



Air Resources Board

State of California



Office Environmental Health
Hazard Assessment

Chairman Robert Sawyer

Governor Arnold Schwarzenegger

Director Joan Denton

Review of the California Ambient Air Quality Standard For Nitrogen Dioxide

Technical Support Document

January 5, 2007

California Environmental Protection Agency

Air Resources Board

and

Office of Environmental Health and Hazard Assessment

The energy challenge facing California is real. Every Californian needs to take immediate action to reduce energy consumption. For a list of simple ways you can reduce demand and cut your energy costs, see our Website: <http://www.arb.ca.gov>.

California Environmental Protection Agency

Printed on Recycled Paper

Project Coordinator and Contributing Author

Norman Kado, Ph.D.

Contributing Authors

Air Resources Board

Andy Delao
Scott Fruin, D.Env., P.E.
Cynthia Garcia
Nancy Hughett
Dongmin Luo, Ph.D., P.E.
Eric McDougal
Nehzat Motallebi, Ph.D.
Ralph Propper
Rajinder Sahota
Dorothy Shimer, M.S.
Dale Shimp
Brent Takemoto, Ph.D.

Office of Environmental Health Hazard Assessment

Daryn Dodge, Ph.D.
Shelley Green, Ph.D.
Janice Kim, M.D., M.P.H.
Bart Ostro, Ph.D.

Author Contractors

Mark W. Frampton, M.D., University of Rochester School of Medicine
Francesco Forastiere, M.D., Ph.D., Rome E Health Authority
Annette Peters, Ph.D., GSF-National Research Center for Environment and Health
Patrick J. Temple, Ph.D., United States Dept. Agriculture, Forest Service

Reviewers

Air Resources Board

Deborah M. Drechsler, Ph.D., Health and Ecosystem Assessment Section
Linda Tombras Smith, Ph.D., Manager, Health and Ecosystem Assessment Section
Richard D. Bode, Chief, Health and Exposure Assessment Branch
Bart E. Croes, P.E., Chief, Research Division
Michael H. Scheible, Deputy Executive Officer

Office of Environmental Health Hazard Assessment

Melanie Marty, Ph.D., Chief, Air Toxicology and Epidemiology Section
Robert Blaisdell, Ph.D., Chief, Exposure Modeling Section
Mark Miller, M.D., M.P.H., Air Toxicology and Risk Assessment Section
George Alexeeff, Ph.D., D.A.B.T., Deputy Director for Scientific Affairs
Val F. Siebal, Chief Deputy Director

Department of Health Services

Michael Lipsett, M.D.

University of California, Irvine

James N. Pitts Jr., Ph.D.

Acknowledgements

Air Resources Board

In addition, staff also thank and acknowledge the following individuals:
Ken Bowers, Richard Corey, Robert Effa, Michael FitzGibbon,
Peggy Jenkins, Karen Magliano, Steve Mara, Eileen McCauley,
Mena Shah, Ken Stroud, William Vance, Tony VanCuren, Bob Weller, Sara Adams

Disclaimer

This report has been reviewed by the staff of the Air Resources Board and the Office of Environmental Health Hazard Assessment. Mention of trade names or commercial products does not constitute endorsement or recommendation for their use. To obtain this document in an alternative format, please contact the Air Resources Board ADA Coordinator at (916) 322-4505, TDD (916) 324-9531, or (800) 700-8326 for TDD calls from outside the Sacramento area. This report is available for viewing or downloading from the Air Resources Board internet site at:

<http://www.arb.ca.gov/research/aaqs/no2-rs/no2-rs.htm>

Table of Contents

1	Introduction and Overview	1-1
1.1	Regulatory.....	1-1
1.1.1	Ambient Air Quality Standards and Children.....	1-1
1.2	Current California Ambient Air Quality Standard for NO ₂	1-1
1.3	History of the Nitrogen Dioxide Air Quality Standard	1-2
1.3.1	Historical Details.....	1-2
1.4	Current National Long-Term Annual Average NO ₂ Standard.....	1-3
1.5	World Health Organization (WHO) Guidelines	1-4
1.6	Environmental Justice.....	1-5
1.7	Public Outreach and Review	1-5
1.8	Air Quality Advisory Committee Review	1-5
1.9	References.....	1-6
2	Physics and Chemistry of Nitrogen Dioxide	2-1
2.1	Introduction	2-1
2.2	Sources of Nitrogen Dioxide	2-1
2.2.1	Direct Emissions.....	2-1
2.2.2	Formation from Reactions in Air.....	2-2
2.3	Relationship of Nitrogen Oxides to Ozone Formation	2-2
2.3.1	Oxides of Nitrogen and the Photostationary-State Relationship for Ozone.....	2-2
2.3.2	VOC Oxidation Cycle	2-3
2.3.3	Nitrogen Dioxide and Radical Sink Reaction	2-4
2.4	Formation of Nitrate-Containing Particles.....	2-4
2.5	Acidity in Ambient Air.....	2-7
2.5.1	Acid Deposition.....	2-7
2.5.2	Acid Fog	2-8
2.6	Formation of Toxic Nitrogen Oxide Derivatives	2-8
2.6.1	Peroxyacetyl Nitrate and Other Organic Nitrates	2-8
2.6.2	Nitro-PAHs.....	2-9
2.6.3	Nitrosamines.....	2-9
2.7	Natural Processes and Nitrogen Dioxide Levels in California	2-10
2.7.1	Background Levels of Nitrogen Dioxide	2-10
2.7.2	Natural Processes that Remove Nitrogen Dioxide.....	2-10
2.8	Spatial and Temporal Variations in Nitrogen Dioxide Levels	2-10
2.8.1	The Role of Weather	2-10
2.8.2	Variations in Nitrogen Dioxide Levels: Seasonal, Diurnal, and Spatial.....	2-11
2.9	Visibility Impairment.....	2-11
2.10	ARB-Sponsored Research Involving NO ₂ Chemistry	2-11
2.10.1	Surface (Heterogeneous) Reactions of NO ₂	2-11
2.10.2	Biological Processes and NO _x Emissions	2-12
2.10.3	Rates for Gas-Phase Nitric Acid Formation	2-12
2.11	References.....	2-12
3	Measurement of Nitrogen Dioxide	3-1
3.1	Introduction	3-1
3.2	Existing Monitoring Methods.....	3-1
3.3	Chemiluminescence Methodology Principle.....	3-2
3.4	Recommendation.....	3-3
3.5	Estimated Costs and Impacts	3-5
3.6	References.....	3-5

4	Sources and Emissions	4-1
4.1	Introduction	4-1
4.1.1	Mobile	4-1
4.1.2	Stationary and Area-Wide	4-1
4.1.3	Spatial and Temporal Distributions	4-1
4.2	Emissions of NO _x	4-1
4.2.1	NO _x Emission Trends and Forecasts	4-2
4.3	References	4-4
5	Exposure to Nitrogen Dioxide	5-1
5.1	Introduction	5-1
5.2	Area Designations for the State Nitrogen Dioxide Standards	5-1
5.2.1	Types of Designations	5-1
5.2.2	Determination of Attainment in California	5-2
5.2.3	Excluding “Highly Irregular or Infrequent” Concentrations	5-2
5.2.4	State Area Designations	5-3
5.2.5	Federal Area Designations	5-3
5.3	Monitoring Network	5-6
5.4	Characterization of Ambient Nitrogen Dioxide Air Quality	5-8
5.4.1	Overview	5-8
5.4.2	Federal Attainment of Nitrogen Dioxide	5-8
5.4.3	Ambient 1-Hour Nitrogen Dioxide Concentrations	5-10
5.4.4	Nitrogen Dioxide Season	5-12
5.4.5	Frequency of Measured 1-Hour Nitrogen Dioxide Concentrations	5-13
5.4.6	Diurnal Variations	5-29
5.4.7	Characterization of Nitrogen Dioxide by Air Basin or Planning Area	5-29
5.5	Analysis of Peak Nitrogen Dioxide Exposure in California	5-41
5.5.1	Introduction	5-41
5.5.2	Calculation of Peak Outdoor Nitrogen Dioxide Exposures	5-41
5.6	Indoor and Personal Exposure to Nitrogen Dioxide	5-52
5.6.1	Introduction	5-52
5.6.2	Nitrogen Dioxide Concentrations	5-52
5.6.3	Factors that Influence Indoor Nitrogen Dioxide Concentrations	5-56
5.6.4	Personal Exposures to NO ₂	5-61
5.6.5	Indoor Chemistry of Nitrogen Dioxide and Related Compounds	5-62
5.6.6	Summary	5-63
5.6.7	Conclusions	5-63
5.7	Summary of Micro-Environmental In-Vehicle Nitrogen Dioxide Measurements	5-63
5.7.1	Mobile Monitoring of NO/NO ₂ in Los Angeles	5-63
5.8	Spatial Variability of NO ₂ Concentrations	5-67
5.9	References	5-68
6	Controlled Human Exposure Studies	6-1
6.1	Summary	6-1
6.2	Introduction	6-2
6.3	Human Clinical Studies of Air Pollution: An Overview	6-2
6.4	Human Clinical Studies of Exposure to NO ₂	6-4
6.4.1	Effects of NO ₂ in Healthy Individuals	6-4
6.4.2	Effects of NO ₂ on Subjects with Asthma	6-8
6.4.3	Effect of NO ₂ on Subjects with Chronic Obstructive Pulmonary Disease	6-14
6.4.4	Effect of NO ₂ on Subjects with Cardiovascular Disease	6-14
6.5	Pollutant Concentration/Dose-Response Function	6-15
6.6	NO ₂ and Other Pollutants	6-15
6.7	Clinical Exposure Studies in Children	6-17
6.8	Other Considerations	6-17

6.9	Summary of Clinical Studies of NO ₂	6-18
6.10	References.....	6-20
6.11	Tables	6-26
7	Epidemiological Studies	7-1
7.1	Outdoor Community-Based Studies: Short-Term Exposure	7-2
7.1.1	Time-Series Studies on Mortality	7-2
7.1.2	Time-Series Studies on Morbidity among Adults (Hospital Admission, Emergency Room Visits 7-4	
7.1.3	Time-Series Studies on Asthma Morbidity among Children (Hospital Admissions and Urgent Care Visits)	7-6
7.2	Panel Studies on Asthmatic Children and Adults with Cardiac Arrhythmias.....	7-8
7.2.1	Asthma	7-8
7.2.2	Cardiovascular Effects	7-10
7.2.3	Summary	7-11
7.3	Studies of Chronic Exposure	7-11
7.3.1	Asthma, Respiratory Diseases, Lung Function	7-12
7.3.2	Cancer.....	7-14
7.3.3	Fetal Effects – Reproductive and Birth Effects.....	7-15
7.3.4	Infant Mortality.....	7-15
7.3.5	Adult Mortality.....	7-16
7.3.6	Indoor Studies	7-16
7.4	Summary and Conclusions.....	7-18
7.5	References.....	7-19
7.6	Tables	7-29
8	Toxicological Studies in Experimental Animals and In Vitro Test Systems	8-1
8.1	Introduction	8-1
8.2	Dosimetry of Nitrogen Dioxide in the Respiratory Tract	8-2
8.2.1	Summary	8-3
8.3	Respiratory Tract Effects	8-4
8.3.1	Morphological Effects	8-4
8.3.2	Summary	8-5
8.3.3	Inflammation and Lung Permeability Changes	8-9
8.3.4	Biochemical Effects	8-13
8.3.5	Lung Host Defense.....	8-19
8.3.6	Effects on Pulmonary Function	8-27
8.3.7	Effects on the Pulmonary Immune Response and Interaction with Allergens	8-31
8.3.8	Summary	8-34
8.4	Systemic Effects	8-38
8.4.1	Summary	8-39
8.5	Effects on Development.....	8-42
8.5.1	Summary	8-46
8.6	Genotoxic, Mutagenic and Carcinogenic Effects.....	8-52
8.6.1	Genotoxicity and Mutagenicity Studies	8-52
8.6.2	Carcinogenicity and Co-carcinogenicity Studies	8-52
8.6.3	<i>In vivo</i> formation of carcinogens from NO ₂	8-53
8.7	Atmospheric Formation of Mutagenic Reaction Products	8-54
8.7.1	Summary	8-54
8.8	Interactions of Nitrogen Dioxide with Other Co-occurring Pollutants	8-56
8.8.1	Non-cancer Toxicological Studies with Co-occurring Pollutants.....	8-56
8.8.2	Genotoxicity/Carcinogenicity Studies with Co-occurring Pollutants.....	8-57
8.8.3	Summary	8-58
8.9	Toxicological Effects in Human In Vitro Test Systems	8-63
8.9.1	Summary	8-65
8.10	Summary of Relevant Effects	8-69

8.11	Effects reported at or below 0.25 ppm.....	8-69
8.12	Effects reported at concentrations above 0.25 ppm.....	8-70
8.13	Conclusions	8-73
8.14	References.....	8-75
9	Effects of Nitrogen Dioxide on Vegetation	9-1
9.1	Introduction	9-1
9.2	Sources and Atmospheric Chemistry of Nitrogen Compounds	9-2
9.2.1	Emissions, Transformations, and Transport of Nitrogen Oxides	9-2
9.2.2	Spatial and Temporal Patters of NO ₂ Concentrations.....	9-4
9.3	Pollutant Uptake	9-5
9.3.1	Uptake of NO _x from the Atmosphere	9-5
9.3.2	Biochemical Reactions	9-6
9.3.3	Assimilated NO ₂ as a Source of Available Plant Nitrogen	9-7
9.4	Effects of NO ₂ on Plants	9-7
9.4.1	Foliar Injury.....	9-7
9.4.2	Physiological, Growth, and Yield Responses.....	9-8
9.5	Ecological Effects of Nitrogen Deposition.....	9-13
9.5.1	The Nitrogen Cycle.....	9-13
9.5.2	Sources of N Deposition to California Ecosystems.....	9-13
9.5.3	Rates of Nitrogen Deposition to California Ecosystems	9-13
9.5.4	Ecological Effects of Nitrogen Deposition in California.	9-17
9.5.5	Critical Loads for Nitrogen Deposition.....	9-18
9.5.6	Oxides of Nitrogen and Global Climate Change	9-19
9.6	References.....	9-21
10	Effects on Visibility	10-1
10.1	Introduction	10-1
10.2	Visibility Reduction Due to NO ₂	10-1
10.2.1	Human Visual Perception	10-1
10.2.2	Extinction of Light in the Atmosphere	10-2
10.2.3	Optical properties of NO ₂	10-4
10.2.4	Coloration of the Atmosphere by NO ₂	10-5
10.2.5	Interactions of NO ₂ with its Environment.....	10-5
10.2.6	Nitrate Visibility Reducing Particles	10-6
10.3	References.....	10-8

Appendices

- A. Office of Environmental Health Hazard Assessment Recommendation for Ambient Air Quality Standard for Nitrogen Dioxide
- B. Findings of the Air Quality Advisory Committee
- C. Staff comments and responses to AQAC Review
- D. Staff comments and responses to Public Comments

Acronyms

AAQS	Ambient Air Quality Standard
ach	air changes per hour
ADAM	Aerometric Data Analysis & Management database; an ARB database that allows users to review historical air quality data.
AGL	above ground level
AIC	Akaike information criterion
AM	alveolar macrophage
ANSA	8-amino-1-naphthalene-sulfonic acid ammonium salt
APHEA	the first “Air Pollution & Health – a European Approach” study, encompassing 11 teams of researchers from 10 European Union countries
APHEA-2	the second “Air Pollution & Health – a European Approach” study, from 29 European Union cities
AQAC	Air Quality Advisory Committee
AQDA	Air Quality Data Action
AQMD	Air Quality Management District
ARB	Air Resources Board
ARDS	acute respiratory distress syndrome
ARR	arrhythmia
β agonist	beta-agonist
BAL	bronchioalveolar lavage
BC	black carbon
BHR	bronchial hyperresponsiveness
B/R ratio	blue/red ratio of light
BS	black smoke
Cal/EPA	California Environmental Protection Agency
CAP	concentrated ambient air particles
CB-DPFs	catalyst-based diesel particle filters
CD4+, CD8+	types of white blood cells
CFR	Code of Federal Regulations
CH ₃ CHO	acetaldehyde
CH ₃ C(O)OONO ₂	peroxyacetyl nitrate
CHF	congestive heart failure
(CH ₃) ₂ NNO	dimethylnitrosamine (also DMN)
CH ₄	methane
CI	confidence interval
CNG	compressed natural gas

CO	carbon monoxide
CO ₂	carbon dioxide
COPD	chronic obstructive pulmonary disease
CVD	cardiovascular disease
DLCO	diffusing capacity of the lung for carbon monoxide
DNA	deoxyribonucleic acid
e	representation of the number (2.7182818...) used as the base for natural logarithms.
EC	elemental carbon
ECP	eosinophilic cationic protein
EPDC	Expected Peak Day Concentration
FEF _x	forced expiratory flow; related to some portion of the FVC curve. Modifiers refer to the amount of the FVC already exhaled when the measurement is made.
FEV	forced expiratory volume; denotes the volume of gas that is exhaled in a given time interval (typically 1 sec) during the execution of a forced vital capacity (see FVC)
FIVC	forced inspiratory vital capacity; the maximal volume of air inspired with a maximally forced effort from a position of maximal expiration.
FRC	functional residual capacity
FVC	forced vital capacity, performed with a maximally forced expiratory effort
FRM	Federal Reference Method
g	gram
GSGOGAT	glutamine synthase/glutamine oxoglutarate aminotransferase cycle
GIS	Global Information System
GSH	glutathione
GWP	global warming potential
ha	hectare
HCHO	formaldehyde
HDM	house dust mites
HiVol sampler	high-volume air sampler
HNO ₃	nitric acid
H ₂ O	water
HO ₂	hydroperoxy radical
HONO	nitrous acid
HOOH	hydrogen peroxide
HOONO	peroxynitrous acid
H&SC	Health & Safety Code (California)
hν	solar radiation
IgE	immunoglobulin E
IHD	ischemic heart disease

IQR	interquartile range
kg	kilogram
km ²	square kilometer
LTAB	Lake Tahoe Air Basin
m	meter
MCAB	Mountain Counties Air Basin
MDAB	Mojave Desert Air Basin
MEF50	mid-expiratory flow
MMEF	maximum mid-expiratory flow; mean forced expiratory flow during the middle half of the forced vital capacity (FVC)
MWe	megawatt electrical output
N ₂	nitrogen gas
N ₂ O	nitrous oxide
NAAQS	National Ambient Air Quality Standards
NaNO ₃	sodium nitrate
NCAB	North Coast Air Basin
NCCAB	North Central Coast Air Basin
NH ₄ ⁺	ammonium ion
NK cells	natural killer cells, a type of white blood cell
nm	nanometer; one-billionth of a meter
NMMAPS	National Morbidity and Mortality Air Pollution Study
NO	nitric oxide
NO ₂	nitrogen dioxide
NO ₃ ⁻	nitrate ion
N ₂ O ₅	dinitrogen pentoxide
NO _x	nitrogen oxides: defined as nitrogen dioxide plus nitric oxide
NO _y etc.	reactive nitrogen species: NO + NO ₂ + HNO ₃ + HONO + N ₂ O ₅ + HNO ₃ + organic nitrates, etc.
NH ₃	ammonia
NH ₄ NO ₃	ammonium nitrate
nitro-PAH	nitro-polycyclic aromatic hydrocarbon
[ν]	(Greek letter nu) light frequency (sec ⁻¹)
O	atomic oxygen
O ₂	molecular oxygen
O ₃	ozone
OC	organic carbon
OEHHA	Office of Environmental Health Hazard & Assessment
OH [·]	hydroxyl radical

OR	odds ratios
PAH	polycyclic aromatic hydrocarbon
PAN	peroxyacetyl nitrate
pH	measure of acidity or alkalinity
PM	particulate matter
PM2.5	particulate matter of 2.5 microns or less in aerodynamic diameter
PM10	particulate matter of 10 microns or less in aerodynamic diameter
PMN	polymorphonuclear (leukocyte)
ppb	parts per billion by volume
ppb-hr	parts per billion hours (i.e., sum of concentration multiplied by duration), a measure of exposure to nitrogen dioxide).
ppm	parts per million by volume
ppm-hr	parts per million hours (i.e., sum of concentration multiplied by duration), a measure of exposure to nitrogen dioxide).
PTSD	Planning and Technical Support Division
QAS	Quality Assurance Section
R	any fragment of an organic molecule
RH	a molecule of a volatile organic chemical or hydrocarbon
R ₂ NH	amines
R ₂ NNO	nitrosamines
RO	alkoxy radical
RO ₂	alkylperoxy radical
SAPALDIA	Swiss Study on Air Pollution and Lung Diseases in Adults
SCCAB	South Central Coast Air Basin
SDAB	San Diego Air Basin
SF ₆	sulfur hexafluoride, a type of tracer gas used for monitoring flow rates of gases and direction of air masses
SFBAAB	San Francisco Bay Area Air Basin
SIP	State Implementation Plan
SJVAB	San Joaquin Valley Air Basin
SO ₂	sulfur dioxide
SoCAB	South Coast Air Basin
SoCalGas	Southern California Gas Company
SRaw	specific airway resistance
SSAB	Salton Sea Air Basin
SVAB	Sacramento Valley Air Basin
TrPD	average number of diesel-powered trucks per day
TSP	total suspended particulates

UFP	ultra-fine particles
μg	microgram, equivalent to one millionth of a gram (= 0.000001 gram)
$\mu\text{g}/\text{m}^3$	micrograms per cubic meter
UK	United Kingdom
μm	micrometer or micron, equivalent to one millions of a meter (=0.000001 meter)
URI	upper respiratory infection
U.S. EPA	United States Environmental Protection Agency
uv	ultraviolet (light)
VOC	volatile organic chemical
WHO	World Health Organization

1 Introduction and Overview

1.1 Regulatory

An “Ambient Air Quality Standard” (AAQS) is a legal definition of clean air. It establishes the maximum allowable levels of air pollutants that can be present in outdoor air for a given averaging time without causing harmful health effects to most people. Health and Safety Code section 39606 authorizes the Air Resources Board (ARB) to adopt standards for ambient air quality that are developed: “in consideration of public health, safety, and welfare, including but not limited to health, illness, irritation to the senses, aesthetic value, interference with visibility, and the effects on the economy”. The objective of an ambient air quality standard is to provide a basis for preventing or abating adverse health or ecological effects due to air pollution (Title 17, California Code of Regulations section 70101). The AAQS should not be interpreted as permitting, encouraging, or condoning the degradation of present air quality that is superior to that stipulated in the standards. Both the ARB and the local and regional air quality management districts work to achieve state and federal ambient air quality standards. Enforcement tools include rules and regulations on stationary sources, such as factory smoke stacks, and for mobile sources such as motor vehicles (Health and Safety Code section 40000, 40001, and 43000 et seq.).

During the adoption of the State AAQS a number of important factors are reviewed and evaluated by ARB, the Office of Environmental Health Hazard and Assessment (OEHHA), the Air Quality Advisory Committee (AQAC), and the public. In consultation with ARB, OEHHA provides detailed analyses of the available health information for each criteria pollutant. Health-based ambient air quality standards are based on the recommendation of OEHHA. The AQAC, a scientific peer review committee, meets to independently evaluate the scientific basis of draft recommendations for revising the California AAQS. The public is involved in the review process through public meetings and may comment on the review through the ARB’s AAQS web site.

As a part of developing an AAQS, a margin of safety is added to account for possible deficiencies in the data and measuring methodology. An important underlying premise of the AAQS evaluation process is to ensure that sensitive sub-populations be protected from adverse health effects. Although many of the studies used in supporting the AAQS have incorporated sensitive populations such as asthmatics in their analyses, most have not specifically included infants or children. California has been in the forefront of studies involving the health effects on children exposed to air pollutants.

