other co-occurring pollutants.

**Other morbidity effects:** A small number of studies have been published in relation to long-term exposure to ambient NO<sub>2</sub>/NO<sub>x</sub> and other morbidity outcomes, including annoyance, diabetes, IBD, neurological diseases/effects, otitis media, osteoporosis and rheumatoid arthritis. The epidemiological evidence remains very limited and/or inconsistent, and further investigation is required before firm conclusions can be reached on these adverse effects from traffic-related air pollution.

In conclusion, the health database on morbidity effects from long-term exposure to NO<sub>2</sub>/NO<sub>x</sub> air pollution has been greatly expanded over the past few years, with new analyses and reanalyses of existing cohort data indicating potential impacts on respiratory health. Results from past and more recent studies support the hypothesis that early childhood exposure to NO<sub>x</sub>/NO<sub>2</sub> air pollution may play an important role in decrements in lung function measures and decreases in lung function growth, as well as in the development of asthma and allergic diseases. Several epidemiological studies, including analyses of the CHS, have found significant associations between long-term exposure to NO<sub>2</sub> and decrements in lung function measures and/or reduction of lung growth. An important new feature of the health dataset is the evidence of effect modification by genetic variants of antioxidant enzymes and genes involved in oxidative pathways or inflammatory responses. In adults, long-term exposure to NO<sub>2</sub> levels appears to increase the incidence of asthma, though this is based on a relatively small number of studies. The dataset for adults in regard to other respiratory health effects is currently quite limited. In the majority of the chronic morbidity studies the possible role of co-pollutants was not investigated. However, in the small number of studies in which co-pollutant models were reported, associations with lung function decrements observed in children remained robust after adjustment for some air pollutants including PM indices, EC and/or gases (O<sub>3</sub> or SO<sub>2</sub>).

Given the often high correlation observed between NO<sub>x</sub>/NO<sub>2</sub> and either PM indices or different traffic indicators it is difficult to disentangle the effect of each air pollutant per se, and this issue remains the major uncertainty in the overall health database relating to long-term exposure to NO<sub>2</sub> levels. Co-pollutant models adjusting for some other key traffic-related air pollutants such as CO or UFP have not been performed.

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# 10. Epidemiological Studies of Reproductive and Developmental Effects Associated with Ambient NO<sub>2</sub>

## 10.1 Introduction

In this chapter, epidemiology studies of the reproductive and developmental effects associated with human exposure to ambient NO<sub>2</sub> are reviewed.

**Chapter organization:** This chapter is organized to present birth weight and other fetal growth measures related to prenatal exposure to ambient NO<sub>2</sub>, followed by studies of preterm birth, preeclampsia, congenital anomalies, and other developmental effects. The individual sections summarize the findings of epidemiological studies of associations between exposure to ambient NO<sub>2</sub> and the adverse reproductive and developmental health outcomes for each category. As well, specific time periods of susceptibility during pregnancy are considered.

**Focus on North American studies:** Reproductive and developmental studies associated with NO<sub>2</sub> air pollution have been conducted all over the world. This chapter focuses on those studies considered particularly relevant to the risks associated with exposure to ambient NO<sub>2</sub> in Canada; therefore, for reasons discussed in detail in the introduction of earlier chapters, the focus will remain mainly on studies conducted in Canada and the US, along with Europe and Australia.

**2008 US EPA ISA as a starting point:** Consistent with previous chapters of this report that describe epidemiological studies of ambient NO<sub>2</sub>, the 2008 US EPA ISA (US EPA, 2008) is used as the basis for summarizing the earlier, although limited, reproductive and developmental literature. In a few cases, there is key literature covered by the ISA, as well as studies that are from the time period of the ISA but were omitted from their report. To retain this information as part of the overall weight of evidence, the summary of these key studies is presented in greater detail with the individual references.

## 10.2 Birth Weight and Other Fetal/Neonatal Growth Measures

The 2008 US EPA ISA identified seven studies investigating the relationship between ambient NO<sub>2</sub> exposures and birth weight (US EPA, 2008). The results of these studies varied somewhat, with most studies reporting adverse effects, some of which reached the statistically significant level. In terms of North American data, Bell et al. (2007, 2008) reported a significant decrease in birth weight in relation to NO<sub>2</sub> exposure (especially among subjects of African-American descent) among infants from a cohort of mothers in Connecticut and Massachusetts; results were significant for the entire-pregnancy exposure (OR = 1.027; 95% CI 1.002, 1.051 per 4.8 ppb increase) and the first-trimester exposure. The associations remained significant in two-pollutant models with either CO or SO<sub>2</sub>. It should however be noted that given the high correlation between NO<sub>2</sub> and both PM<sub>2.5</sub> (r = 0.64) and PM<sub>10</sub> (r = 0.55) the adjustment was only done for pollutant pairs that were uncorrelated; i.e. for NO<sub>2</sub>-CO and NO<sub>2</sub>-SO<sub>2</sub> (correlation coefficients not reported). Conversely, in international studies, an association between NO<sub>2</sub> and decreased birth weight was observed for second-trimester (and in some cases third-trimester) exposures. The 2008 US EPA ISA also notes that the biological mechanism behind an effect of

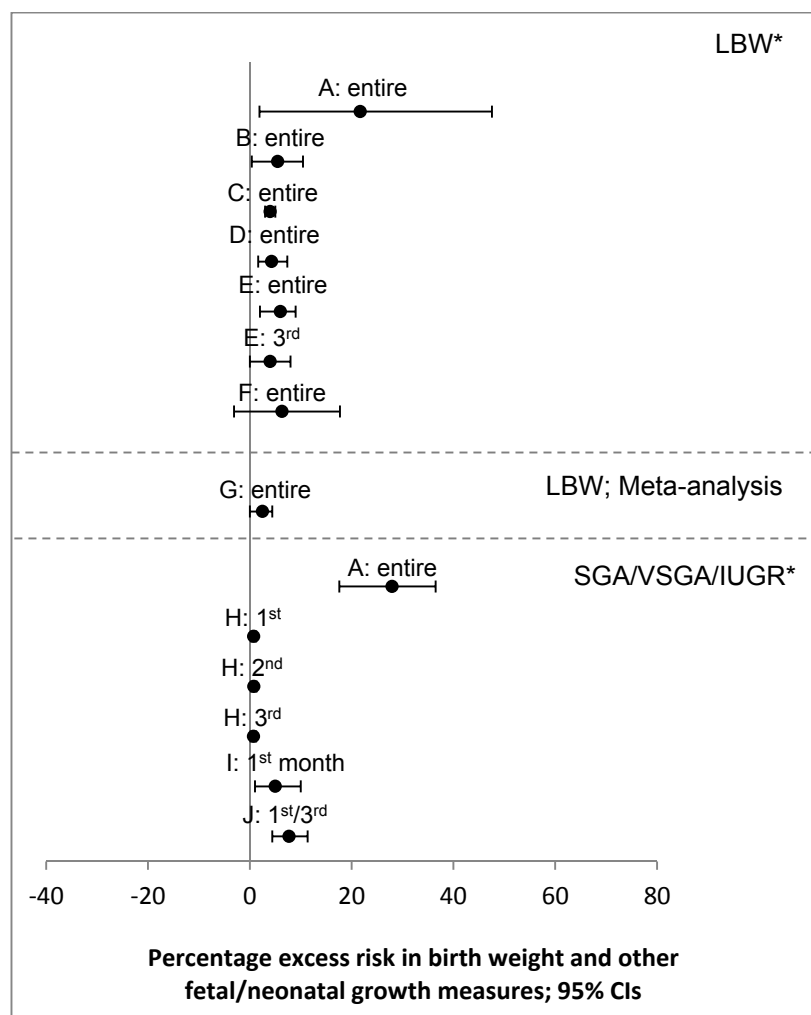
NO<sub>2</sub> during the second trimester is difficult to hypothesize. Finally, NO<sub>2</sub> exposure was strongly correlated with exposure to other pollutant(s), and other pollutant(s) also demonstrated a strong association with birth weight (US EPA, 2008).

More recently, a number of North American studies and a meta-analysis examined the effects of ambient NO<sub>2</sub> exposure on birth weight. The percentage excess risk in birth weight and other measures of fetal/neonatal growth from North American studies per 10 ppb increase in NO<sub>2</sub> concentrations are presented in Figure 10.1. Authors examined changes in birth weight, incidence of LBW (<2,500 g), small for gestational age (SGA—birth weight less than gender-specific 10<sup>th</sup> percentile standardized values for gestational age), very small for gestational age (VSGA—birth weight less than gender-specific 5<sup>th</sup> percentile standardized values for gestational age), and intrauterine growth restriction (IUGR—essentially synonymous with SGA). European and Australian studies have also examined the relationship between birth weight or SGA and NO<sub>2</sub>/NO<sub>x</sub> exposure. In addition, several of these studies also assessed the impact of NO<sub>2</sub> exposure on other fetal and neonatal anatomical growth measures, including (but not limited to) head circumference and femur length.

Brauer et al. (2008) examined LBW among 70,249 single births between 1999 and 2002 in Vancouver, BC. Exposure to air pollution was assigned by three different approaches: two being based on regulatory monitoring network (nearest monitor assignment (within 10 km) and IDW approaches) and the third being LUR models. NO<sub>2</sub> exposure was associated with LBW (OR = 1.11; 95% CI 1.01, 1.23 per 10 µg/m<sup>3</sup> (5.32 ppb) increase based on the IDW method). In general, similar risk estimates were found with the nearest monitor approach (results not presented), while risk estimates with LUR models ( $R^2 = 0.56$ ) were not consistently larger or more precise. Among the other pollutants examined, there were no significantly increased risks of LBW, although high correlations existed between NO, NO<sub>2</sub>, and SO<sub>2</sub>. Proximity to roadways, and especially highways, also was associated with a raised risk of LBW pregnancies.

Several recent studies have been conducted in California. Morello-Frosch et al. (2010) found an association between exposure to NO<sub>2</sub> and risks of reduced birth weight/LBW among a cohort of 3,545,177 singleton births in California between 1996 and 2006. A decreased birth weight of 9.0 g (95% CI -9.6 g, -8.4 g) and an OR of 1.03 (95% CI 1.02, 1.04) for LBW were found per 10 ppb increase in NO<sub>2</sub>. This effect was limited to exposure during the first (-3.0 g; 95% CI -3.9 g, -2.1 g) and third (-7.0 g; 95% CI -7.9 g, -6.0 g) trimesters. Other pollutants (PM, CO, O<sub>3</sub>) also exhibited associations of a similar magnitude with LBW. In two-pollutant analyses with O<sub>3</sub>, SO<sub>2</sub>, PM<sub>10</sub>, PM<sub>2.5</sub> and coarse PM, the NO<sub>2</sub> effect remained statistically significant with the inclusion of each pollutant (risks presented in figures only, no quantitative estimates given). Negative effects of all pollutants on birth weight, except CO, also remained robust in two-pollutant models. NO<sub>2</sub> was highly correlated with PM indices ( $r = 0.72$  with PM<sub>2.5</sub>,  $r = 0.79$  with PM<sub>10-2.5</sub>) and CO ( $r = 0.79$ ) and the authors concluded that the specific pollutant(s) of concern could not be identified. Ghosh et al. (2012) assessed the association between the incidence of term LBW in a cohort of 379,103 infants from Los Angeles County and exposure to NO, NO<sub>2</sub>, and NO<sub>x</sub>, as well as a range of air toxics and criteria pollutants. Various exposure methods were used, including data from air monitoring stations and LUR models ( $R^2 = 0.81$ , 0.86 and 0.85, respectively, for NO, NO<sub>2</sub> and NO<sub>x</sub>). Per 10 ppb increase in all three pollutant concentrations based on monitoring data, the risk of LBW was increased for entire-pregnancy exposure with NO (OR = 1.06; 95% CI 1.03, 1.09), with NO<sub>2</sub> (OR = 1.06; 95% CI 1.02, 1.09) and with NO<sub>x</sub> (OR = 1.06; 95% CI 1.03, 1.10).

**Figure 10.1: Percentage excess risk in birth weight and other fetal/neonatal growth measures and 95% CIs per standardized increment (10 ppb) in long-term ambient NO<sub>2</sub> concentration in single-pollutant models from Canadian and US studies**



- A. Vancouver, BC; Brauer et al. (2008); LBW and SGA
- B. Massachusetts and Connecticut, USA; Bell et al. (2007); LBW
- C. California, USA; Morello-Frosch et al. (2010); LBW
- D. Northeastern and mid-Atlantic, USA; Ebisu and Bell (2012); LBW
- E. Los Angeles County, CA; Ghosh et al. (2012); LBW
- F. Los Angeles County, CA; Wilhelm et al. (2012); LBW only
- G. Meta-analysis; Stieb et al. (2012); LBW; risk estimates from 10 studies were included in this pooled effect estimate for NO<sub>2</sub>, including those from individual studies A, B and C presented above
- H. New Jersey, USA; Rich et al. (2009); VSGA
- I. Vancouver, BC; Liu et al. (2003); IUGR
- J. Three Canadian cities; Liu et al. (2007); IUGR

\*LBW = low birth weight, SGA and VSGA = small and very small for gestational age, IUGR = intrauterine growth restriction, entire = entire pregnancy; 1<sup>st</sup> = first trimester; 2<sup>nd</sup> = second trimester; 3<sup>rd</sup> = third trimester.

Significant associations were noted as well for third-trimester exposure with both NO<sub>2</sub> (OR = 1.04; 95% CI 1.00, 1.08) and NO<sub>x</sub> (OR = 1.05; 95% CI 1.01, 1.09). Similar risk estimates were found with the LUR models. In a cohort of 100,938 term singleton infants born between 2004 and 2006 in Los Angeles County with a mother residing within 5 miles of a monitoring station, entire-pregnancy exposures to NO<sub>2</sub> as well as NO and NO<sub>x</sub> were associated with the incidence of LBW (OR = 1.04; 95% CI 0.98, 1.11 per an IQR increase of 6.4 ppb in NO<sub>2</sub>; OR = 1.08; 95% CI 1.02, 1.13 per an IQR increase of 14.8 ppb in NO; and OR = 1.07; 95% CI 1.01, 1.13 per an IQR increase of 20.5 ppb in NO<sub>x</sub>) (Wilhelm et al., 2012). NO<sub>2</sub>, NO<sub>x</sub> and NO exposure estimates at residential locations were derived with LUR models (R<sup>2</sup> = 0.81, 0.86 and 0.85, respectively, for NO, NO<sub>2</sub> and NO<sub>x</sub>). LBW was also associated with other markers of traffic air pollution (e.g. EC), as well as with PM<sub>2.5</sub>.

Darrow et al. (2011) also observed a relation between NO<sub>2</sub> exposure in the third trimester and birth weight in a cohort of 406,627 live full-term births in Atlanta, GA, from 1994 to 2004. Exposure to air pollutants was assigned using all the monitoring data available. The mean change in birth weight per 5 ppb increase in NO<sub>2</sub> in the third trimester was -4.5 g (95% CI -8.5 g, -0.6 g). As seen in other studies, additional pollutants (SO<sub>2</sub>, several PM components) also demonstrated associations with this outcome.

In a study conducted in the northeastern and mid-Atlantic US between 2000 and 2007 involving 1,207,800 full-term singleton births, the percentage change in odds of LBW increased significantly in relation to NO<sub>2</sub> exposure estimates (4.7%; 95% CI 1.4%, 8.1% per IQR increment of 11 ppb) gathered using data from central monitoring stations (Ebisu and Bell, 2012). Several PM<sub>2.5</sub> chemical components (namely PM<sub>2.5</sub> aluminum, EC, nickel, and titanium) and PM itself were also consistently associated with LBW. Significant associations were also observed in single-pollutant models with other gases including CO and SO<sub>2</sub>. In two-pollutant models, including only co-pollutants with correlations < 0.5, the relationships with PM components remained significant and robust while those for the criteria air contaminants (including NO<sub>2</sub>) did not.

By contrast, Geer et al. (2012) found no association between gestational NO<sub>2</sub> exposure over the entire pregnancy and change in birth weight (1.4 g; 95% CI -2.43 g, 5.24 g per 2.4 ppb increase) in a Texas cohort of 1,548,904 births between 1998 and 2004. No associations were found for trimester-specific exposure to NO<sub>2</sub> or any other pollutants (O<sub>3</sub>, SO<sub>2</sub>).

Studies from outside North America provided variable results when evaluating NO<sub>2</sub> effects on birth weight or fetal growth measures, ranging from relatively large and significant effects through to virtually no apparent relationship.

Based on NO<sub>2</sub> exposure estimates obtained with LUR models ( $R^2 = 0.86$ ), Pereira et al. (2012) observed significant NO<sub>2</sub>-related fetal growth restriction in a cohort of 23,452 births in Perth, Australia, in mid to late pregnancy, with effects being greatest when related to second-trimester exposure (OR = 1.31; 95% CI 1.07, 1.60 per an IQR increase of 16.97 ppb). In a study conducted in the Netherlands, birth weight was significantly decreased with increased exposure to NO<sub>2</sub>, especially in relation to third-trimester exposure (van den Hooven et al., 2012a, 2012b). Adverse associations were also observed for PM<sub>10</sub> but were not as consistent as those for NO<sub>2</sub>. Aguilera et al. (2009) also found a significant adverse association between decreased birth weight and exposure to NO<sub>2</sub> (estimated with LUR models;  $R^2 = 0.75$ ) during the second trimester in a prospective cohort study among a subgroup of pregnant women from a Spanish cohort who spent <2h/d in outdoor environments (-74.7 g; 95% CI -140.4 g, -9.0 g per an IQR increase of 12.0 µg/m<sup>3</sup> (6.4 ppb)). The authors proposed that an effect was seen in this subgroup (rather than the cohort as a whole) due to greater accuracy of actual NO<sub>2</sub> exposure, as this was modelled using the mother's residential address. Significant reductions in birth weight were, however, observed more consistently with exposure to BTEX.

A number of studies (Slama et al., 2007; Lepeule et al., 2010; Estarlich et al., 2011; Iniguez et al., 2012; Rahmalia et al., 2012) found adverse but non-statistically significant effects on birth weight and other fetal growth parameters. A few studies (Hansen et al., 2007; Madsen et al., 2010; Gehring et al., 2011a, 2011b) found no relationships between NO<sub>2</sub> exposures and birth outcomes. Many of these studies examined co-pollutant relationships and modifiers of effect. Of note, PM measures of exposure demonstrated either equal or greater relationships with birth and fetal weight measurements. Accounting for accuracy of the exposure measurements (e.g. time spent at home) provided greater estimates of risks and often moved the relationship into the statistically significant range. While mid- and late-pregnancy exposures generally exhibited



the strongest evidence for a relationship with adverse outcomes, this was not universally the case.

The 2008 US EPA ISA identified three studies specifically comparing weight for gestational age (SGA, VSGA, and IUGR) with national standards. For example, Liu et al. (2003) observed an increased risk for IUGR due to NO<sub>2</sub> exposure in the first month of pregnancy (OR = 1.05; 95% CI 1.01, 1.10 per 10 ppb NO<sub>2</sub> increase) in a Canadian study conducted in Vancouver, BC.

In a cohort of 386,202 singleton term births in Calgary, Edmonton, and Montreal from 1985 to 2000, Liu et al. (2007) examined the association between IUGR and NO<sub>2</sub> exposure (daily avg 24 ppb) based on data collected from ambient monitoring stations. The risk of IUGR was significantly increased due to NO<sub>2</sub> exposure throughout the entire pregnancy and each trimester, with slightly higher effects being seen in the early and late periods of gestation (OR = 1.16; 95% CI 1.09, 1.24 per 20 ppb increase for both first and third trimesters). The greatest association was in the final month of pregnancy. Positive and significant associations were also observed with both CO and PM<sub>2.5</sub> in single-pollutant models. All air pollutants were positively correlated together; NO<sub>2</sub> was highly correlated with CO ( $r = 0.71$ ) and a moderate correlation was observed with PM<sub>2.5</sub> ( $r = 0.41$ ). It should be noted that when three-pollutant modelling was performed including all three individually significant pollutants (NO<sub>2</sub>, CO, and PM<sub>2.5</sub>), the relationships with NO<sub>2</sub> and PM<sub>2.5</sub> were no longer significant; a significant association was only observed with CO exposure during the entire gestation.

In addition to LBW, Brauer et al. (2008) also examined the association between NO<sub>2</sub> exposure and SGA in Vancouver (study characteristics discussed in more detail above). Based on a 10 µg/m<sup>3</sup> (5.32 ppb) increase, exposures to both NO (OR = 1.05; 95% CI 1.03, 1.08) and NO<sub>2</sub> (OR = 1.14; 95% CI 1.09, 1.18) were significantly associated with SGA. There were also significantly increased risks of SGA for all pollutants except O<sub>3</sub>, as well as for residing <50 m from a highway.

In terms of trimester-specific associations for SGA and VSGA, Rich et al. (2009) observed that NO<sub>2</sub> exposure during all three trimesters had an effect on VSGA but not SGA. These outcomes were defined as a fetal growth ratio: i.e. newborn's birth weight divided by median birth weight of corresponding gestational age. VSGA was defined as a fetal growth ratio of <0.75 and SGA as a ratio between ≥0.75 and <0.85, while "reference" births were defined as >0.85. In this cohort of 114,411 singleton term births in New Jersey (1999–2003), the percentage changes in risk of VSGA per 10 ppb increase in NO<sub>2</sub> during the first, second, and third trimesters were 7.0% (95% CI 1.8%, 12.4%), 7.7% (95% CI 2.6%, 13%), and 7.4% (95% CI 2.5%, 12.5%), respectively. Associations were robust in two-pollutant models including PM<sub>2.5</sub>, appeared linear in nature with concentration, and exhibited greater association when pregnancy complications were in evidence. Other pollutants (PM<sub>2.5</sub>, SO<sub>2</sub>, CO) showed little evidence of association with VSGA in single-pollutant models, though PM<sub>2.5</sub> was associated with SGA and this effect also remained significant in two-pollutant models including NO<sub>2</sub>. All pollutant-trimester pairs were uncorrelated except trimester-specific NO<sub>2</sub> and CO concentrations, which were moderately correlated (correlation coefficients not reported).

European and Australian results on incidence of SGA related to NO<sub>2</sub> or NO<sub>x</sub> exposure were relatively inconsistent, with results demonstrating either no association (Hansen et al., 2007; Gehring et al., 2011b; Olsson et al., 2013), or a significant adverse effect sensitive to adjustment for a number of socioeconomic factors (Malmqvist et al., 2011).

In 2012, Stieb et al. (2012a) published a meta-analysis of over 60 cohort, case-control, and ecologic studies examining the association between outdoor air pollution exposure and birth weight and/or preterm birth. The inclusion criteria included non-occupational, non-accidental exposure and live births >20 weeks gestation, and the study considered birth weight and the

adverse pregnancy outcomes of preterm birth, SGA, LBW, and IUGR. Heterogeneity among estimates from primary studies was measured using the  $I^2$  statistic. The pooled estimates per 20 ppb increase in entire-pregnancy exposure to  $\text{NO}_2$  were change in birth weight of -28.1 g (95% CI -44.8 g, -11.5 g;  $I^2 = 84.7\%$ ) based on 10 studies, and an OR for LBW of 1.05 (95% CI 1.00, 1.09;  $I^2 = 78.4\%$ ) also based on 10 studies. Pooled estimates for reduced birth weight/increased risk of LBW were also performed for the different gestational periods (i.e. first, second and third trimester). Pooled estimates of decreases in birth weight per 20 ppb increase in  $\text{NO}_2$  exposure in the first, second and third trimester were -4.2 g (95% CI -19.2 g, 10.8 g;  $I^2 = 90\%$ ), 0.9 g (95% CI -1.3 g, 3.0 g;  $I^2 = 0\%$ ) and -7.9 g (95% CI -29.0 g, 13.3 g;  $I^2 = 93.5\%$ ), respectively. Similar results were obtained for  $\text{CO}$ ,  $\text{PM}_{10}$ , and  $\text{PM}_{2.5}$ . Overall, these results indicate that  $\text{NO}_2$  exposure is associated with reduced birth weight/increased risk of LBW and that effect estimates based on the entire pregnancy exposure were generally largest. It should be noted that there was considerable variability in risk estimates by specific exposure period during pregnancy, and often a high degree of heterogeneity between studies. The authors noted that there were too few estimates in individual exposure periods to conduct analyses to examine sources of heterogeneity. Variation in effects by exposure period and sources of heterogeneity between studies should be further explored.

In addition to birth weight, SGA, and LBW analyses, a number of European studies also examined fetal and neonatal anatomical measurements immediately after birth. In a prospective cohort of 2,337 pregnant women in Spain, birth length (but not head circumference) was significantly decreased in relation to  $\text{NO}_2$  exposure within each trimester (Estarlich et al., 2011). In combined analyses, an increase of  $10 \mu\text{g}/\text{m}^3$  (5.32 ppb) in  $\text{NO}_2$  exposure for the whole pregnancy (estimated by LUR models;  $R^2 = 0.52$  to  $0.75$  for the different study areas) was associated with a decrease in birth length of -0.9 mm (95% CI -1.8 mm, -0.1 mm). The pattern for a decrease in birth size was strongest for second-trimester exposure and for those mothers who spent the majority of their time at home (and therefore presumably had less exposure misclassification). Hansen et al. (2007) found a decrease in crown–heel length at birth ( $\beta = -0.15$  cm; 95% CI -0.25 cm, -0.05 cm per IQR increase of 5.9 ppb during the third trimester), but not head circumference, associated with exposure to  $\text{NO}_2$  estimated from levels at fixed-site monitoring stations in a cohort of 26,217 singleton full-term live births in Brisbane, Australia, between 2000 and 2003. No other pollutants were associated with a reduction in crown–heel length. In an additional prospective cohort study conducted in Rotterdam, the Netherlands, van den Hooven et al. (2012a) measured fetal growth characteristics by ultrasound; fetal head circumference in the third trimester (-0.12 mm; 95% CI -0.17 mm, -0.06 mm), and length in the second (-0.02 mm; 95% CI -0.03 mm, -0.01 mm) and third (-0.02 mm; 95% CI -0.04 mm, -0.01 mm) trimesters were significantly decreased in relation to  $1 \mu\text{g}/\text{m}^3$  (0.53 ppb) increases in  $\text{NO}_2$  exposure. Several studies examined  $\text{NO}_2$  exposure relationships with fetal growth parameters in the INMA (the Spanish Children's Health and Environment or Infancia y Medio Ambiente project) mother–child multi-centre cohort in Spain. Ballester et al. (2010) and Iniguez et al. (2012) utilized the Valencia members of the cohort and found significant  $\text{NO}_2$ -related decreases in a number of measures, including biparietal diameter (BPD), abdominal circumference and femur length, measured during mid-gestation. These reductions were found when evaluated against the entire range of exposure and exhibited characteristics of a linear concentration–response relationship, but they were often more pronounced when examined for concentrations exceeding the cohort median exposure level of  $40 \mu\text{g}/\text{m}^3$  (21.3 ppb). Aguilera et al. (2010) examined traffic-related air pollution ( $\text{NO}_2$ , BTEX) relationships with fetal growth measures in the Sabadell cohort of the Spanish INMA project. They found a number of adverse effects (femur length, head circumference, abdominal circumference) though none in the general analysis reached the statistically significant level (sample size was about one-third smaller than the Valencia sample examined in the studies above). When using a modifier to indicate less

exposure misclassification (<2h/d spent in non-residential outdoor environments), a number of the associations with NO<sub>2</sub> did reach statistical significance (head circumference during weeks 12–20 and growth in abdominal circumference, BPD, and estimated fetal weight during weeks 20–32). Both BTEX and NO<sub>2</sub> provided evidence of similar reductions in these fetal growth parameters, with strongest relationships being for early pregnancy exposures.

By contrast, Hansen et al. (2008), while finding strong adverse relationships for a number of air pollutants, found no significant association between NO<sub>2</sub> levels obtained from the closest monitoring site and any fetal growth measurements during pregnancy in a cohort of pregnant women attending a clinic in Brisbane, Australia.

## 10.3 Preterm Birth

In the 2008 US EPA ISA, six studies were identified that investigated the relationship between NO<sub>2</sub> exposure and preterm delivery (defined as birth at <37 weeks gestation unless otherwise noted); three observed adverse associations, while three reported no association (US EPA, 2008). It should be noted that only two of the above-mentioned six studies were conducted in North America, neither of which found an association between NO<sub>2</sub> exposure and preterm birth (Ritz et al., 2000; Liu et al., 2003). Of the three with positive associations, these associations were reported to have been related to NO<sub>2</sub> exposure during the first and/or third trimesters of pregnancy. These studies were conducted in the Czech Republic (Bobak et al., 2000), Lithuania (Maroziene and Grazuleviciene, 2002), and Korea (Leem et al., 2006).

Seven recently published North American studies and one meta-analysis examined the associations between risk of preterm birth and exposure to NO<sub>2</sub> either during the whole pregnancy or specific periods of gestational exposure. In addition, eight studies of European or Australian cohorts were identified.

Brauer et al. (2008) examined the association between exposure throughout the entire pregnancy to various pollutants, including NO and NO<sub>2</sub>, and the risk of preterm birth (<30 weeks gestation) in Vancouver, BC (study characteristics discussed in more detail in Section 10.2). Most pollutants demonstrated adverse associations with preterm birth in these analyses using different exposure assessment methods, though most did not reach the level of statistical significance. The exceptions were NO and CO, which were not only statistically significant but also exhibited the largest such relationships. ORs of 1.12 (95% CI 0.89, 1.40) and 1.08 (95% CI 0.91, 1.29) were seen per 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> estimated, respectively, with IDW and LUR models (R<sup>2</sup> = 0.56).

In a case-control study of 111,203 singleton births between 2004 and 2006 in Los Angeles County, CA (Wilhelm et al., 2011), entire-pregnancy exposure to NO, NO<sub>2</sub> and NO<sub>x</sub> estimated by LUR models (with respective R<sup>2</sup> values of 0.81, 0.86 and 0.85) were associated with risk of preterm birth with small but statistically significant increases in OR for this outcome (ORs of 1.03–1.04 per IQR). In the same analysis, much higher (and statistically significant) risks were observed for a range of PM metrics (ORs of 1.06–1.21 per IQR) and especially for PAHs (OR = 1.30 per IQR). In a time-series analysis conducted in Atlanta, GA, consisting of 476,489 singleton births between 1994 and 2004 (Darrow et al., 2009), no associations were observed between exposure to NO<sub>2</sub> (or any other criteria pollutant) during the periods examined (first month of gestation, 1 week before birth, and 6 weeks before birth) with the risk of preterm birth. However, when considering only subjects with a maternal residence within 4 miles of a monitor, exposure to NO<sub>2</sub> during the 6 weeks before birth was significantly associated with preterm delivery (OR = 1.06; 95% CI 1.02, 1.09 per 5 ppb increase). Trasande et al. (2013) observed an increased risk for preterm birth (OR = 1.02; 95% CI 1.01, 1.04 per 1 ppb increase in NO<sub>2</sub>, based on air monitoring data within a 10-mile radius of the births) among a cohort of 222,359 births

from the US national Kids' Inpatient Database, linking exposure to air pollutants during the month of birth with adverse birth outcomes and neonatal health care utilization. This result remained significant when analyzed in three-pollutant models involving PM<sub>2.5</sub> and CO, and it was correlated with a significant increase in hospital charges but, conversely, a significant decrease in length of hospital stays. The correlations between NO<sub>2</sub> and the different air pollutants were all negative except for CO ( $r = 0.12$ ). It should be noted that while exposure to PM<sub>2.5</sub> during the month of birth was found to be inversely associated with preterm birth a positive and significant association was seen with preterm LBW.

In two publications utilizing the same cohort of 81,186 singleton births between 1997 and 2006 in Los Angeles and Orange counties, CA (Wu et al., 2009, 2011), exposure to NO<sub>x</sub> was associated with an increased risk of preterm (<37 weeks), moderate preterm (<35 weeks), and very preterm (<30 weeks) delivery, with the risk estimates increasing through the severity of categorization (very preterm > moderate preterm > preterm). Exposures to traffic-related pollution originating from traffic emissions within 3 km of each residence were determined using dispersion modelling in the first study, while four different techniques (ambient monitoring data, traffic density, LUR models and dispersion modelling) were used in the second study. The final LUR models yielded R<sup>2</sup> values of 0.81, 0.86 and 0.85 for NO, NO<sub>2</sub> and NO<sub>x</sub>, respectively. In the first study, entire pregnancy exposure to NO<sub>x</sub> was associated with preterm (OR = 1.06; 95% CI 1.03, 1.09), moderate preterm (OR = 1.13; 95% CI 1.09, 1.18), and very preterm delivery (OR = 1.25; 95% CI 1.17, 1.33), respectively, per 5.65 ppb increase. Effects exhibited an exposure-dependent relationship, with the fourth quartile of exposures demonstrating highly elevated risks in all categories, but especially for very preterm births. Significant relationships were also seen for PM<sub>2.5</sub>, which was highly correlated with NO<sub>2</sub> ( $r = 0.91$ ), though risks were consistently lower than for NO<sub>x</sub>. In the follow-up study (Wu et al., 2011) NO<sub>2</sub> and NO<sub>x</sub> exposure during the entire pregnancy, the first and second trimesters, and the last month of pregnancy was associated with an increased risk of preterm birth. Again, the greatest risk estimate was for very preterm delivery, with significantly elevated ORs of 1.43–1.46, based on the IQR of NO<sub>2</sub> concentrations (11.7 ppb). Elevated ORs were also found for CO and to a lesser extent for PM<sub>2.5</sub> and PM<sub>10</sub>. Adjustment for other preterm birth risk factors generally increased ORs for NO<sub>2</sub> and other pollutants. Estimates of effect were shown to be sensitive to the method of exposure estimation, with some forms of LUR modelling providing results similar to those from monitors, while LURs adjusted for temporal considerations provided much attenuated results.

Ritz et al. (2007) found no significant association between NO<sub>2</sub> exposure during the first trimester and preterm birth (OR = 1.05; 95% CI 0.94, 1.17 per 10 ppb increase) in a case-control survey nested within a birth cohort conducted in Los Angeles and consisting of 2,543 women. Significant associations were observed for CO exposure, as well as for high exposures to PM<sub>2.5</sub>.

In the meta-analysis performed by Stieb et al. (2012) (discussed in detail in Section 10.2), a positive but non-significant association was found between risk of preterm birth and NO<sub>2</sub> exposure during the entire pregnancy (OR = 1.16; 95% CI 0.83, 1.63 per 20 ppb increase;  $I^2 = 53.3\%$ ) based on five studies. Pooled estimates for risk of preterm birth per 20 ppb increase in NO<sub>2</sub> exposure in the first, second and third trimester were 0.87 (95% CI 0.64, 1.17;  $I^2 = 89.1\%$ ), 1.03 (95% CI 0.77, 1.39;  $I^2 = 21.6\%$ ) and 1.06 (95% CI 0.96, 1.18;  $I^2 = 19.5\%$ ), respectively. Similar relationships were found for most other pollutants (CO, PM; not O<sub>3</sub>). The authors noted that the pooled estimates were most precise based on third-trimester exposures.

European and Australian results on preterm birth are less consistent than those from the North American studies.



Olsson et al. (2012) observed an association between NO<sub>2</sub> exposure during the last week of pregnancy and the incidence of preterm delivery (OR = 1.06; 95% CI 1.02, 1.11 per 10 µg/m<sup>3</sup> (5.32 ppb) increase) among a cohort of 115,588 births in Sweden conceived during 1988–1995. Adjustment for other risk factors did not influence the outcome, nor did two-pollutant modelling with O<sub>3</sub>, which was negatively correlated (r = -0.34). Associations were also seen for O<sub>3</sub> related to first- and second-trimester exposures, which were modified by season. No associations were found between preterm birth and NO<sub>2</sub> exposure during the first and second trimester. In a follow-up study by the same team (Olsson et al., 2013) including all deliveries during 1998 to 2006, a small and non-significant adverse association was found, though in this case between NO<sub>x</sub> (instead of NO<sub>2</sub>) exposure during the first trimester and preterm birth (OR = 1.02; 95% CI 0.99, 1.06 per 10 µg/m<sup>3</sup> (5.32 ppb) increase). As in the earlier study, significant associations were observed in relation to O<sub>3</sub> exposure, which were more pronounced in asthmatic than non-asthmatic mothers. Llop et al. (2010) observed an association between preterm birth and exposure to NO<sub>2</sub> (based on increments of 1 µg/m<sup>3</sup> (0.53 ppb)), during the entire pregnancy (OR = 1.29, 95% CI 1.13, 1.46), as well as for the second (OR = 1.11, 95% CI 1.03, 1.21) and third trimesters (OR = 1.10, 95% CI 1.00, 1.21) in a prospective cohort study conducted in Valencia, Spain, although this appeared to be confined to high exposures [ $>46.2 \mu\text{g}/\text{m}^3$  ( $>24.58 \text{ ppb}$ )]. Similar but greater risks were also observed for benzene exposures, based on higher exposure levels.

Jalaludin et al. (2007) examined a number of exposure periods and air pollutants for a three-year cohort of all births in Sidney, Australia. While results for different exposure periods provided widely varying estimates of effect, most did not indicate adverse relationships. The adverse associations were observed for most pollutants for the 3-month period preceding birth, when analysis was restricted to those living within 5 km of a monitoring station, though SO<sub>2</sub> dominated the risk estimate. Malmqvist et al. (2011) found no adverse association between NO<sub>x</sub> exposure or traffic density and preterm birth (ORs varied between 0.85 and 0.91 for the different exposure quartiles) in a cohort of approximately 80,000 births in Sweden. Results were sensitive to adjustment for a number of socioeconomic factors, but in none of the models were adverse relationships found. Gehring et al. (2011a, 2011b) also found no relationships between preterm birth and exposure to NO<sub>2</sub> and/or PM<sub>2.5</sub>, though they note the studies' statistical power to observe such effects was relatively low given the small study population and the low prevalence of preterm birth in the cohort.

## 10.4 Preeclampsia

In the 2008 US EPA ISA, no studies investigating NO<sub>2</sub>-related preeclampsia or pregnancy-induced hypertension were identified (US EPA, 2008). More recently, three North American and five international studies (four in Europe and one in Australia) have been published specifically examining associations between preeclampsia and hypertension in pregnancy and exposure to NO<sub>2</sub>/NO<sub>x</sub>.

The risk of preeclampsia in relation to NO<sub>2</sub> exposure was examined in a retrospective cohort study in Los Angeles and Orange counties, CA, by Wu et al. (2009) (study details discussed in Section 10.3). Entire-pregnancy exposure to NO<sub>x</sub> was associated with an increased risk of preeclampsia in the study population as a whole (OR = 1.11; 95% CI 1.06, 1.16 per 5.65 ppb increase). In the follow-up study performed in Los Angeles County and utilizing the same cohort (details also discussed in Section 10.3), the risk of preeclampsia was examined for NO and NO<sub>x</sub>, in addition to NO<sub>2</sub>, and the association was also assessed by exposure during specific periods of gestation (Wu et al., 2011). Based on data gathered from ambient monitoring stations, exposure to all three pollutants during the last month before birth was related to an increased risk of preeclampsia (ORs = 1.08; 95% CI 1.03, 1.14 per 7.5 ppb increase in ambient



NO<sub>2</sub> and 1.06; 95% CI 1.01, 1.10 per 25.6 ppb NO<sub>x</sub> increase). The higher risks of preeclampsia during this exposure window were robust to the adjustment for PM<sub>2.5</sub> in two-pollutant models, which was highly correlated with ambient NO<sub>2</sub> ( $r = 0.78$ ) and NO<sub>x</sub> ( $r = 0.69$ ). Larger effects were generally observed in Los Angeles County than in Orange County; the authors suggested that this may have been due to better exposure measures, given that subjects lived closer to monitoring stations. The authors concluded the risk of this pregnancy complication was associated with all measures of traffic-related air pollution exposure.

Conversely, in a prospective cohort study in Allegheny County, PA, between 1997 and 2001 involving 1,684 pregnant women recruited during early pregnancy, NO<sub>2</sub> was not found to be associated with an increase in blood pressure (data not shown) (Lee et al., 2012). By contrast, both PM<sub>10</sub> and O<sub>3</sub> exposures were related to blood pressure changes in the second half of pregnancy.

In support of the North American findings, three of the five European and Australian studies identified observed an increased risk of preeclampsia and/or elevation in blood pressure with NO<sub>2</sub> exposure.

In a cohort of 23,452 singleton births in Perth, Australia, between 2000 and 2006, exposure to traffic-related NO<sub>2</sub> levels (estimated by LUR models;  $R^2 = 0.86$ ) was significantly associated with risk of preeclampsia (OR = 1.53; 95% CI 1.07, 2.19 per IQR increase of 5.63 ppb) in the third trimester (OR = 3.26, 95% CI 1.48, 7.16 per IQR increase of 16.64 ppb) (Pereira et al., 2013). Risk was higher when the results were adjusted for other factors (e.g. age, SES) affecting the outcome; risk was also significantly increased for those mothers with pre-existing diabetic conditions. No other air pollutants were measured. Malmqvist et al. (2013) observed an increased risk of acquiring preeclampsia related to NO<sub>x</sub> exposure in all three trimesters among a cohort of 81,110 pregnant women from southern Sweden. The risks for preeclampsia increased with increasing quartiles of third-trimester NO<sub>x</sub> exposures; an OR of 1.51 (95% CI; 1.32, 1.71) was observed for the highest (>22 µg/m<sup>3</sup> (>12.1 ppb)) exposure quartile compared with the lowest one (2.5–8.9 µg/m<sup>3</sup> (1.3–4.7 ppb)). Adjusting the results to account for other risk factors increased the estimate of effect. Road traffic density as an exposure factor also provided indications of effect, but these were lower and less consistent than for NO<sub>x</sub>. The authors also observed an increased risk of gestational diabetes (subsection 9.3.4.2). In a prospective cohort in the Netherlands, van den Hooven et al. (2011) did not observe an increased risk of clinically diagnosed pregnancy-induced hypertension or preeclampsia associated with NO<sub>2</sub> among approximately 7,000 mothers. Based on a 10 µg/m<sup>3</sup> (5.32 ppb) increase in NO<sub>2</sub> levels, systolic blood pressure was, however, significantly increased throughout pregnancy, especially in the first ( $\beta$  for the difference in systolic blood pressure = 1.19 mmHg; 95% CI 0.54 mmHg, 1.83 mmHg) and second ( $\beta$  = 1.32 mmHg; 95% CI 0.76 mmHg, 1.95 mmHg) trimesters. The authors noted that although these small changes in systolic blood pressure may not be clinically relevant on an individual level, they may be more relevant on a population level. They also observed relationships with PM in the third trimester, and higher risks for those with gestational hypertension or clinical preeclampsia. Hampel et al. (2011) observed a decrease in systolic blood pressure in pregnant women related to NO<sub>2</sub> exposure in an additional prospective cohort study conducted in France. While PM<sub>10</sub> exposure was linked to an increase in systolic blood pressure in the first trimester, the data also demonstrated a decrease in the same measurement similar to that seen with NO<sub>2</sub> exposure in later pregnancy. Olsson et al. (2013) found no adverse association between city-average NO<sub>2</sub> exposure in the first trimester and preeclampsia in a prospective cohort of 120,755 pregnant mothers in Stockholm, Sweden, while they observed an association with O<sub>3</sub>.

## 10.5 Congenital Anomalies

In the 2008 US EPA ISA (US EPA, 2008), no studies examining congenital anomalies and NO<sub>2</sub> exposure were discussed. Two studies have been identified that came from the time period covered in the 2008 assessment but were not included in their document; neither showed an association between NO<sub>2</sub> exposure and birth defects. In a study published by Ritz et al. (2002) utilizing the California Birth Defects Monitoring Program, exposure to NO<sub>2</sub> was not related to an increased risk of cardiac and orofacial defects in a cohort of neonates and fetuses delivered in Southern California between 1987 and 1993 (CO and O<sub>3</sub> were so associated). In an additional study of live births and fetal deaths between 1997 and 2000 in Texas, Gilboa et al. (2005) found that no increased risk of selected cardiac birth defects and oral clefts was related to NO<sub>2</sub> exposure during weeks 3–8 of pregnancy, with the majority of ORs being near null.

Since the release of the 2008 US EPA ISA, six studies—three North American, two European and one Australian—as well as a European meta-analysis have been published regarding the association between congenital malformations and NO<sub>2</sub> exposure. These examined specifically cardiac malformations and cleft lip/palate.

In a retrospective cohort study conducted in Atlanta, GA, between 1986 and 2003 involving 715,500 liveborn and stillborn infants of at least 20 weeks gestation, the incidence of cardiovascular malformations was investigated in relation to 5-week (weeks 3–7 after conception) NO<sub>2</sub> exposure as well as exposure to other criteria pollutants (Strickland et al., 2009). In the initial overall analysis the authors found a number of non-significant adverse associations with all pollutants, for a variety of specific malformations; only one (between PM<sub>10</sub> and patent ductus arteriosus) was at the statistically significant level. When using an increased exposure period (weeks 1–9 of pregnancy), the authors found more frequent evidence of malformations related to all pollutants, with significantly increased risks of atrial (RR = 1.58; 95% CI 1.19, 2.09) and ventricular (RR = 1.20; 95% CI 1.02, 1.41) septal defect per 5.7 ppb increase in NO<sub>2</sub>. In addition, with less stringent control in the analysis for seasonal and long-term temporal variation, the authors also noted an increased risk of patent ductus arteriosus (RR = 1.40; 95% CI 1.07, 1.83).

A study by Marshall et al. (2010) provided little consistent evidence associating cleft malformations with maternal exposure to NO<sub>2</sub>. In a case-control study of 12,925 control, 414 cleft lip, and 303 cleft palate only cases among singleton births of >20 weeks gestation in New Jersey between 1998 and 2003, exposure to NO<sub>2</sub> (based on the closest air monitoring site at birth) during weeks 5–10 of gestation was not related to the incidence of cleft lip with or without cleft palate or cleft palate only (for Q1 (<20 ppb) vs. Q4 (>30 ppb): OR = 1.3; 95% CI 0.9, 1.08 for cleft lip +/- cleft palate and OR = 0.6; 95% CI 0.4, 1.0 for cleft palate only). Associations between O<sub>3</sub> and these same congenital anomalies were observed, but this was not the case for traffic density.

In a case-control study conducted in eight counties in the San Joaquin Valley, CA, between 1997 and 2006, the association between NO<sub>2</sub> exposure (using monitored data assigned by geocode to place of residence) and incidence of specific malformations was assessed in livebirth, stillbirth, and pregnancy termination cases (n = 806 cases and 849 controls) (Padula et al., 2013). The highest quartile of NO<sub>2</sub> exposure (20.54–38.94 ppb) was associated with neural tube defects as a whole (OR = 1.47; 95% CI 1.12, 2.69) and also with spina bifida (OR = 1.73; 95% CI 1.01, 2.97). The highest quartile of NO exposure (20.20–67.34 ppb) was also associated with neural tube defects, anencephaly, and spina bifida. No significant associations were found for cleft lip with or without cleft palate (OR = 0.69; 95% CI 0.47, 1.02), cleft palate only (OR = 0.90; 95% CI 0.53, 1.53), or gastroschisis (OR = 0.75; 95% CI 0.46, 1.22). The ORs

were consistent with a concentration-dependent relationship, increasing across quartiles of exposure. Relationships were also seen with CO but were less evident with PM.

In several European and Australian studies, variable associations were observed between NO<sub>2</sub> exposures and congenital anomalies (chromosomal and non-chromosomal), including heart defects and cleft lip/palate. Dolk et al. (2010) used annual average concentrations at the census ward level with 9 years of data from the English congenital anomaly register and found elevated though marginally non-significant associations with chromosomal anomalies. An OR of 1.11 (95% CI 0.95, 1.30) was found per 26.30 µg/m<sup>3</sup> (14.0 ppb) increase in NO<sub>2</sub> levels; similar associations were found for PM<sub>10</sub> and SO<sub>2</sub>. For specific malformations, only minor and non-significant associations were found for these three evaluated pollutants. Hansen et al. (2009) examined malformations in all births in Brisbane, Australia, over 7 years in relation to average air pollution in the 3–8 week period of pregnancy. In many cases, inverse associations were observed for malformations, though there were several adverse but non-significant ones with NO<sub>2</sub>. Associations were stronger for SO<sub>2</sub>, especially when exposures were assigned by the nearest monitor. Dadvand et al. (2011), also using exposures during weeks 3–8 of pregnancy (estimated with data gathered from closest monitoring stations), found no significant associations between NO<sub>2</sub> and congenital anomalies (OR = 0.995; 95% CI 0.988, 1.003 per 1 µg/m<sup>3</sup> (0.53 ppb) increase) in a study conducted in Northeast England. Exposure to NO and CO, however, significantly increased the risk of all congenital heart defects and several specific categories of defect. Effect estimates were much larger for CO, though the relationships with NO showed less variability.