1.1.1 Ambient Air Quality Standards and Children

The Children’s Environmental Health Protection Act (Senate Bill 25, Escutia; Stats. 1999, Ch. 731) required the ARB, in consultation with OEHHA, to evaluate all existing health-based standards for their adequacy to protect human health, including that of infants and children (Health and Safety Code section 39606 [d] and [e]) (ARB, 2000). The ensuing evaluation of existing AAQS found that health effects may occur in infants, children and other groups of the population exposed to certain pollutants. Particulate matter (including sulfates), ozone and nitrogen dioxide (NO₂) were prioritized for review (ARB, 2000). The ARB has since adopted more health-protective standards for both particulate matter and ozone based on this review process and now is reviewing the nitrogen dioxide standard. The standards for carbon monoxide, sulfur dioxide, hydrogen sulfide, and lead may be considered for later review.

1.2 Current California Ambient Air Quality Standard for NO₂

The current California Ambient Air Quality Standard for NO₂ is 0.25 ppm averaged over one hour. This value is not to be exceeded. In Title 17, California Code of Regulations section 70200, the most relevant health and welfare effects from NO₂ exposure are listed. The short-term exposure effect cited is: a potential to aggravate chronic respiratory disease and respiratory symptoms in sensitive groups. Further, there is risk to public health implied by pulmonary and extra-pulmonary biochemical and cellular changes and pulmonary structural changes, which are observed in short-term animal tests at or above the concentration of the standard. The welfare effect cited is: contribution to atmospheric discoloration. See the ‘Most Relevant Effects’ column for 1987 in the NO₂ history table below for further details.

1.3 History of the Nitrogen Dioxide Air Quality Standard

The history of the NO₂ ambient air quality standard is summarized in Table 1 and chronologically detailed in the following text.

History of the One-Hour Nitrogen Dioxide Ambient Air Quality Standard in California			
Year	Standard (parts per million)	Most Relevant Effects	Comments
1955	3	1st Alert: Close approach to maximum allowable concentration for the population at large. Still safe but approaching a point where preventative action is required.	Developed for Los Angeles Air Pollution Control District.
	5	2nd Alert: Air Contamination level at which a health menace exists in a preliminary stage.	
	10	3rd Alert: Air Contamination level at which a health menace exists.	
1959	0.15 "oxidant"	At this time, NO ₂ thought to be one component of "oxidant." Set at the lowest concentration at which eye irritation, vegetative damage and visibility reduction had been reported.	First statewide oxidant standard
1966	3	Bronchoconstriction	First statewide nitrogen dioxide standard.
	0.25	Atmospheric discoloration	
1969	0.25	Health basis for standard: A risk to public health is implied by harmful health effects observed in experimental animals. Welfare basis for standard: atmospheric irritation.	
1985	0.25	Method of analysis changed from the "Saltzman" method to gas phase luminescence.	
1987	0.25	a. 1. Potential to aggravate chronic respiratory disease and respiratory symptoms in sensitive groups. 2. Risk to public health implied by pulmonary and extra-pulmonary biochemical and cellular changes and pulmonary structural changes, which are observed in short-term animal tests at or above the concentration of the standard. 3. Contribution to atmospheric discoloration.	a. The standard is intended to prevent adverse health effects. b. The standard imposes an upper limit on adverse effects on welfare, including atmospheric discoloration by NO ₂ .
1992	0.25	The 1987 standard was reviewed and upheld by the Air Resources Board	

1.3.1 Historical Details

1955

The first ambient (outdoor) air quality standard in California was set in the Los Angeles area because of the severity of smog there. The Los Angeles Air Pollution Control District adopted a three-stage alert system for NO₂ and three other pollutants in June of 1955. This system was designed to prevent a possible air pollution disaster in Los Angeles County.

1959

At this time, NO₂ was considered to be one component of "oxidant," a term used to describe a group of chemicals (not including molecular oxygen) capable of oxidizing a reagent (potassium iodide). In 1959, the California Legislature instructed the Department of Public Health to develop and publish statewide air quality standards for California (Assembly Bill 1386). On December 4th of that year, the State Department of Public Health's Advisory Committee on Air Sanitation set the first ambient air quality standards for California. The Health Department established three levels of standards: Adverse, Serious and Emergency. While state air quality standards for other pollutants were set for all three levels, the oxidant standard was established for only the adverse level.

1966

In January of 1966, the Department of Public Health established separate health and welfare standards for NO₂: a one-hour standard of 0.25 ppm, designed to prevent or mitigate atmospheric discoloration; and a separate health-based one-hour standard of 3.0 ppm based on the limited information on health effects at that time.

1967

In 1967, the Mulford-Carrell Act established the Air Resources Board (ARB), and authorized the agency to set air quality standards for the State of California.

1969

On September 17, 1969, ARB adopted a stricter, more health-protective one-hour standard for NO₂ based on two types of data: effects of NO₂ on laboratory animals and effects of NO₂ on atmospheric discoloration. At the time, it was felt that human health effects data alone could not support a standard due to the paucity and poor quality of experimental research on humans. However, at doses slightly higher than the level of the standard, the short-term NO₂ exposure resulted in harmful health effects on experimental animals, such as increased susceptibility to experimental infection, which implied a risk to the public's health. ARB chose to adopt a combination health and welfare standard. The 0.25 one-hour standard was the same as the pre-ARB standard based on discoloration alone.

1985

On April 30, 1985, ARB adopted a new measurement method for NO₂, changing the measurement method from the "Saltzman method" to gas phase chemiluminescence.

1987

On January 9, 1987, ARB formally adopted an amended NO₂ standard with an expanded health basis. This standard sought to prevent or alleviate the potential aggravation of chronic respiratory disease and respiratory symptoms associated with NO₂ exposure in sensitive groups. Supportive data from short-term exposure studies in laboratory animals showed harmful health effects at or above the concentrations of the standard, indicating a potential risk to public health. Investigators reported that biochemical, cellular, and structural changes in lungs and biochemical and cellular changes in other organ systems resulted from experimental exposure to NO₂.

1992

In 1992, the ARB reviewed the scientific literature on health effects of NO₂. Since the 1987 review, there was additional data to support effects of low-level NO₂ exposure on sensitive human populations. Collectively, results suggested that a subgroup of asthmatics had increased bronchial reactivity to NO₂ at 0.25 ppm but there were inconsistencies in the studies. Epidemiological studies (especially those using the presence of an un-vented gas stove as a surrogate for NO₂) suggested an increased risk of respiratory symptoms or disease in children exposed to NO₂. However the exposure assessments in these studies were not sufficient to indicate a specific level and averaging time for a short-term standard. The toxicological studies in animals suggested allergic and inflammatory responses in the lungs, possible immune effects, and biochemical changes in other organs. Overall, it was concluded that the epidemiological studies, controlled exposure studies of humans and animals provided evidence for adverse effects of NO₂, but there was limited evidence of adverse effects resulting from exposure to NO₂ at concentrations below the standard. Thus, the NO₂ 1-hr standard of 0.25 ppm was retained.

1.4 Current National Long-Term Annual Average NO₂ Standard

The current national air quality standard for NO₂ was initially adopted in 1971 and was last reviewed in 1995 (US EPA 1993, 1995). It is an annual standard of 0.053 ppm (100 µg/m³) calculated as the arithmetic mean of the 1-hour NO₂ concentrations. The value is based in part, on epidemiological studies where investigators reported decreased lung function (FEV₁) for children (ages 7 to 8) living in areas with relatively high (greater than 0.06 ppm) annual average NO₂ levels (Shy et al. 1970 a,b). However, follow-up studies by the same investigator could not support these initial findings (Shy et al. 1973, 1978; Pearlman et al. 1971). Emphasis was placed on animal studies exposed to relatively high concentrations

of NO₂. Investigators reported damage to host defense mechanisms, as well as emphysematous-like lesions in the lungs. Investigators have also reported that NO₂ exposure caused an increase in the animal's susceptibility to infection resulting from immune system effects (US EPA 1995). The U.S. EPA indicated, that "based on the data available in 1985, retaining the annual NAAQS of 0.053 ppm was seen as a means of providing protection from long-term health effects and some measure of protection against possible short-term health effects (50 FR 25541, June 19, 1985). In 1995, the staff paper cited evidence for small changes in pulmonary function in asthmatics between 0.2 and 0.5 ppm and increased airway responsiveness to asthmatics at rest within the range of 0.2-0.3 ppm. A meta-analysis of studies in children living in homes with gas stove provided support for increased risk for developing respiratory disease but it was difficult to use these studies to establish a quantitative relationship between estimated exposure and symptoms for use in determining a standard. Thus, an annual average standard of 0.053 ppm was retained during the last review.

1.5 World Health Organization (WHO) Guidelines

The World Health Organization (WHO) has published Air Quality Guidelines for Europe (WHO 2000a,b, 2003, 2005) which are not ambient air quality standards, but are "the basis for protecting public health from adverse effects of air pollutants, eliminating or reducing exposure to hazardous air pollutants, and to guide national and local authorities in their risk management decisions." (WHO 2000a,b). The WHO guidelines include both toxic air pollutants, such as benzene, and criteria pollutants such as NO₂.

Based on the review of the literature, the WHO indicated that the lowest observable acute effect level for NO₂ was near 0.2 to 0.3 ppm based on clinical studies showing increased airway responsiveness in asthmatics). However, it was difficult to determine "... a clearly defined concentration-response relationship for NO₂ exposure..." (WHO, 2000a,b). The WHO also indicated that it would propose a 50% margin of safety because of additional evidence of possible effects below 0.2 ppm. These include a statistically significant increase in response to a bronchoconstrictor (increased airway responsiveness) with exposure to 190 µg/m³ (0.1 ppm) in one study (Orehek et al. 1976) and a pooled analysis suggesting changes in airway responsiveness in asthmatics below 365 µg/m³ (0.2 ppm). On the basis of these human clinical data, WHO (2000a,b) proposed a 1-hour guideline of 200 µg/m³ (0.106 ppm).

For long-term chronic exposure, the WHO reported that "although there is no particular study or set of studies that clearly support selection of a specific numerical value for an annual average guideline, the database nevertheless indicates a need to protect the public from chronic NO₂ exposure." Epidemiological studies of exposures to NO₂ from indoor sources suggested increased risk of lower respiratory illness in children, but the exposures could not be readily extrapolated to the outdoor situation. The WHO 2000 report stated, "Outdoor epidemiological studies have found qualitative evidence of ambient exposures being associated with increased respiratory symptoms and lung function decreases in children (annual average concentrations of 50–75 µg/m³ (0.026– 0.040 ppm or higher) and consistent with findings from indoor studies, although they do not provide clear exposure-response information for NO₂. In these epidemiological studies, NO₂ has appeared to be a good indicator of the pollutant mixture. Furthermore, animal toxicological studies show that prolonged exposures can cause decreases in lung host defenses and changes in lung structure." The WHO recommended an annual value of 40 µg/m³ (21 ppb) (WHO 1997, 2000a,b) but acknowledged that there were difficulties in ascribing the observed effects solely to NO₂ because of other co-pollutants in the ambient air that were correlated with NO₂.

The WHO recently published an update of its guidelines (WHO, 2005) and reaffirmed the WHO 2000 guideline values of 40 µg/m³ (21 ppb) for annual mean and 200 µg/m³ (0.106 ppm) for 1-hour mean. The WHO 2005 report also recognized that although epidemiological studies have attempted to focus on health risks of NO₂, "the contributing effects of other, highly correlated co-pollutants (such as fine PM) were often difficult to rule out." Nonetheless, the WHO 2005 report found that "these (epidemiological) associations cannot be completely explained by co-exposure to PM, other components in the mixture (such as organic carbon and nitrous acid vapour) might explain part of the association, and ... there may be direct toxic effects of chronic NO₂ exposures at lower levels." The WHO report stated that since many of the unmeasured co-pollutants are correlated with NO₂, "it seems reasonable to retain a prudent annual average limit value for NO₂. In addition, the annual guideline value may help to control complex mixtures of combustion-related pollution (mainly from road traffic)." Based on this rationale, the WHO reaffirmed the annual value for NO₂ of 40 µg/m³ (21 ppb).

1.6 Environmental Justice

Environmental justice is defined as “the fair treatment of people of all races, cultures, and incomes with respect to the development, adoption, implementation, and enforcement of environmental laws, regulations, and policies” (Senate Bill 115, Solis; Stats 19999, Ch. 690; Government Code 65040.12(c)). The Board approved the Environmental Justice Policies and Actions on December 13, 2001, to establish a framework for incorporating environmental justice into ARB’s programs consistent with the directives of State law.

ARB’s environmental justice policies apply to all communities in California, but environmental justice issues have been raised more in the context of low-income and minority communities. These communities may experience higher exposures to some pollutants, such as NO₂, as a result of the cumulative impacts of air pollution from roadways and stationary facilities located in their neighborhoods.

To mediate these possible exposures in the future, local air pollution districts and community members need to work together in their land use evaluations to further reduce pollution exposure, including exposure to NO₂. The ARB has developed a guideline document on land use with respect to air quality entitled, “Air Quality Land Use Handbook: A Community Health Perspective” (ARB 2005). Because NO₂ is emitted from a variety of sources including those from vehicles and industry, land use considerations should involve the review of these NO₂-emitting sources. The ARB handbook recommends that planning agencies strongly consider proximity to these sources when considering new locations for “sensitive” land uses, such as homes, medical facilities, daycare centers, schools, and playgrounds. The handbook is available from the ARB website at <http://www.arb.ca.gov/ch/handbook.pdf>.

1.7 Public Outreach and Review

ARB and OEHHA staff will conduct public workshops on the development of the NO₂ standard and invite the public to openly address questions and provide comments, including those related to environmental justice.

A draft Staff Report and companion Technical Support Document containing staff’s preliminary findings entitled “Review of California Ambient Air Quality Standard for Nitrogen Dioxide” were released to the public on April 14, 2006. Community outreach for the standard review process involves a number of approaches to help effectively disseminate information, including mailings, web “list serve” announcements, public meetings, and workshop presentations. The web list serve notifies the public of scheduled public meetings and workshops, invitations to submit comments on the Staff Report and Technical Support Document, and the availability of these documents. Public workshops on the proposed NO₂ standard are currently planned for Sacramento and El Monte.

Individuals or parties interested in signing up for electronic mail via e-mail “list serve” to receive notifications and information on related air quality issues, may enroll free of charge at the following internet location: www.arb.ca.gov/research/aaqs/aaqs.htm. Additional information on the standards review process is also available at the NO₂ review schedule website at: www.arb.ca.gov/research/aaqs/no2-rs/no2-rs.htm.

1.8 Air Quality Advisory Committee Review

An external independent scientific peer review committee, AQAC, was appointed by the President of the University of California in December 2004. Members have expertise in fields such as chemistry, toxicology, physiology, biochemistry, biology, atmospheric processes, medicine, and environmental health effects. The AQAC will meet in the spring of 2006 to review and discuss the draft NO₂ Staff Report, Technical Support Document, staff recommendations, and public comments. The purpose of this review is to ensure that the scientific basis of the recommendations for the NO₂ standard is based on sound scientific knowledge, methods, and practices. The AQAC meetings are public and allow time for oral public comments.

1.9 References

- ARB, Air Resources Board and Office of Environmental Health Hazard Assessment (2000). Adequacy of California Ambient Air Quality Standards: Children's Environmental Health Protection Act. Staff Report. Sacramento, CA. Available at <http://www.arb.ca.gov/ch/programs/sb25/airstandards.htm>
- ARB. Proposed Air Quality Land Use Handbook: A Community Health Perspective, April 2005.
- Orehek J, Massari JP, Gayrard P, Grimaud C, Charpin J. 1976. Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. *J Clin Invest* 57: 301-7.
- Pearlman ME, Finklea JF, Creason JP, Shy CM, Young MM, Horton RJM. 1971. Nitrogen dioxide and lower respiratory illness. *Pediatrics* 47:391-398.
- Shy CM, Creason JP, Perlman ME, McClain KE, Benson FB, Young MM. 1970a. The Chattanooga school children study: effects of community exposure to nitrogen dioxide. 1. Methods, description of pollutant exposure, and results of ventilatory function testing. *J. Air Pollut. Control Assoc.* 20:539-545.
- Shy CM, Creason JP, Perlman ME, McClain KE, Benson FB, Young MM. 1970b. The Chattanooga school children study: effects of community exposure to nitrogen dioxide. II. Incidence of acute respiratory illness. *J. Air Pollut. Control Assoc.* 20:582-588.
- Shy CM, Niemeyer L, Truppi L, English M. (1973). Re-evaluation of the Chattanooga school children studies and the health criteria for NO₂ exposure (revised draft). Research Triangle Park, NC: US EPA, Human Studies Laboratory
- Shy CM, Kleinbaum DG, Morgenstern H. (1978). The effect of misclassification of exposure status in epidemiological studies of air pollution effects. *Bull. NY Acad. Med.* 54:1155-1165.
- U.S. EPA. 1993. Air Quality Criteria for Oxides of Nitrogen. Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Research Triangle Park, NC. EPA/600/8-91/049F
- U.S. EPA. 1995. Review of the National Ambient Air Quality Standards for Nitrogen Dioxide. Office of air Quality Planning and Standards, Research Triangle Park, NC. EPA/452/R-95-005.
- WHO. World Health Organization "Air quality guidelines for Europe". WHO Reg Publ Eur Ser. 2000a; (91): V-X, 1-273
- WHO. World Health Organization, Evaluation and use of epidemiological evidence for environmental health risk assessment. Guideline document. Geneva, 2000b. <http://www.euro.who.int/document/e68940.pdf>
- WHO. World Health Organization Air Quality Guidelines Global Update 2005. Report on a working group meeting, Bonn Germany, 18-20 October 2005.
- WHO. World Health Organization "Health Aspects of Air Pollution with Particulate Matter, Ozone and Nitrogen Dioxide". Report from WHO Working Group Meeting, Bonn, 13 - 15 January 2003. <http://www.euro.who.int/document/e79097.pdf>
- WHO. *Nitrogen oxides*. Geneva, 1997 (Environmental Health Criteria, No. 188).

2 Physics and Chemistry of Nitrogen Dioxide

2.1 Introduction

NO₂ is a pungent gas that, along with fine airborne particulate matter, contributes to the reddish-brown haze characteristic of smoggy air in California. NO₂ is one of the nitrogen oxides (NO_x) that is emitted from high-temperature combustion processes, such as those occurring in automobiles and power plants. (NO_x is defined as nitric oxide [NO] + NO₂.) Home heaters and gas stoves also produce substantial amounts of NO₂ in indoor settings.

Exposure to NO₂ is associated with respiratory symptoms, episodes of respiratory illness, and reduced lung function. NO₂ reacts with the cells of the lung linings or their membranes, damaging them in the process. Animal lungs suffer biochemical, structural, and cellular changes when exposed to NO₂ at levels somewhat higher than the California 1-hour standard (0.25 ppm). Recent research in controlled human studies suggests that NO₂ exposure might worsen the effect of allergens in asthmatics. As a result of their review of criteria pollutant standards under the Children's Environmental Health Act (SB25) (<http://www.arb.ca.gov/research/aags/caags/ad-aags/ad-aags.htm>) in 2000, the ARB and OEHA staff recommended that the current NO₂ ambient air quality standard should be a high priority for review. See Chapter 6 - 8 for a more complete discussion of health effects from exposure to NO₂.

The NO₂ observed in the atmosphere is derived both from direct combustion emissions and from emissions of NO followed by its subsequent chemical conversion to NO₂. In sunlight NO₂ is a precursor in the formation of several other air pollutants, such as ozone (O₃), nitric acid (HNO₃), and nitrate [(NO₃)⁻]-containing particles. NO₂ levels in air vary with direct emission levels, and as changing conditions (e.g., sunlight) shift its relationship with other NO_x compounds in a complex chemical linkage. Not only is NO₂ the sole source of anthropogenic O₃, it is also the key agent in the formation of several toxic chemicals, such as HNO₃ and fine particles.

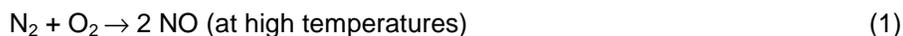
This chapter discusses the formation and removal of NO₂ from the atmosphere, and the key role of NO₂ formation in toxic air contaminants, particulate matter, and atmospheric acidity. Nitrogen oxides play an important role in the formation of other air pollution problems. Also discussed are research findings from ARB-sponsored research on NO₂ chemistry and emissions. The ARB sponsors general research in atmospheric processes that affect air pollution. For more information, see <http://www.arb.ca.gov/research/apr/past/atmospheric.htm#Projects>.

For more extensive information on the physics and chemistry of NO₂ as an air pollutant, the reader is referred to Finlayson-Pitts and Pitts (2000), Seinfeld and Pandis (1998), and Warneck (2000).

2.2 Sources of Nitrogen Dioxide

2.2.1 Direct Emissions

Nitrogen is the most abundant element in the air. During combustion, nitric oxide (NO) is formed from reaction (1) between atmospheric oxygen (O₂) and nitrogen, which exists primarily as atmospheric nitrogen (N₂), but is also organically bound in some fuels.



Nitric oxide may react further with oxygen to form NO₂:



The rate of reaction (2) varies with the square of the concentration of NO (second order). At concentrations generally found in ambient air, this reaction is extremely slow, and is therefore not the primary pathway of atmospheric formation of NO₂. However, the reaction can be significant in plumes where there are high emissions of NO and high temperatures. Usually, less than ten percent of the total NO_x emissions from combustion sources is in the form of NO₂, with NO accounting for most of the NO_x emitted.

The major reduction of NO_x emissions from light-duty motor vehicles with the introduction in the late 1970's of the 3-way catalyst resulted in a dramatic drop in NO₂ levels in ambient air. Chapter 5 provides data on recent trends for NO₂ levels in California's ambient air.

Catalyst-based diesel particle filters (CB-DPFs) are post-combustion devices that reduce emissions of particulate matter (PM). The catalyst oxidizes exhaust NO to NO₂ in order to facilitate the oxidation of carbon in the soot filter. The NO to NO₂ conversion can vary from approximately 20% to 70% (Ayala et al. 2002). Their use may lead to higher ambient levels of NO₂ in urban near-source locations.

2.2.2 Formation from Reactions in Air

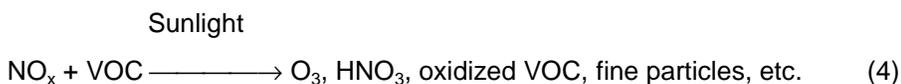
The major source of NO₂ in air is the oxidation of NO emitted from combustion processes. Nitric oxide is oxidized by hydroperoxy (HO₂) and alkylperoxy (RO₂) free radicals, both of which are reactive intermediates formed by the VOC-NO_x chemistry in air:



The chemistry leading to the formation of HO₂ and RO₂ is discussed in more detail below.

2.3 Relationship of Nitrogen Oxides to Ozone Formation

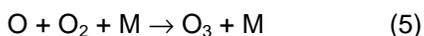
The formation of ozone is a complex process, but the overall reaction mechanism can be summarized as



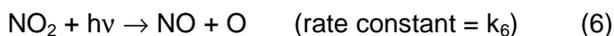
Key elements are summarized below.

2.3.1 Oxides of Nitrogen and the Photostationary-State Relationship for Ozone

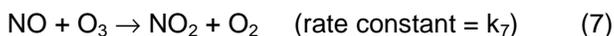
The formation of ozone in the troposphere ("anthropogenic O₃") results from only one known reaction: addition of atomic oxygen (O) to molecular oxygen (O₂) in the presence of a third "body" (M) that can absorb the excess energy. (M is any molecule, which in air is primarily nitrogen or oxygen, that can absorb energy from the reaction. Without this removal of energy as the bond between O and O₂ forms, dissociation of the O₃ generated occurs immediately so that there is no net reaction.)



The oxygen atoms are produced from photolysis of NO₂ by the ultraviolet portion (wavelength [λ] = 290 - 430 nm) of solar radiation (hν) that reaches the earth's surface.