Finally, a meta-analysis was identified (which included many of the studies above), involving original epidemiological studies (seven for NO<sub>2</sub>) examining congenital anomaly risk in single-pollutant models, specifically restricting the exposure window to either weeks 3–8 or month 2 of gestation (Vrijheid et al., 2011). Exposure to NO<sub>2</sub> was significantly associated with an increased risk for tetralogy of Fallot (OR = 1.20; 95% CI 1.02, 1.42) and for coarctation of the aorta (OR = 1.17; 95% CI 1.00, 1.36) for a 10 ppb increase in continuous exposure analysis. Both SO<sub>2</sub> and PM<sub>10</sub> also displayed significant associations with these outcomes, though they were somewhat weaker than those for NO<sub>2</sub>.

## 10.6 Other Developmental Effects

### 10.6.1 Neonatal Effects

In the 2008 US EPA ISA (US EPA, 2008), one study was identified that investigated hospitalization for respiratory disease in the early neonatal period. Although it was not discussed in any detail in the ISA, Dales et al. (2006) observed a 2.94% increase (95% CI 1.93%, 3.95% per IQR increase of 10 ppb NO<sub>2</sub>) in respiratory hospitalization among a cohort of neonates 1–27 d of age in 11 large Canadian cities. Pearson correlations between NO<sub>2</sub> and other air pollutants varied between cities from 0.20 to 0.67 with SO<sub>2</sub>, 0.13 to 0.76 with CO, -0.26 to 0.69 with PM<sub>10</sub> and -0.55 to 0.05 with O<sub>3</sub>. As compared to the other pollutants examined (PM<sub>10</sub>, O<sub>3</sub>, SO<sub>2</sub>, and CO), NO<sub>2</sub> had the strongest association, remaining significant in four-pollutant analyses with all four gases, or in models with these gases and every-sixth-day PM<sub>10</sub>. The associations with the other gases also remained significant in the four- and five-pollutant models. A positive, but non-significant, association was found for PM<sub>10</sub>.

Since the release of the 2008 US EPA ISA, eight studies (four North American and four European) have been published that examined neonatal health effects associated with prenatal exposure to NO<sub>2</sub>. In a Canadian case-control study conducted in southwestern BC between 1999 and 2000, the association between prenatal NO<sub>2</sub> exposure and incidence of asthma diagnosis up to 3–4 years of age was examined in a cohort of 37,401 singleton normal-weight

term births (3,482 were diagnosed as asthmatics) (Clark et al., 2010). NO<sub>2</sub> exposure (estimated with IDW method) during the entire pregnancy was significantly associated with asthma diagnosis (OR = 1.10; 95% CI 1.05, 1.15 per increase of 10 µg/m<sup>3</sup> (5.32 ppb) NO<sub>2</sub>). A significant relationship was also found based on the NO<sub>2</sub> exposure during first year of life (subsection 9.3.1.3). Similar relationships were also seen for NO, CO, PM metrics and a point source indicator.

In an additional North American study, Mortimer et al. (2008) analyzed pulmonary function among 232 asthmatic children aged 6–11 residing in Fresno, CA, in relation to prenatal exposure to NO<sub>2</sub>. Significant associations with two measures of lung function were observed for second-trimester exposure: per IQR increase in NO<sub>2</sub> (IQR not reported) among African-Americans, FVC was decreased by 7.1%, while among the whole cohort FEV<sub>1</sub> was decreased by 1.2% for exposure to NO<sub>2</sub> expressed as the average from 6 a.m. to 6 p.m. CO and PM<sub>10</sub> also displayed adverse significant relationships.

Two European publications also provide some evidence of prenatal exposures to NO<sub>2</sub> having subsequent adverse respiratory effects in infants. Latzin et al. (2009) observed an increase in respiratory rate (1.50 breaths/min; 95% CI 0.52 breaths/min, 2.49 breaths/min), a decrease in tidal volume (-0.78 mL; 95% CI -1.30 mL, -0.27 mL) and an increase in pulmonary inflammatory markers (0.98 ppb in eNO; 95% CI 0.45 ppb, 1.51 ppb) in 5-week-old infants in Bern, Switzerland, in relation to increases of 1 µg/m<sup>3</sup> (0.53 ppb) in prenatal exposure to NO<sub>2</sub> in a model adjusted for other risk factors. Exposure to prenatal exposure to PM<sub>10</sub> was also associated with higher respiratory need in newborns but not with inflammatory processes. In a cohort of 12–18-month-old children in Spain (Aguilera et al., 2013), doctor-diagnosed LRTIs were significantly adversely associated with prenatal NO<sub>2</sub> exposure (obtained with LUR models; R<sup>2</sup> = 0.52 to 0.75 for the different study areas) especially in relation to estimated second-trimester exposure (OR = 1.08; 95% CI 1.02, 1.15 per 10 µg/m<sup>3</sup> (5.32 ppb) increase). A similar association was reported with benzene exposure. A significant association was also observed between NO<sub>2</sub> exposure in the first year of life and ear infections, though the authors note a high correlation between pre- and postnatal exposures (subsection 9.3.1.2). For the INMA cohort in Valencia, Spain, Esplugues et al. (2011) found a significant association between exposures to NO<sub>2</sub> in the first trimester (estimated with LUR models; R<sup>2</sup> = 0.73) and LRTI in the first year of life. However, after adjustment for other risk factors, the OR remained at the same level, but the confidence interval widened and the relationship became statistically non-significant (OR = 1.18; 95% CI 0.94, 1.48 per 10 µg/m<sup>3</sup> (5.32 ppb) increment in NO<sub>2</sub> levels). NO<sub>2</sub> exposure in the first year of life was significantly associated with persistent cough (subsection 9.3.1.2). No other pollutants were studied.

Becerra et al. (2013) studied the influence of exposure to air pollution during pregnancy on the development of autism at ages 3–5 (7,603 cases) in a case-control study conducted in Los Angeles, CA. Entire-pregnancy exposure to NO<sub>2</sub> (estimated based on data from LUR models; R<sup>2</sup> = 0.86) was significantly related to risk of autism development (OR = 1.07; 95% CI 1.03, 1.12 per 5.41 ppb increase). Adjusting for other autism risk factors increased the ORs for most pollutants, with NO, NO<sub>2</sub>, O<sub>3</sub> and PM<sub>2.5</sub> exhibiting adverse and significant associations after such adjustment. Two-pollutant models also increased the likelihood of significant associations being observed in almost all cases; associations remained robust for NO<sub>2</sub> following adjustment for O<sub>3</sub> (r = -0.23), PM<sub>10</sub> (r = 0.23), PM<sub>2.5</sub> or CO (r = 0.26). Trimester-specific analyses did not reveal any periods of increased sensitivity. In another case-control study also conducted in California, Volk et al. (2013) observed an increased risk of autism in children aged 2–5, for entire-pregnancy exposure to NO<sub>2</sub> (OR = 1.81; 95% CI 1.23, 2.65 per 14.1 ppb increase), for all trimester-specific exposures, and for the first year of life. Entire-pregnancy and first-year-of-life exposures, which were highly correlated, provided the highest ORs (subsection 9.3.4.4).



Finally, Guxens et al. (2012) found adverse but non-significant associations between prenatal NO<sub>2</sub> exposure (estimated by LUR models; R<sup>2</sup> = 0.52 to 0.75 for the different study areas) and infant mental development in the Spanish INMA cohort. For a doubling in NO<sub>2</sub> levels the regression coefficients (β) for mental development in children aged 11–23 months were -0.95 (95% CI -3.90, 1.89) and -5.15 (95% CI -8.04, -2.27), respectively, for all four regions combined and for the industrial region. This relationship was significantly adverse (β = -4.13; 95% CI -7.06, -1.21) among mothers consuming low amounts of fruits and vegetables (a known risk factor), with risk estimates increasing in all regions. Relationships were similar for benzene exposures.

### 10.6.2 Live Birth Rates and Sudden Infant Death Syndrome

In the 2008 US EPA ISA, it was noted that one study involving Sudden Infant Death Syndrome (SIDS) and NO<sub>2</sub> exposure was found (Dales et al., 2004), but further details of the results were omitted. Dales and colleagues observed a 15.23% (95% CI; 6.74%, 24.39%) increase in SIDS incidence per 10.86 ppb increase in NO<sub>2</sub> levels in 12 Canadian cities between 1984 and 1999. These results remained robust when adjusting for sociodemographic factors, temporal trends, and weather. Associations were also observed for SO<sub>2</sub> but not for O<sub>3</sub> or PM.

Although no additional publications were found involving NO<sub>2</sub> exposure and SIDS specifically, in more recent literature, two North American studies were identified that examined live birth rates and NO<sub>2</sub> prenatal exposure.

Faiz et al. (2012) studied the incidence of stillbirth as it related to NO<sub>2</sub> exposure among a cohort of 718,974 singleton births (3,034 stillbirths) between 20 and 42 weeks gestation and with a birth weight of ≥500g in New Jersey. In the overall cohort, exposure to NO<sub>2</sub> (gathered from the closest monitoring station within 10 km of the maternal residence) was significantly associated with stillbirth for the entire pregnancy (OR = 1.27; 95% CI 1.04, 1.55) and the first trimester (OR = 1.16; 95% CI 1.03, 1.31) per 10 ppb increase in NO<sub>2</sub>. When the cohort was restricted to mothers residing within 5 km of a monitoring station, the ORs for the first, second, and third trimester were 1.26, 1.26, and 1.14, respectively (p < 0.01). Associations of a similar magnitude were also observed for CO and SO<sub>2</sub>, while none were significant for PM<sub>2.5</sub>.

In a unique study using data from *in vitro* fertilization (IVF) centres in the northeastern USA during the years 2000 to 2007 (Legro et al., 2010), the association of live birth rates following IVF and exposure to NO<sub>2</sub> was examined among 7,403 women undergoing their first IVF cycle. An increase of 10 ppb in NO<sub>2</sub> exposure (using monitored data assigned by geocode to place of residence) for specific phases of the IVF cycle was negatively associated with live births during the periods from medication start to oocyte retrieval (OR = 0.80; 95% CI 0.71, 0.91); from oocyte retrieval to embryo transfer (OR = 0.87; 95% CI 0.79, 0.96) and from embryo transfer to pregnancy test (OR = 0.76; 95% CI 0.66, 0.86). These results remained significant when modelling for interactions with O<sub>3</sub>, while O<sub>3</sub> results were no longer significant.

### 10.6.3 Other Effects

Since 2008, one North American and seven European studies have been published examining a variety of reproductive and developmental effects that do not directly fit into the above-mentioned categories. These include placental growth and function, circulating CRP levels, lymphocytes, vitamin D levels in fetal cord blood, maternal annoyance during pregnancy, and semen quality.

In a prospective cohort study conducted in Allegheny County, PA, between 1997 and 2001 involving 1,696 pregnant women recruited during early pregnancy (Lee et al., 2011), no association between NO<sub>2</sub> exposure and serum CRP levels (considered a marker of systemic



inflammation) was found at 22 weeks gestation (data not shown), though there were such relationships with PM<sub>10</sub>, PM<sub>2.5</sub>, and O<sub>3</sub>.

The levels of CRP in maternal and fetal cord blood were assessed in 6,508 mothers and singleton live birth infant pairs in a prospective cohort study conducted in Rotterdam, the Netherlands, between 2001 and 2005 (van den Hooven et al., 2012c). No associations were observed in maternal blood CRP levels for either NO<sub>2</sub> or PM<sub>10</sub>. However, fetal cord CRP levels were adversely associated with maternal NO<sub>2</sub> exposure for all exposure periods examined (1, 2 and 4 weeks before birth, and entire pregnancy). The relationships exhibited clear dose–response patterns, with the top two quartiles of exposure being significantly different from the first quartile (OR = 2.85; 95% CI 1.25, 6.47 in the third quartile (39.6–42.3 µg/m<sup>3</sup> (21.06–22.5 ppb)); OR = 3.42; 95% CI 1.36, 8.58 in the fourth quartile (>42.3 µg/m<sup>3</sup> (>22.5 ppb))). In another study from van den Hooven et al. (2012b) using subjects from the same large prospective cohort, the team examined dysfunction in placental growth and function (measured with angiogenic growth factors), considered to increase the risk of many pregnancy complications, such as adverse effects on fetal growth and preeclampsia. Based on modelled exposures assigned to home address, maternal soluble fms-like tyrosine kinase (sFlt) and placental growth factor were both reduced for all exposure periods examined (2.1% and 2.8%, respectively, in relation to full pregnancy exposure). Fetal sFlt and PIGF were also significantly affected, with the former being increased (8.9%) and the latter decreased (14.6%) for full pregnancy exposure. PM<sub>10</sub> relationships were similar. Placental weight was also significantly decreased in relation to exposure to NO<sub>2</sub> in the last 2 months of pregnancy.

In a prospective cohort study conducted in the cities of Poitiers and Nancy, France, the relationship between NO<sub>2</sub> exposure (estimated with a complex urban dispersion model) and placental weight was assessed for 888 singleton births (Rahmalia et al., 2012). Among the births in Nancy, an increase of 10 µg/m<sup>3</sup> (5.32 ppb) in NO<sub>2</sub> exposure (specifically in the third trimester) was significantly associated with a decrease in placental weight (-23.2 g; 95% CI -38.8 g, -7.5 g) and the placental fetal weight ratio (-0.58%; 95% CI -0.96%, -0.20%). Among births in the less polluted city of Poitiers, opposite or null results were observed. Similar but more pronounced associations were observed for PM<sub>10</sub> exposure.

In a single study examining the possible effect of NO<sub>2</sub> exposure on the immune systems of newborns, Baiz et al. (2011) measured lymphocyte cell counts from fetal cord blood in 370 women from a prospective cohort study in France in 2002–2005. Third-trimester NO<sub>2</sub> exposure (estimated from regional ambient monitoring) was significantly related to decreased counts of natural killer cells (0.09%; 95% CI 0.01%, 0.17% per 10 µg/m<sup>3</sup> (5.32 ppb) increase). Associations with PM<sub>10</sub> were more pervasive during all trimesters and displayed higher and more significant effect sizes. In a further prospective cohort study conducted by the same group, the cord blood serum levels of 25-hydroxyvitamin D were significantly decreased in relation to entire-pregnancy and third-trimester exposure to NO<sub>2</sub> estimated from complex urban air modelling (Baiz et al., 2012). As in the earlier study, effects were more robustly associated with PM<sub>10</sub>.

Llop et al. (2008) found that perceived annoyance to air pollution was significantly associated with NO<sub>2</sub> exposure in a prospective cohort of 786 pregnant women in Valencia, Spain; the higher the NO<sub>2</sub> concentration the higher the degree of annoyance. Results were similar for noise.

Finally, Boggia et al. (2009) found significantly lower sperm total motility and rapid forward progression among Motorway Company workers in Naples, Italy, occupationally categorized as exposed to NO<sub>2</sub> as compared with those categorized as not exposed to NO<sub>2</sub>. By contrast, Rubes et al. (2010) found no association between exposure to air pollution (including NO<sub>2</sub>, NO,

NO<sub>x</sub> and other criteria air pollutants) and sperm quality among 47 policemen working in downtown Prague, Czech Republic. Some marginal associations were found with PAHs.

## 10.7 Summary and Considerations

North American studies provide evidence of a relatively consistent significant association between NO<sub>2</sub> exposure and birth weight, specifically when examining changes in average birth weight, incidence of LBW, SGA/VSGA and IUGR (Figure 10.1). Periods of susceptibility during pregnancy have been observed in a number of these studies, with later pregnancy (i.e. the third trimester) potentially being a particularly vulnerable period, though associations are observed with other trimesters and whole pregnancy exposures.

A limited number of epidemiological studies have performed co-pollutant models in order to assess the independent effect of NO<sub>2</sub>. The NO<sub>2</sub>-related effects remained significant in some, but not all, cases, and significant associations were seen with a number of other pollutants, especially PM. Given the high correlation observed between the various air pollutants in several of these studies it is difficult to identify the specific pollutant(s) of concern. While more variable, most European and Australian publications also demonstrate significant associations between NO<sub>2</sub> exposure and decreases in birth weight, though some find little or no association. A limited international dataset also found some associations of NO<sub>2</sub> exposure with other fetal growth measures, namely birth length, head circumference, and biparietal diameter.

In support of an effect of NO<sub>2</sub> exposure on birth weight, a recently conducted meta-analysis revealed an association between NO<sub>2</sub> exposures and decreases in birth weight, although the authors pointed out that risk estimates were highly heterogeneous, results by exposure period were variable, and other pollutants also demonstrated significant associations. Overall, evidence indicates that exposure to NO<sub>2</sub> may have an impact on fetal growth but that uncertainties remain, due primarily to differentiating the impact of NO<sub>2</sub> from other pollutants.

A limited number of studies reviewed in the 2008 US EPA ISA had previously provided equivocal evidence of an effect of NO<sub>2</sub> on preterm birth. A larger body of work is now available, with new North American studies providing evidence of a consistent, usually statistically significant adverse relationship. As was the case for LBW, these relationships are also evident for other pollutants, especially PM and CO. European and Australian studies, while providing somewhat more variable results, also offer an indication of adverse effects associated with NO<sub>2</sub>. As for North American studies, other pollutants (including VOCs and O<sub>3</sub>) are also associated. There is some indication that issues with exposure estimates may be impacting the results observed; a study performed in Los Angeles County, CA, found an effect on mothers residing within 4 miles of a monitor. European studies also exhibit this phenomenon. In the recent meta-analysis a small but non-significant association was noted between NO<sub>2</sub> exposure and incidence of preterm birth, while other pollutants were more strongly associated with the outcome. The period of susceptibility remains unclear, with some evidence accumulating that entire-pregnancy or late gestational exposure to NO<sub>2</sub> results in the greatest risk. More recently, a limited number of studies have been published examining the association between NO<sub>2</sub> exposure and incidences of preeclampsia in pregnancy, a significant proportion of which provide support for such an association, possibly through anti-angiogenic mechanisms. Other pollutants (PM and O<sub>3</sub>) and indicators of traffic exposure were also associated with this outcome. It is possible that late gestational exposure results in the greatest risk, but further research is required.

In terms of congenital anomalies, there is evidence of an air pollution effect, though the dataset for NO<sub>2</sub> is limited and an equal number of studies find significant or no effects. While other pollutants appeared to be associated with orofacial abnormalities such as cleft palate, no such

associations were seen for NO<sub>2</sub>. However, a European meta-analysis found an increased risk of specific cardiac anomalies (tetralogy of Fallot and coarctation of the aorta) with NO<sub>2</sub> exposure. It is difficult to draw any conclusions due to the generally inconsistent results and the limited size of this dataset.

A small number of studies have been published on the neonatal effects of prenatal exposure to NO<sub>2</sub>. Evidence is accumulating of a link to postnatal respiratory complications (such as respiratory hospitalization, asthma development, decreased pulmonary function, and LRTI) soon after birth and during early childhood. Very limited numbers of studies have examined other outcomes and their association with NO<sub>2</sub> exposure, including the risk of autism, SIDS and stillbirths. Studies of markers of placental dysfunction, indicators of systemic inflammation, inhibition of the immune system, and vitamin D deficiency provide some mechanistic basis for the effects seen above, but they require further elaboration. It should be noted that most of these effects were also observed for PM<sub>10</sub>, and thus the agent of concern remains uncertain.

In conclusion, there is a growing body of epidemiological evidence implicating NO<sub>2</sub> exposure and air pollution in general in a variety of adverse reproductive and developmental effects. Relatively strong evidence is accumulating for effects on fetal growth, such as LBW, SGA, and IUGR, as well as for preterm birth. The primary uncertainty that remains is the role of NO<sub>2</sub> as compared to other air pollutants. Further investigation is required to elucidate the details and pollutant-specific associations with various endpoints. Variation in effects observed by exposure period as well as sources of heterogeneity between studies should be further explored.

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# 11. Epidemiological Studies of Health Effects Associated with Indoor NO<sub>2</sub>

Indoor environments are characterized by a different suite of combustion sources and pollutant profiles from those found outdoors. The measured ambient NO<sub>2</sub> concentration is often considered to be a marker of vehicle emissions, the primary ambient source of NO<sub>2</sub> in most urban centres. NO<sub>2</sub> in the indoor environment is the result of both infiltration of ambient NO<sub>2</sub> and NO<sub>2</sub> produced by combustion sources within the home, typically unvented or poorly vented gas appliances, such as heaters or stoves (Chapter 3).

Given these differences in combustion sources and pollutant profiles, epidemiological studies investigating health effects related to indoor NO<sub>2</sub> exposure are considered less relevant to this assessment of ambient NO<sub>2</sub>, though they can provide important supporting evidence for the types of health effects observed in epidemiological studies in outdoor environments. The health effects associated with exposure to NO<sub>2</sub> in indoor epidemiological studies are considered in detail in the Residential Indoor Air Quality Guideline Science Assessment Document for this pollutant (Health Canada, 2015) and form an important part of the basis for their assessment. The findings in indoor epidemiological studies are briefly summarized in the next few paragraphs, based on the study accounts in this recent indoor air assessment.

The health effects of exposure to NO<sub>2</sub> in the indoor environment have been investigated in a substantial number of epidemiological studies. Over 20 observational studies, as well as 2 intervention studies that evaluated the effects of reducing NO<sub>2</sub> exposure, were identified and evaluated (Health Canada, 2015). Numerous epidemiological studies have found positive associations between indoor NO<sub>2</sub> concentrations and respiratory symptoms, but generally little or no effect on lung function parameters. The positive associations with respiratory symptoms are most consistently observed in studies of asthmatic children exposed to indoor NO<sub>2</sub> levels that are somewhat higher than the levels typically encountered in Canadian homes (Belanger et al., 2006; Nitschke et al., 2006; Kattan et al., 2007; Hansel et al., 2008; Belanger et al., 2013). Studies investigating the relationship between personal exposure to NO<sub>2</sub> (which is generally more strongly related to indoor NO<sub>2</sub> levels than to those outdoors) and health effects also generally support the connection between NO<sub>2</sub> exposure and respiratory effects (Infante-Rivard, 1993; Pilotto et al., 1997; Mukala et al., 1999; Linaker et al., 2000; Chauhan et al., 2003; Delfino et al., 2006). Several large cohort studies with infants (not restricted to asthmatics; it is not possible to reliably diagnose the condition in this age class) have not reported increased respiratory symptoms in association with exposure to NO<sub>2</sub> (Samet et al., 1993; Emenius et al., 2003; Sunyer et al., 2004) despite relatively high NO<sub>2</sub> concentrations in some participating homes. Asthmatics appear to be more vulnerable to the adverse effects of NO<sub>2</sub> exposure than healthy individuals, something that was also noted in controlled human exposure studies, where asthmatics experienced symptoms at lower levels of exposure than did healthy adults (Chapter 7). One epidemiological study conducted in Spain has suggested the possibility of neurological effects (on cognition and attention behaviour) associated with infant exposure to NO<sub>2</sub> and/or other indoor co-pollutants of gas combustion (Morales et al., 2009).

In studies in which only the presence/use of a gas stove is recorded and NO<sub>2</sub> levels are not measured, the results of large cross-sectional and longitudinal studies have given inconsistent results, with some indication of an association between increased respiratory symptoms and slight decrements in lung function in children when gas stoves are present in the home. Furthermore, no specific respiratory symptom is consistently affected across studies. The

potential for exposure misclassification is greater in these types of study and may explain in part the inconsistencies in the database.

The indoor concentrations observed in these epidemiological studies, though measured over relatively short time periods, are often assumed to be representative of ongoing exposure over months or years. However, the results of two randomized intervention studies (Pilotto et al., 2004; Marks et al., 2010), in which NO<sub>2</sub> levels were reduced in Australian classrooms through replacement of an unflued gas heater, support a relationship between reduction in exposure to NO<sub>2</sub> and its co-pollutants emitted from gas appliances and an improvement in respiratory symptoms in the short term, including in asthmatic children.

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# 12. Risk Characterization

## 12.1 Introduction

Risk characterization serves an integrative function in an assessment. That is, the risk characterization seeks to integrate the scientific information on the nature and concentrations of the pollutant to which receptors are exposed (in this case exposure to ambient NO<sub>2</sub> in Canada); the nature of the health effects induced by exposure to the pollutant (NO<sub>2</sub>-related health effects reported in epidemiological studies and in experimental studies in humans and laboratory animals); and the quantitative relationship(s) between exposure of receptors and the responses they exhibit (i.e. the concentration–response relationships). Together, this information is used to draw inferences about the risk that the pollutant poses to the receptors.

This risk characterization is organized as follows:

- In Section 12.2, summaries of the key information on various categories of health effects associated with exposure to NO<sub>2</sub> that have been observed in epidemiological, controlled human exposure, and animal toxicological studies are presented in an integrated fashion. For each of these, cross-reference is made to the more detailed presentation of the information in the main body of this report. Then the weight of evidence for the category of health effect is evaluated to draw a conclusion with respect to the extent to which the findings support a causal relationship between exposure to ambient NO<sub>2</sub> and the category of health effect, using the framework described in subsection 12.2.1.
- In Section 12.3, the evidence that certain subgroups are at increased risk from exposure to ambient NO<sub>2</sub> as a result of increased sensitivity or increased exposure to the pollutant is summarized and evaluated.
- Section 12.4 summarizes the key information on several exposure-related issues. Initially, there is a summary of the current ambient sources and levels, including the spatial and temporal distribution of ambient NO<sub>2</sub> in Canada. In addition, key information on the relationship between ambient NO<sub>2</sub> concentrations (generally used in epidemiological studies as a surrogate for community exposure to ambient NO<sub>2</sub>) and personal exposure to NO<sub>2</sub> is summarized. This is followed by a comparison of the concentrations at which the key health effects of NO<sub>2</sub> are observed with the ambient levels of NO<sub>2</sub> in Canada for various NO<sub>2</sub> metrics (daily 1-h max, 24-h avg, and long-term avg). Finally, the shape of the concentration–response curve relating NO<sub>2</sub> levels to health effects is discussed.
- Section 12.5 discusses key uncertainties with respect to a number of issues in the assessment and the underlying database, as well as their implications.
- Section 12.6 presents a summary of key findings and insights arising from the preceding sections of the risk characterization.

The above range of topics is designed to address a range of issues relevant to policy, including the types of health effects associated with ambient NO<sub>2</sub> and the weight of evidence for each of these; population subgroups that appear to be at increased risk for NO<sub>2</sub>-related health effects; current ambient sources and levels of NO<sub>2</sub> in Canada, and the relationship between ambient NO<sub>2</sub> levels and personal exposures to NO<sub>2</sub>; microenvironments where ambient NO<sub>2</sub> levels are elevated; how the concentrations at which health effects are observed compare with ambient NO<sub>2</sub> levels in Canada; and the shape of the concentration–response relationship for key NO<sub>2</sub>-related health effects. As an aid to the reader, the policy-relevant questions that are being addressed are presented in text boxes at the beginning of the corresponding sections.

## 12.2 Summary and Weight of Evidence for Selected Categories of Health Effects Associated with Ambient NO<sub>2</sub>

*What effects are associated with NO<sub>2</sub> in the epidemiological, controlled human exposure and animal toxicological studies?*

*What is the weight of evidence for NO<sub>2</sub>-related health effects?*

### 12.2.1 Framework for Weight of Evidence for Causal Determination

This subsection introduces the framework for weight of evidence for causal determination that is applied in subsequent subsections. Namely, subsections 12.2.2 and 12.2.3 summarize and evaluate the collective results from epidemiological, animal toxicological, and controlled human exposure studies to assess the weight of evidence for various categories of health effects from short-term and long-term exposure to ambient NO<sub>2</sub>, respectively, and to draw conclusions with respect to how likely it is that the relationships are causal. The epidemiology studies provide the most extensive and highly relevant evidence of the adverse health effects of NO<sub>2</sub> air pollution, inasmuch as they investigate responses in the general population (including susceptible subgroups) to exposure to NO<sub>2</sub> as a component of the ambient mix of pollutants in “real world” settings. They can also often examine more severe health outcomes and/or very large numbers of people. However, these studies are observational rather than experimental, and the information on both exposure and effects is generally only available for the population rather than for individuals. As a consequence of such limitations, there can be uncertainty as to whether the effects reported in the epidemiology studies are in fact due to ambient NO<sub>2</sub> alone, or if NO<sub>2</sub> is a marker, in whole or in part, for other air pollutants, or if the association is due to some other factor.

By contrast, the experimental studies in humans provide compelling evidence that exposure to NO<sub>2</sub> truly causes various respiratory health effects. However, for ethical and practical reasons the controlled human exposure studies are generally limited to examining short-term, mild, reversible alterations in health endpoints, typically in small groups of relatively healthy individuals who do not include those who may be most at risk (e.g. those with severe pre-existing disease). They are therefore unable to capture the full range of severities of effects and the profiles of affected populations, and they do not have the statistical power to identify relatively small risks.

In the following subsections, epidemiological studies of ambient NO<sub>2</sub> have been weighted more heavily than animal toxicological or controlled human exposure studies for several reasons: 1) epidemiological studies provide the most direct approach for assessing the health effects of “real world” complex mixtures of air pollutants to which people are exposed; 2) human populations are highly heterogeneous as compared with laboratory animal populations and encompass a large range of susceptibilities, disease/illness status and exposures; and 3) no species extrapolation is necessary. However, the results from animal toxicological studies and especially controlled human exposure studies are still quite relevant and shed light on results

from epidemiological studies, particularly with respect to pathophysiological mechanisms underlying observed effects.

To evaluate the weight of evidence that the epidemiological associations between health outcomes and ambient NO<sub>2</sub> are causal, it is necessary to examine the various lines of evidence in combination and to assess the collective evidence using established criteria for causal determination. In this subsection, the evidence for various categories of health outcomes is reiterated in an integrated fashion, by reporting the findings from the available epidemiological, controlled human exposure, and/or animal toxicological studies together. This collective evidence is then evaluated for various categories of health outcomes in light of considerations that have traditionally been used to form judgments as to how likely it is that the observed associations are causal.

These considerations include:

- the *strength of association*, including the magnitude and precision of the risk estimates and their statistical significance;
- the *robustness* of the associations to model specifications and adjustment for potential confounders such as weather, temporal trends, and co-occurring pollutants;
- the *consistency* of reported associations across studies and study designs conducted by different researchers in different locations and times;
- the *coherence* of the relationship between exposure to NO<sub>2</sub> and related endpoints within and across animal toxicology, controlled human exposure, and various types of epidemiological studies; and
- the *biological plausibility* of the associations in light of what is known regarding NO<sub>2</sub> dosimetry and the types of effects observed and associated potential mechanisms of action, based largely on animal toxicology and controlled human exposure studies.

Using the US EPA framework for Weight of Evidence for Causal Determination from the Integrated Science Assessment for ozone (Table 12.1), the above considerations are used to draw conclusions with respect to the weight of evidence for a given health effect or related set of health effects: i.e. to conclude whether the relationship between ambient NO<sub>2</sub> and the health effect category is causal, likely to be causal, suggestive, but not sufficient to infer a causal relationship, etc.

## 12.2.2 Short-term Effects

### 12.2.2.1 Respiratory Morbidity

A number of earlier and more recent epidemiological studies have investigated the association between short-term exposure to ambient NO<sub>2</sub> and respiratory effects, including lung function, AHR, respiratory symptoms, pulmonary inflammation, and respiratory medical utilizations. The strongest evidence of respiratory health effects from short-term exposure to ambient NO<sub>2</sub> comes from epidemiological studies of hospital admissions and ERVs for respiratory causes and from studies of volunteers exposed to near-ambient levels of NO<sub>2</sub> for periods of a few hours or less in controlled human exposures.

**Table 12.1: Weight of evidence for causal determination—health effects\***

Relationship	Description
<b>Causal relationship</b>	Evidence is sufficient to conclude that there is a causal relationship with relevant pollutant exposures (i.e. doses or exposures generally within one to two orders of magnitude of current levels). That is, the pollutant has been shown to result in health effects in studies in which chance, bias, and confounding could be ruled out with reasonable confidence. For example: a) controlled human exposure studies that demonstrate consistent effects; or b) observational studies that cannot be explained by plausible alternatives or are supported by other lines of evidence (e.g. animal studies or mode of action information). Evidence includes multiple high-quality studies.
<b>Likely to be a causal relationship</b>	Evidence is sufficient to conclude that a causal relationship is likely to exist with relevant pollutant exposures, but important uncertainties remain. That is, the pollutant has been shown to result in health effects in studies in which chance and bias can be ruled out with reasonable confidence, but potential issues remain. For example: a) observational studies show an association, but co-pollutant exposures are difficult to address and/or other lines of evidence (controlled human exposure, animal, or mode of action information) are limited or inconsistent; or b) animal toxicological evidence from multiple studies from different laboratories demonstrates effects, but limited or no human data are available. Evidence generally includes multiple high-quality studies.
<b>Suggestive, but not sufficient to infer a causal relationship</b>	Evidence is suggestive of a causal relationship with relevant pollutant exposures, but is limited. For example: a) at least one high-quality epidemiologic study shows an association with a given health outcome but the results of other studies are inconsistent; or b) a well-conducted toxicological study, such as those conducted in the National Toxicology Program, shows effects in animal species.
<b>Inadequate to infer a causal relationship</b>	Evidence is inadequate to determine that a causal relationship exists with relevant pollutant exposures. The available studies are of insufficient quantity, quality, consistency, or statistical power to permit a conclusion regarding the presence or absence of an effect.
<b>Not likely to be a causal relationship</b>	Evidence is suggestive of no causal relationship with relevant pollutant exposures. Several adequate studies, covering the full range of levels of exposure that human beings are known to encounter and considering at-risk populations, are mutually consistent in not showing an effect at any level of exposure.

\*Modified from US EPA (U.S. Environmental Protection Agency). 2013. Integrated science assessment for ozone and related photochemical oxidants. (EPA/600/R-10/076F). Research Triangle Park, NC: US Environmental Protection Agency, National Center for Environmental Assessment. <http://cfpub.epa.gov/ncea/isa/recordisplay.cfm?deid=247492>



In a large number of population-based epidemiological studies, ambient NO<sub>2</sub> was positively and significantly associated with increased risks for total respiratory and asthma hospitalizations and ERVs, when potential confounders were accounted for in the analysis (subsection 8.3.1; Figures 8.2 and 8.3). These studies were conducted in cities all over the world, including in Canada and in other countries with ambient NO<sub>2</sub> levels that are similar to those experienced in Canada. These associations were often observed in analyses for participants of all ages, but were generally greater and more consistent in children and older adults for hospital admissions and ERVs for all respiratory conditions combined, and in children for asthma (subsection 8.3.1, Figures 8.2 and 8.3), perhaps reflecting the age-related prevalence of the conditions underlying these different groupings of medical visits. Significant associations were found using various model specifications and study designs, and they were robust to adjustment for PM or other gaseous pollutants (including certain traffic-related co-pollutants (CO, PM<sub>2.5</sub> and UFPs) in some studies) in two-pollutant or multi-pollutant models in most studies, though not in all. The strongest single-day associations were mostly for short-term lags of 2–3 d or less. Risk estimates for respiratory ERVs were sometimes stratified by season and were greatest and most consistent in warm-season analyses (when time spent outdoors and infiltration are maximal), especially for children’s asthma ERVs, whereas there was little evidence of an effect during the cold season (Figure 8.3). Most of the studies estimated risks in relation to 24-h avg NO<sub>2</sub>, but positive findings were also associated with ambient NO<sub>2</sub> over shorter periods, primarily for daily 1-h max NO<sub>2</sub> but also for other sub-daily averaging periods; this is not unexpected given the strong correlations among these various time metrics. Findings for hospital utilizations for other respiratory conditions or for other respiratory medical visits are very limited, though short-term ambient NO<sub>2</sub> was associated with asthma outpatient visits in two large studies in Ontario.

Controlled human exposure studies, also known as clinical studies, document the health effects from inhalation of specific concentrations of pollutants under experimental conditions. The studies considered in this assessment (Chapter 7) were those with inhalation exposures to relatively low concentrations (1000 ppb or less; i.e. within an order of magnitude of peak ambient concentrations) for up to 6 h. In these studies, among healthy adults exposed by inhalation to NO<sub>2</sub> levels in this range, with or without exercise, there was generally no effect on respiratory symptoms, lung function, or AHR with bronchial challenge, though there were equivocal immunological effects and pulmonary inflammation in some healthy adults in individual studies (Section 7.1). Conversely, adults with asthma or with COPD exhibited decreased lung function and increased AHR (a hallmark of asthma) with non-specific bronchial challenge (cold air, histamine or methacholine) at approximately 3-fold lower levels of NO<sub>2</sub> in a number of studies, though not in all (Section 7.2). Inflammatory mediators were also increased in the BALF of exercising asthmatic adults by short-term exposure to higher concentrations of NO<sub>2</sub> in the one study that examined this endpoint. An inflammatory response was the most sensitive endpoint in some asthmatic adults with allergies exposed to NO<sub>2</sub> and challenged with an allergen, histamine or carbachol, with decreased lung function and increased AHR also being observed in some studies, indicating that NO<sub>2</sub> can potentiate allergen-induced airway responses in some allergic asthmatics. While it is clear from this literature that subjects with asthma and COPD are more susceptible to adverse respiratory effects induced by NO<sub>2</sub>, there was little indication of age- or gender-sensitivity to this pollutant in the small number of studies with older adults and children.

These controlled human exposure studies demonstrate a relationship between short-term exposure to NO<sub>2</sub> and adverse respiratory effects in asthmatics or COPD subjects, but the dose–response at concentrations of 1000 ppb or less is somewhat uncertain. Inconsistencies in findings between studies may be the result of a number of factors, including the large variation in individual responsiveness to NO<sub>2</sub>, the limited number of exposure levels tested in most

studies, the small sample sizes characteristic of this literature, and differing exposure protocols. The lowest concentration to increase AHR in asthmatic adults in response to non-specific bronchial challenge was 100 ppb NO<sub>2</sub> for 1 h in one study (though most studies showing significant effects were in the range of 300 ppb or greater). In a meta-analysis by the US EPA of the combined individual subject data from a number of these experimental studies, the fraction of resting subjects with increased AHR in response to non-specific bronchial challenge was significantly greater following exposure to 100 ppb NO<sub>2</sub> for 1 h, and with 2- to 3-fold higher exposures after ½ h. Overall, the US EPA concluded that exposure to NO<sub>2</sub> at levels of less than 300 ppb, and as low as 100 ppb, for 1 h was linked to nonspecific AHR in people with asthma (Section 7.3).

In several earlier and more recent Canadian or US panel studies there were significant NO<sub>2</sub>-related decrements in lung function in schoolchildren tested by spirometry (subsection 8.4.1.1; Figure 8.4). A number of these studies examined asthmatics, but similar findings were also reported in groups of subjects drawn from the general population. Associations were often greatest for lags of a few days, but they were seen within a few hours in some studies, and in studies of asthmatics were primarily limited to subjects not taking asthma control medications. In a study of California schoolchildren with asthma, personal NO<sub>2</sub> was related to similar decrements in FEV<sub>1</sub> to those observed with NO<sub>2</sub> measured at a central site (Figure 8.4). However, lung function decrements were also associated with other pollutants in most of these studies, and in co-pollutant modelling in a limited subset of these studies the evidence for an independent effect of NO<sub>2</sub> was mixed.

Experimental studies in animals (as well as in humans with the controlled exposures presented above) demonstrate that short-term exposure to NO<sub>2</sub> causes increased AHR, a hallmark of asthma, in response to bronchial challenge (Chapters 6 and 7). There is support for these findings in a recent epidemiology study in which ambient NO<sub>2</sub> was related to increases in AHR in asthmatic children from Mexico City (subsection 8.4.1.2). The association was limited to children not currently using asthma control medications and to those with mild intermittent asthma, and it appeared to be independent of other pollutants.

A number of epidemiological studies have investigated whether ambient NO<sub>2</sub> is related to risks for respiratory symptoms or asthma medication use. In most Canadian and US panel studies of asthmatic schoolchildren, there were consistent and mostly significant associations between ambient NO<sub>2</sub> and increases in asthma-related symptoms, including one or more of wheeze, cough, chest tightness, total asthma symptoms, slow play days, asthma-related school absences, and rescue medication use (subsection 8.4.1.4; Figure 8.5). In most of the studies that used co-pollutant models, the risks for NO<sub>2</sub> were independent of those for other pollutants, including traffic-related pollutants such as PM<sub>2.5</sub>, CO and UFPs in the small number of cases where these were investigated. In studies of panels of schoolchildren drawn from the general population, findings again highlighted effects in asthmatics, with associations between NO<sub>2</sub> and asthma-related symptoms and/or increased risks being restricted to asthmatics. Risks for respiratory symptoms were most pronounced in asthmatic children with allergies and in those who were not using asthma control medications. In panel studies of asthmatic adults, ambient NO<sub>2</sub> was also associated with respiratory symptoms and asthma medication use or sales (subsection 8.4.1). However, these epidemiological findings are only weakly supported by the results of controlled human exposure studies, as only a few of the experimental studies found increases in respiratory symptoms in adults or children with asthma when exposed to NO<sub>2</sub> (Chapter 7).

There is additional support for NO<sub>2</sub>-related increases in respiratory symptoms from indoor epidemiological studies in which there have been significant associations between ongoing exposure to NO<sub>2</sub> in the home and increased respiratory symptoms (Chapter 11). These

increased risks are most consistently observed in studies of asthmatic children exposed to indoor NO<sub>2</sub> levels that are somewhat higher than the levels typically encountered in Canadian homes, but that overlap ambient levels in Canada. Two randomized Australian intervention studies, in which NO<sub>2</sub> levels were reduced in classrooms through replacement of unflued gas heaters, provide strong support for a relationship between reduction in exposure to NO<sub>2</sub> (and other pollutants emitted from gas combustion appliances) and improvements in respiratory symptoms in the short term, particularly in children. The indoor studies may, however, be less relevant because the emissions from combustion sources in the indoor environment are somewhat different than those from ambient sources.

In a number of earlier and more recent panel studies of schoolchildren, both asthmatics and non-asthmatics, there were NO<sub>2</sub>-related increases in biomarkers of airway inflammation and oxidative stress (subsection 8.4.1). There were somewhat consistent findings with eNO, which was positively and often significantly associated with personal or ambient NO<sub>2</sub> in a number of Canadian and US studies. There have also been positive findings for some other little-studied biomarkers, including TBARS, isoprostane, IL-8 and the pH of EBC. However, there were associations with other pollutants in most of these studies, and these were often more consistent than those for NO<sub>2</sub>. In addition, the NO<sub>2</sub>-related risks were often not robust to adjustment for other pollutants in those studies in which co-pollutant models were applied.

In experimental studies in animals (Chapter 6), inhalation of NO<sub>2</sub> at greater than ambient levels induced a range of respiratory effects: airway inflammation; increased markers of oxidative stress and lipid peroxidation; increased inflammatory response to HDMA; increased inherent responsiveness of the airway to non-specific bronchial challenge; impairment of components of the lung host defence system, including mucociliary transport and alveolar macrophages; and increased susceptibility to both viral and bacterial infection.

In summary, the strongest evidence of respiratory effects from short-term exposure to ambient NO<sub>2</sub> comes from results of studies relating to asthma exacerbations. These findings across disciplines provide a consistent and coherent suite of asthma respiratory outcomes associated with ambient NO<sub>2</sub>, including increased hospital admissions and ERVs for asthma in population-based epidemiology studies in children; increases in asthma-related symptoms and medication use in panel studies of asthmatic schoolchildren; and increases in asthma medication use and sales in panels of asthmatic adults. There is also strong support for NO<sub>2</sub>-related increases in risks for respiratory symptoms from studies of asthmatic children exposed to NO<sub>2</sub> in indoor classroom or residential settings, and for decreases in symptoms shortly after reductions in exposure to NO<sub>2</sub> and related pollutants emitted by gas heaters in two well-designed intervention studies. Additional coherence as well as biological plausibility is provided by the results of experimental studies in which NO<sub>2</sub> induced airway inflammation and AHR and also potentiated airway responses to allergens in animals and humans, and in epidemiological studies of these endpoints. The epidemiological associations with respiratory symptoms, lung function and AHR were often limited to children not using asthma control medications, and respiratory effects were often most pronounced in the warm season (when time spent outdoors and infiltration are generally greater), both of which strengthen biological plausibility. For most asthma-related outcomes, the epidemiological findings were generally not highly sensitive to study designs, model specifications, and adjustment for potential confounders, including co-occurring pollutants. Associations were also observed in cities from various regions of the world, in spite of differences in climatic conditions, pollutant mixtures, and socioeconomic factors. While the increased risks reported for the various respiratory outcomes are modest, they nonetheless demonstrate strength of association, because they show a concentration–response relationship, are statistically significant in most studies, and (since the entire population is exposed) represent large numbers of people and substantial impacts on public health.

The epidemiological associations between ambient NO<sub>2</sub> and this suite of effects related to asthma exacerbation, with support from results of experimental studies in animals and humans, thus exhibit consistency, coherence, biological plausibility, robustness and strength of association. However, some uncertainty remains about the possible role of other co-occurring pollutants, either measured or unmeasured, in the NO<sub>2</sub>-related effects. By contrast, the controlled human exposure studies show effects on airway inflammation and responsiveness that are caused by NO<sub>2</sub>, though there is considerable variability in the dose–response relationship at the near-ambient exposure levels employed in these studies. Considering the epidemiological and experimental evidence together, the overall evidence indicates that there is **a causal relationship** between short-term exposures to ambient NO<sub>2</sub> at current levels and respiratory morbidity related to asthma exacerbations (including airway inflammation and AHR, increases in respiratory symptoms and asthma medication use, and asthma hospitalizations and ERVs).

#### 12.2.2.2 Cardiovascular Effects

A number of earlier and more recent epidemiological studies have examined the association between short-term exposure to ambient NO<sub>2</sub> and effects on the cardiovascular system, including mortality, hospitalizations, ERVs, and effects on cardiovascular function and biomarkers of disease risk. This category of effects has also been investigated to a limited extent in experimental studies in humans and animals.

The strongest evidence for cardiovascular effects related to short-term exposure to ambient NO<sub>2</sub> is for mortality (Section 8.2). In a time-series study of mortality in 12 Canadian cities over almost 20 years, and in several European and Australian multi-city time-series or case-crossover studies, there were significantly increased risks of mortality from cardiovascular causes related to ambient NO<sub>2</sub>. There is also support for these findings in the consistent associations between NO<sub>2</sub> and all-natural cause or CP mortality, inasmuch as deaths from cardiovascular causes make up a large fraction of these broader categories of mortality. The association of NO<sub>2</sub> with cardiovascular mortality was generally not sensitive to adjustment for other pollutants in those studies that conducted co-pollutant models. In several studies, mortality risk was most pronounced in the warm season and in older adults. NO<sub>2</sub>-related risks were increased for various subsets of cardiovascular deaths, including cardiac, cerebrovascular and IHD mortality across studies.

There were also positive and significant associations between ambient NO<sub>2</sub> and CVD, cardiac or circulatory hospitalizations in a number of studies reviewed in the 2008 US EPA ISA and in this assessment. For more specific causes, there were positive associations with admissions for MI in several studies, and also for IHD or for ischemic stroke in some of the studies that examined these outcomes. Findings were generally similar for ERVs, with increased NO<sub>2</sub>-related risks for visits for all CVD and/or more specific causes, including IHD and angina/MI, in a number of studies (subsection 8.3.2). However, in many of these studies these outcomes were also related to other pollutants, most often PM and/or CO, and either only single-pollutant models were run or NO<sub>2</sub>-related risks were often (though not always) sensitive to adjustment for these co-pollutants. The association of ambient NO<sub>2</sub> with cardiovascular ERVs was often more pronounced in the warm season and in older adults.

In panel studies of cardiovascular function in subjects exposed to ambient air pollution (subsection 8.4.2), there were NO<sub>2</sub>-related decreases in HRV (predictive of mortality after MI) in only about half of the studies; even among the positive findings the specific HRV measures affected differed. There are also reports that ambient NO<sub>2</sub> is associated with increased blood pressure, vasodilation, and ST-segment depression (a non-specific marker of MI) in adults, as well as with bradycardia in high-risk infants, though there are very few studies of any of these endpoints and the results are somewhat inconsistent. The cardiac function endpoints were

almost always related to other pollutants (most often particulates) in these studies, and the association with NO<sub>2</sub> was sensitive to adjustment for other pollutants in the small number of studies that conducted co-pollutant models, with the exception of changes in HRV in a Canadian study of urban cyclists, and ST-segment depression in a US study of patients with CAD.

Similarly, panel studies generally found an association between ambient or personal NO<sub>2</sub> and increases in biomarkers related to inflammation (e.g. IL-6, TNF-RII, CRP, 8-OHdG) and coagulation (e.g. fibrinogen) (subsection 8.4.3.1), which are linked to increased risks of CVD. However, in virtually all of these studies other pollutants, most often PM and CO, were also associated with alterations in these biomarkers, and co-pollutant models were not reported in any of them.

There is only limited evidence for cardiovascular effects following controlled exposure of humans to NO<sub>2</sub> at greater than ambient levels for periods of a few hours or less (Section 7.4). Some measures of HRV were decreased (along with decreased QT variability index and increased HDL cholesterol) following exposure to NO<sub>2</sub> in a study of exercising healthy volunteers, but not in another study of CHD patients with similar exposures. Hemoglobin and hematocrit were decreased by NO<sub>2</sub> in two earlier studies. The results of one more recent study suggested that NO<sub>2</sub> exposure may stimulate the release of soluble factors into systemic circulation, where they may activate endothelial cells to produce inflammatory markers, which has been linked to atherosclerosis. Across the small number of controlled human exposure studies there was little or no indication of effects of NO<sub>2</sub> on most other cardiovascular endpoints, including cardiac output, stroke volume, heart rate, blood pressure, O<sub>2</sub> saturation, frequency of ectopic beats or arrhythmias, and circulating levels of pro-inflammatory mediators and coagulation factors.