Reaction 7 converts ozone back to oxygen and NO back to NO₂:



Ozone is not formed appreciably at night because the key step, reaction (6), requires light. It is also one of the reasons why ozone levels are high at mid-year, when radiation is at its most intense. Ozone accumulates over several hours, depending on emission rates of the precursors and the meteorological conditions. This chemistry leads to ozone concentrations which are related to the concentrations of NO and NO₂ in the following manner, which is known as the "photostationary-state":

$$[\text{O}_3]_{\text{photostationary-state}} = (k_6/k_7) \times [\text{NO}_2]/[\text{NO}] \quad (8)$$

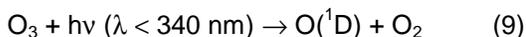
(brackets denote concentration)

If reactions (5) - (7) were all that occurred, the ozone concentrations would be determined solely by the NO and NO₂ concentrations and the rate of photochemical decomposition of NO₂ in reaction (6). However, much larger concentrations of ozone are observed in urban areas than expected based on the

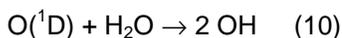
photostationary state, suggesting that there is an additional pathway for efficiently converting NO to NO₂ which then forms O₃. This is discussed in more detail in the following section.

2.3.2 VOC Oxidation Cycle

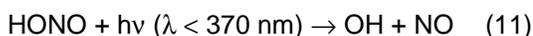
Hydrocarbons and other VOCs are oxidized in the atmosphere by a series of free radical-initiated reactions to form such compounds as carbon monoxide (CO), carbon dioxide (CO₂), and water (H₂O). Intermediate steps in this overall oxidation process typically involve cyclic stages driven by hydroxyl radical (OH) attack on the parent hydrocarbon, on partially oxidized intermediate compounds, and on other VOCs. The hydroxyl radical is ubiquitous in the ambient air. It is formed by photolysis from ozone in the presence of water vapor (reactions 9 and 10).



(¹D denotes a form of electronic excitation)

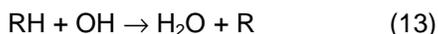


and directly from the photo-decomposition of nitrous acid (11), hydrogen peroxide (12), and other sources:



In urban areas, HONO is generally the major source of OH in the early morning, and also when averaged throughout the day.

In the sequence shown below, R can be virtually any organic fragment (e.g. alkyl radical). The oxidation process usually starts with reaction 13, involving OH attack on a hydrocarbon or a VOC (RH):



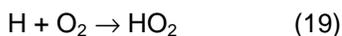
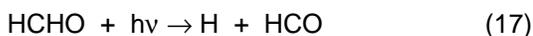
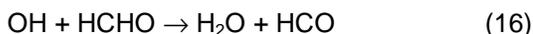
This is followed by reaction (14) with oxygen in the air to generate the alkylperoxy radical (RO₂).



The key reaction in the VOC oxidation cycle is the conversion of NO to NO₂. This takes place through the fast radical transfer reaction of the alkylperoxy radical with NO.



Hydroperoxy radicals (HO₂) are generated by similar reactions, and like RO₂, HO₂ radicals react with NO to yield NO₂. This is the critical step in the reaction mechanism. For example, formaldehyde (HCHO), a common intermediate in the oxidation of a variety of VOCs, forms HO₂ via reactions such as the following:



Reactions (18) and (19) generate HO₂, and the hydroxyl radical (consumed in reaction 16) is regenerated in reaction (20). Reactions (15) and (20) produce the NO₂ required for ozone formation. The ARB has been sponsoring research into the elucidation of these reactions.

2.3.3 Nitrogen Dioxide and Radical Sink Reaction

A critical reaction, with significant health implications, is reaction (21) leading to the formation of nitric acid.

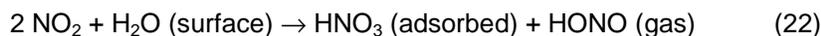


(abbreviated version: $\text{NO}_2 + \text{OH} \rightarrow \text{HNO}_3$)

This reaction not only generates HNO_3 , but also acts as a sink for the very reactive hydroxyl free radical. Nitric acid may exist as a vapor, or deposit on particles and other surfaces exposed to polluted air. (Ambient levels of nitric acid affect visibility, and exposure may lead to significant health effects in children.) This is an important reaction because it removes both NO_x and OH radicals from the atmospheric system, and terminates the chain reactions that lead to ozone formation.

In urban areas, NO levels are relatively high in the early morning due to primary emissions. After sunrise, which initiates the formation of OH and HO_2 radicals photochemically (from HONO, O_3 , HOOH, HCHO, etc.), the VOC- NO_x oxidation cycle described above begins. Subsequent NO to NO_2 conversion by the peroxy radical reactions (15) and (20) causes NO_2 to become the dominant NO_x species. When the NO_2 to NO ratio becomes large enough, ozone builds up. Meanwhile, NO_2 levels decrease via photolysis and reactions such as (21). Winds disperse and dilute NO_2 and ozone. During the day, NO_2 is also diluted by the increase in the inversion height. During the night, NO and ozone combine to form NO_2 and oxygen via reaction (3) until either the NO or ozone is consumed.

Nitrous acid (HONO) is emitted from fossil fuel combustion, such as from power plants. However, HONO is present at night in polluted ambient air in California, primarily as the result of a heterogeneous reaction between gaseous NO_2 and films of water on surfaces (a heterogeneous reaction involves a gas and a condensed phase):



Nitrous acid is released to the gas phase while the nitric acid formed simultaneously remains adsorbed to the surface. At sunrise, HONO photo-dissociates rapidly to generate OH, which reacts rapidly with VOCs to form HO_2 and RO_2 , setting off the photochemical reactions that lead to ozone and other air pollutants. The nitric acid has been thought to remain on the surface and thereby permanently removed from the gaseous VOC- NO_x cycle. Recent research has been conducted to determine the extent to which nitric acid is a permanent sink for NO_x . This topic is discussed further in Section 3.10.1.

2.4 Formation of Nitrate-Containing Particles

The information in this section is derived from "Chemistry of the Upper and Lower Atmospheres," Finlayson-Pitts and Pitts (2000), and two ARB staff reports: "The Effects of Oxides of Nitrogen on California Air Quality" (ARB 1985), and "Public Hearing to Consider Amendments to the Ambient Air Quality Standards for Particulate Matter and Sulfates" (ARB 2002). The latter report may be obtained from the following website:

<http://www.arb.ca.gov/research/aaqs/std-rs/pm-final/pm-final.htm>.

Nitrogen oxides emitted into polluted urban air are converted to particulate nitrate both by photochemical reactions during daylight hours and by "dark" reactions at night. After formation of NO_2 , the next step in the nitrate formation process is the oxidation of NO_2 to nitric acid. Nitric acid reacts with the only gaseous base found in the atmosphere, ammonia (NH_3), to generate ammonium nitrate (NH_4NO_3) particles in air:



("↔" here denotes a reversible reaction)

Significant ammonia emissions in California come from agricultural activities such as cattle feedlots, and as a result, particulate nitrate levels tend to be higher downwind of such activities. Reaction of HNO_3 with sea salt particles in coastal areas also generates particulate nitrate in the form of sodium nitrate (NaNO_3).

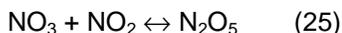
In summary, fresh NO_x emissions, which consist primarily of nitric oxide (NO), undergo reactions with ozone and peroxy radicals to form NO_2 . The NO_2 can be directly converted to nitric acid via the gas phase

reaction (21) with OH. This is the principal formation mechanism for nitric acid in the daytime. The concentration of the OH radical, which is the key species in the photochemical oxidation cycle, is controlled by the amount of sunlight and the ambient concentrations of ozone, water vapor, NO, NO₂, and reactive VOCs.

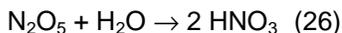
The gaseous nitrate radical, NO₃, is formed at night by the reaction of NO₂ with O₃:



Sunlight causes the nitrate radical to photolyze rapidly during the day, so it is available for other reactions mostly at night. An important reaction of NO₃ is with NO₂ to form dinitrogen pentoxide (N₂O₅). This is a reversible reaction, since N₂O₅ thermally decomposes back to NO₂ + NO₃:



At night, N₂O₅ reacts with water on surfaces (e.g., airborne particles, buildings, vegetation etc.) to form nitric acid (it is uncertain whether reaction with water in the gas phase makes a significant contribution):

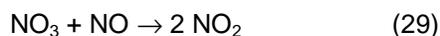
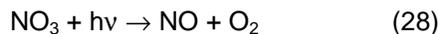


Another way that nitric acid is formed at night is by the reaction of the nitrate radical with certain VOCs such as formaldehyde:



Finally, in the presence of water adsorbed on surfaces, NO₂ reacts as described earlier to generate nitric acid on surfaces, while releasing HONO to the gas phase.

During daylight hours and below the mixing layer at night, the concentration of nitrate radical is low because of the very fast rates of nitrate radical photolysis and scavenging of nitrate radicals by NO.



Although conversion rates for NO₂ to nitric acid vary widely throughout a 24-hour period (OH daytime and O₃ at night), these rates are significant during both daytime and nighttime hours.

Nitric acid has a high vapor pressure, but is a very "sticky" molecule. It therefore readily adsorbs on surfaces, particularly when even small amounts of water are present on the surface, which is always the case under atmospheric conditions.

Nitric acid also reacts with ammonia, reaction (23) above, to form particulate ammonium nitrate (NH₄NO₃). Equilibrium often exists in the atmosphere between ammonia, nitric acid, and NH₄NO₃. This reaction is believed to be the primary source of fine (<2.5 μm diameter) nitrate particles in California's urban air. The reaction equilibrium depends upon both temperature and relative humidity. High humidity and low temperature favor NH₄NO₃ formation. Solid NH₄NO₃ becomes aqueous (deliquesces) when the ambient relative humidity rises above 62%.

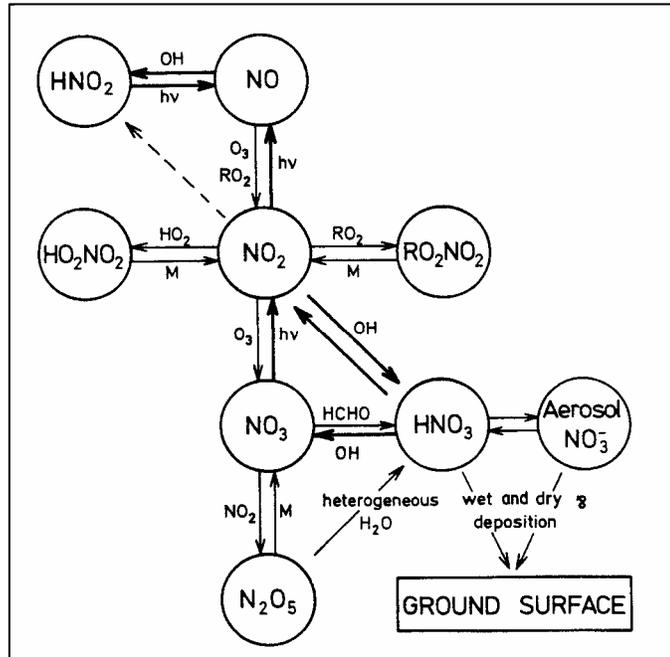


Figure 2.1. Oxidation scheme for nitrogen oxides and related compounds (Warneck 2000)

Figure 2.1 illustrates the chemical pathways involving nitrogen oxides in the atmosphere (Warneck 2000). Photochemically-induced reaction pathways are indicated by bold arrows. These processes are active only during the day, whereas the others occur at all times.

Ambient concentrations of secondary particles are not necessarily proportional to the quantities of their precursor emissions, since the rates at which they form and their gas/particle equilibria may be controlled by factors other than the concentration of the precursor gases. The rate of NO_x oxidation and the branching ratio between inorganic and organic nitrates depends on the specific environmental conditions in addition to reactant concentrations (Seinfeld and Pandis 1998). The partitioning of inorganic nitrate between gaseous nitric acid, ammonium nitrate, and nonvolatile nitrate depends on factors such as relative humidity, temperature, and ammonia concentration, in a nonlinear manner.

Secondary ammonium nitrate is generally the largest contributor to the $\text{PM}_{2.5}$ mass during the winter at most California urban sites. The results of several studies (Magliano et al. 1999, Kim et al. 2000) indicate that during some episodes of high particle concentrations in California, ammonium nitrate – formed secondarily from NO_x and ammonia emissions – can account for over half of the $\text{PM}_{2.5}$ mass. The formation of secondary particles (which are a major proportion of fine PM in California) from gas-phase precursors is a complex, nonlinear process. Consequently, a one-to-one relationship between precursor emissions and ambient secondary PM concentrations is not expected. However, in general, decreased NO_2 emissions would lead to lower ambient PM concentrations in California.

2.5 Acidity in Ambient Air

This section summarizes information on atmospheric acidity in California. Additional information may be obtained from the ARB document "Atmospheric Acidity Protection Program: Final Assessment" (2000).

2.5.1 Acid Deposition

The term "acid deposition" is used to describe the transfer of acid substances from the ambient air to receptors. Although acid deposition is often called "acid rain," it includes any form of precipitation, including rain, snow, and fog, with a pH of 5.5 or below, as well as deposition onto surfaces in the absence of precipitation, so-called "dry deposition". (Note: pH values below 7 are acidic; vinegar has a pH of 3). Dry acid deposition of particles and gases appears to be more significant than wet deposition in California due to the limited amounts of rainfall in large parts of the state.

Acid deposition usually results from emissions of NO_x and sulfur dioxide from anthropogenic sources. Although acid rain can fall virtually anywhere, ecological damage in environmentally sensitive areas downwind of industrial and urban emissions is a major concern. This includes areas that have a reduced capacity to neutralize acid inputs because of low-alkalinity soils and areas that contain species with a low tolerance to acid conditions. Acid deposition in other parts of the world has been linked to the acidification of terrestrial and aquatic systems, risks to public health, damage to plants and materials, and effects on atmospheric visibility. Acid deposition in California may have significant adverse effects on the environment, economy, and public health. Some areas of the state are potentially susceptible to damage from acid deposition. Acid deposition in the state is due primarily to emission sources within California.

In California (unlike much of the United States), emissions of NO_x (rather than sulfur dioxide) are responsible for most of the atmospheric acidity and nitrogen oxide deposition to urban landscapes, and also to forests. A significant proportion of the NO_x emitted in the South Coast Air Basin (SoCAB) is deposited (both wet and dry) as nitrate (Blanchard 1994). Summertime concentrations and deposition of nitric acid vapor and particle nitrate in California are among the highest in the nation.

Long-term exposure to ambient levels of acids in ambient air, including nitric acid, has been shown to pose a chronic health risk, alone or in combination with other airborne pollutants. For example, investigators from the Cal/EPA Children's Health Study (Cal/EPA 2004) showed that acid vapor, NO_2 , and fine PM were associated with decreased rates of lung function growth in children living in the most polluted communities of Southern California (Gauderman et al. 2004). Long-term effects of NO_x on human health have been demonstrated to be significant, and effects on aquatic and forest ecosystems are also a concern.

The long-term effects of episodic acidification are largely unknown. Chronic acidification of high elevation surface waters in the Sierra Nevada has not been found, but episodic depressions in acid neutralizing capacity do occur. While no large-scale or widespread adverse ecological impacts have been detected, many high elevation aquatic ecosystems are nitrogen-limited and potentially at risk from current levels of atmospheric nitrogen deposition. Nitrogen oxide saturation has occurred in forested watersheds in the San Bernardino Mountains, which may lead to nitrate contamination of drinking water sources. In future years, atmospheric NO_x deposition could lead to forest soil nitrogen saturation in other areas such as the San Gabriel Mountains and southern Sierra Nevada.

In Lake Tahoe, phytoplankton growth is no longer co-limited by the availability of nitrogen and phosphorus; rather, growth is now limited by phosphorus alone, due to the deposition of atmospheric NO_x (Jassby et al. 1994). A recent report for the ARB (Cohen and Murphy 2005) concluded that:

During summer months, the Sacramento region is the dominant source of reactive nitrogen in the plume on the western slope of the Sierra Nevada, but this plume rarely reaches the Tahoe Basin.

HNO_3 deposition is sufficiently fast that very little remains in the plume by the time it reaches high elevation sites near the western rim of the Lake Tahoe Basin.

Urban areas to the west of Lake Tahoe cannot be identified as important sources of nitric acid.

Organic nitrates are significantly elevated in the plume compared to background conditions but their contribution to NO_x deposition remains poorly understood.

2.5.2 Acid Fog

Fog is a potentially important pathway for acid deposition. Fog occurs frequently along the coast of California during the summer, in the Central Valley during the winter, and throughout the year in the mountainous areas of the state. Recent studies of the chemical composition of fog water in southern California have shown significantly greater acidity in fog water than that observed in rainwater. In the 1980's, the ARB sponsored fog water sampling programs at seven sites in California. Fog water collected in the western portion of the South Coast Air Basin (SoCAB) was found to be highly acidic, with pH values ranging down to 1.7 (similar to lemon juice) at Corona del Mar in the coastal portion of Orange County. That sample contained a high level of nitrate, suggesting that NO_x sources may have contributed the major portion of the acidity. Acid fog has been associated with harmful air pollution episodes, and reported to adversely affect materials, crops, and forests.

Fog water collected at non-urban, coastal sites was less acidic due, in part, to the alkalinity of sea salt found in marine atmospheres. In addition, in the eastern part of the SoCAB and the southern San Joaquin Valley, fogs were generally not as acidic due to high levels of acid-neutralizing ammonia. Estimates of total annual deposition of nitrate and hydrogen ion from stratus clouds striking mountainsides are of comparable magnitude to the deposition from rainfall in certain mountainous areas of the South Coast Air Basin.

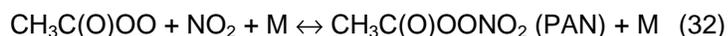
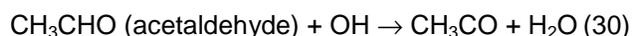
As is the case for rain, nitric acid is a more important contributor to fog acidity than is sulfuric acid. Across the state, the nitrate-to-sulfate ratios in fog are typically about 3:1, but local emissions influence measured concentration ratios. For example, the 3:1 ratio typifies areas where motor vehicle emissions of NO_x dominate (e.g., Los Angeles), but may be close to 1:1 at sites in the southern San Joaquin Valley where sulfur emissions from oil production are significant. Concentrations of ammonium, nitrate, and sulfate ions are commonly 100 times higher in fog than in rain. High concentrations of chemical components in fog correlated well with the occurrence of photochemical smog events, as well as the physical processes of condensation and evaporation.

2.6 Formation of Toxic Nitrogen Oxide Derivatives

2.6.1 Peroxyacetyl Nitrate and Other Organic Nitrates

Significant amounts of NO_x can be converted to organic nitrates known as peroxyacyl nitrates. The simplest member of this group of compounds is peroxyacetyl nitrate or PAN, which has the formula $\text{CH}_3\text{C}(\text{O})\text{OONO}_2$. PAN is the most abundant organic nitrate in urban air; levels have reached 35 ppb, but current California levels are probably below 10 ppb (Finlayson-Pitts and Pitts 2000, p. 594, and Grosjean et al. 1996). PAN is a very strong eye irritant for humans, is mutagenic in certain bacterial assays, and is highly toxic to plants, certain commercial field crops, and animals.

PAN is created through the following series of reactions:



The thermal decomposition of PAN (reaction 32) is very temperature-sensitive. As temperature rises, PAN decomposes back to NO_2 and the peroxyacetyl radical. PAN can act as a NO_x "reservoir", capable of transporting NO_x over long distances. PAN can also act to accelerate photochemical smog formation, by decomposing to yield NO_2 and reactive VOCs as temperatures rise in the morning. Related compounds (collectively called peroxyacyl nitrates), such as peroxybenzoyl nitrate, are also found, but in lower concentrations, in ambient air. A deficit exists in observable amounts of reactive nitrogen species (NO_y) in ambient air, and peroxyacyl nitrates may comprise a significant part of the missing NO_y . The term NO_y refers to the sum of oxides of nitrogen: $\text{NO}_y = \text{NO} + \text{NO}_2 + \text{HNO}_3 + \text{HONO} + \text{N}_2\text{O}_5 + \text{HNO}_3 + \text{organic nitrates, etc.}$

2.6.2 Nitro-PAHs

Polycyclic aromatic hydrocarbons (PAHs) include well-known carcinogens found in several sources, and are formed during the incomplete combustion of organic matter, e.g., gaseous and particulate diesel exhaust. Atmospheric NO₂ reacts with PAHs found in California's air to produce nitro-PAHs, as has been summarized in Finlayson-Pitts and Pitts (2000). Many of the nitro-PAHs found in ambient air have very high bacterial mutagenicity (in the "Ames assay"), and several of these are also known to be human cell mutagens. Some of PAHs and nitro-PAHs are known carcinogens.

The simplest PAH is naphthalene, which consists chemically of two fused benzene rings. According to the National Toxicology Program (2004), naphthalene is reasonably anticipated to be a human carcinogen, based on sufficient evidence from studies in experimental animals. Naphthalene and its derivatives are the most prevalent PAHs in ambient air, mostly as the result of their presence in vehicle exhaust. Some of the most prevalent nitro-PAHs in ambient air include 1- and 2-nitronaphthalene and methylnitronaphthalenes, which can react *in vivo* to form highly carcinogenic naphthylamines. Radical-initiated reactions of naphthalene and alkylnaphthalenes (also emitted in vehicle exhaust) produce these nitro-PAHs in ambient air. Dimethyl/ethyl-nitronaphthalenes, formed from atmospheric reactions of dimethyl/ethyl-naphthalenes present in vehicle exhaust (including diesel exhaust), have been identified in southern California's atmosphere.

Combustion sources emit nitro-PAHs into the ambient air, but nitro-PAHs are also formed in the ambient air. For example, several nitro-PAHs have been detected in diesel exhaust. However, some nitro-PAHs (e.g., 2-nitrofluoranthene and 2-nitropyrene) are not usually found in diesel exhaust, but have been detected in ambient air at many locations. These nitro-PAHs probably arise from gas-phase radical-initiated photochemical reaction of the parent PAHs, and subsequent attachment of the nitro-PAHs to carbon particles. Ambient concentrations of nitro-PAHs formed from atmospheric reactions greatly exceed those of nitro-PAHs formed from combustion.

Diesel exhaust particulate also contains dinitropyrenes and 3-nitrobenzanthrone, both nitro-PAHs of potential health concern. They are both highly mutagenic in the Ames assay. Often the isomers produced by atmospheric reactions are distinct from those present in vehicle emissions and, therefore, may impart new health effects to ambient particles. Also PAH-quinones, which are likely to be products of atmospheric reactions of PAHs and nitro-PAHs, have been implicated in a variety of toxic mechanisms. A better understanding of the atmospheric chemistry of diesel and other vehicle exhaust is needed to know whether controls will decrease not only emissions of toxic compounds but also the toxicity of reaction products inhaled by the Californians at downwind locations.

Mutagenicity studies on atmospheric samples containing nitro-PAHs show that nitro-PAHs are responsible for much of the total bacterial mutagenicity. Naphthalene derivatives account for much of the mutagenicity of the vapor phase of ambient air in California. Much of the mutagenicity of particulate matter in the southern California's ambient air can be accounted for by nitropyrenes, nitrofluoranthenes, and nitro-PAH-lactones. These nitro-PAH-lactones include nitrophenanthrene lactones and nitropyrene lactones (2- and 4-nitrodibenzopyranone). In general, mutagenic activities (per volume of air) of the nitro-PAH daughter products can significantly exceed those of the parent PAHs. This suggests that the contribution of nitro-PAHs should be considered in an overall health risk assessment of airborne PAHs (Yaffe et al. 2001).

Some of the information in this section was obtained from Arey (2004), and from two ARB-sponsored research projects conducted at the University of California, Riverside. Information about each of these projects is available at the following websites: <http://www.arb.ca.gov/research/abstracts/93-307.htm>, <http://www.arb.ca.gov/research/abstracts/a132-075.htm>.

2.6.3 Nitrosamines

Amines (R₂NH) react with nitrous acid (HONO) to form nitrosamines (R₂NNO), several of which are highly carcinogenic (reaction 33).



The simplest nitrosamine is dimethylnitrosamine, also called N-nitrosodimethylamine, which has the formula (CH₃)₂NNO. Amines are present in all living tissues and most foodstuffs. As described in section

3.4 (reaction 20), nitrous acid is formed (along with nitric acid) from the reaction of NO₂ with water. Although nitrosamines are emitted into the air in California, the lifetime of dimethylnitrosamine (due to photolysis) is estimated to be 5 minutes (<http://www.arb.ca.gov/toxics/tac/factshts/nnitrsod.pdf>), and other nitrosamines are expected to have similarly short lifetimes. This limits the concern about possible health effects from exposure to airborne nitrosamines. However, nitrosamines could be adsorbed into ambient particles, which would allow for increased atmospheric lifetimes.

The formation of nitrosamines after exposure to NO₂ *in vivo* in the presence of added amines has been well demonstrated (WHO 1997). However, it is unclear how much nitrosamines are formed without adding amines.

2.7 Natural Processes and Nitrogen Dioxide Levels in California

2.7.1 Background Levels of Nitrogen Dioxide

Background NO₂ is defined as ambient NO₂ that results from uncontrollable processes. In general, background NO₂ levels result from natural emissions; long-range transport is small and unlikely to alter peak concentrations. It is believed that the most significant contributors to background NO₂ in California are biomass burning, lightning discharges, and soil emissions. Minor sources include oceans, oxidation of ammonia, transport from the stratosphere, and high-flying aircraft. Natural sources contribute about a third of the total emissions. Conventionally, biomass burning is also considered “a natural source”; although some biomass burning results from human intervention, wildfires are generally not “controllable”.

Nitric oxide is produced by many organisms (including humans), but especially by bacteria. The NO that results can be released to the air, where conversion to NO₂ takes place via the reactions summarized in previous sections. Two kinds of bacteria produce NO: nitrifiers and de-nitrifiers. The emission of NO_x from soil is associated with the biological nitrogen cycle and is produced during nitrification and denitrification processes. Variables include the soil temperature and moisture content, substrate availability, and local soil microbial communities.

Nitrifying bacteria oxidize ammonia in soil to substances such as hydroxylamine, nitrous oxide, nitric oxide, nitrite, and nitrate. Ammonia occurs in soil as the result of the action of nitrogen-fixing bacteria, or by the application of ammonia-releasing fertilizer. De-nitrifying bacteria use nitrate to take the place of oxygen in metabolism, and can produce NO₂, nitrite, NO, N₂O, and nitrogen. There appear to be fewer species of de-nitrifying bacteria than nitrifiers, probably because use of nitrate as an electron acceptor produces less energy for growth than the use of oxygen. Therefore, de-nitrifying bacteria are active only under low-oxygen conditions. In general, nitrification as a source of NO_x from soils is more important than denitrification. More information on microbial nitrification and de-nitrification is available at

<http://www.arb.ca.gov/research/abstracts/96-304.htm>.

Further information on natural and long-range sources that contribute to background NO₂ levels may be found in Chapter 5 of this report.

2.7.2 Natural Processes that Remove Nitrogen Dioxide

Leaves and needles have surface area that can allow for deposition of air pollutants such as NO₂. In the process, nitrous and nitric acids can form on the leaf surfaces from the NO₂, damaging the vegetation. Several factors affect pollutant removal, including how long a parcel of air is in contact with the leaf, and the amount of leaf area. Because this deposition has an affect on air quality, the ARB is interested in this phenomenon. More information is available on the web at

<http://www.arb.ca.gov/research/ecosys/tree-aq/tree-aq.htm>.

2.8 Spatial and Temporal Variations in Nitrogen Dioxide Levels

2.8.1 The Role of Weather

In the troposphere, the air is usually warmest near the ground. Warm air has a tendency to rise and cold air to sink, causing the air to mix, which disperses ground-level pollutants. However, if cooler air gets layered beneath warm air, no mixing occurs -- the air is stable or stagnant. The region in which temperature is so inverted is called an inversion layer. Pollutants released within an inversion tend to get

trapped there. If an inversion lies near the ground, people can be exposed to high pollutant levels. Mountain chains, such as those downwind of California's coastal cities and the Central Valley, help to trap air and enhance the air quality impact of inversions. Cooler air draining into the State's valleys and air basins also enhances inversion formation. Air pollution that is generated near mountains tends to move up-slope during the day, and down-slope during the night. As a result, air pollution levels may increase with altitude.

The direction and strength of the wind also affect levels. Based on worldwide climate patterns, western coasts at California's latitude tend to have high-pressure areas over them, especially in summer. By preventing the formation of storms, and by promoting the sinking of very warm air, these high-pressure areas are associated with light winds and temperature inversions.

2.8.2 Variations in Nitrogen Dioxide Levels: Seasonal, Diurnal, and Spatial

Since NO₂ plays a photochemical role in ozone formation, its levels are lower in the summer when ozone is highest. The combination of less chemical reactivity and a higher frequency of lower atmosphere inversions, results in the highest concentrations of NO₂ occurring in the cooler months.

NO₂ forms from NO in the presence of sunlight on a scale of minutes. Since the intensity of sunlight and the strength of the emissions of NO_x vary throughout the day, the concentration of NO₂ varies throughout the day as well. In urban areas, mobile sources produce the most NO_x emissions as part of their fuel combustion process. The highest NO₂ levels thus occur during the morning and afternoon rush hours. The levels peak during the morning commute hours and decrease slightly only to peak again, to a lesser extent, during the evening commute hours.

Ambient NO_x levels can vary from non-detectable near combustion sources, where nitric oxide (NO) is emitted into the air, to several ppm in downwind plumes. Nitrogen oxides can be transported from upwind to downwind air basins, and the extent of such transport is an area of concern for modeling of photochemical oxidant formation. A particular concern is the extent of such NO_x transport aloft.

Additional information on this topic may be found in Chapter 5, *Characterization of Ambient Nitrogen Dioxide Air Quality*.

2.9 Visibility Impairment

California has a standard for "Visibility-Reducing Particles", which refers to "... in sufficient amount to reduce the prevailing visibility to 10 miles ...", to be applied only when the relative humidity is less than 70%. In the Lake Tahoe Air Basin, the standard is 30 miles visibility. Particulate nitrate and NO₂ can contribute significantly to visibility impairment. In fact, nitrate is one of the main forms of PM that cause visibility impairment. Most of the nitrate particles are found in the size range (0.1 to 2 μm) that is the most effective in scattering light, thereby reducing visibility. (The formation of nitrates was discussed in Section 2.4.) Ambient NO₂ can impart an orange-brown appearance to the atmosphere. However, at typical ambient NO₂ concentrations, this light absorption does not appreciably reduce the distance one can see.

Additional information on this topic may be found in Chapter 10, *Effects on Visibility*.

2.10 ARB-Sponsored Research Involving NO₂ Chemistry

2.10.1 Surface (Heterogeneous) Reactions of NO₂

This section concerns two reactions of NO₂ that occur on surfaces. Information on ARB-sponsored research in this area may be obtained from the following website: <http://www.arb.ca.gov/research/abstracts/00-323.htm>.

1) Nitrous acid (HONO) is the major source at dawn of hydroxyl (OH) radicals, which initiate reactions that generate ozone and other pollutants. Although HONO is emitted from various sources, HONO is also formed from the hydrolysis of NO₂ on wet surfaces:



Based on controlled chamber studies, the reaction rate depends on the surface to volume ratio, which indicates the reaction is heterogeneous, and is first order in the concentration of NO₂.

2) It had been assumed that once nitric acid is formed from NO_x, e.g., by the scavenging of OH by NO₂ (reaction 16), the nitrogen is no longer available for photochemical activity. However, deposited nitric acid may be converted back into NO_x through a chemical reaction of NO with HNO₃ in films of water, and perhaps through photolysis of the adsorbed nitric acid as well. To the extent this "renoxification" occurs, air pollutants (such as ozone and PM) may not respond to VOC and NO_x controls in the manner predicted by models that omit this chemistry. This topic represents an active area of research.

2.10.2 Biological Processes and NO_x Emissions

Biofilters break down organic compounds with bacteria, and are used commercially to reduce emissions of VOCs from several sources. Both production and removal of NO_x have been demonstrated in vapor-phase biofilters, based on laboratory experiments. Therefore, under certain operating conditions, NO_x may be emitted from biofilters designed to remove volatile organic compounds from contaminated air. Locally anaerobic conditions can occur in which nitrous oxide (N₂O) and NO are produced and released to the atmosphere. ARB-sponsored research found that nitrogen-containing compounds (e.g., NH₃, N₂O, NO or NO₂) were emitted during normal and/or "upset" biofilter operations, but differences between inlet and outlet levels were not statistically significant. On the other hand, oxidation of N₂O and NO to nitrate ion [(NO₃)⁻] can be carried out in biofilters using microbial nitrification. More information on microbial re- and de-nitrification is available at the following website:

<http://www.arb.ca.gov/research/abstracts/96-304.htm>.

Small amounts of NO_x are also emitted from crops, apparently related to fertilizer application. The rate and timing of NO_x flux from soil depends on various soil, climatic, and management factors. NO_x fluxes vary widely, apparently related to proximity in time to a fertilizer application and to soil moisture characteristics. More information on the emission of NO_x from plants is available on the web at: <http://www.arb.ca.gov/research/abstracts/94-732.htm>. This topic is also discussed in Chapter 9, "Effects of Nitrogen Dioxide on Vegetation".

2.10.3 Rates for Gas-Phase Nitric Acid Formation

Urban airshed models are used to assess control strategies for NO_x and VOCs. In such models, ozone formation involves reactions that generate free radicals, primarily through photolysis reactions, conversion and regeneration of radicals, and removal of radicals through termination steps. As discussed above, an important termination step (for ozone formation from NO_x) is the formation of nitric acid, a relatively long-lived product, from two reactive precursors:



However, a minor channel in this reaction produces peroxyxynitrous acid, HOONO:



In order to determine the relative rates of these reactions, the ARB sponsored a project with the California Institute of Technology entitled "Gas-Phase Formation Rates of Nitric Acid and its Isomers under Urban Conditions" (Okumura 2005). More information is available at <http://www.arb.ca.gov/research/rsc/Feb02Adv.htm>.

2.11 References

- ARB. 1985. The effects of oxides of nitrogen on California air quality, Report Number: TSD-85-01.
- ARB. 2000. Atmospheric Acidity Protection Program: Final Assessment
<http://www.arb.ca.gov/research/acid-dep/AAPPPrpt.pdf>
- ARB 2002. Public Hearing to Consider Amendments to the Ambient Air Quality Standards for Particulate Matter and Sulfates.

- Arey J. 2004. Commentary: A Tale of Two Diesels. *Environmental Health Perspectives* 112(8):812-813.
- Ayala A, Kado NY, Okamoto RA, Holmén BA, Kuzmicky PA, Kobayashi R, Stiglitz KE. 2002. Diesel and CNG heavy-duty transit bus emissions over multiple driving schedules: Regulated pollutants and project overview, *Journal of Lubricants and Fuels*, SAE Transactions, Vol. 111, pp. 735-747.
- Blanchard CL, Michaels H. 1994. Regional Estimates of Acid Deposition Fluxes in California. Final Report. California Air Resources Board.
- Cal/EPA. 2004. The California Environmental Protection Agency's Children's Environmental Health Program Biennial Report for 2002 – 2003.
- Cohen RC, Murphy J. 2005. Keeping Tahoe blue: Quantifying atmospheric nitrogen oxides in the Lake Tahoe Basin, Final Report for Air Resources Board, Contract No. 01-327, January 2005.
- Finlayson-Pitts BJ, Pitts JN. 2000. *Chemistry of the Upper and Lower Atmosphere*, Academic Press, San Diego, CA.
- Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters J. 2004. The effect of air pollution on lung development from 10 to 18 years of age. *N Engl J Med* 351(11):1057-67
- Grosjean D, Grosjean E, Fraser, MP, Cass GR. 1996. Air Quality Model Evaluation Data for Organics. 3. Peroxyacetyl Nitrate and Peroxypropionyl Nitrate in Los Angeles Air, *Environmental Science and Technology* 30(9): 2704 -2714.
- Jassby AD, Reuter JE, Axler RP, Goldman CR, Hackley SH. 1994. Atmospheric deposition of nitrogen and phosphorus in the annual nutrient load of Lake Tahoe (California-Nevada). *Water Resources Research* 30(7): 2207-2216.
- Kim BM, Teffer S, Zeldin MD. 2000. Characterization of PM_{2.5} and PM₁₀ in the South Coast Air Basin of southern California: Part 1- Spatial variations, *Journal Air & Waste Management Association*, 50: 2034-2044.
- Magliano KL, Hughes VM, Chinkin LR, Coe, DL, Haste TL, Kumar N, Lurmann FW. 1999. Spatial and temporal variations in PM₁₀ and PM_{2.5} source contributions and comparison to emissions during the 1995 integrated monitoring study, *Atmospheric Environment*, 33: 29 4757-4773.
- National Toxicology Program. 2004. Eleventh Report on Carcinogens: Substance Profile, Naphthalene, CAS No. 91-20-3.
- Okumura M, Sander SP. 2005. Gas-Phase Formation Rates of Nitric Acid and its Isomers under Urban Conditions. Final Report. California Air Resources Board.
- Seinfeld JH, Pandis SN. 1998. *Atmospheric Chemistry and Physics*, John Wiley and Sons, New York, NY.
- Warneck P. 2000. *Chemistry of the Natural Atmosphere* (second edition), Academic Press, San Diego, CA. p. 544.
- WHO. 1997. Nitrogen dioxide (second edition). International Programme on Chemical Safety. Environmental Health Criteria 188. World Health Organization, Geneva Switzerland.
- Yaffe D, Cohen Y, Arey J, Grosovsky AJ. 2001. Multimedia analysis of PAHs and Nitro-PAH daughter products in the Los Angeles Basin, *Risk Analysis*, Vol. 21, No. 2, 2001, p. 275.

3 Measurement of Nitrogen Dioxide

3.1 Introduction

NO₂ gas is formed in ambient air through the oxidation of nitric oxide (NO). The sum of the concentrations of NO and NO₂ in ambient air is generally defined as the concentration of nitrogen oxides (NO_x).

The recommended methodology to quantify hourly NO₂ concentrations for California's ambient air monitoring network is gas-phase chemiluminescence. Ambient concentrations of NO and NO_x are determined directly by chemiluminescence technology. However, NO₂ concentrations are determined indirectly by subtracting NO concentrations from NO_x concentrations.

3.2 Existing Monitoring Methods

Five NO₂ monitoring technologies are listed in the current United States Environmental Protection Agency (U.S. EPA) "List of Designated Reference and Equivalent Methods":

Sodium Arsenite Method for NO₂, Manual Equivalent Methods.

TGS-ANSA Method for NO₂ (triethanolamine, guaiacol, and sodium metabisulfate [TGS]; sulfanilamide; and 8-amino-1-naphthalene-sulfonic acid ammonium salt [ANSA]), Manual Reference Method.

Optical Long Path Automated Reference Method.

Optical Open Path Automated Equivalence Method.

Chemiluminescence Automated Reference Methods.

A Federal Reference Method (FRM) is defined in Title 40 of the Federal Code of Regulations (40 CFR), Section 53.1, as a method that samples and analyzes the ambient air for an air pollutant that is specified as a reference method in 40 CFR, Parts 50 and 53. 40 CFR, Part 50, Appendix F lists gas phase chemiluminescence as the reference method for determining NO₂. Furthermore, 40 CFR, Part 53, Section 53.2 (b), states that an automated NO₂ reference method must utilize the measurement principle and calibration procedure as specified in Appendix F in 40 CFR, Part 50 and meet the requirements specified in Part 53, Subpart B.

Accuracy and precision of the NO₂ measurements are reflected in the field audit data. (ARB, 2004; ARB, 2006). Accuracy is represented as an average percent difference of measurements of a NIST standard introduced through the probe used for NO₂ sampling. The average percent difference is the combined differences from the certified value of all the individual audit points. For 2002, 2003, 2004, and 2005, the percent differences were: 1.1, 0.9, -0.7, and -2.1, respectively, representing 96, 77, 78, and 79 analyzers, respectively. These are operating within the ARB's control limits (+/-15%) (ARB, 2004, 2006). The standard deviation (statistical variability) of these measurements reflect the precision of the method. For 2002, 2003, 2004, and 2005 the standard deviation of the method as evaluated by audit was 5, 5.3, 4.5, and 4.5 percent, respectively (ARB, 2004, 2006).

A Federal Equivalent Method (FEM) is defined (40 CFR, Part 50, Section 50.1) as a method for measuring the concentration of an air pollutant in ambient air that has been designated as an equivalent method (to the FRM) in accordance with 40 CFR, Part 53.

The California ambient air standard for NO₂ (California Code of Regulations, Title 17, Section 70200) stipulates that gas phase chemiluminescence is the method to be used to measure NO₂. The standard also allows an equivalent method to be used as described in the first footnote to the "Table of Standards" in Section 70200:

"Any equivalent procedure which can be shown to the satisfaction of the Air Resources Board to give equivalent results at or near the level of the air quality standard may be used."

Manual Equivalent Methods present in the U.S. EPA "List of Designated Reference and Equivalent Methods" are not recommended for use in California's ambient monitoring network. Manual gaseous methods possess significant limitations due to the level of labor, analysis time, potential interferences,

and operational costs. The ARB has not utilized manual Equivalent Methods for more than 25 years, therefore these manual methods have not been included in the list of recommended NO₂ methods.

Long path and open path methods are also not currently used in California's ambient air monitoring network. For these methods, ARB staff do not know the quality of the analytical data, reliability of the instruments, or the overall value to California's current monitoring network. Therefore, open path and long path methods are not included in the list of recommended NO₂ methods.

Since January 1980, chemiluminescence methods have proven robust while producing data with acceptable precision and accuracy. With the additional benefit of method continuity among air monitoring agencies throughout California, staff recommends only "federal designated automated chemiluminescence methods" as the approved method for determining compliance with California's NO₂ Ambient Air Quality Standard.

The U.S. EPA-designated NO₂ chemiluminescence automated reference methods are in accordance with 40 CFR, Part 53. Each method is currently acceptable for use in state or local air quality surveillance systems under 40 CFR, Part 58. Each method must be operated according to manufacturer's manual procedures along with CFR quality assurance procedures. Manufacturer or user modifications to a U.S. EPA designated "reference" method may invalidate its designative status (refer to 40 CFR, Part 58, Appendix C, Section 2.8, for user modification approval).

3.3 Chemiluminescence Methodology Principle

Chemiluminescence results from a chemical reaction in which light is emitted from a species or compound that is in an excited state. Chemiluminescent NO-NO₂-NO_x monitors directly measure the concentrations of NO and NO_x. The concentration of NO₂ is calculated by subtracting the measured NO concentration from the measured NO_x concentration.

NO is measured by mixing ozone into the NO sample flow path. In the mixing chamber, all NO is oxidized to NO₂, resulting in temporarily excited NO₂ molecules. As the excited NO₂ molecules release photons of energy, a photomultiplier tube measures the emitted light.



* = excited state of species

hν = light

[h] = Planck's constant (6.62 x 10⁻²⁷ erg sec)

[ν] = light frequency (sec⁻¹)

NO_x is measured by diverting a separate sample flow through a thermal converter prior to the addition of ozone, as mentioned above. In the thermal converter, NO₂ is reduced to NO. Analysis is performed using the same photomultiplier tube with the aid of a sample flow splitter.

Procedures for testing performance characteristics of automated NO₂ methods are located in 40 CFR, Part 53, Subpart B. In Section 53.20 of Subpart B, Table B-1 displays the automated method performance specifications and the corresponding testing procedure. In Section 53.23, analyzer interference is defined as a positive or negative response caused by a substance other than the one being measured. Table B-3 in Section 53.23(d) lists the interferences and test concentrations for each automated method. The compounds and concentrations listed to test NO₂ chemiluminescence analyzer interferences are:

0.1 ppm ammonia

0.5 ppm sulfur dioxide

0.5 ppm nitric oxide

20,000 ppm water vapor

3.4 Recommendation

Staff recommends that the Board continue to endorse the chemiluminescence method as the approved method in California for determining compliance with California's Ambient Air Quality Standard for NO₂. By reference, therefore, staff recommends all federally approved chemiluminescence methods be incorporated as "California Approved Samplers" for NO₂. This will result in no change in air monitoring practices, but will align state monitoring requirements with federal requirements. Specifically, we recommend that a new part be added to the California Administrative Code 70100.1 to read in part:

"NO₂ Monitoring Methods. The method for determining compliance with the NO₂ ambient air quality standard shall be the chemiluminescence Federal Reference Method for the determination of NO₂ in the atmosphere (40 CFR, Part 50, Appendix F). California Approved Samplers for NO₂ are set forth in the Air Monitoring Quality Assurance Manual, Volume IV, Part D: Monitoring Methods for NO₂."

The current U.S. EPA "List of Designated Reference and Equivalent Methods" may be obtained through the U.S. EPA Technology Transfer Network Ambient Monitoring Technology Information Center web site at <http://www.epa.gov/ttn/amtic/criteria.html>.