In toxicology studies of laboratory animals, short-term exposure to NO<sub>2</sub> caused a range of cardiovascular effects (Section 6.2), including slight histological changes in the heart; decreased hematocrit, hemoglobin and RBC counts, and increased turnover of RBCs; and alterations in several markers indicative of oxidative stress in the heart, aorta and RBCs, of inflammation and increased vasoactivity (including ET-1, a potent vasoconstrictor) in the heart, and of cardiac myocyte apoptosis.

In summary, in the population-based epidemiological studies reviewed for this assessment, there were consistent and significant NO<sub>2</sub>-related increased risks for cardiovascular mortality, hospital admissions, and ERVs, as well as for more specific causes, particularly MI and IHD. There is also support for the biological plausibility of these findings from the results of panel studies and individual controlled human exposure studies in which there were NO<sub>2</sub>-related decreases in HRV, changes in ventricular repolarization, and increases in biomarkers related to inflammation and/or coagulation, as well as in effects from NO<sub>2</sub> in experimental studies in animals that can underlie CVD (e.g. inflammation, oxidative stress) or can cause serious cardiovascular events (e.g. vasoconstriction). However, the findings in the panel and controlled human exposure studies are somewhat inconsistent, and the size of the dataset and the extent to which the spectrum of NO<sub>2</sub>-related cardiovascular effects has been elucidated is limited, both of which factors weaken the coherence of the database. As well, in many of the epidemiological studies of cardiovascular morbidity, the outcomes were also related to other pollutants, and NO<sub>2</sub>-related risks were sometimes attenuated by adjustment for other pollutants or co-pollutant models were not run. Considering the uncertainty as to whether NO<sub>2</sub> is independently related to the cardiovascular morbidity, and the limited supporting data from panel, controlled human exposure, and animal toxicological studies, the overall evidence is **suggestive, but not sufficient to infer a causal relationship** between short-term exposure to ambient NO<sub>2</sub> and cardiovascular effects.



### 12.2.2.3 Mortality

In large numbers of epidemiology studies of various designs, there were positive and (especially in those of strongest design (i.e. multi-city studies)) statistically significant associations between short-term levels of ambient NO<sub>2</sub> and total non-accidental, CP, cardiovascular, and respiratory mortality (Section 8.2). These associations were observed in cities from various regions of the world, encompassing different climatic regimes, pollutant mixes, and socioeconomic conditions. The risk estimates for short-term lags (in the range of 0–2 d singly or combined) in Canadian and US studies in all-season analyses mostly ranged from 1.7% to 3.5% per 20 ppb 24-h NO<sub>2</sub> (Figure 8.1). Cardiovascular and respiratory mortality risks were increased by a similar or greater amount than for total mortality, and there were also significant increases in more specific causes of death, including from IHD, stroke, and COPD. NO<sub>2</sub>-related mortality risks were generally robust to model specifications, including adjustment for co-pollutants. This was observed most often in models with common air pollutants including PM<sub>10</sub>, O<sub>3</sub>, and SO<sub>2</sub>. However, effect estimates for NO<sub>2</sub> were also not sensitive to adjustment for traffic-related pollutants including CO, PM<sub>2.5</sub>, and (in a single Canadian study) NO and a wide range of particulate components including copper, zinc and iron, though traffic-related pollutants have not been extensively studied in this regard. Risks for mortality were greater in the warmer months (Figure 8.1) as well as in older adults and in subjects with pre-existing heart and lung conditions.

With respect to other lines of evidence, there is support for the relationship of short-term exposure to NO<sub>2</sub> with total and cardiovascular mortality in the form of consistent NO<sub>2</sub>-related increased risks for cardiovascular hospital admissions and ERVs (discussed in the previous subsection), particularly considering that these are major causes of morbidity and mortality. There is also evidence at several levels that ambient NO<sub>2</sub> may trigger a fatal MI. Epidemiological studies have found increased risks for HA/ERVs or mortality for MI, angina, and/or their underlying cause IHD, as well as S-T segment changes; there is also experimental support for potentially biologically plausible mechanisms, including inflammation and oxidative stress. However, there is only limited and sometimes inconsistent evidence of effects on cardiac function (e.g. HRV), vasoconstriction or other cardiovascular endpoints that could result in mortality. In addition, as noted in the previous subsection, in most studies of cardiovascular morbidity the outcomes were associated with both NO<sub>2</sub> and other pollutants, and there is considerable uncertainty as to whether NO<sub>2</sub> is independently related to these effects. With respect to respiratory effects, the strongest evidence links NO<sub>2</sub> to asthma exacerbations, but asthma is generally managed effectively by means of various health interventions and is not a common cause of death. COPD and respiratory infections are conditions that could more plausibly give rise to increases in respiratory mortality, and there is some limited epidemiological evidence of associations of ambient NO<sub>2</sub> with morbidity from these conditions. NO<sub>2</sub> was related to increased hospital admissions, ERVs and mortality in COPD patients in a small number of studies (Sections 8.2 and 8.3), and decreased lung function in two controlled human exposure studies of subjects with COPD (Section 7.2). As well, pulmonary inflammation is a key event in COPD exacerbation, mediating narrowing of the airways and reducing airflow, and NO<sub>2</sub> is linked to airway inflammation in experimental and epidemiological studies (though not in COPD patients). Concerning respiratory infections, a number of epidemiological studies have reported increases in hospital admissions or ERVs for upper or lower respiratory tract infections, though the effect estimates were often marginal and co-pollutants were not much investigated (subsections 8.3.1.2, 8.3.1.3). An NO<sub>2</sub>-related risk for respiratory infections appears plausible in light of animal experiments showing that inhalation of NO<sub>2</sub> increased susceptibility to pulmonary infection and impaired components of the lung host defence system, including mucociliary activity/transport and alveolar macrophages (Section 6.1.1).

In summary, the associations of ambient NO<sub>2</sub> with total non-accidental mortality, and to a lesser extent cardiovascular and respiratory mortality, display strength of association and consistency,

and they indicate that NO<sub>2</sub> is independently related to these outcomes. The findings were observed in diverse locations from around the world and were robust to study design, model specifications and adjustment for co-pollutants, including traffic-related pollutants in a limited number of studies. However, the coherence of the databases for NO<sub>2</sub>-related effects that may give rise to mortality from cardiovascular and respiratory causes is somewhat limited, though there is evidence for several elements in the sequences of events that could give rise to increased cardiovascular and respiratory mortality, as well as non-specific but plausible mechanisms of action for mortality from these causes. Overall, the evidence indicates that there is **likely a causal relationship** between short-term exposure to ambient NO<sub>2</sub> at current levels and mortality, especially for total non-accidental mortality.

### 12.2.3 Long-term Effects

#### 12.2.3.1 Respiratory Effects

A number of earlier and more recent epidemiological studies have examined the association between long-term exposure to ambient NO<sub>2</sub> and respiratory effects, including lung function and lung function growth, respiratory symptoms, asthma-related outcomes and medical visits due to asthma or development of other chronic respiratory disease.

Many recent epidemiological studies have been performed in multiple locations, including Canada and other countries with ambient NO<sub>2</sub> levels similar to those observed in Canada; these studies used different study designs and lengths of exposure duration (several months to up to several years, representing lifetime exposure), and various methods of exposure assessment and control for key potential confounders. In children, several large longitudinal studies found consistent and mainly significant associations between long-term exposure to ambient NO<sub>2</sub> and decrements in lung function and partially irreversible deficits in lung function growth (subsection 9.3.1.1; Figure 9.5). New analyses of the CHS also suggested that NO<sub>2</sub>-related lung function deficits in children can be modified by psychosocial stress and by genetic variants for antioxidant enzymes involved in detoxification pathways. Some new studies also investigated the effect of long-term NO<sub>2</sub> air pollution on pulmonary function in adults; while most of these studies also found decrements in various lung function indices, the majority did not reach statistical significance. Overall, findings were generally not highly sensitive to study design, but uncertainty remains about whether the effects related to NO<sub>2</sub> are independent of other pollutants.

As illustrated in Figure 9.7 (subsection 9.3.1.3), positive and consistent associations were also observed between long-term exposure to ambient NO<sub>2</sub> and incidence of asthma in children, with significant associations being reported in most of the studies. Results of a recent meta-analysis which combined risk estimates from 17 cohorts also support the findings from individual studies that long-term NO<sub>2</sub> exposure is associated with asthma incidence. Results from an important European birth cohort study also suggested that early life exposure (exposure during infancy) is a critical period that can lead to long-term respiratory consequences: development of childhood asthma and related symptoms. There is also growing evidence of increased asthma incidence in adults in relation to long-term exposure to NO<sub>2</sub> in several recent European multi-city studies; higher risks were also observed for carriers of specific genes implicated in antioxidant defence pathways and increased inflammatory responses.

With respect to respiratory symptoms, in asthmatic children long-term exposure to ambient NO<sub>2</sub> was found to be significantly associated with increased bronchitis symptoms in two CHS studies (subsection 9.3.1.2; Figure 9.6). Several studies also found associations between long-term exposure to NO<sub>2</sub> and wheeze symptoms in children; while most of them did not reach the level of statistical significance, the risk estimates were consistently positive. In adults, weak or no associations have been found in a very small number of studies that investigated the impact of

long-term exposure to  $\text{NO}_x/\text{NO}_2$  on respiratory symptoms, though the prevalence of chronic bronchitis was significantly increased in one study. Evidence for increased respiratory symptoms in asthmatic children is also provided by several studies in indoor environments with indoor  $\text{NO}_2$  levels overlapping ambient levels in Canada (Chapter 11). Further stronger, experimental evidence is also provided by randomized intervention studies, in which levels of  $\text{NO}_2$  and related pollutants were reduced in Australian classrooms through replacement of unflued gas heaters and there was a corresponding improvement in respiratory symptoms in the short term, including in asthmatic children. The indoor studies may, however, be less relevant because the emissions from combustion sources in the indoor environment are somewhat different than from ambient sources.

Some recent epidemiological studies have investigated the relationship between long-term exposure to ambient  $\text{NO}_2$  levels and allergic conditions, including allergic rhinitis, eczema and allergic sensitization to inhalant allergens (subsection 9.3.1.5; Figure 9.8). All of these studies except one have been conducted in Europe, and most of them focused on children. Several of the studies have found positive associations with the various allergic responses, though most of them did not achieve statistical significance. Many of the studies reporting significant results found that specific characteristics of the cohort (e.g. children who had not moved residence, proximity to traffic and oxidative pathway genetics) appeared to confer increased risks for  $\text{NO}_2$ -related effects, possibly accounting for the heterogeneity in overall results.

The independence of the effects related to  $\text{NO}_2$  has not been widely established in long-term exposure studies, given the high collinearity among the various traffic-related air pollutants, and uncertainty still remains. However, robust  $\text{NO}_2$  associations, especially for lung function decrements and chronic bronchitis symptoms in children, have been observed in a number of studies (though not in all) following adjustment for some air pollutants including PM indices, EC and/or gases ( $\text{O}_3$  or  $\text{SO}_2$ ). Co-pollutant models adjusting for some other key traffic-related air pollutants such as CO or UFPs have not been performed. In asthmatic adults, a number of controlled human exposure studies (Section 7.2) have shown decreases in lung function as well as increases in AHR, a hallmark for asthma, in response to non-specific bronchial challenges. These support the biological plausibility of independent effects of  $\text{NO}_2$  on lung function and asthma-related outcomes, though the extent to which these very short-term effects are related to chronic outcomes is not known.

A more limited number of studies investigated the association between long-term exposure to ambient  $\text{NO}_2$  and hospitalizations or ERVs for chronic respiratory diseases (subsections 9.3.1.3 and 9.3.1.4). Risks of hospitalizations for asthma and COPD were increased among participants in the Danish Diet, Cancer and Health cohort study, while increased risks of hospitalization for COPD in residents of Vancouver, BC, were not related to long-term exposure to  $\text{NO}_2$ . In California, inconsistent results were found regarding the relationship between asthma hospitalizations and long-term exposure to  $\text{NO}_2/\text{NO}_x$ ; in Orange County an increased risk of repeated hospital encounters (hospitalizations and/or ERVs) for children with a primary asthma diagnosis was related to local traffic-related air pollutants, including  $\text{NO}_x$ , while in San Joaquin Valley no associations were found between  $\text{NO}_2$  and asthma-related ERVs and/or hospitalizations. Pneumonia hospitalizations in older patients were found to be significantly associated with long-term exposure to  $\text{NO}_2$  in a study in Hamilton, ON. Several other traffic-related air pollutants were found to be associated with these respiratory hospitalizations in some, but not all, of these studies.

Risk estimates for  $\text{NO}_2$ -related respiratory mortality, investigated in relatively few studies, were somewhat inconsistent and/or imprecise in North American studies, but long-term exposure to ambient  $\text{NO}_2$  was found to be associated with increased respiratory mortality in a small number of large European population-based epidemiological studies (subsection 9.2.2; Figure 9.2).

Since the publication of the 2008 US EPA ISA no new animal toxicological studies have examined the relationship between chronic exposure to NO<sub>2</sub> levels and respiratory effects. Findings from earlier experimental animal studies on respiratory effects observed following exposure to NO<sub>2</sub> provide support for the associations between NO<sub>2</sub> exposure and health effects in humans, particularly with respect to antioxidant defences/oxidative stress, respiratory function and morphology, and host defences (Section 6.1). Increased lipid peroxidation and alterations in the GSH antioxidant pathway were found following chronic exposure to NO<sub>2</sub>, suggesting that long-term exposure to NO<sub>2</sub> can modify the oxidant balance in the respiratory tract, leading to initiation of inflammatory responses. Morphological changes in the respiratory tract, particularly in the centriacinar region of the lung and in the bronchiolar epithelium, have been observed in several animal toxicological studies, while chronic exposure to high NO<sub>2</sub> levels can lead to emphysema or can result in permanent alterations resembling emphysema-like disease. Impaired host defence has been demonstrated in several studies in which increased mortality was observed in mice and monkeys following exposure to NO<sub>2</sub> combined with challenges with bacterial or viral infection. NO<sub>2</sub> exposure has been found to enhance Th2 immune responses, which can contribute to the development of asthma. Increased IgE-mediated mast cell histamine release was observed in guinea pigs, though not in mast cells from rats, following subchronic exposure to high concentrations of NO<sub>2</sub>, while short-term exposure to NO<sub>2</sub> increased the number of eosinophils in the tracheal mucosa of guinea pigs. Eosinophil activation was also observed in other rodent species, though not in all. Increased AHR was observed in guinea pigs following both short- or long-term exposure to NO<sub>2</sub>. Taken together, these toxicological results provide support for the above-mentioned associations between long-term exposure to NO<sub>2</sub> and the development of asthma and/or allergic responses in children.

In summary, consistent associations have been observed between long-term exposure to ambient NO<sub>2</sub> and several adverse respiratory effects, and there is some indication of independent effects in a small number of studies. The strongest evidence comes from epidemiological studies relating to asthma incidence and decreases in lung function and partially irreversible deficits in lung function growth in children. Based on a relatively small number of recent studies, long-term exposure to NO<sub>2</sub> levels also appears to increase the incidence of asthma in adults. The findings across disciplines provide a consistent and coherent suite of asthma-related respiratory outcomes associated with ambient NO<sub>2</sub>, including increased wheeze and chronic bronchitis symptoms and increased hospitalizations or ERVs for children with a primary asthma diagnosis. There is also support for NO<sub>2</sub>-related increases in risks for respiratory symptoms from studies of asthmatic children exposed to NO<sub>2</sub> in indoor environments, and for decreases in symptoms shortly after reductions in exposure to NO<sub>2</sub> and related pollutants emitted by gas heaters in two well-designed intervention studies. Several cohort studies have also shown relationships between long-term exposure to NO<sub>2</sub> and the development of allergic responses in children. Furthermore, biological plausibility for the development of asthma or allergic-related disease is provided by the results of epidemiological and experimental studies in which NO<sub>2</sub> was able to induce pulmonary inflammation, AHR and immune responses in animals and/or humans. However, the dataset for biological plausibility is limited, the modes of action are not fully characterized, and some uncertainty still remains about the possible role of other co-occurring traffic-related air pollutants in the NO<sub>2</sub>-related respiratory effects. Thus, based on evidence from several lines of enquiry exhibiting consistency and strength of association, coherence across disciplines as well as some indication of robustness and biological plausibility, there is a basis for concluding that there **is likely a causal relationship** between long-term exposures to ambient NO<sub>2</sub>/NO<sub>x</sub> at current levels and respiratory effects.

### 12.2.3.2 Cardiovascular Effects

The epidemiological database reviewed in the current assessment includes significant new studies investigating the relationship between long-term exposures to NO<sub>2</sub> and cardiovascular effects, as well as updates of previously studied cohorts. Cardiovascular mortality has been better characterized than morbidity in the reviewed literature, and more specific cardiovascular outcomes have also been examined.

As illustrated in Figure 9.3 (Section 9.2), virtually all positive, and for the most part significant, associations have been observed between long-term exposure to ambient NO<sub>2</sub> and mortality due to all CVDs combined. Strong NO<sub>2</sub>-related cardiovascular mortality risks have also been observed in different cohorts from Canada where people were exposed to relatively low levels of NO<sub>2</sub>. Significant associations were also reported in several European cohorts where new published studies confirmed earlier findings. More accurate exposure estimates may have been obtained, since all the Canadian and most European studies used more spatially resolved exposure estimates of outdoor residential levels of NO<sub>2</sub>. Relatively few studies examined more specific causes of cardiovascular mortality, but significant associations were also noted in some studies for mortality due to CHD and IHD. The latter was included in the extended phase III reanalysis of the ACS cohort, which found strong associations between NO<sub>2</sub> concentrations at central monitoring stations and IHD mortality. No associations have been found between long-term exposure to NO<sub>2</sub> and cerebrovascular mortality, which has, however, been studied less frequently. Potential confounding by other co-occurring pollutants was not considered in most of these studies; further, the associations between NO<sub>2</sub> and cardiovascular-related mortality remained robust to co-pollutant adjustment in some of these studies, while in others the effects were attenuated and no longer significant.

The associations between long-term exposures to ambient concentrations of NO<sub>2</sub> and medical visits for cardiovascular causes have been investigated in a smaller number of studies than mortality (subsection 9.3.2.1). Most researchers studied the relationship of traffic air pollutants to stroke incidence or hospitalization, and associations were generally absent or non-significantly positive, except for a significant NO<sub>x</sub>-related increase in ischemic stroke admissions in a study of Swedish diabetic subjects. Positive but still mostly non-significant associations were also observed for NO<sub>2</sub>-related hospital admissions due to other cardiovascular conditions, including heart failure, CHD and MI. In some of these studies, long-term exposure to PM was also reported to be associated with hospital admissions due to cardiovascular events, and none of these studies conducted co-pollutant analyses. Overall, this dataset provides only weak support for the mortality findings.

A very limited number of epidemiological studies also investigated the relationship between long-term exposure to ambient NO<sub>2</sub> and various markers of CVD risk, and inconsistent results have been found (subsection 9.3.2.2). Significant NO<sub>2</sub>-related increases in chronic inflammatory biomarkers have been observed but not in all studies, and other ambient air pollutants, including PM, have also been shown to be associated with various different inflammatory markers. Long-term exposures to traffic-NO<sub>2</sub> levels were also related to increased arterial stiffness independently of other air pollutants in one study. Significant decrements in blood pressure were observed in women, but not in men, while stronger decrements in HRV parameters were also observed in women with self-reported cardiovascular problems in an individual study. Changes in markers of oxidative stress and inflammation have also been observed in animal studies following short-term or long-term exposures to NO<sub>2</sub> (Section 6.2). Chronic exposures of laboratory animals to NO<sub>2</sub> decreased heart rate and induced dyslipidemia, i.e. an abnormal level of lipids in the blood, which is known to be a risk factor for IHD; these effects were, however, only reported in one study. Overall, these findings provide some indication of the means by



which NO<sub>2</sub> may affect CVD, but the biological plausibility of long-term exposure to NO<sub>2</sub> leading to cardiovascular effects has not been well demonstrated.

In summary, fairly consistent associations have been observed between cardiovascular mortality and long-term exposure to ambient NO<sub>2</sub>, and there is some indication of independent effects in a relatively small number of studies. However, the independent relation of NO<sub>2</sub> to either mortality or morbidity has not been widely characterized in these epidemiological studies, given the high collinearity among the various air pollutants and the lack of attempts to examine this issue, and uncertainty remains with respect to possible confounding by co-occurring traffic-related pollutants. The evidence for cardiovascular medical visits lacks strength of association and is not very coherent with the cardiovascular mortality findings. While results from a very limited number of experimental studies indicate that both acute and long-term exposure to ambient NO<sub>2</sub> levels can affect the cardiovascular system, the dataset for biological plausibility is very limited and not consistent, and the chain of events that may lead to cardiovascular mortality or morbidity has not been well characterized. Overall, the evidence is **suggestive, but not sufficient to infer a causal relationship** between long-term exposure to ambient NO<sub>2</sub> and cardiovascular effects.

#### 12.2.3.3 Cancer and Related Effects

A number of earlier and more recent epidemiological studies have examined the association between long-term exposure to ambient NO<sub>2</sub> and cancer or related effects.

The results of a number of large longitudinal epidemiological studies that used different lengths of exposure duration and controlled for several potential confounding factors (including smoking status) have provided some evidence of an association between long-term exposure to NO<sub>2</sub> and lung cancer mortality (Section 9.2; Figure 9.4). Positive associations have been observed between lung cancer mortality and long-term exposures to NO<sub>2</sub>/NO<sub>x</sub> levels in some North American and European studies, but most of them did not reach statistical significance.

A significant association was observed with exposure to NO<sub>x</sub> in a cohort of Norwegian men that might reflect the traffic-related air pollution mixture including PM that was not considered in the analyses. A few significant associations were also observed among women of both the California AHSMOG and Oslo cohorts. These results are, however, not consistent with the findings from the CTS and the German Women's Health Study cohort, where either null or weak associations were found. In addition, there was no indication of a positive association in other high-quality epidemiological studies, such as the extended ACS and NLCS cohorts. In several of these studies, NO<sub>2</sub> was highly correlated with PM<sub>2.5</sub>, which was itself related to death from lung cancer, thus making it difficult to differentiate the impacts of specific pollutants. Virtually all studies only performed single-pollutant models, except for the California AHSMOG study, where the association between NO<sub>2</sub> and lung cancer mortality in the female group disappeared following adjustment for SO<sub>2</sub>.

A more limited number of epidemiological studies evaluated the relationship between long-term exposure to NO<sub>x</sub>/NO<sub>2</sub> and cancer incidence, mostly for lung cancer (subsection 9.3.3). Mainly positive associations between long-term exposures to NO<sub>2</sub> and lung cancer incidence were found, but most of these were non-significant. In some of these cohorts, stronger and significant associations were, however, observed for residences located in proximity to heavy-traffic roads and/or with exposure to higher concentrations of NO<sub>2</sub>. In a Danish cohort, a stronger association was observed among women than men, which provides support to some of the mortality findings.

Positive associations between long-term exposure to ambient NO<sub>2</sub>/NO<sub>x</sub> levels and cancer incidence at other tumour sites have also been observed, including for acute leukemia in children, post-menopausal breast cancer, and cancers of the prostate, cervix and brain

(subsection 9.3.3). However, these associations have only been reported in single epidemiological studies, and there is no specific evidence from animal studies to support the biological plausibility of a link with long-term exposure to NO<sub>2</sub>/NO<sub>x</sub>.

Based on a limited number of experimental studies there is no clear evidence that NO<sub>2</sub> can directly act as a carcinogen (Section 6.3). The few *in vivo* genotoxicity studies have generally been negative, though there are isolated positive findings in lung cells in rats and mice exposed to higher-than-ambient concentrations, and some recent experimental studies have shown the ability of low concentrations of NO<sub>2</sub> to induce genotoxic and mutagenic effects in some *in vitro* models, supporting earlier findings. In animal toxicological studies, there was a small but significant increase in pulmonary tumours following exposure to higher-than-ambient concentrations of NO<sub>2</sub> in a single study in a mouse strain with spontaneously high tumour rates. However, NO<sub>2</sub> at relatively high levels appeared to have co-carcinogenic effects when combined with other inhaled oxidants, supporting the hypothesis that NO<sub>2</sub> may potentially act as a tumour promoter rather than as a complete carcinogen. Another critical effect of NO<sub>2</sub> exposure, under certain conditions, may be to increase dissemination and/or proliferation of tumour metastases. Some toxicological studies also demonstrated a potential relationship between exposure to very high levels of NO<sub>2</sub> and the formation of nitrosamine, a known carcinogenic compound. Overall, these findings provide some indications of the means by which NO<sub>2</sub> may induce cancer-related effects, but the specific mechanisms are currently unclear.