The following method constitutes "California Approved Samplers" for NO₂ for the purposes of determining compliance with California's ambient air quality standard: Gas phase chemiluminescence method for the determination of NO₂ in the atmosphere (40 CFR, Part 50, Appendix F). The specific instruments approved are:

Advanced Pollution Instrumentation, Inc. Model 200 NO₂ Analyzer - *Automated Reference Method*: RFNA-0691-082 "Advanced Pollution Instrumentation, Inc. Model 200 Nitrogen Oxides Analyzer". [Federal Register: Vol. 56, page 27014, 06/12/91]

Beckman Model 952-A NO/NO₂/NO_x Analyzer - *Automated Reference Method*: RFNA-0179-034 "Beckman Model 952-A NO/NO₂/NO_x Analyzer". [Federal Register: Vol. 44, page 7806, 02/07/79]

Bendix Model 8101-B Oxides of Nitrogen Analyzer - *Automated Reference Method*: RFNA-0479-038 "Bendix Model 8101-B Oxides of Nitrogen Analyzer". [Federal Register: Vol. 44, page 26792, 05/07/79]

Bendix/Combustion Engineering Model 8101-C Oxides of Nitrogen Analyzer - *Automated Reference Method*: RFNA-0777-022 "Bendix or Combustion Engineering Model 8101-C Oxides of Nitrogen Analyzer". [Federal Register: Vol. 42, page 37435, 07/21/77]

Columbia Scientific Industries Models 1600 and 5600 Analyzers - *Automated Reference Method*: RFNA-0977-025 "CSI Model 1600 Oxides of Nitrogen Analyzer". [Federal Register: Vol. 42, page 46574, 09/16/77]

Dasibi Model 2108 Oxides of Nitrogen Analyzer - *Automated Reference Method*: RFNA-1192-089 "Dasibi Model 2108 Oxides of Nitrogen Analyzer". [Federal Register: Vol. 57, page 55530, 11/25/92]

DKK-TOA Corporation Model GLN-114E Nitrogen Oxides Analyzer - *Automated Reference Method*: RFNA-0798-121 "DKK-TOA Corporation Models GLN-114E and GLN-114E-1 Nitrogen Oxides Analyzer". [Federal Register: Vol. 63, page 41253, 08/03/98]

Environnement S. A. Model AC31M NO₂ Analyzer - *Automated Reference Method*: RFNA-0795-104 "Environnement S. A. Model AC31M Chemiluminescent Nitrogen Oxide Analyzer". [Federal Register: Vol. 60, page 38326, 07/26/95]

Environnement S. A. Model AC32M NO₂ Analyzer - *Automated Reference Method*: RFNA-0202-146 "Environnement S. A. Model AC32M Chemiluminescent Nitrogen Oxides Analyzer". [Federal Register: Vol. 67, page 15567, 04/02/02]

Horiba Instruments Models APNA-360 or APNA-360-CE NO-NO₂-NO_x Monitor - *Automated Reference Method*: RFNA-0196-111 "Horiba Instruments, Inc. Models APNA-360 or APNA-360-CE Ambient NO-NO₂-NO_x Monitor". [Federal Register: Vol. 61, page 11404, 03/20/96]

Horiba Instruments Model APNA-370 NO₂ Monitor *Automated Reference Method*: RFNA-0506-157 "Horiba Instruments Incorporated Model APNA-370 Ambient NO_x Monitor," standard specification, operated with a full scale fixed measurement range of 0 - 0.50 ppm with the automatic range switching off, at any ambient

temperature in the range of 20 °C to 30 °C, and with a 0.3 micrometer sample particulate filter installed. [Federal Register: Vol. 71, page 25587, 05/01/06]

Meloy Model NA530R Nitrogen Oxides Analyzer - *Automated Reference Method*: RFNA-1078-031 "Meloy Model NA530R Nitrogen Oxides Analyzer". [Federal Register: Vol. 43, page 50733, 10/31/78 and Vol. 44, page 8327, 02/09/79]

Monitor Labs Model 8440E Nitrogen Oxides Analyzer - *Automated Reference Method*: RFNA-0677-021 "Monitor Labs Model 8440E Nitrogen Oxides Analyzer". [Federal Register: Vol. 42, page 37434, 07/21/77; Vol. 42, page 46575, 09/16/77; Vol. 46, page 29986, 06/04/81]

Monitor Labs/Lear Siegler Model 8840 Nitrogen Oxides Analyzer - *Automated Reference Method*: RFNA-0280-042 "Monitor Labs or Lear Siegler Model 8840 Nitrogen Oxides Analyzer". [Federal Register: Vol. 45, page 9100, 02/11/80 and Vol. 46, page 29986, 06/04/81]

Monitor Labs/Lear Siegler Model 8841 Nitrogen Oxides Analyzer - *Automated Reference Method*: RFNA-0991-083 "Monitor Labs or Lear Siegler Model 8841 Nitrogen Oxides Analyzer". [Federal Register: Vol. 56, page 47473, 9/19/91]

Philips Model PW9762/02 NO/NO₂/NO_x Analyzer - *Automated Reference Method*: RFNA-0879-040 "Philips Model PW9762/02 NO/NO₂/NO_x Analyzer". [Federal Register: Vol. 44, page 51683, 09/04/79]

Seres Model NO_x 2000 G Nitrogen Dioxide Analyzer *Automated Reference Method*: RFNA-0706-163 "Seres Model NO_x 2000 G Nitrogen Dioxide Ambient Air Analyzer," operated with a full scale measurement range of 1 - 0.50 ppm, at any ambient temperature in the range of 20°C to 30 °C. [*Federal Register*: Vol. 71, page 42089, 07/25/06]

SIR S.A. Model S-5012 Nitrogen Oxides Analyzer - *Automated Reference Method*: RFNA-0804-152. [Federal Register: Vol. 69, page 47924, 08/06/04]

Teledyne - Advanced Pollution Instrumentation, Inc. Models 200A, 200AU, 200E; Teledyne Analytical Instruments Model 9110A; or Teledyne Monitor Labs sensor-e™ Model TML-41 NO₂ Analyzers - *Automated Reference Method*: RFNA-1194-099 "Teledyne - Advanced Pollution Instrumentation, Inc. Models 200A, 200AU, 9110A, or 200E; Teledyne Analytical Instruments Model 9110A; or Teledyne Monitor Labs, Inc. sensor-e™ Model TML-41 Chemiluminescence Nitrogen Oxides Analyzer". [Federal Register: Vol. 59, page 61892, 12/02/94]

Teledyne Monitor Labs/Casella/Ecotech Models ML9841, ML9841A/EC9841A, Teledyne Monitor Labs/Casella/Ecotech Model ML9841B/EC9841B, or Wedding & Associates Model 1030 NO₂ Analyzers - *Automated Reference Method*: RFNA-1292-090– "Teledyne Monitor Labs, Casella Monitor, or Ecotech Models ML9841, ML9841A/EC9841A, or ML9841B/EC9841B, or Wedding & Associates, Inc. Model 1030 Nitrogen Oxides Analyzers". [Federal Register: Vol. 57, page 60198, 12/18/92]

Thermo Electron/Thermo Environmental Instruments Model 14 B/E – *Automated Reference Method*: RFNA-0179-035 "Thermo Electron or Thermo Environmental Instruments, Inc. Model 14 B/E Chemiluminescent NO/NO₂/NO_x Analyzer". [Federal Register: Vol. 44, page 7805, 02/07/79 and Vol. 44, page 54545, 09/20/79]

Thermo Electron/Thermo Environmental Instruments Model 14 D/E – *Automated Reference Method*: RFNA-0279-037 "Thermo Electron or Thermo Environmental Instruments, Inc. Model 14 D/E Chemiluminescent NO/NO₂/NO_x Analyzer". [Federal Register: Vol. 44, page 10429, 02/20/79]

Thermo Environmental Instruments Models 42, 42C, 42i NO/NO₂/NO_x Analyzer - *Automated Reference Method*: RFNA-1289-074 "Thermo Environmental Instruments Inc. Model 42, Model 42C, or Model 42i NO-NO₂-NO_x Analyzer". [Federal Register: Vol. 54, page 50820, 12/11/89]

3.5 Estimated Costs and Impacts

Automated reference NO₂ chemiluminescence methods are currently utilized throughout California's Ambient Monitoring Network. Therefore, the recommendation to continue with existing NO₂ chemiluminescence methods should not impact routine NO₂ monitoring costs or resources.

3.6 References

ARB (2004). Annual Data Quality Report. For the Monitoring and Laboratory Division's and Local Districts' Air Monitoring Networks. Monitoring and Laboratory Division. Quality Assurance Section

ARB (2006) Performance audits. Gaseous Pollutants.

<http://www.arb.ca.gov/aaqm/qmosqual/sysaudit/audrslts/audrslts.htm>

Code of Federal Regulations, Title 40, Part 50, Appendix F – Measurement Principle and Calibration Procedure for the Measurement of Nitrogen Dioxide in the Atmosphere (Gas Phase Chemiluminescence).

Code of Federal Regulations, Title 40, Part 53, Subpart B – Procedures for Testing Performance Characteristics of Automated Methods for SO₂, CO, O₃ and NO₂.

Code of Federal Regulations, Title 40, Part 58 – Ambient Air Quality Surveillance.

United States Environmental Protection Agency, Office of Research and Development, "List of Designated Reference and Equivalent Methods", December 5, 2006:

<http://www.epa.gov/ttn/amtic/criteria.html>.

4 Sources and Emissions

4.1 Introduction

Oxides of nitrogen (NO_x) are gaseous compounds of nitrogen and oxygen, which contribute to the formation of ozone, PM10, and PM2.5. NO_x consists of nitric oxide (NO) and NO₂. Most NO_x emissions are produced by the combustion of fuels. The sources of NO_x emissions have been grouped into three major categories: mobile sources, stationary sources, and area-wide sources.

4.1.1 Mobile

Mobile sources (including cars, trucks, and off-road mobile equipment) make up about 81 percent of the total statewide NO_x emissions in 2004. About 51 percent of the total NO_x emissions are from on-road motor vehicles (cars, trucks, and buses) and 30 percent are from other mobile sources (off-road equipment, trains, ships, and farm equipment).

4.1.2 Stationary and Area-Wide

Stationary sources of NO_x include both internal and external combustion processes in industries such as manufacturing, food processing, electric utilities, and petroleum refining. These sources contribute about 16 percent of the total statewide NO_x emissions.

Area-wide sources, which include residential fuel combustion, managed burning, and fires, contribute only a small portion of the total NO_x emissions, about 3 percent.

4.1.3 Spatial and Temporal Distributions

Emissions from different types of sources vary regionally in California. To illustrate this, the following table 4.1 shows 2004 NO_x mobile sources emissions statewide and regionally for selected areas. The emissions are expressed as a percentage of the total emissions for each area.

Table 4.1. Percentage NO_x from mobile sources statewide and by basin.

Area	NO _x (%)
Statewide	81
Sacramento Valley	82
San Diego	93
San Francisco Bay Area	84
San Joaquin Valley	69
South Coast	90

NO_x emissions for individual source categories have daily, weekly, and seasonal variations. For most NO_x categories, higher emissions occur during the day rather than at night and higher emissions on weekdays rather than on weekends. NO_x emissions from electric utility fuel combustion are higher in summer, while emissions from fuel combustion for space heating are higher in winter.

4.2 Emissions of NO_x

Typically, emission projections are based on historical emissions (emission inventory data) and use of projected expectations of future economic and population growth as well as emission controls. There is some degree of uncertainty in these approaches (ARB, 2006). Industrial sources report NO_x emissions to local air districts and to the ARB. Other sources of NO_x emissions are estimated by the local air districts and the ARB. Mobile sources (including on-road and other) make up about 81 percent of the total statewide NO_x emissions. Area-wide sources, which include residential fuel combustion, managed burning and disposal, commercial cooking, and fires, contribute only a small portion of the total NO_x emissions.

4.2.1 NO_x Emission Trends and Forecasts

The NO_x emission trends are summarized in Figure 4-1 for the emissions statewide. NO_x emission standards for on-road motor vehicles were introduced in 1971 and followed in later years by the implementation of more stringent standards and the introduction of three-way catalysts.

NO_x emissions from on-road motor vehicles have declined by over 25 percent from 1990 to 2000, and NO_x emissions are projected to decrease by an additional 50 percent between 2000 and 2020. This has occurred as vehicles meeting more stringent emission standards enter the fleet, and all vehicles use cleaner burning gasoline and diesel fuel or alternative fuels. Stationary source NO_x emissions dropped by 44 percent between 1980 and 1995. This decrease has been largely due to a switch from fuel oil to natural gas and the implementation of combustion controls such as low- NO_x burners for boilers, and catalytic converters for both external and internal combustion stationary sources. State Implementation Plan (SIP) and conformity inventory forecasts may differ from the forecasts presented in this almanac. For additional information on these forecasts, refer to the ARB SIP web page at www.arb.ca.gov/planning/sip/sip.htm.

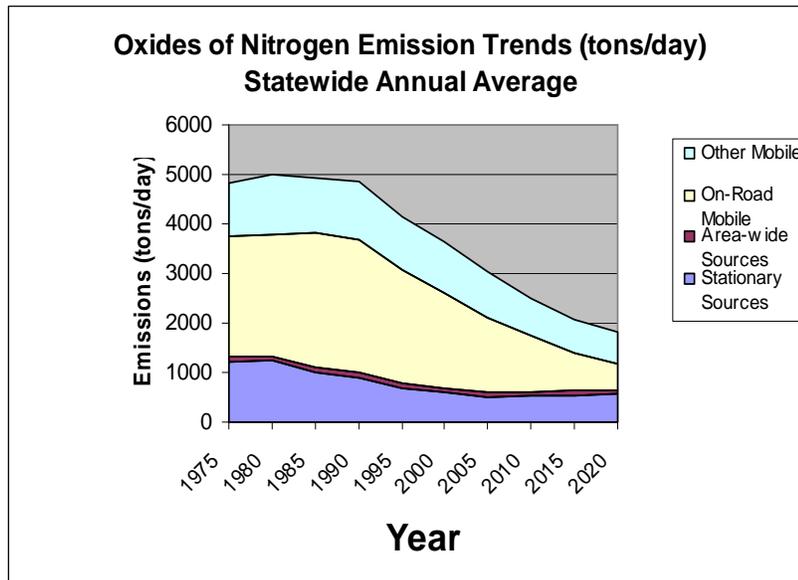


Figure 4.1. Emission trends (tons/day) illustrating statewide annual averages.

The NO_x emission trends (tons/day, annual average) are summarized in Table 4.2 as well as some additional detail regarding the types of mobile emissions.

Table 4.2. Emission trends of NO_x by emission sources (ARB, 2005).

NO_x Emission Trends (tons/day, annual average)										
Emission Source	1975	1980	1985	1990	1995	2000	2005	2010	2015	2020
All Sources	4811	4982	4945	4871	4128	3629	3026	2499	2059	1811
Stationary Sources	1228	1250	1009	909	696	602	506	519	538	556
Area-wide Sources	83	88	91	89	87	90	93	89	88	89
On-Road Mobile	2435	2459	2721	2675	2301	1915	1518	1127	757	532
Gasoline Vehicles	2149	1975	1936	1789	1535	1113	757	536	371	266
Diesel Vehicles	286	484	784	885	766	802	761	590	386	266
Other Mobile	1065	1185	1125	1199	1044	1022	908	764	675	634
Gasoline Fuel	43	48	52	61	60	67	74	68	62	60
Diesel Fuel	941	1052	988	1043	899	868	748	614	528	483
Other Fuel	82	85	85	95	85	87	86	83	85	90

The emissions by air basin are illustrated in Figure 4.2. The South Coast Air Basin has the highest emissions of NO_x based on the 2004 emissions inventory.

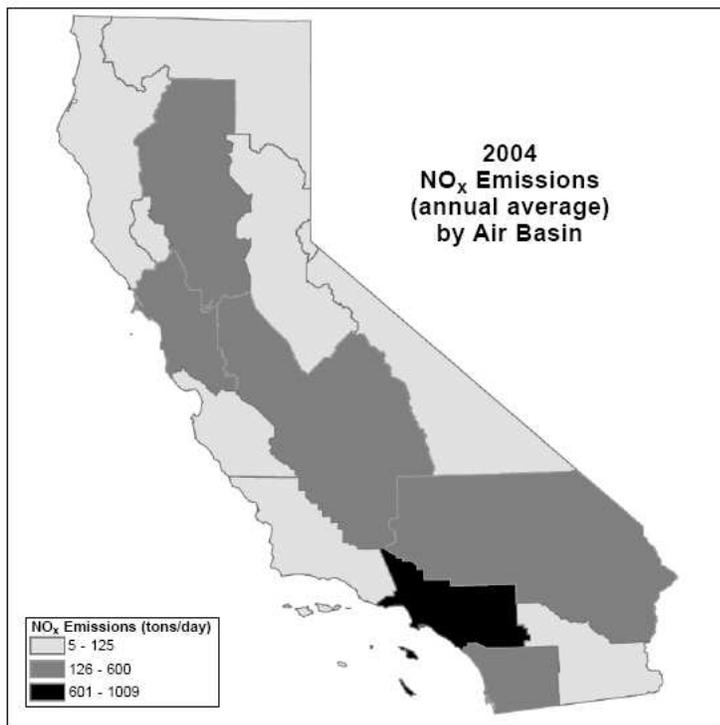


Figure 4.2 2004 NO_x emissions by air basin. Legend indicates tons/day by shading.

4.3 References

ARB 1997. Stationary Source Division and Mobile Source Control Division, Sources and Control of Oxides of Nitrogen Emissions. August 1997. (<http://www.arb.ca.gov/noxdoc/noxdoc.pdf>)

ARB 2005. The California Almanac of Emissions and Air Quality 2005 Edition.

ARB 2006. The California Almanac of Emissions and Air Quality 2006 Edition.

5 Exposure to Nitrogen Dioxide

5.1 Introduction

This chapter describes ambient NO₂ concentrations throughout California. The main focus of discussion is the current State 1-hour standard since it is more stringent than the federal annual standard, though the entire state is presently designated unclassified or attainment for both standards. The federal standard is an annual arithmetic mean of 0.053 ppm and the State 1-hour standard is 0.25 ppm. Much of the information relates to current air quality; in particular, NO₂ data collected during three recent years – 2002 through 2004.

Significant improvements in NO₂ air quality have been achieved. Since the early 1980's, maximum 1-hour NO₂ concentrations have decreased by more than 50 percent. This is due primarily to implementation of tighter controls on both mobile and stationary sources. Although many of these controls were implemented to reduce ozone, they also resulted in NO₂ emission reductions.

Section 5.2 describes the current area designations for the State 1-hour NO₂ standard. The area designations indicate which areas of the State attain the health-based standards. Section 5.3 gives a brief description of California's monitoring network, which collects the data used to assess air quality status and trends. Section 5.4 gives a characterization of ambient NO₂ air quality. Information in this section includes discussions of current air quality and the frequency of maximum daily concentrations, along with historical trend data for each air basin or planning area. Section 5.5 characterizes the numbers of people currently exposed to different peak 1-hour NO₂ concentrations within the State. Section 5.6 characterizes exposures to NO₂ in indoor settings, including homes. Finally, Section 5.7 characterizes exposures to NO₂ inside vehicles.

5.2 Area Designations for the State Nitrogen Dioxide Standards

California Health & Safety Code (H&SC) section 39607(e) requires the Air Resources Board (ARB) to establish and periodically review criteria for designating the attainment status of areas with respect to the State ambient air quality standards. The area designation process occurs separately for each pollutant for which an ambient air quality standard is specified in section 70200 of Title 17, California Code of Regulations. The ARB originally adopted State designation criteria in June 1989. The ARB subsequently amended the designation criteria in June 1990, May 1992, December 1992, November 1993, November 1995, September 1998, and January 2004. H&SC section 39608 requires the ARB to use the designation criteria in assessing the designation status of areas in California.

State area designations indicate whether an area meets the State's health-based standards for ambient air quality. For both State and federal area designations, the size of an area designated for NO₂ is generally an air basin. However, for the State standards, the ARB may designate a smaller area if an area within an air basin has distinctly different air quality, attributable to sources and conditions not affecting the entire air basin. This determination is based on analyses of air quality data, meteorology, topography and the distribution of population and emissions. The ARB will use political boundary lines, such as those of counties, to the extent possible. The smaller designated area must include those sources whose emissions contribute to a violation of the standard.

5.2.1 Types of Designations

There are three basic designation categories: *nonattainment*, *attainment*, and *unclassified*. A *nonattainment designation* indicates that the air quality violates a State standard. There is a subcategory of the nonattainment designation called nonattainment-transitional. This designation is given to areas that still violate the State standard, but are making progress and are close to attainment. In contrast to nonattainment, an *attainment designation* indicates that the air quality does not violate the State standard. Finally, an *unclassified designation* indicates that there are insufficient data for determining attainment or nonattainment status.

The United States Environmental Protection Agency (U.S. EPA) has a similar process for designating and classifying areas with respect to the federal NO₂ standards. However, the U.S. EPA uses only two designation categories. Similar to the State designations, areas with air quality that violates the federal

standard are designated as *nonattainment*. However, areas with air quality that do not violate the standard and areas with insufficient data for determining nonattainment are combined in a category called *unclassified/attainment*.

Both the ARB and the U.S. EPA designate areas based on recent ambient air quality data. These data must satisfy specific siting and quality assurance procedures established by the ARB and U.S. EPA. In general, area designations for both the State and federal standards are based on data collected during the previous three years.

5.2.2 Determination of Attainment in California

After each calendar year, ARB determines the attainment status of areas in the state using the most recent three years of available data for a given pollutant. A statistical procedure utilizes this data to calculate a “design value” which largely determines attainment status when compared to the level of the relevant standard. For California’s short-term standards (averaging time of 24 hours or less) the design value is a calculated value and is also called the Expected Peak Day Concentration or EPDC. This design value represents the concentration expected to be exceeded once per year on average if the rates of emissions in the future continued as they were for the years used in making the designations. Accordingly, the design value or EPDC estimates the 364/365th or 99.73rd percentile of an ongoing conceptual distribution of daily pollutant concentrations. Daily pollutant levels above the design value are excluded from consideration when making area designations.

The EPDC is constructed to be a robust design value that is not unduly affected by unusual short-term events, such as a diesel truck idling near an NO₂ monitor. The use of three years of data helps moderate the influence of year-to-year differences in meteorological conditions.

5.2.3 Excluding “Highly Irregular or Infrequent” Concentrations

The California Clean Air Act of 1988 included H&SC section 39607(e), which requires the ARB to consider instances where there might be “highly irregular or infrequent” violations of a standard. The ARB has developed methods that exclude these atypical violations from the designation process.

The State area designation process has several provisions for excluding certain types of high values that may not be reasonable to control through the regulatory process. These excluded values are identified as concentrations affected by *highly irregular or infrequent events*. While a concentration identified as a highly irregular or infrequent event “exceeds” the level of the State standard, such an exceedance is not considered a “violation” of the standard. This is important because only a “violation” can trigger a nonattainment designation. Under State law, there are three types of highly irregular or infrequent events: extreme concentration events, exceptional events, and unusual concentration events.

An *extreme concentration event* is identified by a statistical procedure and is not necessarily tied to any specific, identifiable event. However, adverse meteorology, such as an extreme temperature inversion that traps emissions in a small volume of air near the ground, is one potential cause of an extreme concentration event. For short-term standards, measured daily concentrations higher than the design value (EPDC) are identified as extreme concentration events, which are excluded from the area designation process (The process of setting a standard can be thought of as part of “risk identification” and the process of setting attainment criteria as part of “risk management.”). Because meteorology is a potential cause of an extreme concentration event and meteorology varies from year-to-year, an area may have several values excluded during years with adverse meteorology, and no values excluded during years with more normal meteorology. This is a natural consequence of the procedure for calculating the design value (EPDC).

In contrast to an extreme concentration event, an *exceptional event* is a specific, identifiable event that causes an exceedance of a standard, but is considered unreasonable to control through the regulatory process. Exceptional events may be identified for both State and federal designation purposes. For example, short-term construction equipment activity near a monitor may result in high NO₂ concentrations.

Finally, an *unusual concentration event* is an anomalous exceedance of a State standard that cannot be identified as an extreme concentration event or an exceptional event. Unusual concentration events can

be identified only for areas designated as attainment or unclassified at the time the exceedance occurs. Furthermore, this type of event is usually identified in areas with limited monitoring data, where we do not have a long-term record for determining what is “characteristic” for the area. In identifying such events, the ARB’s Executive Officer must make specific findings based on relevant information. An area may retain its attainment or unclassified designation by excluding an exceedance affected by an unusual concentration event for up to three consecutive years. However, if an exceedance occurs during the fourth year, the area is redesignated as nonattainment, unless the exceedance can be excluded as an extreme concentration event or an exceptional event.

5.2.4 State Area Designations

The State NO₂ standard is 0.25 ppm for one hour, not to be exceeded. The State area designations for 2003 shown in Figure 5.1 are based on a site-by-site comparison of the maximum measured 1-hour concentration during a three-year period with the peak 1-hour indicator value.

For the purpose of evaluating long-term NO₂ air quality trends and population exposures, the maximum concentration usually is not the best measure, because maximum concentrations can be highly influenced by year-to-year variations in meteorology. In contrast to the maximum values, two calculated statistics that provide more stable measures of long-term trends are the *peak indicator value* (also called the design value or the EPDC) and a *moving 3-year mean*. The *peak indicator value* represents the maximum concentration expected to occur once per year, on average. This indicator is based on a statistical calculation using three years of ambient monitoring data and is calculated for each monitoring site in an area. The highest peak indicator value among all sites in an area is generally used when evaluating area-wide air quality. A moving 3-year mean of the annual maximum measured concentrations also tends to be a more stable trend indicator, when compared to the measured maximum concentration. Although the moving 3-year mean is not as robust as the peak indicator, the 3-year mean does tend to dampen some of the year-to-year variation caused by meteorology. This yields data that are more suitable for trend analysis, when compared with data for individual years.

As described previously, the peak indicator is a statistically derived value representing the maximum concentration expected to occur once per year, on average. Measured concentrations that are higher than the peak indicator value are excluded from the designation process as extreme concentration events. If appropriate, other exceedances may also be evaluated to determine if they are affected by an exceptional event or unusual concentration event (refer to previous discussion) and may be excluded. If any non-excluded measurement is higher than the State standard, it is a violation of the State standard, and the area is designated as nonattainment.

Figure 5.1 shows the area designations for the State NO₂ standard for 2003. At a public hearing in January 2004, the ARB designated that all basins within the state were in attainment of the State 1-hour standard.

5.2.5 Federal Area Designations

There is one federal standard for NO₂: an annual arithmetic mean of 0.053 ppm. For an area to be designated as attainment for this standard, the annual arithmetic mean concentration in a calendar year has to be less than or equal to 0.053 ppm, rounded to three decimal places. The annual mean must be based on hourly data that are at least 75% complete or upon data derived from manual methods that are at least 75% complete for the scheduled sampling days in each calendar quarter. As mentioned earlier, areas that either do not violate the standard or do not have sufficient data to determine compliance with the standard are combined together in a designation category called unclassified/attainment.