In conclusion, the overall database remains fairly small and the associations between long-term exposure to ambient NO<sub>2</sub> and both lung cancer incidence and mortality are generally positive and statistically significant in some, though not all, epidemiological studies. Hence, while there is some limited indication of consistency and coherence for NO<sub>2</sub>-related increases in pulmonary tumours in the epidemiological and toxicological databases, there is little strength of association. As noted above, there is also considerable uncertainty in isolating the potential effects of NO<sub>2</sub> from those of other substances in the complex mixture of outdoor air pollutants. Furthermore, the dataset for biological plausibility is limited and the modes of actions are not well characterized. Therefore, the evidence from epidemiological and toxicological studies is very weak and is **suggestive, but not sufficient to infer a causal relationship** between long-term exposure to ambient NO<sub>x</sub>/NO<sub>2</sub> and cancer.

#### 12.2.3.4 Total Mortality

The epidemiological database on mortality from long-term exposure to NO<sub>2</sub>/NO<sub>x</sub> air pollution has been greatly enhanced over the past few years with new cohorts, as well as with reanalysis or updates of existing cohorts. In general, inconsistent associations were observed between long-term exposure to ambient NO<sub>2</sub> and mortality among large US cohorts (Section 9.2; Figure 9.1). No association was found in several reanalyses of the ACS cohort, including the extended phase III reanalysis, and weak or no associations were reported in both the California AHSMOG and the Washington University–EPRI veterans' study cohorts. By contrast, positive and significant associations were observed in other well-designed epidemiological studies, such as the Harvard Six Cities Study cohort and a US cohort of men working in the trucking industry. Most of the new cohort studies have been performed in Europe, where consistent positive and significant associations between long-term exposure to ambient NO<sub>2</sub> and all-cause mortality were found in a variety of countries (Section 9.2; Figure 9.1). The European studies may have provided more accurate exposure estimates than the community-level air pollution estimates typically used in the US studies. Positive associations were also observed with both respiratory and cardiovascular mortality in several epidemiological studies (Section 9.2; Figures 9.2 and 9.3); fairly consistent associations have been observed with cardiovascular mortality, while risk estimates for respiratory mortality were usually less precise and mostly non-significant. Positive associations have also been observed between lung cancer mortality and long-term exposures

to NO<sub>2</sub>/NO<sub>x</sub> levels in some North American and European cohort studies, but most of them did not reach statistical significance (Section 9.2; Figure 9.4).

Most of these epidemiological studies of mortality only conducted single-pollutant models, due to the collinearity among the measured pollutants, especially between NO<sub>2</sub> and PM<sub>2.5</sub>. In the small number of studies where co-pollutant analyses were performed, results were somewhat inconsistent, with the effects of NO<sub>2</sub> remaining robust in some cases and not in others. Some studies have reported significant associations between long-term exposure to NO<sub>2</sub> and mortality following the inclusion of some traffic variables; i.e. the risk for NO<sub>2</sub> itself remained robust, supporting the hypothesis that NO<sub>2</sub> not only may serve as an indicator of traffic but is also independently associated with some health outcomes. In other studies, traffic-related indicators were found to be more strongly associated with mortality endpoints. It should be noted that PM also continues to be one of the dominant factors explaining the long-term effects of air pollution on premature mortality in several of these studies.

The associations between long-term exposure to ambient NO<sub>2</sub> and medical visits for respiratory or cardiovascular conditions that are linked to mortality (subsections 9.3.1 and 9.3.2, respectively) have been investigated in a more limited number of studies. Hospitalizations for COPD in relation to NO<sub>2</sub> concentrations were observed among participants in the Danish Diet, Cancer and Health cohort study, while increased risks of hospitalization due to COPD in residents of Vancouver, BC, were not related to long-term exposure to NO<sub>2</sub>. In Hamilton, ON, pneumonia hospitalizations in older patients were also found to be significantly associated with long-term exposure to NO<sub>2</sub>. Several other traffic-related air pollutants, including BC and PM<sub>2.5</sub>, were found to be associated with these respiratory hospitalizations in some, but not all, of these studies. Positive but mostly non-significant associations were observed between long-term exposures to ambient concentrations of NO<sub>2</sub>/NO<sub>x</sub> and hospital admissions for cardiovascular conditions, including stroke, heart failure, CHDs and MI, as well as with measures of cardiovascular markers.

The effect of NO<sub>2</sub> exposure on resistance to infectious agents has been examined in a small number of experimental studies (subsection 6.1.1). Increased mortality due to infectious agents was observed in some, but not all, animal studies following chronic exposure to high NO<sub>2</sub> concentrations, which is suggestive in light of the increase in pneumonia hospitalizations in the above-mentioned study in Hamilton.

In summary, generally only weak and inconsistent associations have been observed between long-term exposure to ambient NO<sub>2</sub> and all-cause mortality among adults in large American cohorts of multiple US cities. Fairly consistent associations have, however, been found in Europe, where more accurate NO<sub>2</sub> exposure levels may have been estimated. Uncertainty still remains with respect to the role of NO<sub>2</sub> as compared to the other air pollutants in the traffic air pollution mixture. Given the multicollinearity between air pollutants, results from co-pollutant models are difficult to interpret, and no studies adjusted for key traffic-related air pollutants such as EC or UFPs. While there is some indication of coherence and biological plausibility among the various lines of evidence, few studies have examined the spectrum of morbidity effects associated with long-term exposure to ambient concentrations of NO<sub>2</sub> that could lead to mortality. Some support is provided by the evidence of short-term effects on both the respiratory and cardiovascular systems (subsections 8.3.1 and 8.3.2, respectively) but the specific biological pathways by which long-term exposure to ambient NO<sub>2</sub> may lead to mortality are not well characterized. Overall, the evidence of an increased risk for all-cause mortality associated with long-term exposure to ambient NO<sub>2</sub>/NO<sub>x</sub> is **suggestive, but not sufficient to infer a causal relationship**.

### 12.2.3.5 Reproductive and Developmental Effects

The body of evidence relating long-term exposure to ambient NO<sub>2</sub> and reproductive and developmental effects has grown considerably over the past few years as a result of several new studies becoming available. The recent literature included investigations of several endpoints, including LBW (<2500 g), SGA/IUGR (birth weight <10<sup>th</sup> percentile for gestational age), VSGA (birth weight <5<sup>th</sup> percentile for gestational age), preterm births (<37 weeks gestation), preeclampsia (hypertension during pregnancy) as well as infant mortality. A smaller number of studies of birth defects, other reproductive endpoints, and neonatal effects were also identified.

Several epidemiological studies using various exposure assignment methods found significant associations between NO<sub>2</sub> exposures and fetal growth. As shown in Figure 10.1, relatively strong and consistent North American evidence is accumulating for effects on endpoints that include LBW, SGA/IUGR, and VSGA. While more variable, associations between NO<sub>2</sub> exposures and decreases in birth weight were also observed in several European and Australian publications, though some found little to virtually no association. In a limited number of studies, some effects of NO<sub>2</sub> exposure on ultrasound measurements of fetal growth taken at different periods of pregnancy, such as head circumference, BPD and birth length, were also found. Results from a meta-analysis of more than 60 cohort, case-control and ecological studies also demonstrated significant associations between reduced birth weight and several air pollutants including NO<sub>2</sub>, though risk estimates were highly heterogeneous. In the majority of the North American studies other co-occurring pollutants demonstrated equal or even stronger associations, but associations with NO<sub>2</sub> remained robust to adjustment for co-pollutants in several studies. Some studies found that the associations between NO<sub>2</sub> and birth outcomes appeared to be stronger in the third trimester than in other periods of pregnancy, though there were also significant findings during the entire pregnancy period or in other trimesters. Variation in effects observed by exposure period as well as sources of heterogeneity between studies is an important uncertainty in this dataset and should be further explored.

Evidence of an association, usually statistically significant, was found between NO<sub>2</sub>/NO<sub>x</sub> exposures and preterm birth in a number of earlier and more recent epidemiological studies. In most of these studies, significant associations were noted with other air pollutants, especially PM and CO. As was the case for LBW, the period of susceptibility remains unclear, with some evidence accumulating for whole pregnancy or late gestational exposure to NO<sub>2</sub>. In the meta-analysis discussed above, a positive but non-significant association was found between incidence of preterm birth and exposure to NO<sub>2</sub>, while other pollutants were more strongly associated with this outcome.

The epidemiological evidence remains very limited and inconsistent in terms of NO<sub>2</sub>-related risks for preeclampsia, congenital anomalies, neonatal respiratory and neurodevelopmental effects, and a variety of other reproductive and developmental effects, including SIDS, stillbirth and sperm quality (subsection 9.3.4.4 and Sections 10.4, 10.5, and 10.6). Evidence is building for relationships between several of these outcomes and air pollution in general, though to date it does not reveal consistent associations with NO<sub>2</sub> or other specific pollutants.

A very limited number of epidemiological studies investigated the relationship of ambient NO<sub>2</sub> exposures with inflammatory markers in biological fluids, and inconsistent results have been found (subsection 10.6.3). No association was found between NO<sub>2</sub> exposure and serum CRP levels (a marker of systemic inflammation) in some new prospective cohort studies, while significant associations were observed between human umbilical cord blood CRP levels and higher quartiles of NO<sub>2</sub> exposure in a single study. In another prospective cohort study, NO<sub>2</sub> exposure in the third trimester was found to be associated with a decrease in counts of lymphocyte cells, a component of the innate immune system, in fetal cord blood. As for some

other outcomes, these studies tend to identify more than one pollutant as being associated with the effect, and at this time, there is too limited a dataset to draw conclusions.

Toxicological studies employing rodent models have shown that exposure to NO<sub>2</sub> at environmentally relevant concentrations can adversely affect pre- and postnatal development (subsection 10.6.3). Prenatal exposures to relatively low concentrations of NO<sub>2</sub> reduced the weights of rat fetuses and progeny. Impairments in postnatal development were also noted in some studies; neurobehavioural effects, such as retarded neuromotor development or disturbances in motor coordination, were observed in rats following *in utero* NO<sub>2</sub> exposure, while postnatal exposures to low concentrations of NO<sub>2</sub> adversely affected the respiratory system of neonates. Acute NO<sub>2</sub> exposure did not alter spermatogenesis, germinal cells or interstitial cells in a single rat toxicological study. However, chronic exposure to high concentrations of NO<sub>2</sub> alone or in combination with SO<sub>2</sub> was found to disturb the estrual cycle in female albino rats, though the number of dams that became pregnant after mating with an unexposed male was not affected.

In summary, relatively consistent significant associations were observed between prenatal exposure to ambient NO<sub>2</sub> and measures of fetal growth and birth weight in Canadian and American studies, with somewhat more variable findings in European and Australian studies. Evidence of an association between NO<sub>2</sub>/NO<sub>x</sub> exposures and preterm birth was also noted in several North American studies, while international studies revealed still more variable relationships. There are additional uncertainties in this dataset: findings were not always associated with a particular exposure period, the associations with NO<sub>2</sub> were not always robust to adjustment for other pollutants, and other pollutants sometimes demonstrated similar or more robust relationships. Inconsistent associations have also been reported between exposure to NO<sub>2</sub> and several other developmental and reproductive effects in the small number of available studies. Several mechanisms underlying NO<sub>2</sub>-related effects on reproductive and developmental outcomes have been proposed, including systemic inflammation, impaired immune function, and vascular dysfunction. The dataset pertaining to biological plausibility is, however, very limited (though NO<sub>2</sub> reduced fetal, birth and neonatal weights in rodents in some studies), and the chain of events that may lead to reproductive and developmental effects is largely unknown. Overall, the evidence is considered **suggestive, but not sufficient to infer a causal relationship** between long-term exposure to ambient NO<sub>2</sub>/NO<sub>x</sub> and reproductive and developmental effects, particularly for fetal growth and birth weight.

## 12.3 Subgroups with Increased Sensitivity or Exposure to Ambient NO<sub>2</sub>

*What population groups appear to be at increased risk of the health effects of NO<sub>2</sub>?*

Available evidence indicates that a variety of factors can affect individuals' responses to ambient NO<sub>2</sub>. Some of these are innate factors that may affect the sensitivity of individuals to exposure to NO<sub>2</sub>, such as certain pre-existing diseases. Other factors may render individuals more vulnerable to the effects of NO<sub>2</sub> by increasing their exposure; for example, spending considerable time in microenvironments with elevated levels of ambient NO<sub>2</sub>. In this section, the evidence that there are subgroups with increased sensitivity or exposure to ambient NO<sub>2</sub> is



summarized, principally for those categories of health effects for which the weight of evidence is relatively strong: respiratory effects, and mortality associated with short-term ambient NO<sub>2</sub>.

Individuals with pre-existing respiratory diseases may be sensitive to the effects of NO<sub>2</sub> on the respiratory system, and several lines of evidence indicate that asthmatics are a susceptible subgroup. In a number of controlled human exposure studies, short-term exposure of asthmatic adults to NO<sub>2</sub> decreased lung function, potentiated allergen-induced airway responsiveness and inflammation, and increased AHR following non-specific bronchial challenge at concentrations at least 3 times less than those that affected healthy adults (Section 7.2). In numerous epidemiology studies, short-term exposure to ambient NO<sub>2</sub> was consistently and independently associated with asthma-related hospitalizations and ERVs (subsection 8.3.1), as well as with respiratory symptoms and medication use (subsection 8.4.1.4). Although few of these studies stratified analyses separately for asthmatics and non-asthmatics, in panel studies of adolescents from New York schools the risks for wheeze and for chest tightness were increased in asthmatics but not in non-asthmatics, whereas inflammatory biomarkers did not differ between these groups (subsections 8.4.1.3 and 8.4.1.4). In studies of long-term effects, ambient NO<sub>2</sub>-related risks were higher in asthmatics than in non-asthmatics for lung function decrements in German schoolchildren (subsection 9.3.1.1), for hospital admissions for asthma and COPD in a Danish study (subsection 9.3.1.3), and for stroke mortality in a Danish cohort (Section 9.2).

COPD patients also appear to be more sensitive to NO<sub>2</sub> than the population at large, though the evidence base is smaller than that for asthmatics. In two controlled human exposure studies, short-term exposure of adults with COPD to NO<sub>2</sub> decreased lung function at levels about 3-fold lower than those affecting healthy adults in other studies (Section 7.2). Compared with the general population, after short-term ambient NO<sub>2</sub> exposure patients with COPD also had increased risks for respiratory mortality in Rome (subsection 8.2.2) and for COPD-related ERVs among adults aged >65 in Helsinki (subsection 8.3.1.3). This was also the case for asthma-related hospitalizations in relation to long-term ambient NO<sub>2</sub> levels among participants in the Danish Diet, Cancer and Health cohort study (subsection 9.3.1.3).

Age is also clearly related to susceptibility. Physiologically, children may be more at risk from ambient NO<sub>2</sub> because their lungs and immune systems are still maturing, and they generally are more exposed because they spend more time outdoors and are more active than other age groups. In addition, asthma is more prevalent in children than in adults. Very few controlled human exposure studies included children, but in these studies their lung function decrements and AHR responses to histamine were similar to those in adults (Section 7.2). However, stratified analyses in numerous epidemiology studies showed that (compared with other age groups) children had increased short-term NO<sub>2</sub>-related risks of hospital admissions for asthma and for all respiratory causes combined, as well as for ERVs for asthma (subsection 8.3.1). In Canadian and Australian studies in which a detailed age breakdown was examined, the risk for asthma ERVs was greatest in very young children and was increased to a lesser extent in older children. Similarly, risks in relation to long-term exposure to ambient NO<sub>2</sub> were most pronounced in very young children in epidemiological studies of asthma-related outcomes in Orange County, CA, and in Munich, Germany (subsection 9.3.1.3); they also showed higher risks for development of allergic sensitization to pollen in a Swedish population-based birth cohort (subsection 9.3.1.5).

Older adults also appear to be more susceptible to a number of the effects from exposure to ambient NO<sub>2</sub>, probably at least in part because this is the age group in which many of the diseases that predispose to NO<sub>2</sub>-related effects are most common. Epidemiological studies of short-term NO<sub>2</sub>-related mortality have consistently revealed that risks for all-cause (and in some studies respiratory) mortality were greater in older adults than in all-age analyses (Section 8.2). In a number of studies of hospital admissions, short-term exposure to ambient NO<sub>2</sub> was again

associated with increased risks of hospitalization for respiratory causes in older adults compared with younger people (Section 8.3). Older adults were also at greater risk in relation to short-term ambient NO<sub>2</sub> in single studies of asthma ERVs in Edmonton, AB, of COPD ERVs in Helsinki, Finland, and of doctors' house calls for URD and LRD in France (Section 8.3), as well as for decrements in lung function associated with long-term exposure in England (subsection 9.3.1.1). Although the weight of evidence for cardiovascular effects is considered weaker than that for respiratory effects (subsections 12.2.2.2 and 12.2.3.2), it is noted that older adults also had increased risks for adverse cardiovascular outcomes in epidemiological studies. These included associations between short-term ambient NO<sub>2</sub> and cardiovascular mortality in studies from several countries (Section 8.2) and hospitalizations for CVD, cardiac disease and IHD in two European studies (Section 8.3), as well as for stroke mortality in relation to long-term ambient NO<sub>2</sub> in a Danish cohort (Section 9.2). It should be noted that the absolute effect of ambient NO<sub>2</sub> would be substantially greater than the increased relative risk estimates would indicate, due to the increasing proportion of older adults in the Canadian population and the higher underlying rates of cardiovascular mortality and morbidity in this age group.

There is little evidence that gender influences the risk of short-term effects of ambient NO<sub>2</sub>, though there is some indication that females may be more sensitive than males to long-term exposure. In a small number of epidemiological studies, the risk of asthma hospitalizations or ERVs in relation to short-term ambient NO<sub>2</sub> was sometimes increased in males, sometimes in females, or was not significantly modified by gender (Section 8.3). By contrast, long-term ambient NO<sub>2</sub>/NO<sub>x</sub> was fairly consistently associated with higher risks in females than in males for a wide range of health effects, including decreased lung function in a children's cohort in Rome, and increases in persistent wheeze in a Swedish birth cohort, in the prevalence of asthma in a study in Hamilton, ON, and in the risk of repeated hospital encounters for asthma in Orange County, CA (subsection 9.3.1). Females also appeared to be more sensitive to long-term NO<sub>2</sub>-related decreases in HRV and blood pressure, increases in vascular damage, and risks for diabetes and for lung cancer (subsections 9.3.2, 9.3.3 and 9.3.4, respectively). However, each of these endpoints was examined in relation to gender in only one or two studies; consequently there is only very limited evidence that gender modulates NO<sub>2</sub>-related effects.

There is emerging evidence that genetics may play a role in inter-individual differences in sensitivity to ambient NO<sub>2</sub> exposure, especially for long-term effects. In several epidemiological studies, polymorphisms of various genes involved in the oxidative stress defence pathways modulated the risks of various effects in cohorts of children in relation to long-term ambient NO<sub>2</sub>, including decrements in lung function, incident asthma, and allergic sensitization to common allergens (subsection 9.3.1). Similar polymorphisms in genes related to oxidative stress or inflammation modified the risks of new-onset and prevalent asthma in adults in a multi-city European study (subsection 9.3.1.3). With respect to acute effects, in individual studies genetic polymorphisms did not significantly affect the relationship of short-term ambient NO<sub>2</sub> with ventricular repolarization in older men from Boston, MA, or with IL-6, a pro-inflammatory biomarker, in MI survivors in six European cities (subsection 8.4.2).

Subpopulations can also be more at risk of the effects associated with ambient NO<sub>2</sub> if they receive greater exposures than the population at large. As noted in Chapters 3 and 4, on-road vehicle emissions are an important source of ambient NO<sub>2</sub>; hence concentrations of NO<sub>2</sub> are increased in on-road, near-road and in-vehicle microenvironments, especially on major roadways. People who spend substantial amounts of time in such locations can have elevated exposures to NO<sub>2</sub>. These would include people who spend a long time in vehicles commuting or in the course of their work, who work or commute in proximity to major roadways or who reside, work, attend school, etc. in buildings near such roadways. In the epidemiology studies reviewed

for this assessment, measures of exposure to traffic (living close to major roads, traffic density or traffic intensity) were associated with increased NO<sub>2</sub>-related risks for a range of health outcomes: all-cause and CP mortality, development/prevalence of asthma, and lung cancer incidence (subsections 9.2.2, 9.3.1.3, and 9.3.3, respectively). People engaged in vigorous physical activity would also have increased inhalation exposure to NO<sub>2</sub> by virtue of their increased minute volume and oral breathing

There is evidence from epidemiology studies that social factors can also modify effects associated with ambient NO<sub>2</sub>. Measures of lower SES, including lower incomes and less education, increased short-term NO<sub>2</sub>-related risks for respiratory hospitalizations in 10 Canadian cities (Section 8.3). Similarly, in less-educated subpopulations risks associated with long-term ambient NO<sub>2</sub> were increased for stroke mortality in a Danish cohort (subsection 9.2.2) and for measures of vascular damage in a study of young Dutch adults (subsection 9.3.2). However, in other single epidemiological studies, high SES or education was associated with greater NO<sub>2</sub>-related effects on lung function in Roman children (subsection 9.3.1.1) and on lung cancer incidence in the Danish Diet, Cancer and Health cohort study (subsection 9.3.3). Risks associated with NO<sub>2</sub> were also increased for asthma incidence in children exposed to elevated levels of violence in a pregnancy cohort from East Boston, MA, and for pro-inflammatory mediators, eosinophil counts, respiratory symptoms and PEFR in asthmatic children with higher stress levels in a small study in Vancouver, BC (subsection 9.3.1.3).

In summary, the available evidence indicates that certain subgroups of the population are at increased risk for health effects related to ambient NO<sub>2</sub>. Individuals with certain pre-existing diseases are more sensitive: particularly asthmatics and perhaps people with COPD. Age is also clearly related to susceptibility; in epidemiology studies, children (and especially asthmatics) were more at risk of respiratory effects associated with both short- and long-term exposure to NO<sub>2</sub>, while older adults had increased risks for respiratory and cardiovascular effects with short-term NO<sub>2</sub> exposure. People with increased exposures because they spend substantial time in microenvironments with elevated levels of ambient NO<sub>2</sub>, such as in proximity to major roadways, or because they are engaged in vigorous physical activity, may also be at greater risk of NO<sub>2</sub>-related health effects.

## 12.4 Exposure and Concentration–Response Aspects for Ambient NO<sub>2</sub>

### 12.4.1 Ambient Sources, Levels and Exposures

*What are the current ambient sources and levels of NO<sub>2</sub> in Canada?  
What is the spatial and temporal distribution of ambient NO<sub>2</sub> in  
Canada? What is the relationship between NO<sub>2</sub> levels measured at  
stationary monitoring sites and personal exposure to NO<sub>2</sub>?*

Emissions of NO<sub>x</sub> arise primarily from combustion sources. Based on the NPRI, in 2011 the major ambient releases of NO<sub>x</sub> in Canada were from mobile sources (50% of total emissions), predominantly off-road and on-road diesel engines (Section 3.2). Substantial amounts of NO<sub>x</sub> were also emitted from industrial sources (30%), the majority from the upstream petroleum

sector. Lesser quantities were released by the non-industrial category (12%), mostly from combustion-generated electrical power, and by natural sources (7%).

As noted in Section 3.2, NO<sub>x</sub> is made up of NO and NO<sub>2</sub>. Most of the epidemiological information relates to NO<sub>2</sub>, which is the focus of this assessment. The entire population is exposed to ambient NO<sub>2</sub>, both outdoors and in indoor environments into which ambient NO<sub>2</sub> has infiltrated. Information on ambient concentrations of NO<sub>2</sub> in Canada is provided primarily by the NAPS network of monitoring stations. As described in Section 3.3, ambient NO<sub>2</sub> levels display marked variations in space and time on several scales, some of which highlight the important contribution of traffic emissions to exposure to ambient NO<sub>2</sub>.

With respect to spatial variability, the daily 1-h max, 24-h avg and annual average concentrations measured at NAPS stations vary by site type, with the highest levels occurring at transportation- and industry-influenced sites (including point source sites) (Section 3.3; Table 12.2). Concentrations for other site types generally decrease as population size decreases, moving from large urban through medium urban, small urban and non urban monitoring stations in turn (Table 12.2), likely as a result of the parallel reduction in emissions from traffic, residential heating, and other population-related sources. There is also large variability in ambient NO<sub>2</sub> as a function of distance from roads; large gradients in NO<sub>2</sub> concentrations near roadways have been observed in several studies, and concentrations have been found to be strongly correlated (or inversely correlated) with distance from the roadway, traffic volume, road length, etc. LUR models show that, at the urban scale, ambient NO<sub>2</sub> can often be reliably predicted by roadway proximity and other traffic characteristics (Section 4.6).