Figure 5.2 shows the current area designations for the federal annual NO₂ standard. Similar to the State designations, Figure 5.2 shows that the entire state is designated as attainment, and has been so for the last several years. In 1998, South Coast became the last nonattainment area for the federal standard to be redesignated as unclassified/attainment.

Figure 5.1 2003 Area Designations for the State 1-Hour Nitrogen Dioxide Standard

2003 Area Designations for State Ambient Air Quality Standards NITROGEN DIOXIDE

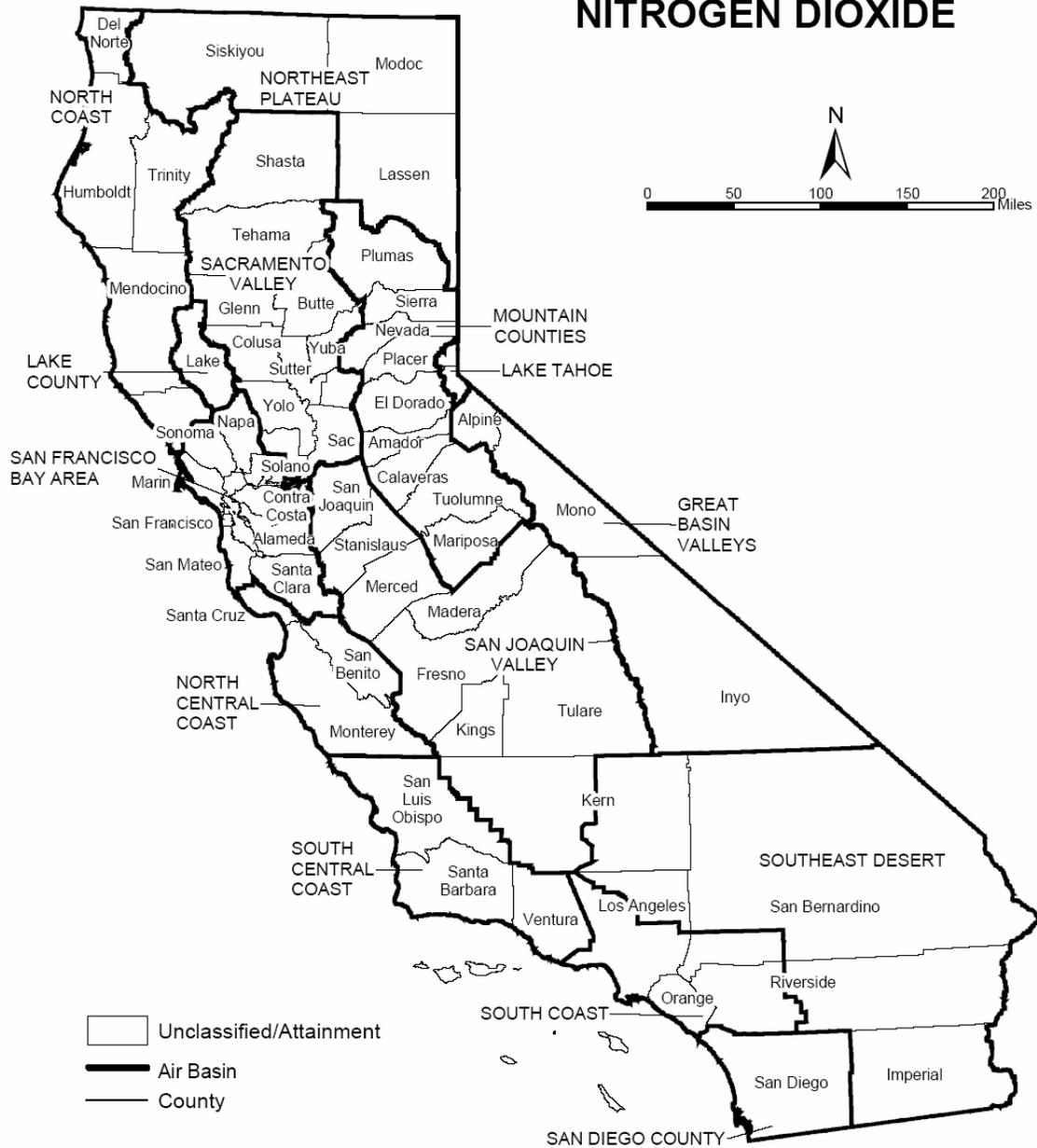


Source Date:
October 2003
Emission Inventory Branch, PTSD

November 17, 2003

Figure 5.2 2003 Area Designations for the Federal Annual Nitrogen Dioxide Standard

Area Designations for National Ambient Air Quality Standards NITROGEN DIOXIDE



Source Date:
October 2003
Emission Inventory Branch, PTSD

November 17, 2003

5.3 Monitoring Network

As shown in Figure 5.3, California's NO₂ monitoring network has over 100 monitors located throughout the State. These monitors are generally operated by the ARB or by local air pollution control or air quality management districts (districts). At each site, a monitor provides continuous hourly averages of ambient NO₂ concentrations. The measured data are used to determine the attainment status of the various areas of California and also, the severity of the pollution problem in each area.

The monitoring program is designed to ensure that the data collected comply with established siting and operating procedures and regulations set forth by the U.S. EPA. Conforming data are considered good quality data or data for record. The quality of the data is ensured through a program of nightly quality control checks of the NO₂ instrument and an annual audit of each monitoring site.

The nightly quality checks are performed at every NO₂ monitoring site to verify that instrument precision, zero, and calibration meet monitoring standards. Precision checks confirm the linear response of the NO₂ instrument. The zero check confirms the instrument's ability to maintain a stable reading. The calibration check confirms the instrument's ability to respond to a known concentration of gas.

To ensure the data collected are representative and accurate, the ARB conducts annual audits of the NO₂ monitoring sites. Each audit includes an assessment of whether the site meets federal siting criteria and whether the equipment is reporting accurate data. Each year, a thorough evaluation is made at each station for criteria including sampler model, objective, monitoring scale, residence time, station temperature, obstacles, traffic, local sources, and dominant influence with respect to emissions.

Pollutant-specific monitor siting criteria are set forth by the U.S. EPA in 40 Code of Federal Regulations (CFR) 58. While most ambient air monitoring stations carefully adhere to regulations during the initial site setup, changes can occur over time, and these changes are sometimes overlooked. These changes might include scaling or source problems, obstacles, and instrument temperature requirements. The second element of the annual audit is an accuracy determination. Accuracy of the monitor is determined by a through-the-probe performance audit to verify the accuracy of the automated methods and to ensure the integrity of the sampling system.

A site fails its audit when the criteria do not meet specifications and/or when an accuracy check exceeds the ARB's established criteria of plus or minus 15 percent for NO₂ data. If a site fails, the ARB issues an Air Quality Data Action (AQDA) request to determine the cause of the problem found during an audit, and initiates corrections. The problem is usually resolved after a review of calibrations, precision checks, and audit results. The ARB submits the AQDA to the site operator, if the site is an ARB site; or to the district Air Pollution Control Officer if a district operates the site.

Over the period of 1994 through 2003, the ARB Quality Assurance Section (QAS) conducted approximately 900 audits of NO₂ analyzers located at air monitoring stations throughout California. Data from the QAS audits were used in calculating air quality accuracy estimates, including average percent differences, standard deviations, and 95 percent probability limits. The estimates were calculated using U.S. EPA analysis and reporting methods found in 40 CFR, Part 58, Appendix A. Improved QAS audit techniques and procedures, along with comprehensive quality control programs, have resulted in lower overall percent differences. These results reflect the effectiveness of properly designed and executed quality assurance/quality control programs.

Figure 5.3 Nitrogen Dioxide Monitoring Sites in California
(National Air Monitoring Program and State Air Monitoring Program)



5.4 Characterization of Ambient Nitrogen Dioxide Air Quality

5.4.1 Overview

This section discusses air quality with reference to ambient NO₂ in each of California's air basins and/or planning areas, based on measured and statistically derived values. Included is a brief description of NO₂ measurements as they pertain to the annual federal standard. Most of the discussion will focus on summary information about the magnitude and frequency of monitored concentrations for the State 1-hour averaging time. In addition, information is provided regarding the seasonal and diurnal variations and a characterization of NO₂ concentrations in each area. Finally, we present information on peak NO₂ values distributed across the population for each area. These discussions include current NO₂ statistics, as well as historical trends.

NO₂ is monitored continuously at over 100 sites in California. The data for each monitoring site are reported as 1-hour average concentrations. These 1-hour data can be summarized as daily, seasonal, or annual arithmetic mean concentrations. In addition, these data are used in determining the number of days during which measured concentrations exceed the State NO₂ standard.

For the purpose of evaluating long-term NO₂ air quality trends and population exposures, the maximum concentration usually is not the best measure, because maximum concentrations can be highly influenced by year-to-year variations in meteorology. In contrast to the maximum values, two calculated statistics that provide more stable measures of long-term trends are the *peak indicator value* and the *moving 3-year mean*. The peak indicator represents the maximum concentration expected to occur once per year, on average. This indicator is based on a statistical calculation using three years of ambient monitoring data and is calculated for each monitoring site in an area. The highest peak indicator value among all sites in an area is generally used when evaluating area-wide air quality. A moving 3-year mean of the annual maximum measured concentrations also tends to be a more stable trend indicator, when compared to the measured maximum concentration. Although the moving 3-year mean is not as robust as the peak indicator, the 3-year mean does tend to dampen some of the year-to-year variation caused by meteorology. This yields data that are more suitable for trend analysis, when compared with data for individual years.

Data presented in this subsection are evaluated using the maximum measured concentrations, as well as the peak indicator and the number of days the State standard was exceeded for each air basin. In most cases, the data used reflect data for record extracted from the ARB ADAM or U.S. EPA AIRS databases.

5.4.2 Federal Attainment of Nitrogen Dioxide

As mentioned previously, there is no federal 1-hour standard for NO₂. The federal NO₂ annual arithmetic mean standard is 0.053 ppm. The entire state has been designated as unclassified/attainment for the past decade. Table 5.1 lists the annual arithmetic means for selected areas in California for the past three years.

Table 5.1 Annual Arithmetic Mean Nitrogen Dioxide Concentrations (ppb) ^a

Area	2002	2003	2004	2005
Statewide	40.2	35.3	33.7	30.9
Lake Tahoe	11.7	10.0	NA	NA
Mojave Desert	25.0	23.7	22.7	22.3
North Central Coast	6.9	6.2	6.8	7.6
North Coast	9.5	8.9	8.8	7.8
Sacramento Valley	19.6	17.9	16.7	16.4
Salton Sea	16.2	16.2	15.1	14.8
San Diego	22.2	20.8	23.3	24.6
San Francisco Bay Area	19.1	18.3	17.4	18.5
San Joaquin Valley	23.9	23.1	18.9	20.9
South Central Coast	16.7	15.2	13.9	15.0
South Coast	40.2	35.3	33.7	30.9

^a Annual average concentrations of NO₂ for a specific basin are estimated using the site reporting the highest annual average concentration within that basin. The annual average for a specific site is calculated by adding all hourly concentrations at that site and dividing by the total number of observations.

All concentrations are well below the federal annual arithmetic mean standard, and all further discussion focuses on the more stringent state standard.

5.4.3 Ambient 1-Hour Nitrogen Dioxide Concentrations

Table 5.2 shows the maximum measured 1-hour NO₂ concentration and the number of days with measured 1-hour concentrations exceeding the State 1-hour standard of 0.25 ppm. The table includes statistics for the years 2002 through 2004, which were extracted from the ARB ADAM database in May 2005. It is important to note that the counts of exceedance days reflect area-wide totals. In other words, each day with an exceedance is counted as one day, regardless of the number of individual sites with concentrations exceeding the standard.

During 2002 through 2004, the State standard was not exceeded in any of the air basins with the exception of one exceedance in the South Coast Air Basin in 2002. This measurement, a 1-hour concentration of 0.262 parts per million, rounds to 0.26 ppm, which is an exceedance of the State NO₂ standard. Because State designations are based on three years of data, the 0.26 ppm measurement was evaluated for designation purposes during three separate years. In all cases, the 0.26 ppm measurement was higher than the rounded EPDC value, and therefore, was excluded from the State designation process as an extreme concentration event. As a result, the South Coast Air Basin maintained its attainment designation. In 2003, the maximum 1-hour value in the South Coast Air Basin had dropped significantly to 0.163 ppm.

In general, the South Coast, Salton Sea, San Diego, Sacramento Valley, and Mojave Desert Air Basins have higher maximum 1-hour values than the other regions. Mountain Counties did not have a sufficient amount of data for 2004 to produce any meaningful summary statistics for comparison. There are currently no sites collecting NO₂ data in the Mountain Counties region. As shown in Table 5.2, the maximum 1-hour values in the Mountain Counties region for 2002 and 2003 are less than half the state 1-hour standard.

Almost all regions have experienced noticeable variability in maximum 1-hour values over the past three years. This is expected and can be partly attributed to year-to-year meteorological variability. With the exception of the one exceedance in the South Coast, maximum 1-hour concentrations are well below the state standard of 0.25 ppm.

Table 5.2 Measured NO₂ Statistics for 2002-2004 for California Air Basins

Basin	Year	Maximum 1-Hour	Days Exceeding State 1-Hour Std
Lake Tahoe	2002	0.088	0
	2003	0.059	0
	2004	0.068	0
Mojave	2002	0.101	0
	2003	0.095	0
	2004	0.103	0
Mountain Counties	2002	0.043	0
	2003	0.019	0
	2004	NA	NA
North Central Coast	2002	0.049	0
	2003	0.053	0
	2004	0.139	0
North Coast	2002	0.08	0
	2003	0.053	0
	2004	0.037	0
Sacramento Valley	2002	0.09	0
	2003	0.102	0
	2004	0.146	0
Salton Sea	2002	0.138	0
	2003	0.189	0
	2004	0.108	0
San Diego	2002	0.126	0
	2003	0.148	0
	2004	0.125	0
San Francisco Bay Area	2002	0.08	0
	2003	0.081	0
	2004	0.073	0
San Joaquin Valley	2002	0.107	0
	2003	0.092	0
	2004	0.083	0
South Central Coast	2002	0.064	0
	2003	0.103	0
	2004	0.071	0
South Coast	2002	0.262	1
	2003	0.163	0
	2004	0.157	0

Notes: Days exceeding State 1-hour standard are distinct area-wide days, meaning the exceedance day is counted only once, even if multiple sites experienced an exceedance on the same day. The State 1-hour NO₂ standard is exceeded when the concentration is equal to or greater than 0.25 ppm. Data Source: ADAM 2005.

NA = No data available.

5.4.4 Nitrogen Dioxide Season

NO₂ is formed in the ambient air through the oxidation of NO. NO_x, the generic term for NO₂ and NO, play a major role in the formation of ozone, particulate matter, and haze. The major sources of man-made NO_x emissions are high-temperature combustion processes such as those that occur in automobiles and power plants. Since NO₂ plays a photochemical role in ozone formation, its levels are lower in the summer when ozone is highest. The combination of less chemical reactivity and a higher frequency of lower atmosphere inversions, results in the highest concentrations of NO₂ occurring in the cooler months.

Figure 5.4 and Table 5.3 show the average 1-hour maximum concentration for each month during 1990 through 2004. The graph and table include information for each of California's largest air basins or planning areas.

As shown by the graph, the statewide maximum 1-hour average is driven by the maximum 1-hour average in the South Coast region.

Figure 5.4 Seasonal Distribution of the 1-Hour Nitrogen Dioxide Concentration (1990-2004)

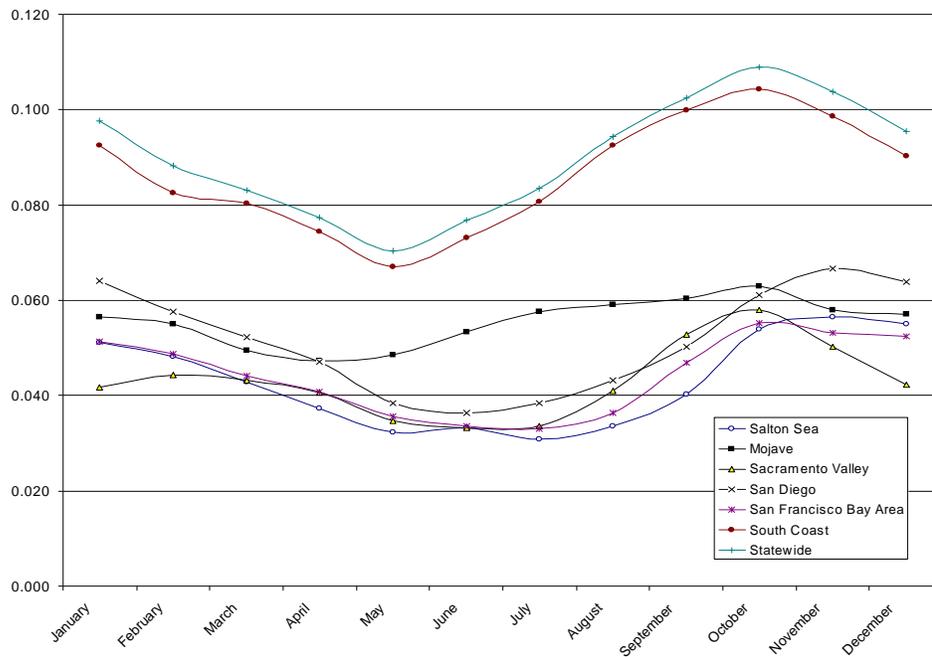


Table 5.3 Monthly Average of Maximum 1-Hour Nitrogen Dioxide Concentration Parts Per Million (1990-2004)

MONTH	AIR BASIN						
	Salton Sea	Mojave	Sacramento Valley	San Diego	San Francisco Bay Area	South Coast	Statewide
January	0.051	0.057	0.042	0.064	0.051	0.092	0.098
February	0.048	0.055	0.044	0.058	0.049	0.082	0.088
March	0.043	0.050	0.043	0.052	0.044	0.080	0.083
April	0.037	0.047	0.041	0.047	0.041	0.074	0.077
May	0.032	0.048	0.035	0.038	0.036	0.067	0.070
Jun	0.033	0.053	0.033	0.036	0.034	0.073	0.077
July	0.031	0.058	0.034	0.038	0.033	0.081	0.083
August	0.034	0.059	0.041	0.043	0.036	0.093	0.094
September	0.040	0.060	0.053	0.050	0.047	0.100	0.103
October	0.054	0.063	0.058	0.061	0.055	0.104	0.109
November	0.056	0.058	0.050	0.067	0.053	0.099	0.104
December	0.055	0.057	0.042	0.064	0.052	0.090	0.095

Note: The seasonality is represented by the monthly average of daily maximum values that were measured at one or more monitoring sites in an air basin or planning area for the years 1990 to 2004.

Statewide concentration averages are higher than specific air basin concentrations since on some days within a given month, higher NO₂ levels have been observed in an air basin other than the South Coast. Data Source: ADAM 2005

5.4.5 Frequency of Measured 1-Hour Nitrogen Dioxide Concentrations

The following graphs and tables present frequency distributions of 1-hour NO₂ concentrations for each of the years 2002, 2003, and 2004. The frequency distributions represent a summary of the maximum daily NO₂ concentrations measured at all the sites within each air basin or planning area, as well as for all sites within the State. Maximum NO₂ concentration levels are aggregated into bins or concentration ranges of 0.01 ppm in size (for example, daily maximum concentrations in the range of 0.00 ppm to 0.01 ppm, 0.01 ppm to 0.02 ppm, 0.02 ppm to 0.03 ppm). The original input data were taken from the ARB air quality CD (ARB 2005).

Figures 5.5 through 5.7 show frequency information on a statewide basis. In contrast, Tables 5.5 through 5.7 present information for each area of California, as well as for the State as a whole. Each table provides information for an individual year: 2002, 2003, and 2004. The tabular information includes the frequency of the various ranges of maximum daily 1-hour concentrations measured in each area, expressed both as a count and as a percentage of the total. In addition, the tables also show the cumulative frequency (again, expressed as a count and as a percentage), from the lowest range to the highest range. The frequency graphs and tables provide information on the frequency of high

concentrations for each area, as well as the most frequent, or predominant concentrations levels. This information provides insight about the impact of setting the standards at various levels.

As shown in Figures 5.5 through 5.7, the majority of maximum daily 1-hour NO₂ concentrations during 2002 to 2004 were below the level of the State 1-hour standard. An average of 93 percent of the daily maximums were in the range of 0.00 ppm to 0.05 ppm. During all three years, an average of 99.9 percent of the daily maximum concentrations were at or below 0.13 ppm, half the level of the State standard.

When looking at the data for individual air basins or planning areas, the results are similar. We do, however, see some variation in the ranges of concentrations represented. For example, the maximum concentrations represented in the South Coast Air Basin range from 0.00 ppm to 0.27 ppm for the 1-hour values, with only one exceedance of the state 1-hr standard of 0.25 ppm. In contrast, the concentrations for the Lake Tahoe Air Basin range from 0.00 ppm to 0.09 ppm for the 1-hour values. However, all individual air basins and planning areas show a relatively large percentage of concentrations in the 0.00 ppm to 0.08 ppm range (one-third the state 1-hr standard), and all areas had at least 50 percent of their maximum 1-hour concentrations at or below 0.05 ppm during 2002 through 2004.