**Table 12.2: Ranges of mean and maximum measured ambient NO<sub>2</sub> concentrations by NAPS site type (2009–2011)**

Site type*	Daily 1-h max (ppb)		24-h avg (ppb)		Long-term avg (ppb)
	Mean	Max	Mean	Max	
<b>T</b>	23–40	60–109	11–22	33–68	11–22
<b>S</b>	9–35	98–230	4–19	40–56	4–19
<b>LU</b>	5–30	27–109	2–17	14–63	2–17
<b>MU</b>	7–23	37–80	2–12	15–44	2–12
<b>SU</b>	3–23	17–73	1–11	5–47	1–11
<b>NU</b>	2–21	27–88	1–8	6–33	1–8

\*T = transportation-influenced, S = potentially industrial source-influenced, LU = large urban, MU = medium urban, SU = small urban, NU = non urban. Detailed definitions appear in subsection 3.3.1.

With respect to long-term temporal trends, both 1-h max and annual average ambient concentrations of NO<sub>2</sub> at various types of NAPS sites decreased steadily between 1997 and 2011 (Section 3.4), attributable to reductions in emissions from on-road vehicles and electric power generation. All site types also exhibited a common seasonal pattern, with wintertime maxima and summertime minima, the low summer levels being consistent with increased mixing heights and photochemical oxidation of NO<sub>2</sub> and decreased emissions from residential heating compared with winter. Concentrations of ambient NO<sub>2</sub> also varied throughout the day, with two peak concentrations corresponding to morning and afternoon/evening rush hours. Weekend concentrations were generally lower than those measured on weekdays and the diurnal peaks were shorter on weekends, likely as a combined result of reduced traffic (especially diesel truck traffic) and the lack of rush hour traffic on weekends.

As presented in Chapter 4, the entire population is exposed to NO<sub>2</sub> originating from ambient sources, both when people are outdoors and when they are in indoor environments into which ambient NO<sub>2</sub> has infiltrated. As they go through their day, some people also spend time in locations that have higher NO<sub>2</sub> concentrations as a result of releases from non-ambient sources (e.g., indoors in homes with gas stoves).

This assessment is being conducted to support the development of an ambient standard for NO<sub>2</sub>, and is based in large part on the extensive epidemiological evidence linking ambient concentrations of NO<sub>2</sub> to a wide range of health effects (Chapters 8, 9, 10). In this context, a key issue is the ability of NO<sub>2</sub> concentrations measured by the monitoring network to serve as an indicator of personal exposure to NO<sub>2</sub> of *ambient origin*, as opposed to the total personal exposure to NO<sub>2</sub> from all sources that is measured in most exposure assessment studies.

Studies of the relationship between personal exposures to NO<sub>2</sub> and concentrations measured by ambient monitoring networks have generally shown positive and often statistically significant correlations or regressions between short-term ambient concentrations and total personal exposures. Usually the ambient component of personal exposure to air pollutants is not directly measurable, but the total personal exposure can be regarded as the personal exposure of ambient origin if there are no indoor sources. In those studies where indoor sources of NO<sub>2</sub> were absent, the correlation of personal exposure with ambient concentration was moderate to strong, and was increased 2- to 3-fold compared with that observed in the presence of indoor sources. In addition, the association between total personal exposure and ambient NO<sub>2</sub> was greater in the warm season (when people are generally more exposed to ambient air pollutants because building infiltration and time spent outdoors are greater) in a number of studies. These findings were confirmed in a recent meta-analysis of a large number of exposure assessment studies.

Overall, the results of these studies indicate that, although the concentrations measured by the ambient monitoring network may not account for differences between individuals in exposure to NO<sub>2</sub> of ambient origin, they appear to be a reasonable surrogate for exposure at a population level. In addition, day-to-day variations in exposure of the population to NO<sub>2</sub> of ambient origin are likely to track changes in the concentrations measured at a central site/sites. It is these variations over time and the ability to represent population average personal exposure, rather than the absolute magnitude of the exposure itself, that are the basis for the associations between ambient NO<sub>2</sub> levels and the health effects reported in short-term epidemiological studies. Therefore, ambient concentrations are a useful and appropriate exposure measure for epidemiological studies of the health effects of NO<sub>2</sub> air pollution.

The relationship between ambient concentrations and personal exposure to ambient NO<sub>2</sub> will vary as a result of the influence of a number of factors, including spatial and temporal variability in NO<sub>2</sub> concentrations, time-activity patterns, building ventilation, and perhaps measurement artifacts and analytical methods; this results in exposure measurement error and potential bias in risk estimates. The bias can be either upward or downward, but is expected to most often underestimate risks and to make it more difficult to detect a health effect. Therefore, this source of uncertainty is not expected to change the principal conclusion from the epidemiological studies: that several categories of adverse health effects are consistently and independently associated with ambient NO<sub>2</sub> concentrations.



## 12.4.2 Comparison of Ambient Concentrations in Canada and in Key Epidemiological Studies

*How do the concentrations at which health effects occur compare with ambient NO<sub>2</sub> levels in Canada?*

In this subsection, the health risk associated with exposure to ambient NO<sub>2</sub> in Canada is characterized by comparing the concentrations at which health effects are observed in key epidemiological studies with the levels measured at monitoring stations in the NAPS network across Canada.

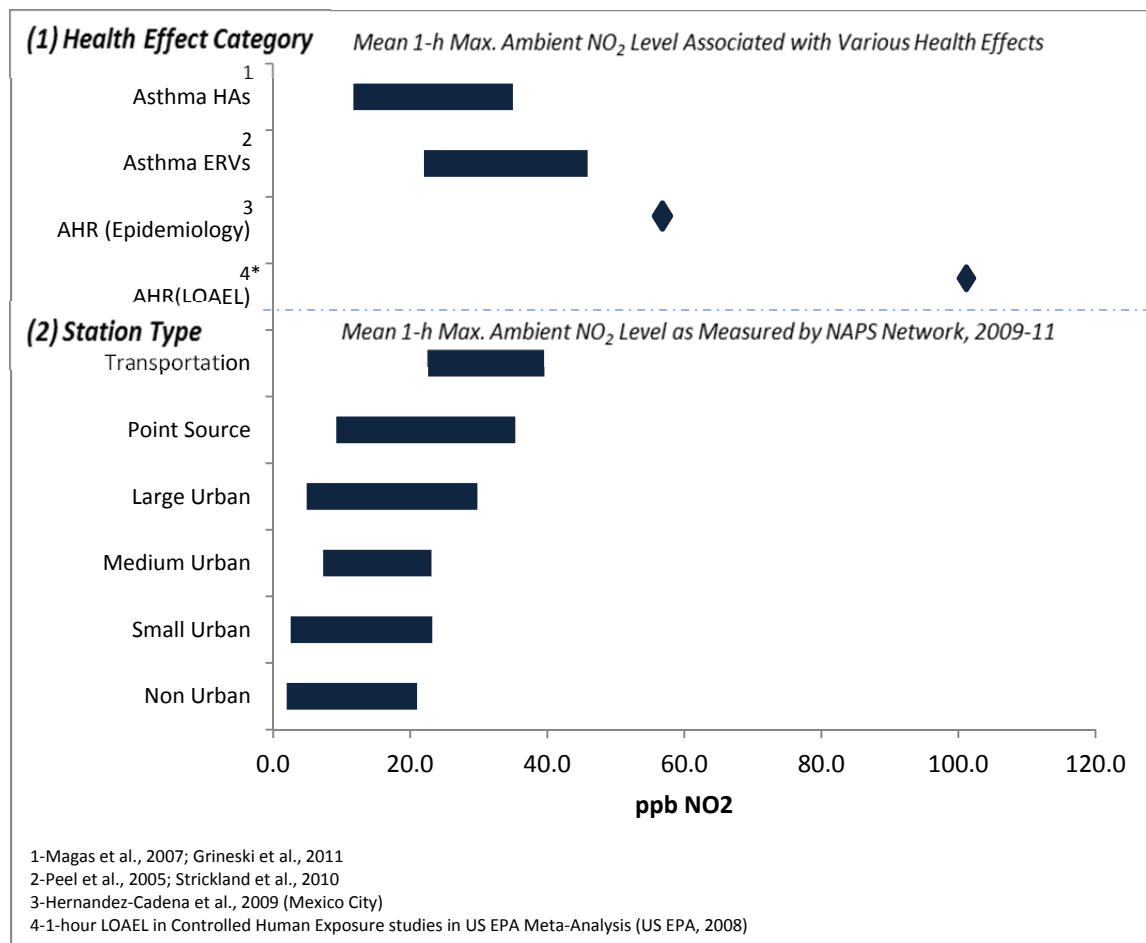
To make the comparison most relevant, it has been restricted to those health effect categories for which the weight of evidence analysis presented in subsections 12.2.2 and 12.2.3 concluded that the relationship between exposure to ambient NO<sub>2</sub> and the category is either **causal** or **likely to be causal**. These categories include mortality associated with short-term exposure to ambient NO<sub>2</sub> and respiratory morbidity associated with each of short-term and long-term exposure.

The key health effect studies selected were principally epidemiological studies of ambient NO<sub>2</sub>-related effects conducted in Canada and the US. They were further limited to those studies that reported a significant association between ambient NO<sub>2</sub> and the above health effect categories, which provided effect estimates for NO<sub>2</sub> for the same metrics as are commonly used for ambient standards (daily 1-h max, 24-h avg, and long-term average) and for which ambient levels were reported (including data in figures). In addition, for those studies that reported associations for short-term exposures, studies were only included if the findings for NO<sub>2</sub> were robust to adjustment for other pollutants, or if exclusively single-pollutant models were run and health outcomes were significantly related solely to NO<sub>2</sub> and not to other pollutants. These latter criteria were not applied in selecting long-term studies, because almost none of the long-term exposure studies adjusted for co-pollutants, given the high collinearity among the various air pollutants.

The ambient levels presented for each of the health effect papers were limited to mean or median concentrations, rather than upper percentiles (e.g. 98<sup>th</sup>), because the papers did not consistently report any specific percentile. The ambient levels in Canada were those reported for the NAPS network stations for 2009–2011. The mean concentration of NO<sub>2</sub> measured at each NAPS station over this 3-year period was calculated, and the results are presented by NAPS station type.

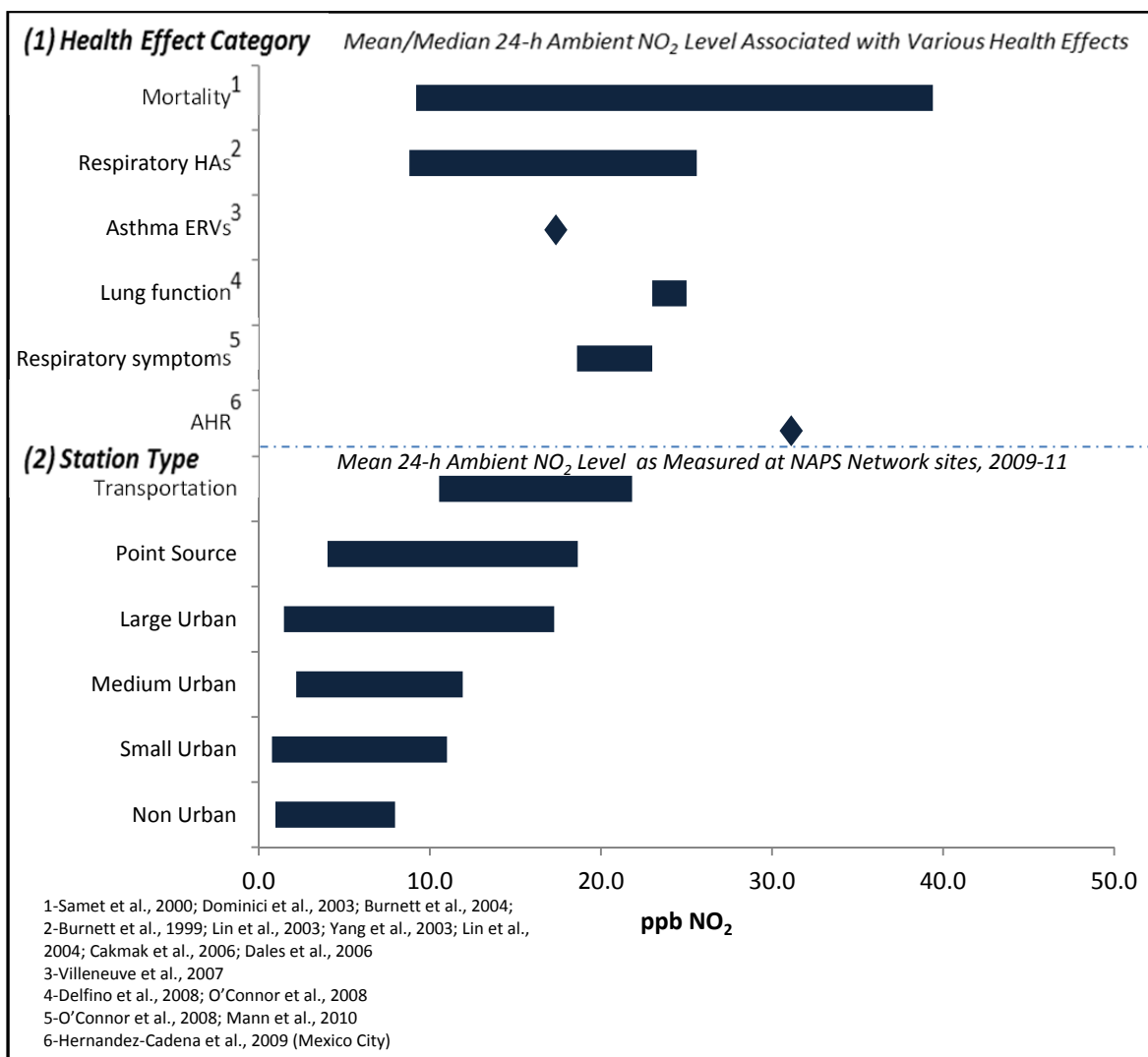
The results of this analysis are presented in Figure 12.1 for the daily 1-h max NO<sub>2</sub>, in Figure 12.2 for the 24-h avg NO<sub>2</sub>, and in Figure 12.3 for NO<sub>2</sub> as the long-term (annual/multi-year) average. In each figure, the top panel presents the mean or median NO<sub>2</sub> levels associated with various categories of health effects, generally presented in the same order as they are discussed in this assessment. The lower panel presents the mean concentrations of NO<sub>2</sub> measured at the NAPS stations, grouped by station type. In instances where there is more than one data point, they are presented as a bar that represents the range of mean/median concentrations, whereas if there is only a single data point, it is presented as a diamond. The individual studies from which the NO<sub>2</sub> levels associated with health effects were extracted are listed in footnotes to each figure.

**Figure 12.1: Comparison between mean daily 1-h max ambient NO<sub>2</sub> levels (1) associated with various health effects in selected Canadian/US epidemiology studies and (2) measured at Canadian NAPS monitoring stations**



The dataset for 1-h max is relatively small (Figure 12.1). Nonetheless, the ambient daily 1-h max concentrations associated with both hospitalizations and ERVs for asthma substantially overlap those measured in the NAPS network for all station types, including non urban, various sizes of urban communities, and sites impacted by nearby transportation and potentially industrial sources. In addition, the figure includes data on the NO<sub>2</sub> levels associated with increases in AHR, which is a hallmark of asthma. Mean ambient levels in Canada (especially at large urban, and transportation-influenced and potentially industrial source-influenced sites) approach those associated with AHR in a panel study of asthmatic children from Mexico City. They are less than the concentration of 100 ppb associated with increased AHR in a meta-analysis of controlled human exposure studies conducted by the US EPA in its 2008 assessment. However, the maximum levels measured at several NAPS transportation- and potentially industrial source-influenced sites during 2009–11 exceeded both the 100 ppb effect level for increased AHR from these experimental studies and the 57 ppb level associated with increased AHR in the asthmatic children from Mexico City (Table 12.2).

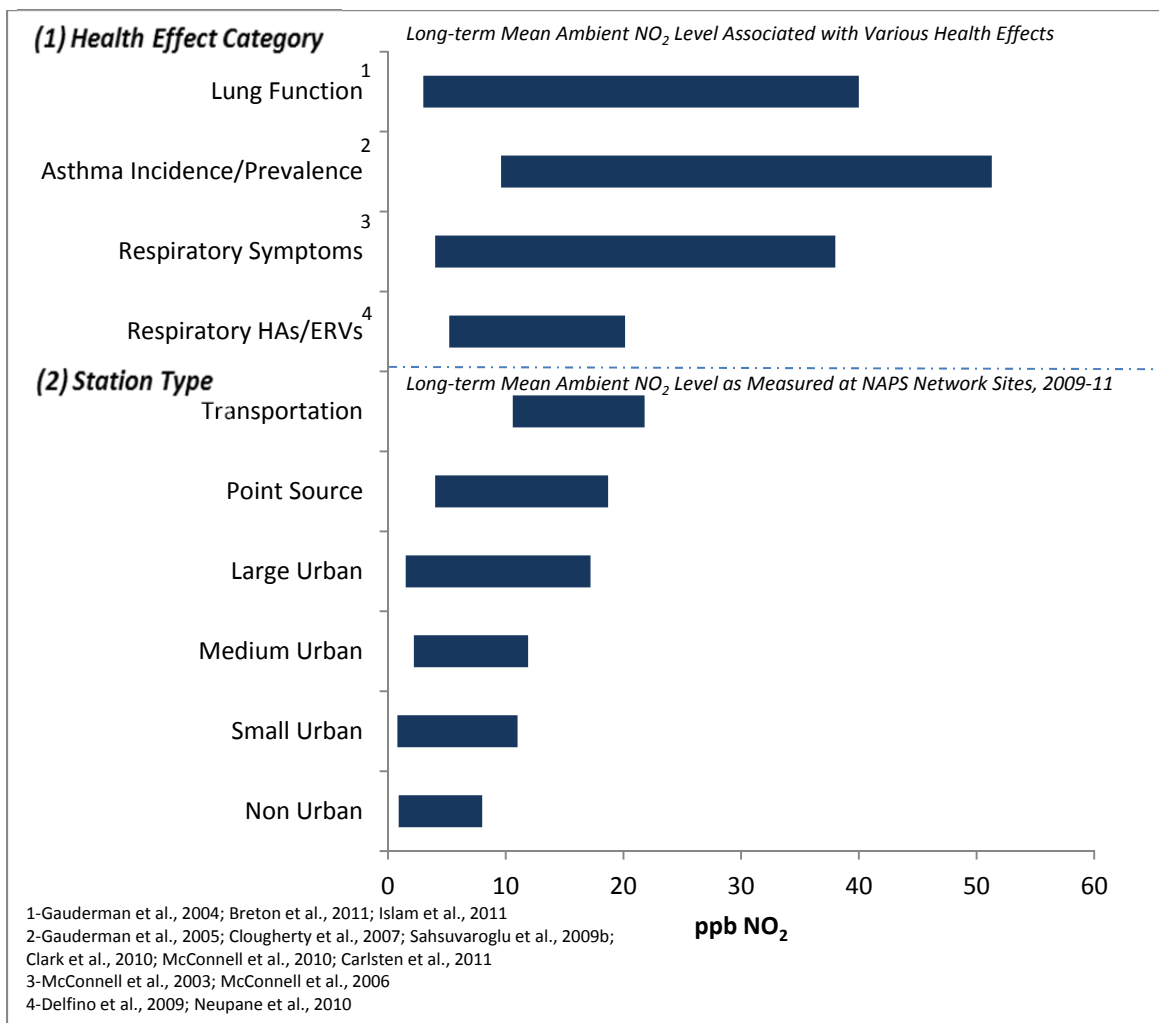
**Figure 12.2: Comparison between mean 24-h avg ambient NO<sub>2</sub> levels (1) associated with various health effects in selected Canadian/US epidemiology studies and (2) measured at Canadian NAPS monitoring stations**



The ambient 24-h NO<sub>2</sub> levels associated with increased risks for mortality (total, cardiovascular and respiratory), respiratory hospitalizations, and asthma ERVs overlap the concentrations measured at most NAPS site types to a considerable extent (Figure 12.2). In addition, in panel studies there were NO<sub>2</sub>-related decrements in lung function and increases in respiratory symptoms and AHR at ambient concentrations that are similar to or slightly greater than those measured at NAPS transportation-influenced sites.

Finally, in similar analyses for long-term exposures, there was a considerable degree of overlap between the long-term average NO<sub>2</sub> concentrations associated with respiratory effects in epidemiology studies and the 3-year average concentrations measured at all types of NAPS sites (Figure 12.3). This was the case for a range of effects, including decreases in lung function and increases in each of asthma incidence/prevalence, respiratory symptoms, and respiratory hospital admissions and ERVs.

**Figure 12.3: Comparison between long-term mean ambient NO<sub>2</sub> levels (1) associated with various health effects in selected Canadian/US epidemiology studies and (2) measured at Canadian NAPS monitoring stations**



In summary, ambient levels of NO<sub>2</sub> measured in Canada overlap those associated with health effects in epidemiology studies of relatively strong design and for which there is considerable weight of evidence (causal/likely to be causal). For those health effects examined in population-based studies with relatively great statistical power (i.e. mortality, hospitalizations, and ERVs), the ambient levels overlap those measured at all NAPS site types, ranging all the way from non urban to transportation-impacted sites. Therefore, adverse health effects are occurring in epidemiological studies at ambient NO<sub>2</sub> levels that are commonly experienced in Canada. A coherent range of effects is associated with each of the daily 1-h max, 24-h avg, and long-term average NO<sub>2</sub> levels, and none of these exposure durations is more or less clearly related to health effects, even though the dataset for 1-h max NO<sub>2</sub> is relatively small.

### 12.4.3 Shape of the Concentration–Response Relationship

*What is the shape of the curve relating ambient NO<sub>2</sub> concentrations to health effects?*

The shape of the curve relating ambient NO<sub>2</sub> to various health outcomes has implications for the setting of ambient air quality standards, as well as for estimating the health impacts from exposure to this air pollutant and the risk management to address these impacts. This aspect of the association between NO<sub>2</sub> and health effects, including the potential for the existence of threshold levels below which health effects are not observed, has been investigated in a number of studies.

With respect to short-term mortality, a very small number of multi-city studies have investigated the shape of the NO<sub>2</sub>–mortality curve (Section 8.2). In a study of mortality in 12 Canadian cities over 20 years, conducted in the course of developing an AQHI, a linear model of concentration–response provided a better fit than quadratic or cubic polynomials (which imply a threshold or change in slope) for NO<sub>2</sub> and other pollutants. Similarly, in another study using data from 72 US NMMAPS cities, using a sub-sampling approach and flexible models to investigate the shape of the concentration–response relationship, the result was consistent with linearity.

Concerning medical utilizations, the 2008 US EPA ISA (US EPA, 2008) reported the results of several studies that examined the shape of the C–R curve between ambient NO<sub>2</sub> and hospitalizations or ERVs for respiratory and cardiac disease. One study showed a steeper (log-linear) relationship with CHF admissions at lower concentrations of NO<sub>2</sub>, whereas several other studies reported that the data were best described by a linear function. Subsequently, two US studies reviewed in this assessment investigated the shape of the relationship between NO<sub>2</sub> and children’s asthma ERVs (subsection 8.1.3.1.3). In a study from Atlanta, GA, the risks for asthma ERVs exhibited a dose–response relationship across quintiles of warm-season ambient NO<sub>2</sub> concentrations, and using LOESS concentration–response analyses, there was no apparent threshold across the entire exposure range, which extended down to roughly 10 ppb 1-h max NO<sub>2</sub>. Similarly, in a study of asthma ERVs and hospitalizations in Medicaid children from Detroit, MI, there was an association with NO<sub>2</sub> using conventional linear models that did not assume a threshold, whereas there was no significant evidence of either a threshold or even of an increased risk related to NO<sub>2</sub> using GAM and case-crossover threshold models.

Most of the recent long-term epidemiological studies (Chapter 9) have not formally evaluated the concentration–response relationship or performed analyses to identify a threshold level for the effects of ambient NO<sub>2</sub>. In a Canadian study of a high-risk birth cohort, the risk of asthma increased monotonically with increasing NO and PM<sub>2.5</sub> exposures, but no clear dose–response trend was found for NO<sub>2</sub>. A few European studies have used categorical analyses to examine the relationship between health effects and ambient NO<sub>2</sub> concentrations. The risks for respiratory effects were increased across higher tertiles or quartiles of NO<sub>2</sub> air pollution levels, including for asthma incidence in young Swedish adults as well as for asthma hospital admissions of participants in the Danish Diet, Cancer and Health cohort study. These results support earlier findings of linear relationships between ambient NO<sub>2</sub> concentrations and decrements in lung function and deficits in lung function growth that were observed in children from California and Mexico City. The Rome, Italy, longitudinal cohort study of more than 1 million residents also found increased risk estimates for several mortality outcomes across the quintile NO<sub>2</sub> distributions. Similar findings were also noted in a cohort from Oslo, Norway, where



concentration–response relationships were also studied for cause-specific mortality. Based on quartile-exposure analyses, the effects appeared to increase at daily NO<sub>2</sub> levels above 21 ppb in the youngest men (aged 51–70) while a linear dose–response relationship was observed for the oldest men (aged 71–90) with NO<sub>2</sub> levels between 10 and 32 ppb (subsection 9.2.2).

Overall, most studies that have examined the shape of the concentration–response relationship for the health effects associated with ambient NO<sub>2</sub> have found that the relationship is consistent with a linear model and that no biological threshold can be identified based on current evidence.

It should be noted that the general lack of a clearly identifiable threshold at a population level based on epidemiological studies is consistent with the wide variation between individuals in susceptibility to the respiratory effects of NO<sub>2</sub> that was observed in the controlled human exposure studies. Although individual thresholds may exist, they are likely to differ widely, particularly considering that the population-based epidemiology studies (unlike the controlled human exposure studies) include subjects who have the most severe pre-existing disease and are therefore perhaps most likely to be affected by pollutants at low concentrations. Due to these large differences in sensitivity within the general population, a common threshold is not likely to be observable at a population level in epidemiological studies. In addition, a number of the disease conditions that are affected by NO<sub>2</sub> (e.g. asthma) are common in the general population and are the combined result of multiple risk factors. If exposure to NO<sub>2</sub> is exacerbating these diseases, its effects would be expected to be incremental to the existing background of the disease in the population, without exhibiting a threshold. However, it is also recognized that other factors may also make it difficult to identify a threshold at a population level; low data density in the lower NO<sub>2</sub> concentration range, response measurement error, exposure measurement error, and a shallow slope are some of the factors that complicate the ability to determine the shape of the concentration–response curve. Regardless of the reasons for the general lack of an evident threshold in the epidemiological studies, overall the current evidence indicates that if a general population threshold level exists for the health effects of ambient NO<sub>2</sub>, it is likely to be near the lower limit of ambient concentrations.

## 12.5 Uncertainties in Health Assessment of Ambient NO<sub>2</sub>

Despite the general coherence of the database concerning NO<sub>2</sub> health effects, especially with the emergence of a considerable body of epidemiological evidence in recent years, there remain important uncertainties in the understanding of the exposure to and health effects of NO<sub>2</sub>. This section discusses the more important uncertainties in the database that has been used to characterize the risks associated with ambient NO<sub>2</sub>. For each issue discussed, the uncertainties and their implications are briefly summarized.

**Role of co-pollutants:** Vehicle exhaust is a major source of numerous combustion pollutants, including NO<sub>2</sub>. Because ambient NO<sub>2</sub> and other related pollutants originate to a significant degree from this common source, they are often highly correlated and it is difficult to ascertain from epidemiological studies the extent to which effects are independently related to ambient NO<sub>2</sub> or whether NO<sub>2</sub> is a marker for the effects of other traffic-related pollutants. In fact, many studies reported high correlations between NO<sub>2</sub> and various air pollutants, including CO and PM. Nonetheless, in those short-term epidemiology studies of mortality or respiratory morbidity that included analyses with other pollutants in conjunction with NO<sub>2</sub>, the associations with NO<sub>2</sub> were generally robust to adjustment for one or more of PM indicators and other gaseous air pollutants. This was observed most often in models with common air pollutants including PM<sub>10</sub>, O<sub>3</sub>, and SO<sub>2</sub>. However, effect estimates for NO<sub>2</sub> were often also not sensitive to adjustment for traffic-related pollutants including CO, PM<sub>2.5</sub>, and in a small number of studies, NO, particulate

metals, EC, OC or UFPs, though traffic-related pollutants have not been extensively studied in this regard. In addition, the recent epidemiological findings have for the most part strengthened the evidence that effects are independently associated with NO<sub>2</sub>. Further, intervention studies linked improvements in respiratory symptoms to reduced exposure to NO<sub>2</sub> from indoor combustion sources, suggesting that NO<sub>2</sub> is not solely a marker for an ambient air pollution mixture from outdoor sources (most likely traffic emissions). Finally, the results of controlled human exposure studies and toxicological studies in animals conclusively demonstrate independent effects of NO<sub>2</sub> on respiratory health that are strongly coherent with the findings of the epidemiological studies. Hence, a number of lines of evidence collectively indicate that NO<sub>2</sub> itself is responsible for the respiratory morbidity, especially asthma exacerbation, that is related to short-term exposure to this air pollutant. However, there is more uncertainty about the independence of short-term exposure to NO<sub>2</sub> in relation to other categories of health effects for which the weight of evidence with respect to causality is weaker.

The possible role of co-pollutants was not investigated in most of the studies of long-term exposure to ambient NO<sub>2</sub>. However, in the small number of studies in which co-pollutant models were performed, associations with lung function decrements remained after adjustment for several pollutants including PM indices, EC and/or gases (O<sub>3</sub> or SO<sub>2</sub>). Given the high correlation observed between NO<sub>x</sub>/NO<sub>2</sub> and either PM indices or different traffic indicators, it is difficult to disentangle the effect of each air pollutant per se; this issue remains the major uncertainty in the overall health database relating to long-term exposure to NO<sub>2</sub> levels. Additional co-pollutant models adjusting for key traffic-related air pollutants, including CO and UFPs, are needed to determine whether effects related to long-term exposure to ambient NO<sub>2</sub> are independent of other pollutants.

**Central site monitoring as a measure of exposure:** Many of the reviewed epidemiological studies rely on a single central monitor, or the averaging of several, to characterize the pollution levels in a given community, using the resultant NO<sub>2</sub> concentration as an indicator of population exposure. Given the high spatial heterogeneity in ambient NO<sub>2</sub> concentrations, both within the airshed at large and between microenvironments, a mismatch of individual exposures and their health status can result. The relationship between ambient concentration and personal exposure to ambient NO<sub>2</sub> will vary as a result of individual-, seasonal-, city-, or region-specific differences, resulting in measurement error and potential bias in risk estimates. The bias can be either upward or downward, though it is expected to most often underestimate risks and make it more difficult to detect a health effect. Several studies performed in Atlanta that investigated the potential bias from using fixed area monitors on the resulting estimates of short-term risk indicated that the spatial heterogeneity of air pollutants was a much greater source of measurement error than instrument imprecision. For NO<sub>2</sub>, most results suggested that this measurement error markedly attenuated the risk estimates, even resulting in a loss of statistical significance.

Studies investigating the relationship between ambient NO<sub>2</sub> and personal exposure to NO<sub>2</sub> have yielded mixed results due to several factors, including the spatial heterogeneity of NO<sub>2</sub>, the prevalence of indoor sources, the factors influencing infiltration (e.g. season) as well as the time spent by subjects in different microenvironments. Overall, the data indicate that ambient NO<sub>2</sub>, as estimated at central site monitors, is generally an adequate surrogate for personal exposure to NO<sub>2</sub> of ambient origin.

**Concentration–response relationships, thresholds:** For those health endpoints associated with ambient NO<sub>2</sub> in the epidemiological studies, an important question in characterizing risk is the shape of the concentration–response curve, and the issue of potential population threshold levels. In the small number of studies that have examined the shape of the concentration–response relationship for short-term NO<sub>2</sub>-related mortality or hospitalizations,

there was an approximately linear concentration–response relationship with no clear evidence for a threshold. Most of the recent long-term epidemiological studies have not formally evaluated the concentration–response relationships or performed analyses to identify a threshold level for the effects of ambient NO<sub>2</sub>, though dose–response relationships have been observed in categorical analyses in a limited number of chronic epidemiological studies. These results need to be confirmed through additional studies.

There is a need to better characterize the dose–response curve in a controlled human exposure setting, specifically related to concentration of NO<sub>2</sub> and resultant respiratory effects in both healthy and asthmatic individuals. Potential health effects at near-ambient NO<sub>2</sub> concentrations should also be analyzed in controlled human exposure studies to support epidemiological observations and facilitate identification of threshold concentrations, if any.

**Respiratory effects of long-term exposure in adults:** The database on the relationship between long-term exposure to ambient NO<sub>2</sub> and respiratory effects in adults is relatively small. Some new studies have investigated the impact of long-term NO<sub>2</sub> air pollution on pulmonary function in adults, finding inconsistent results. Weak or no associations have also been found in a very small number of studies investigating the impact of long-term exposure to NO<sub>x</sub>/NO<sub>2</sub> on different respiratory symptoms in adults. Additional chronic epidemiological studies are needed, especially in Canada and the US, in order to determine the potential effect of this pollutant on adults and/or older adults.

**Cardiovascular effects:** Further research is needed to better characterize the cardiovascular effects of short-term and long-term exposure to NO<sub>2</sub> air pollution. The dataset from the panel epidemiological studies is currently small and inconsistent; it should be expanded with more powerful studies of cardiovascular function and biomarkers of CVD risk. Controlled human exposure studies on cardiovascular effects at lower NO<sub>2</sub> concentrations (i.e. under 1 ppm) are limited and additional studies should be performed. The database on the relationship between long-term exposure to ambient NO<sub>2</sub> and cardiovascular morbidity is also currently limited and somewhat inconsistent. Moreover, most of the epidemiological studies only performed single-pollutant models and in several of these, associations were related to PM air pollution. In addition, the chain of events that may lead to NO<sub>2</sub>-related cardiovascular mortality or morbidity has not been well characterized; more research is needed to better understand potential mechanisms of action.

**Reproductive and developmental outcomes:** As there appears to be some impact of NO<sub>2</sub> on birth weight and preterm birth, further examination of periods of susceptibility during pregnancy (i.e. is there a trimester of greatest concern) is needed. The epidemiological evidence remains very limited and/or inconsistent in terms of NO<sub>2</sub> exposure, showing a variety of other reproductive and developmental effects, including preeclampsia, congenital anomalies, and neonatal respiratory effects; further investigation is required. The primary uncertainty that remains regarding the adverse reproductive and developmental effects observed is the role of NO<sub>2</sub> as compared to the other air pollutants. In addition, the chain of events that may lead to reproductive and developmental effects is largely unknown and should also be investigated in future studies. Given the potential for lifelong sequelae of adverse reproductive outcomes, the impacts of air pollution and the specific role that NO<sub>2</sub> plays in these is of some importance. Additionally there is uncertainty as to the public health consequences of these usually small changes in outcomes, such as birth weight, and this needs to be better understood as the health database advances.

**Emerging effects of NO<sub>2</sub>:** A small number of studies have investigated the relationship between short-term exposure to ambient NO<sub>2</sub> and a variety of other adverse effects, including medical visits for conditions including appendicitis, headache, depression, suicide

attempts/suicidal thoughts, and otitis media. A limited number of studies have also been published in relation to long-term ambient NO<sub>2</sub>/NO<sub>x</sub> exposure effects on other morbidity outcomes, including annoyance, diabetes, IBD, neurological diseases/effects, otitis media, osteoporosis and rheumatoid arthritis. Each of these outcomes was, however, only examined in a very small number of studies, and the effects were also significantly related to most other particulate and gaseous pollutants. The epidemiological evidence for these effects remains very limited and/or inconsistent, and further investigation is required before firm conclusions can be reached.

**Differences in sensitivity to NO<sub>2</sub>:** Several lines of evidence clearly indicate that asthmatics, children, and older adults are more sensitive to the health effects of ambient NO<sub>2</sub>. However, additional research is required to determine whether other pre-existing diseases or factors increase the risk of NO<sub>2</sub>-related health effects, including COPD, CVD, diabetes, gender, obesity, race/ethnicity, smoking status, SES and other social factors such as psychological stress.

It is also clear from controlled human exposure studies that there are substantial differences in responsiveness to short-term exposure to NO<sub>2</sub> between individuals, but the reasons for these differences in sensitivity are not known. There is a need to define the characteristic(s) of mild asthmatics that result in their being “NO<sub>2</sub> responders” as compared to “NO<sub>2</sub> non-responders” in controlled human exposure studies investigating AHR in response to bronchial challenge, including exposure to concentrations lower than 100 ppb.

**Genetic effect modification:** An important new feature of the health dataset, and one that has consequences for more than just NO<sub>2</sub>, is the emerging evidence that polymorphisms in some genes (e.g. genetic variants of antioxidant enzymes and genes involved in oxidative pathways or inflammatory responses) can influence the association between air pollutant exposures and morbidity effects. The observed interactions between genes and the environment seemed to indicate a potential role of these in some potentially at-risk populations. Additional research in this area would contribute to a fuller understanding of these gene–environment interactions.

## 12.6 Conclusions

This concluding section presents a summary of key findings and insights arising from the preceding sections of the risk characterization for ambient NO<sub>2</sub>. These are presented for various key subject areas, including the weight of evidence for various categories of health effects, subpopulations with increased sensitivity or exposure, exposure-related issues, the public health impacts of ambient NO<sub>2</sub>, and the implications of some key elements of the dataset for the development of CAAQS.

**Short-term respiratory effects:** In short-term controlled studies of asthmatic adults, exposure to near-ambient levels of NO<sub>2</sub> elicited a range of adverse respiratory effects, including decreased lung function, increased AHR, and airway inflammation. Most of these effects, as well as increases in asthma-related respiratory symptoms, were also associated with ambient NO<sub>2</sub> in epidemiological studies of asthmatic children. Respiratory symptoms in asthmatic children were also related to indoor NO<sub>2</sub> in several epidemiological studies, and interventions to reduce NO<sub>2</sub> from gas appliances in classrooms decreased respiratory symptoms. The mechanisms by which these effects occur have been investigated in both humans and animals and provide biologically plausible pathways for these effects.

Ambient NO<sub>2</sub> concentrations were significantly and independently associated with increased respiratory and asthma hospitalizations and asthma ERVs in numerous population-based epidemiology studies. These findings are strongly coherent with the experimental and

epidemiological evidence for lung function decrements, increased respiratory symptoms, airway inflammation, and increased AHR in asthmatics, and they provide an indication of the public health impacts at a population level arising from the effects on the airways seen in experimental and epidemiological studies.

Thus several lines of evidence indicate that ambient NO<sub>2</sub> is associated with asthma exacerbations. The epidemiological associations with short-term asthma-related endpoints exhibit strength of association, consistency, robustness, and coherence. In conjunction with the experimental findings in animals and humans, the overall evidence indicates that **there is a causal relationship** between short-term exposure to ambient NO<sub>2</sub> at current levels and increased asthma-related morbidity (including airway inflammation and AHR, respiratory symptoms, and asthma hospitalizations and ERVs).

**Short-term cardiovascular effects:** In population-based epidemiological studies, there were consistent and significant associations of ambient NO<sub>2</sub> with increased cardiovascular mortality, hospitalizations, and ERVs. However, the morbidity outcomes were often related to other pollutants, and NO<sub>2</sub>-related risks were often attenuated by adjustment for co-pollutants or only single-pollutant models were conducted.

In some panel studies and controlled human exposure studies, there were NO<sub>2</sub>-related decreases in HRV, changes in ventricular repolarization, and increases in inflammatory and/or coagulatory biomarkers. However, the findings in this small dataset were somewhat inconsistent, and the spectrum of NO<sub>2</sub>-related cardiovascular effects has not been well characterized.

Given the questions surrounding the independence of the NO<sub>2</sub>-related effects and the limited supporting data, the overall evidence is **suggestive, but not sufficient to infer a causal relationship** between short-term exposure to ambient NO<sub>2</sub> and cardiovascular effects.

**Mortality related to short-term exposure:** In numerous epidemiological studies of various designs, short-term ambient NO<sub>2</sub> was independently associated with increases in total non-accidental, CP, cardiovascular, and respiratory mortality. These associations were observed in cities from various regions of the world, encompassing different climatic regimes, pollutant mixes, and socioeconomic conditions. However, the coherence of the epidemiological findings with respect to NO<sub>2</sub>-related morbidity that could give rise to mortality from cardiovascular and respiratory causes is somewhat limited.

Therefore, even though the associations with total non-accidental, CP, and to a lesser extent cardiovascular and respiratory mortality display strength of association, consistency, and robustness, considering the lack of coherence it is concluded that there is **likely a causal relationship** between short-term exposure to ambient NO<sub>2</sub> at current levels and these categories of mortality.

**Long-term respiratory morbidity:** In epidemiological studies, long-term exposure to ambient NO<sub>2</sub> was associated with adverse respiratory effects, especially in children, including decrements in lung function and deficits in lung function growth. In children, several cohort studies also showed relationships between long-term exposure to NO<sub>2</sub> and the development of asthma and/or allergic responses. Long-term exposure to NO<sub>2</sub> levels also appears to increase the incidence of asthma in adults. However, some uncertainty remains about the possible role of other co-occurring pollutants in the NO<sub>2</sub>-related respiratory effects.

The epidemiological associations with respiratory health endpoints exhibit consistency, strength of association, and coherence across disciplines, as well as some indication of robustness and biological plausibility. However, considering the questions surrounding the possible role of co-



pollutants, the overall evidence indicates that there is **likely a causal relationship** between long-term exposures to ambient NO<sub>2</sub>/NO<sub>x</sub> at current levels and respiratory effects related to the development of asthma or allergic-related disease.

**Other long-term effects:** Overall, the limited available evidence is **suggestive, but not sufficient to infer a causal relationship** between long-term exposure to ambient NO<sub>2</sub> and each of cardiovascular effects, cancer and related effects, mortality, and reproductive and developmental endpoints. For each of these, there are significant ambient-NO<sub>2</sub>-related associations in some epidemiology studies, but the database is lacking in a number of respects, and more research is needed.

**Emerging effects:** A number of other emerging NO<sub>2</sub>-related effects warrant further examination, including those on the CNS and on other morbidity outcomes (diabetes, appendicitis, IBD, otitis media, osteoporosis and rheumatoid arthritis) to determine whether such effects are consistently observed and occur at relevant concentrations. The emerging evidence that polymorphisms in some genes can influence the association between air pollutant exposures and morbidity effects indicates a potential role for such genetic effect modification in some at-risk populations, and additional research in this area would contribute to a fuller understanding of these gene–environment interactions.

**Subgroups with increased sensitivity or exposure to ambient NO<sub>2</sub>:** Individuals with certain pre-existing diseases appear to be sensitive to exposure to ambient NO<sub>2</sub>. Several lines of evidence from controlled human exposure and epidemiological studies indicate that asthmatics are a susceptible subgroup. There is some evidence (albeit more limited than for asthma) that people with COPD also appear to be more sensitive to NO<sub>2</sub>.

Age is also clearly related to susceptibility. The results of epidemiological studies indicate that children, especially asthmatics, are more at risk of respiratory health outcomes from both short- and long-term exposure to NO<sub>2</sub>. Older adults appear to be more sensitive to short-term effects of NO<sub>2</sub> on respiratory hospital admissions, ERVs and other medical visits, as well as all-cause and respiratory mortality. Older adults also had increased risks for cardiovascular mortality and morbidity in epidemiological studies.

Concentrations of ambient NO<sub>2</sub> are higher near local sources, especially in on-road, near-road, and in-vehicle microenvironments for roadways with heavy traffic. People who spend substantial amounts of time in such locations can have elevated exposures to NO<sub>2</sub>. These would include people who have long commutes or drives during the course of their work, or who work, reside, attend school, etc. in close proximity to major roadways. People engaged in vigorous physical activity would also inhale greater amounts of NO<sub>2</sub>.

**Exposure:** The entire population is exposed to NO<sub>2</sub> of ambient origin, both outdoors and in indoor environments into which ambient NO<sub>2</sub> has infiltrated. For epidemiological studies, where estimating population average exposure is required, the ambient monitoring network provides a useful and appropriate measure of population exposure to NO<sub>2</sub> of ambient origin.

The relationship between ambient concentration and personal exposure to ambient NO<sub>2</sub> will vary as a result of the influence of a number of factors resulting in exposure measurement error and potential bias in the risk estimates. NO<sub>2</sub> ambient monitors may be less representative of community exposures than are ambient monitors for PM<sub>2.5</sub> or O<sub>3</sub> as a consequence of the greater spatial heterogeneity of ambient NO<sub>2</sub>. The potential bias arising from this exposure measurement error can be either upward or downward, but it is expected to most often underestimate risks and thus make it more difficult to detect a health effect. Therefore, this source of uncertainty should not change the principal conclusions, based largely on

epidemiological studies, that several categories of adverse health effects are consistently and independently associated with ambient NO<sub>2</sub> concentrations.

**Public health impacts:** The effects associated with NO<sub>2</sub> have been observed in epidemiological studies in Canada and in other countries at NO<sub>2</sub> concentrations that occur in Canada. For those health outcomes for which the weight of evidence and statistical power are greatest (i.e. mortality, respiratory/asthma hospitalizations and asthma ERVs), the mean or median ambient levels at which effects are observed overlap those measured at all NAPS site types, ranging from non urban to transportation- and potentially industry-impacted sites. Therefore, adverse health effects in epidemiological studies are occurring at ambient NO<sub>2</sub> concentrations that are commonly experienced in Canada and that are much lower than current ambient air quality objectives and standards in Canada and the US.

In most of the studies that examined the shape of the concentration–response relationship for short-term NO<sub>2</sub>-related mortality or medical visits, there was an approximately linear relationship, with no clear evidence of a threshold. Overall, the current evidence indicates that if a general population threshold exists for the health effects of NO<sub>2</sub>, it is likely to be near the lower limit of ambient NO<sub>2</sub> concentrations. Consequently, the available evidence indicates that any increment in concentrations of ambient NO<sub>2</sub> presents an increased risk for serious health effects, up to and including mortality.

Although the risks for ambient NO<sub>2</sub>-related health risks are relatively small by traditional epidemiological standards, the entire population is exposed, and the subpopulations that have increased sensitivity or exposure to NO<sub>2</sub> (including children, older adults, individuals with asthma or COPD, people engaged in vigorous physical activity and those spending substantial amounts of time near major roadways) comprise a considerable proportion of the population. In addition, the health impacts that have been the focus of most assessments, including mortality, hospitalizations, and ERVs, represent serious outcomes. Nonetheless, these are just the “tip of the iceberg” in the pyramid of health effects associated with ambient NO<sub>2</sub>, and the unmeasured morbidity also has important public health impacts and costs. As a result, the public health impacts of ambient NO<sub>2</sub> are substantial and are expected to remain important as the population ages and the pool of older adults increases, especially given the higher underlying death and disease rates in this age group.

**Exposure duration:** With respect to the durations of exposure that are associated with health effects, the types of health effects and the consistency of the findings are much the same for daily 1-h max and 24-h avg ambient NO<sub>2</sub>. There is also some indication of the same kinds of health effects for other sub-daily averages (e.g. 3-h). These similarities are not unexpected, given that these various short-term exposure metrics are highly correlated. In addition, the overlap between ambient levels in Canada and the concentrations associated with health effects in the epidemiological studies is similar for daily 1-h max, 24-h avg, and even annual average NO<sub>2</sub> (Figures 12.1, 12.2 and 12.3). In short, the information on health effects associated with ambient NO<sub>2</sub> does not itself provide strong support for any one exposure metric over the other as the basis for the form of the CAAQS. However, the differences between the types of health effects that are related to short-term versus long-term ambient NO<sub>2</sub> suggest that standards are needed for each of these durations to protect against the associated health effects.

**Support for development of new CAAQS:** This risk assessment was conducted to inform the development of new CAAQS for NO<sub>2</sub> to replace the current NAAQOs. It is recommended that new CAAQS be developed for ambient NO<sub>2</sub> with consideration of the following key conclusions from the health risk assessment:

- there is strong evidence that ambient NO<sub>2</sub> causes both short-term and long-term respiratory effects and short-term mortality, as well as suggestive evidence linking it to a wide range of other adverse health outcomes
- these effects have been observed in epidemiological studies at NO<sub>2</sub> concentrations that commonly occur in Canada, well below the levels of existing NAAQOs and other ambient standards, such as provincial/territorial guidelines and the US National Ambient Air Quality Standards
- in studies examining the shape of the concentration-response curve, there is an approximately linear relationship between ambient NO<sub>2</sub> concentrations and health effects, with no clear evidence of a threshold; hence, based on the balance of the evidence it should be assumed that any increment in concentrations of ambient NO<sub>2</sub> presents an increased risk for health effects, up to and including mortality
- the health evidence supports the establishment of both short-term and long-term standards to protect against the full suite of health effects associated with ambient NO<sub>2</sub>.