Figure 5.5 2002 Statewide Frequency of Maximum Daily 1-Hour Nitrogen Dioxide Concentrations (PPM)

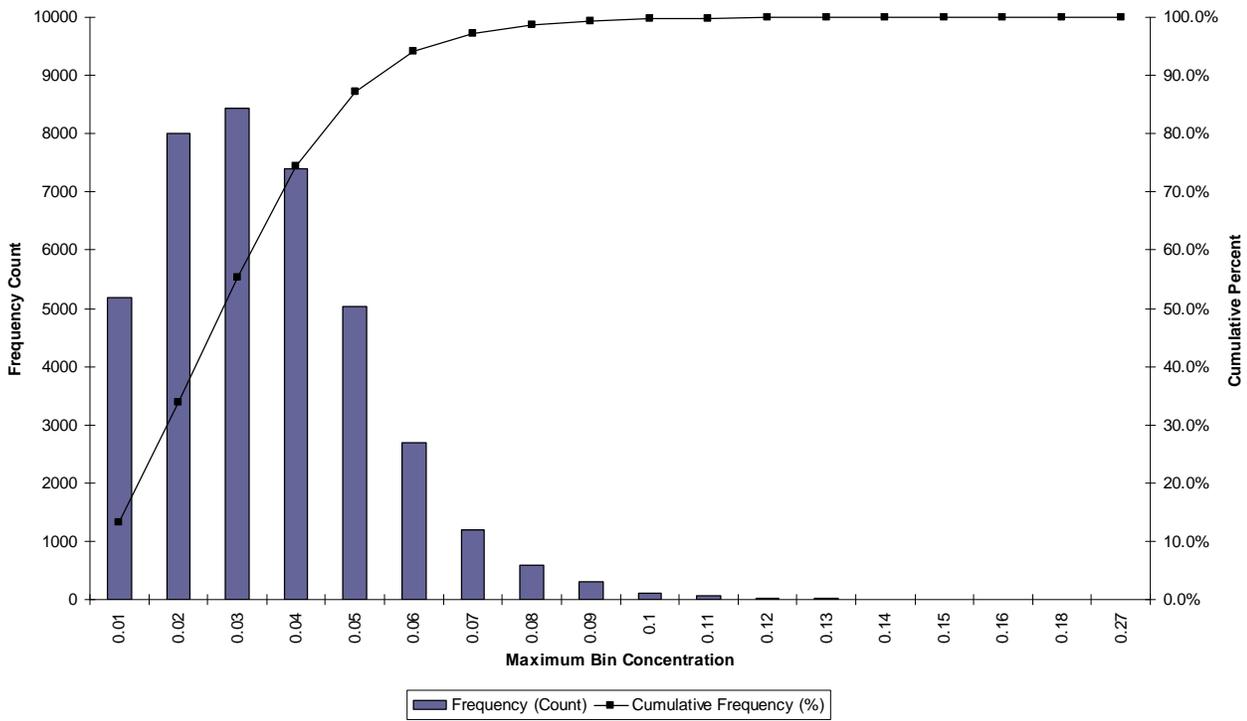


Figure 5.6 2003 Statewide Frequency of Maximum Daily 1-Hour Nitrogen Dioxide Concentrations (PPM)

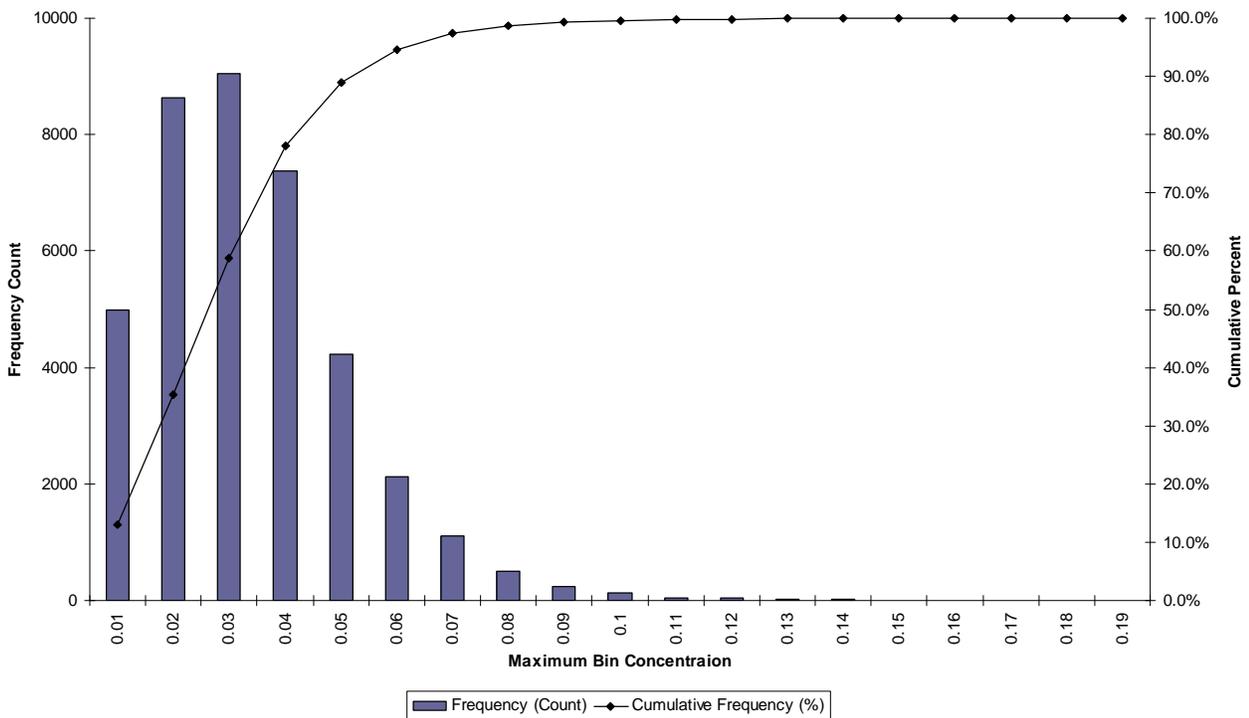


Figure 5.7 2004 Statewide Frequency of Maximum Daily 1-Hour Nitrogen Dioxide Concentrations (PPM)

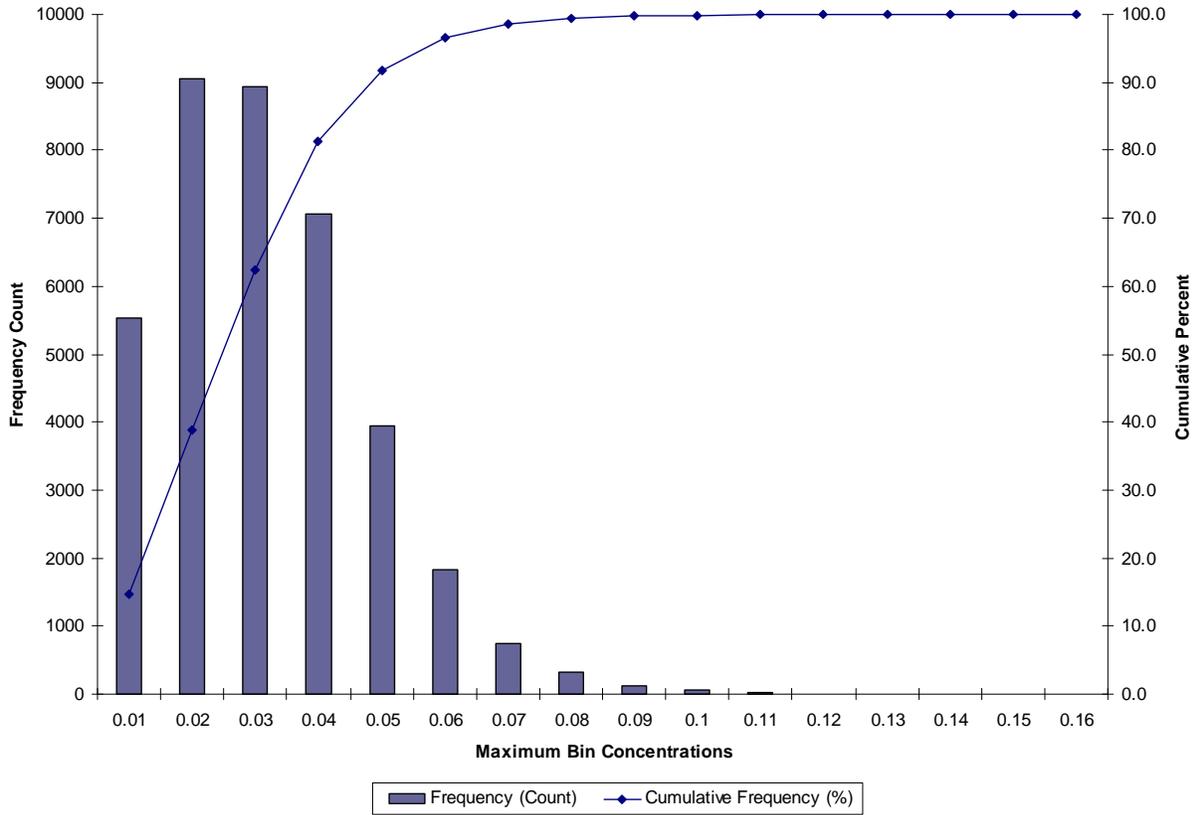


Table 5.4 Frequency of 1-Hour Nitrogen Dioxide Concentrations Measured During 2002

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
California Statewide	0	0.01	5185	13.3%	5185	13.3%
	0.01	0.02	8009	20.5%	13194	33.8%
	0.02	0.03	8433	21.6%	21627	55.4%
	0.03	0.04	7403	18.9%	29030	74.3%
	0.04	0.05	5035	12.9%	34065	87.2%
	0.05	0.06	2684	6.9%	36749	94.1%
	0.06	0.07	1188	3.0%	37937	97.1%
	0.07	0.08	592	1.5%	38529	98.6%
	0.08	0.09	301	0.8%	38830	99.4%
	0.09	0.1	114	0.3%	38944	99.7%
	0.1	0.11	62	0.2%	39006	99.8%
	0.11	0.12	25	0.1%	39031	99.9%
	0.12	0.13	22	0.1%	39053	100.0%
	0.13	0.14	6	0.0%	39059	100.0%
	0.14	0.15	3	0.0%	39062	100.0%
	0.15	0.16	2	0.0%	39064	100.0%
	0.17	0.18	1	0.0%	39065	100.0%
0.26	0.27	1	0.0%	39066	100.0%	
Lake Tahoe	0	0.01	312	46.5%	312	46.5%
	0.01	0.02	157	23.4%	469	69.9%
	0.02	0.03	115	17.1%	584	87.0%
	0.03	0.04	66	9.8%	650	96.9%
	0.04	0.05	16	2.4%	666	99.3%
	0.05	0.06	2	0.3%	668	99.6%
	0.06	0.07	1	0.1%	669	99.7%
	0.07	0.08	1	0.1%	670	99.9%
	0.08	0.09	1	0.1%	671	100.0%
Mojave Desert	0	0.01	222	12.4%	222	12.4%
	0.01	0.02	308	17.3%	530	29.7%
	0.02	0.03	224	12.5%	754	42.2%
	0.03	0.04	361	20.2%	1115	62.5%
	0.04	0.05	312	17.5%	1427	79.9%
	0.05	0.06	193	10.8%	1620	90.8%
	0.06	0.07	105	5.9%	1725	96.6%
	0.07	0.08	50	2.8%	1775	99.4%
	0.08	0.09	8	0.4%	1783	99.9%
	0.09	0.1	1	0.1%	1784	99.9%
	0.1	0.11	1	0.1%	1785	100.0%

Table 5.4 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
Mountain Counties	0	0.01	26	22.0%	26	22.0%
	0.01	0.02	64	54.2%	90	76.3%
	0.02	0.03	25	21.2%	115	97.5%
	0.03	0.04	1	0.8%	116	98.3%
	0.04	0.05	2	1.7%	118	100.0%
North Central Coast	0	0.01	298	41.9%	298	41.9%
	0.01	0.02	268	37.6%	566	79.5%
	0.02	0.03	107	15.0%	673	94.5%
	0.03	0.04	36	5.1%	709	99.6%
	0.04	0.05	3	0.4%	712	100.0%
North Coast	0	0.01	163	22.3%	163	22.3%
	0.01	0.02	336	46.0%	499	68.4%
	0.02	0.03	189	25.9%	688	94.2%
	0.03	0.04	37	5.1%	725	99.3%
	0.04	0.05	1	0.1%	726	99.5%
	0.05	0.06	2	0.3%	728	99.7%
	0.07	0.08	2	0.3%	730	100.0%
Sacramento Valley	0	0.01	316	8.9%	316	8.9%
	0.01	0.02	977	27.5%	1293	36.4%
	0.02	0.03	985	27.7%	2278	64.1%
	0.03	0.04	679	19.1%	2957	83.2%
	0.04	0.05	334	9.4%	3291	92.6%
	0.05	0.06	185	5.2%	3476	97.8%
	0.06	0.07	61	1.7%	3537	99.5%
	0.07	0.08	12	0.3%	3549	99.9%
	0.08	0.09	4	0.1%	3553	100.0%
Salton Sea	0	0.01	168	13.0%	168	13.0%
	0.01	0.02	237	18.4%	405	31.4%
	0.02	0.03	231	17.9%	636	49.3%
	0.03	0.04	269	20.9%	905	70.2%
	0.04	0.05	213	16.5%	1118	86.7%
	0.05	0.06	105	8.1%	1223	94.9%
	0.06	0.07	36	2.8%	1259	97.7%
	0.07	0.08	15	1.2%	1274	98.8%
	0.08	0.09	4	0.3%	1278	99.1%
	0.09	0.1	4	0.3%	1282	99.5%
	0.1	0.11	1	0.1%	1283	99.5%
	0.11	0.12	2	0.2%	1285	99.7%
	0.12	0.13	3	0.2%	1288	99.9%
	0.13	0.14	1	0.1%	1289	100.0%

Table 5.4 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Diego	0	0.01	113	3.9%	113	3.9%
	0.01	0.02	474	16.4%	587	20.3%
	0.02	0.03	710	24.6%	1297	44.9%
	0.03	0.04	656	22.7%	1953	67.6%
	0.04	0.05	508	17.6%	2461	85.2%
	0.05	0.06	241	8.3%	2702	93.5%
	0.06	0.07	119	4.1%	2821	97.6%
	0.07	0.08	43	1.5%	2864	99.1%
	0.08	0.09	11	0.4%	2875	99.5%
	0.09	0.1	8	0.3%	2883	99.8%
	0.1	0.11	4	0.1%	2887	99.9%
	0.11	0.12	1	0.0%	2888	100.0%
0.12	0.13	1	0.0%	2889	100.0%	
San Francisco Bay Area	0	0.01	391	7.6%	391	7.6%
	0.01	0.02	1364	26.6%	1755	34.2%
	0.02	0.03	1492	29.1%	3247	63.3%
	0.03	0.04	1204	23.5%	4451	86.7%
	0.04	0.05	529	10.3%	4980	97.1%
	0.05	0.06	122	2.4%	5102	99.4%
	0.06	0.07	26	0.5%	5128	99.9%
	0.07	0.08	3	0.1%	5131	100.0%
San Joaquin Valley	0	0.01	181	2.6%	181	2.6%
	0.01	0.02	1343	19.6%	1524	22.2%
	0.02	0.03	2012	29.3%	3536	51.6%
	0.03	0.04	1701	24.8%	5237	76.4%
	0.04	0.05	949	13.8%	6186	90.2%
	0.05	0.06	422	6.2%	6608	96.4%
	0.06	0.07	164	2.4%	6772	98.7%
	0.07	0.08	59	0.9%	6831	99.6%
	0.08	0.09	21	0.3%	6852	99.9%
	0.09	0.1	4	0.1%	6856	100.0%
	0.1	0.11	2	0.0%	6858	100.0%

Table 5.4 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Central Coast	0	0.01	2868	39.9%	2868	39.9%
	0.01	0.02	2037	28.3%	4905	68.2%
	0.02	0.03	1398	19.4%	6303	87.6%
	0.03	0.04	635	8.8%	6938	96.4%
	0.04	0.05	199	2.8%	7137	99.2%
	0.05	0.06	46	0.6%	7183	99.8%
	0.06	0.07	11	0.2%	7194	100.0%
South Coast	0	0.01	127	1.6%	127	1.6%
	0.01	0.02	444	5.5%	571	7.0%
	0.02	0.03	945	11.6%	1516	18.6%
	0.03	0.04	1758	21.6%	3274	40.2%
	0.04	0.05	1969	24.2%	5243	64.4%
	0.05	0.06	1366	16.8%	6609	81.2%
	0.06	0.07	665	8.2%	7274	89.4%
	0.07	0.08	407	5.0%	7681	94.4%
	0.08	0.09	252	3.1%	7933	97.5%
	0.09	0.1	97	1.2%	8030	98.7%
	0.1	0.11	54	0.7%	8084	99.4%
	0.11	0.12	22	0.3%	8106	99.6%
	0.12	0.13	18	0.2%	8124	99.9%
	0.13	0.14	5	0.1%	8129	99.9%
	0.14	0.15	3	0.0%	8132	100.0%
	0.15	0.16	2	0.0%	8134	100.0%
	0.17	0.18	1	0.0%	8135	100.0%
0.26	0.27	1	0.0%	8136	100.0%	

Table 5.5 Frequency of 1-Hour Nitrogen Dioxide Concentrations Measured During 2003

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
California Statewide	0	0.01	4984	13.0%	4984	13.0%
	0.01	0.02	8623	22.4%	13607	35.4%
	0.02	0.03	9054	23.5%	22661	58.9%
	0.03	0.04	7377	19.2%	30038	78.0%
	0.04	0.05	4225	11.0%	34263	89.0%
	0.05	0.06	2129	5.5%	36392	94.6%
	0.06	0.07	1100	2.9%	37492	97.4%
	0.07	0.08	492	1.3%	37984	98.7%
	0.08	0.09	243	0.6%	38227	99.3%
	0.09	0.1	122	0.3%	38349	99.6%
	0.1	0.11	51	0.1%	38400	99.8%
	0.11	0.12	43	0.1%	38443	99.9%
	0.12	0.13	20	0.1%	38463	99.9%
	0.13	0.14	11	0.0%	38474	100.0%
	0.14	0.15	5	0.0%	38479	100.0%
	0.15	0.16	2	0.0%	38481	100.0%
	0.16	0.17	2	0.0%	38483	100.0%
	0.17	0.18	2	0.0%	38485	100.0%
0.18	0.19	1	0.0%	38486	100.0%	
Lake Tahoe	0	0.01	358	49.4%	358	49.4%
	0.01	0.02	189	26.1%	547	75.4%
	0.02	0.03	104	14.3%	651	89.8%
	0.03	0.04	57	7.9%	708	97.7%
	0.04	0.05	13	1.8%	721	99.4%
	0.05	0.06	4	0.6%	725	100.0%
Mojave Desert	0	0.01	227	12.9%	227	12.9%
	0.01	0.02	322	18.3%	549	31.1%
	0.02	0.03	282	16.0%	831	47.1%
	0.03	0.04	322	18.3%	1153	65.4%
	0.04	0.05	261	14.8%	1414	80.2%
	0.05	0.06	188	10.7%	1602	90.9%
	0.06	0.07	100	5.7%	1702	96.5%
	0.07	0.08	49	2.8%	1751	99.3%
	0.08	0.09	10	0.6%	1761	99.9%
	0.09	0.1	2	0.1%	1763	100.0%
Mountain Counties	0	0.01	39	75.0%	39	75.0%
	0.01	0.02	13	25.0%	52	100.0%

Table 5.5 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
North Central Coast	0	0.01	312	47.7%	312	47.7%
	0.01	0.02	229	35.0%	541	82.7%
	0.02	0.03	96	14.7%	637	97.4%
	0.03	0.04	13	2.0%	650	99.4%
	0.04	0.05	1	0.2%	651	99.5%
	0.05	0.06	3	0.5%	654	100.0%
North Coast	0	0.01	129	17.7%	129	17.7%
	0.01	0.02	441	60.5%	570	78.2%
	0.02	0.03	136	18.7%	706	96.8%
	0.03	0.04	20	2.7%	726	99.6%
	0.04	0.05	2	0.3%	728	99.9%
	0.05	0.06	1	0.1%	729	100.0%
Sacramento Valley	0	0.01	283	8.6%	283	8.6%
	0.01	0.02	884	26.8%	1167	35.4%
	0.02	0.03	1114	33.8%	2281	69.2%
	0.03	0.04	624	18.9%	2905	88.1%
	0.04	0.05	236	7.2%	3141	95.3%
	0.05	0.06	99	3.0%	3240	98.3%
	0.06	0.07	39	1.2%	3279	99.5%
	0.07	0.08	12	0.4%	3291	99.8%
	0.08	0.09	4	0.1%	3295	99.9%
	0.1	0.11	2	0.1%	3297	100.0%
Salton Sea	0	0.01	186	13.6%	186	13.6%
	0.01	0.02	317	23.1%	503	36.7%
	0.02	0.03	287	20.9%	790	57.6%
	0.03	0.04	287	20.9%	1077	78.6%
	0.04	0.05	184	13.4%	1261	92.0%
	0.05	0.06	58	4.2%	1319	96.2%
	0.06	0.07	29	2.1%	1348	98.3%
	0.07	0.08	6	0.4%	1354	98.8%
	0.08	0.09	1	0.1%	1355	98.8%
	0.09	0.1	3	0.2%	1358	99.1%
	0.1	0.11	1	0.1%	1359	99.1%
	0.11	0.12	1	0.1%	1360	99.2%
	0.12	0.13	4	0.3%	1364	99.5%
	0.13	0.14	1	0.1%	1365	99.6%
	0.14	0.15	1	0.1%	1366	99.6%
	0.15	0.16	1	0.1%	1367	99.7%
	0.16	0.17	1	0.1%	1368	99.8%
	0.17	0.18	2	0.1%	1370	99.9%
0.18	0.19	1	0.1%	1371	100.0%	

Table 5.5 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Diego	0	0.01	92	3.2%	92	3.2%
	0.01	0.02	496	17.3%	588	20.5%
	0.02	0.03	719	25.1%	1307	45.6%
	0.03	0.04	704	24.6%	2011	70.2%
	0.04	0.05	458	16.0%	2469	86.2%
	0.05	0.06	235	8.2%	2704	94.4%
	0.06	0.07	92	3.2%	2796	97.6%
	0.07	0.08	31	1.1%	2827	98.7%
	0.08	0.09	17	0.6%	2844	99.3%
	0.09	0.1	11	0.4%	2855	99.7%
	0.1	0.11	3	0.1%	2858	99.8%
	0.11	0.12	1	0.0%	2859	99.8%
	0.12	0.13	2	0.1%	2861	99.9%
	0.13	0.14	1	0.0%	2862	99.9%
	0.14	0.15	2	0.1%	2864	100.0%
San Francisco Bay Area	0	0.01	340	7.4%	340	7.4%
	0.01	0.02	1413	30.8%	1753	38.2%
	0.02	0.03	1485	32.4%	3238	70.6%
	0.03	0.04	1028	22.4%	4266	93.0%
	0.04	0.05	240	5.2%	4506	98.2%
	0.05	0.06	56	1.2%	4562	99.5%
	0.06	0.07	22	0.5%	4584	99.9%
	0.07	0.08	2	0.0%	4586	100.0%
	0.08	0.09	1	0.0%	4587	100.0%
San Joaquin Valley	0	0.01	241	3.3%	241	3.3%
	0.01	0.02	1609	22.1%	1850	25.4%
	0.02	0.03	2372	32.5%	4222	57.9%
	0.03	0.04	1766	24.2%	5988	82.2%
	0.04	0.05	782	10.7%	6770	92.9%
	0.05	0.06	297	4.1%	7067	97.0%
	0.06	0.07	137	1.9%	7204	98.8%
	0.07	0.08	60	0.8%	7264	99.7%
	0.08	0.09	23	0.3%	7287	100.0%
	0.09	0.1	2	0.0%	7289	100.0%

Table 5.5 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Central Coast	0	0.01	2689	37.0%	2689	37.0%
	0.01	0.02	2298	31.6%	4987	68.6%
	0.02	0.03	1451	20.0%	6438	88.5%
	0.03	0.04	606	8.3%	7044	96.9%
	0.04	0.05	178	2.4%	7222	99.3%
	0.05	0.06	43	0.6%	7265	99.9%
	0.06	0.07	4	0.1%	7269	99.9%
	0.07	0.08	3	0.0%	7272	100.0%
	0.1	0.11	1	0.0%	7273	100.0%
South Coast	0	0.01	88	1.1%	88	1.1%
	0.01	0.02	412	5.2%	500	6.3%
	0.02	0.03	1008	12.8%	1508	19.1%
	0.03	0.04	1950	24.7%	3458	43.9%
	0.04	0.05	1870	23.7%	5328	67.6%
	0.05	0.06	1145	14.5%	6473	82.1%
	0.06	0.07	677	8.6%	7150	90.7%
	0.07	0.08	329	4.2%	7479	94.9%
	0.08	0.09	187	2.4%	7666	97.3%
	0.09	0.1	104	1.3%	7770	98.6%
	0.1	0.11	44	0.6%	7814	99.1%
	0.11	0.12	41	0.5%	7855	99.7%
	0.12	0.13	14	0.2%	7869	99.8%
	0.13	0.14	9	0.1%	7878	99.9%
	0.14	0.15	2	0.0%	7880	100.0%
	0.15	0.16	1	0.0%	7881	100.0%
	0.16	0.17	1	0.0%	7882	100.0%

Table 5.6 Frequency of 1-Hour Nitrogen Dioxide Concentrations
Measured During 2004

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
California Statewide	0	0.01	5538	14.7	5538	14.7
	0.01	0.02	9063	24.1	14601	38.8
	0.02	0.03	8933	23.7	23534	62.5
	0.03	0.04	7071	18.8	30605	81.2
	0.04	0.05	3950	10.5	34555	91.7
	0.05	0.06	1827	4.8	36382	96.6
	0.06	0.07	743	2.0	37125	98.5
	0.07	0.08	327	0.9	37452	99.4
	0.08	0.09	123	0.3	37575	99.7
	0.09	0.1	55	0.1	37630	99.9
	0.1	0.11	28	0.1	37658	100.0
	0.11	0.12	8	0.0	37666	100.0
	0.12	0.13	5	0.0	37671	100.0
	0.13	0.14	2	0.0	37673	100.0
	0.14	0.15	1	0.0	37674	100.0
0.15	0.16	1	0.0	37675	100.0	
Lake Tahoe	0	0.01	282	57.6	282	57.6
	0.01	0.02	106	21.6	388	79.2
	0.02	0.03	54	11.0	442	90.2
	0.03	0.04	29	5.9	471	96.1
	0.04	0.05	13	2.7	484	98.8
	0.05	0.06	4	0.8	488	99.6
	0.06	0.07	2	0.4	490	100.0
Mojave Desert	0	0.01	246	13.7	246	13.7
	0.01	0.02	334	18.7	580	32.4
	0.02	0.03	237	13.2	817	45.6
	0.03	0.04	369	20.6	1186	66.3
	0.04	0.05	301	16.8	1487	83.1
	0.05	0.06	187	10.4	1674	93.5
	0.06	0.07	90	5.0	1764	98.5
	0.07	0.08	21	1.2	1785	99.7
	0.08	0.09	3	0.2	1788	99.9
0.1	0.11	2	0.1	1790	100.0	

Table 5.6 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
North Central Coast	0	0.01	299	44.2	299	44.2
	0.01	0.02	236	34.9	535	79.1
	0.02	0.03	105	15.5	640	94.7
	0.03	0.04	33	4.9	673	99.6
	0.04	0.05	2	0.3	675	99.9
	0.13	0.14	1	0.1	676	100.0
North Coast	0	0.01	178	24.4	178	24.4
	0.01	0.02	404	55.4	582	79.8
	0.02	0.03	128	17.6	710	97.4
	0.03	0.04	19	2.6	729	100.0
Sacramento Valley	0	0.01	379	11.1	379	11.1
	0.01	0.02	1201	35.3	1580	46.5
	0.02	0.03	998	29.3	2578	75.8
	0.03	0.04	515	15.1	3093	90.9
	0.04	0.05	206	6.1	3299	97.0
	0.05	0.06	73	2.1	3372	99.1
	0.06	0.07	19	0.6	3391	99.7
	0.07	0.08	7	0.2	3398	99.9
	0.08	0.09	2	0.1	3400	100.0
	0.14	0.15	1	0.0	3401	100.0
Salton Sea	0	0.01	106	7.6	106	7.6
	0.01	0.02	272	19.6	378	27.2
	0.02	0.03	338	24.3	716	51.5
	0.03	0.04	340	24.5	1056	76.0
	0.04	0.05	215	15.5	1271	91.5
	0.05	0.06	91	6.6	1362	98.1
	0.06	0.07	14	1.0	1376	99.1
	0.07	0.08	9	0.6	1385	99.7
	0.08	0.09	1	0.1	1386	99.8
	0.09	0.1	1	0.1	1387	99.9
	0.1	0.11	2	0.1	1389	100.0

Table 5.6 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
San Diego	0	0.01	123	4.2	123	4.2
	0.01	0.02	611	20.9	734	25.1
	0.02	0.03	737	25.2	1471	50.4
	0.03	0.04	671	23.0	2142	73.3
	0.04	0.05	445	15.2	2587	88.6
	0.05	0.06	201	6.9	2788	95.4
	0.06	0.07	81	2.8	2869	98.2
	0.07	0.08	33	1.1	2902	99.3
	0.08	0.09	13	0.4	2915	99.8
	0.09	0.1	4	0.1	2919	99.9
	0.1	0.11	1	0.0	2920	100.0
	0.12	0.13	1	0.0	2921	100.0
San Francisco Bay Area	0	0.01	605	12.8	605	12.8
	0.01	0.02	1403	29.7	2008	42.6
	0.02	0.03	1456	30.9	3464	73.4
	0.03	0.04	942	20.0	4406	93.4
	0.04	0.05	259	5.5	4665	98.9
	0.05	0.06	42	0.9	4707	99.7
	0.06	0.07	11	0.2	4718	100.0
	0.07	0.08	1	0.0	4719	100.0
San Joaquin Valley	0	0.01	273	4.1	273	4.1
	0.01	0.02	1771	26.8	2044	31.0
	0.02	0.03	2247	34.0	4291	65.0
	0.03	0.04	1437	21.8	5728	86.8
	0.04	0.05	566	8.6	6294	95.3
	0.05	0.06	214	3.2	6508	98.6
	0.06	0.07	67	1.0	6575	99.6
	0.07	0.08	25	0.4	6600	100.0
	0.08	0.09	2	0.0	6602	100.0

Table 5.6 (continued)

Air Basin / Planning Area	Lower Limit Conc (ppm)	Upper Limit Conc (ppm)	Frequency (Count)	Frequency (%)	Cumulative Frequency	Cumulative Frequency (%)
South Central Coast	0	0.01	2861	40.0	2861	40.0
	0.01	0.02	2200	30.8	5061	70.8
	0.02	0.03	1421	19.9	6482	90.7
	0.03	0.04	519	7.3	7001	98.0
	0.04	0.05	126	1.8	7127	99.7
	0.05	0.06	14	0.2	7141	99.9
	0.06	0.07	3	0.0	7144	100.0
	0.07	0.08	1	0.0	7145	100.0
South Coast	0	0.01	186	2.4	186	2.4
	0.01	0.02	525	6.7	711	9.1
	0.02	0.03	1212	15.5	1923	24.6
	0.03	0.04	2197	28.1	4120	52.7
	0.04	0.05	1817	23.3	5937	76.0
	0.05	0.06	1001	12.8	6938	88.8
	0.06	0.07	456	5.8	7394	94.6
	0.07	0.08	230	2.9	7624	97.6
	0.08	0.09	102	1.3	7726	98.9
	0.09	0.1	50	0.6	7776	99.5
	0.1	0.11	23	0.3	7799	99.8
	0.11	0.12	8	0.1	7807	99.9
	0.12	0.13	4	0.1	7811	100.0
	0.13	0.14	1	0.0	7812	100.0
	0.15	0.16	1	0.0	7813	100.0

5.4.6 Diurnal Variations

NO₂ forms from nitrogen oxide in the presence of sunlight on a scale of minutes. Since the intensity of sunlight and the strength of the emissions of nitrogen oxides vary throughout the day, the concentration of NO₂ varies throughout the day as well. In urban areas, mobile sources produce the most nitrogen oxide emissions as part of their fuel combustion process. The highest NO₂ levels thus occur during the morning and afternoon rush hours. The levels peak during the morning commute hours and decrease slightly only to peak again, to a lesser extent, during the evening commute hours.

5.4.7 Characterization of Nitrogen Dioxide by Air Basin or Planning Area

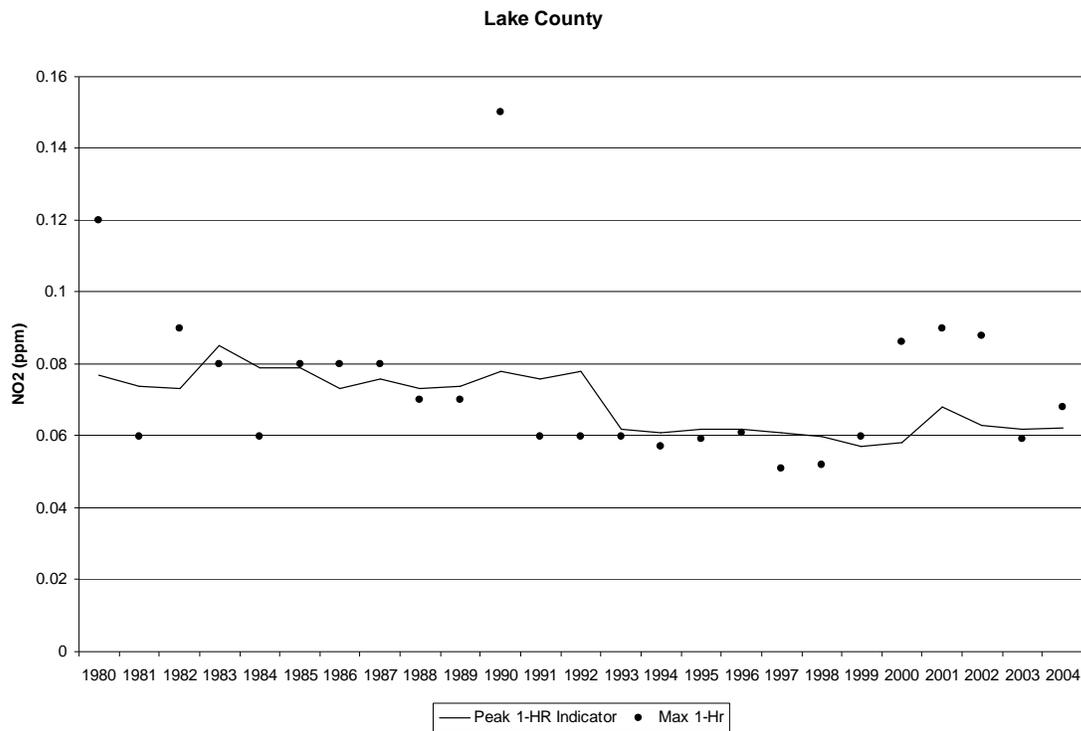
NO₂ levels in ambient air have decreased substantially over the last two decades in most areas of California, despite significant population and economic growth. Statewide, all areas of California are currently unclassified or attainment of the State 1-hr standard. In contrast, the statewide population increased 54 percent, and the average number of vehicle miles traveled each day statewide increased 120 percent from 1980 to 2004. The following subsections describe the trends in 1-hour concentrations for each air basin or planning area of California.

5.4.7.1 Lake Tahoe Air Basin

The Lake Tahoe Air Basin (LTAB) consists of the eastern portions of El Dorado and Placer counties. Less than 1 percent of the State's population lives in the LTAB. This area is well within attainment of the State 1-hr standard.

NO₂ levels are relatively low in the LTAB. During 1980 through 2003, the peak 1-hour indicator varied between 0.085 ppm and 0.057 ppm as illustrated in Figure 5.8. In contrast, the maximum 1-hour concentration was more variable, ranging between 0.12 ppm and 0.051 ppm. This is not unexpected, since the maximum 1-hour concentration is less stable than the peak indicator and therefore, more affected by year-to-year changes in meteorology. The maximum 1-hr concentration has been less than half the State 1-hr standard since 1991.

Figure 5.8 Lake Tahoe

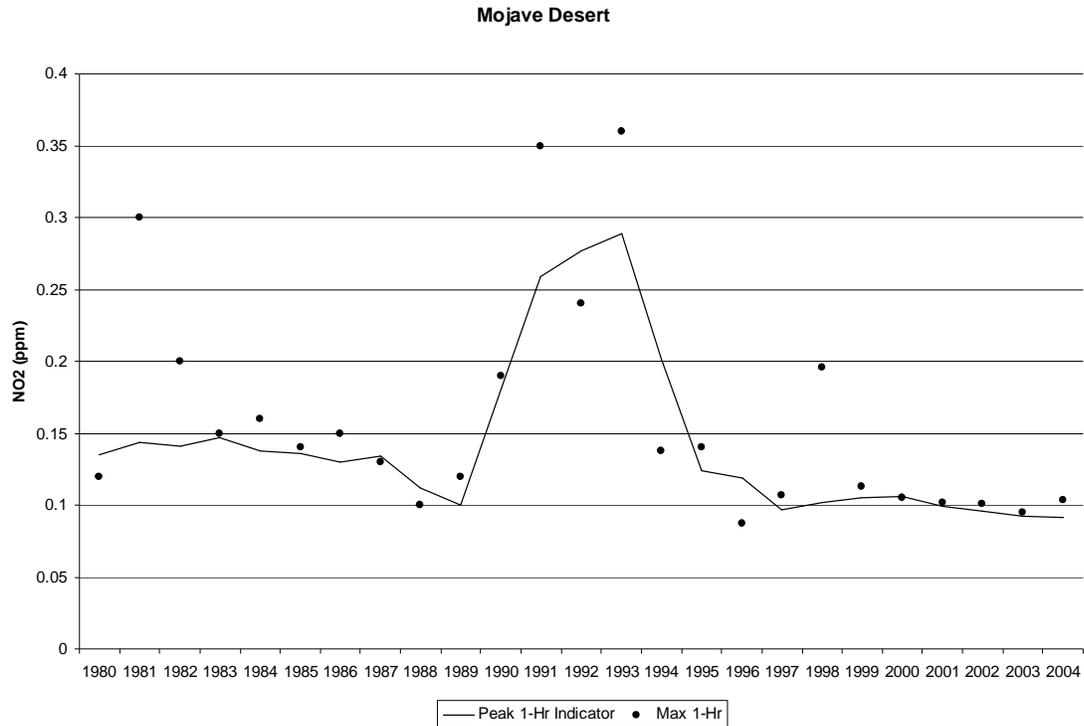


5.4.7.2 Mojave Desert Air Basin

The Mojave Desert Air Basin (MDAB) covers a large part of California's high desert. The air basin includes the eastern half of Kern County, the northeastern portion of Los Angeles County, all of San Bernardino County except for the southwestern corner, and the eastern third of Riverside County. Less than 3 percent of California's population resides in the MDAB.

From 1980 to 2004 both the peak indicator and maximum concentration showed large variations in concentration as illustrated in Figure 5.9. Most notable, between the years of 1990 and 1994, both statistics showed significant increases, some that exceeded the State 1-hr standard. All of these high exceedances occurred at the long-term San Bernardino site. We do not have any information as to what caused these high values. The peak indicator ranged from 0.289 ppm to 0.096 ppm. The maximum concentration ranged from 0.36 ppm to 0.095 ppm. This area has not had an exceedance since 1993 and maximum concentrations have been less than half the State 1-hr standard since 1999.

Figure 5.9 Mojave Desert



5.4.7.3 Mountain Counties Air Basin

For the purposes of this document, the Mountain Counties Air Basin (MCAB) comprises the central and northern portions of the Sierra Nevada mountain region, including Amador, Calaveras, Mariposa, Nevada, Plumas, Sierra, and Tuolumne counties (the MCAB portions of Placer and El Dorado counties are included in the Sacramento Metropolitan Area). The MCAB is thinly populated, its communities separated from one another by the region's complex terrain. A little less than 1 percent of the State's population lives in the MCAB. There are sparse NO₂ data for this region. The very limited data (only four data points) between 1981 and 2004 are all well below half the State 1-hr standard of 0.25 ppm.

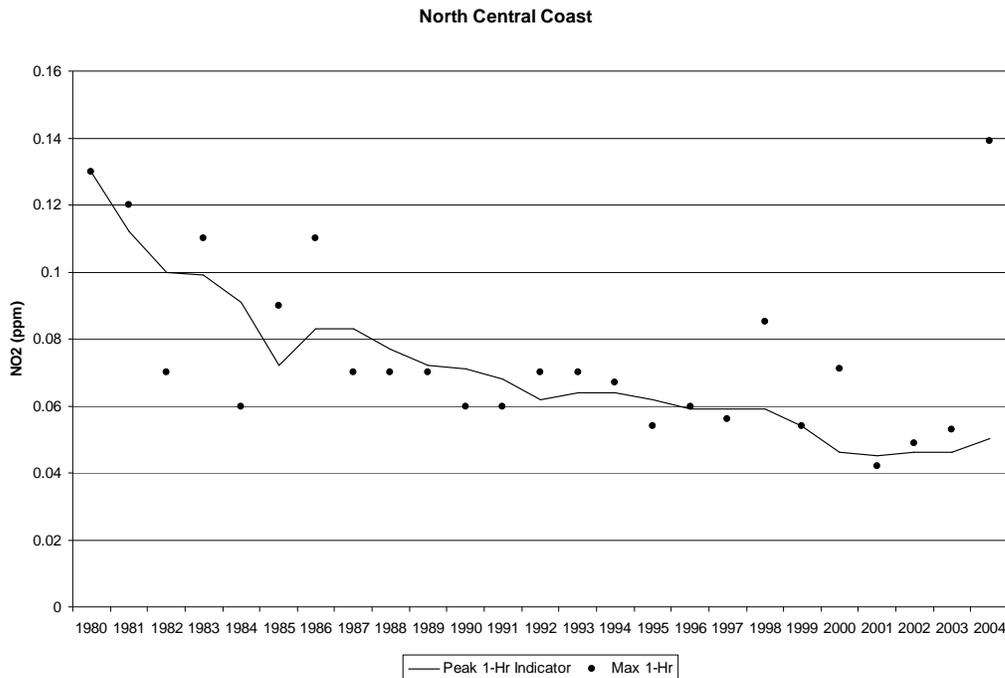
No Figure presented due to the limited number of data points.

5.4.7.4 North Central Coast Air Basin

The North Central Coast Air Basin (NCCAB) is located on the coast of central California and includes all of Monterey, San Benito and Santa Cruz counties. About 2 percent of the State's population lives in the NCCAB.

The NCCAB enjoys relatively clean air and has never had an exceedance of the State 1-hr standard since monitoring began in 1980. There has been a 63% decrease in the peak indicator from 1980 to 2003 and a decrease of 56% in the maximum concentration within the same time period (figure 5.10). In 2004, there was an unusually high concentration of 0.139 ppm at the long-term Salinas site, which is still just over one half the state standard of 0.25 ppm. Since 1981, maximum concentrations have been below half the State 1-hr standard.

Figure 5.10 North Central Coast

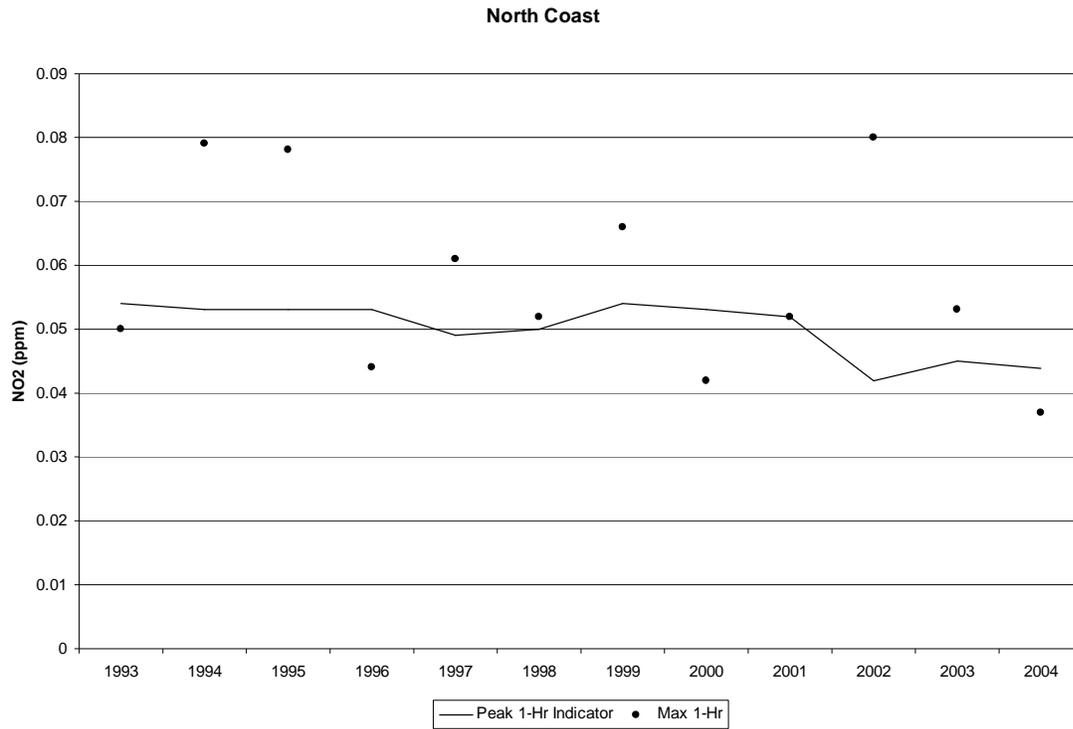


North Coast Air Basin

The North Coast Air Basin (NCAB) includes all of Del Norte, Humboldt, Mendocino, and Trinity counties, as well as the northern portion of Sonoma County. Slightly less than 1 percent of the State's population lives in the NCAB.

The NCAB has not had any NO₂ exceedances in the last ten years. Values for both the peak indicator and maximum concentration are well below half the State 1-hr standard. Looking at Figure 5.11, there appears to be a slight decrease in the peak indicator but a lot of variability associated with the max concentration. This may be attributed to meteorological variability from year to year. All values are well below the state standard.

Figure 5.11 North Coast

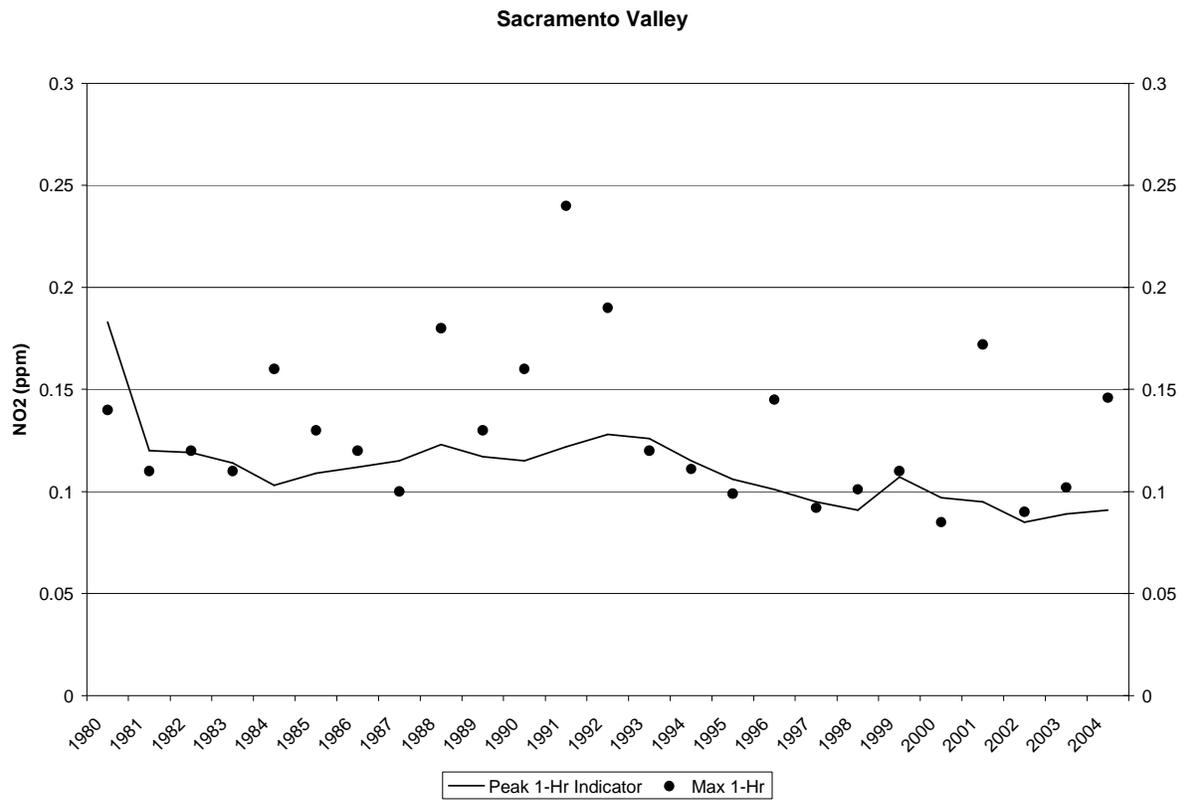


5.4.7.5 Sacramento Valley

The Sacramento Valley Air Basin (SVAB) includes Butte, Colusa, Glenn, Sacramento, Shasta, Sutter, Tehama, Yolo, and Yuba counties, the western urbanized portion of Placer County and the eastern portion of Solano County. In terms of population, the Sacramento Metro Area is home to about 8 percent of California's citizens.

Peak 1-hour NO₂ levels in the SVAB have declined about 50% from 1980 to 2004 (Figure 5.12). The peak 1-hour indicator has shown considerable variability between 1980 and 2004, peaking in the early 1990's. In 2004, the peak 1-hour value had an elevated value of 0.146 ppm. In 1991, the SVAB came close to exceeding the State 1-hr standard with 0.24 ppm, otherwise the peak indicator suggests a steady decline in NO₂ levels and both the peak 1-hr and max 1-hr values are expected to remain well below the State 1-hr standard.

Figure 5.12 Sacramento Valley

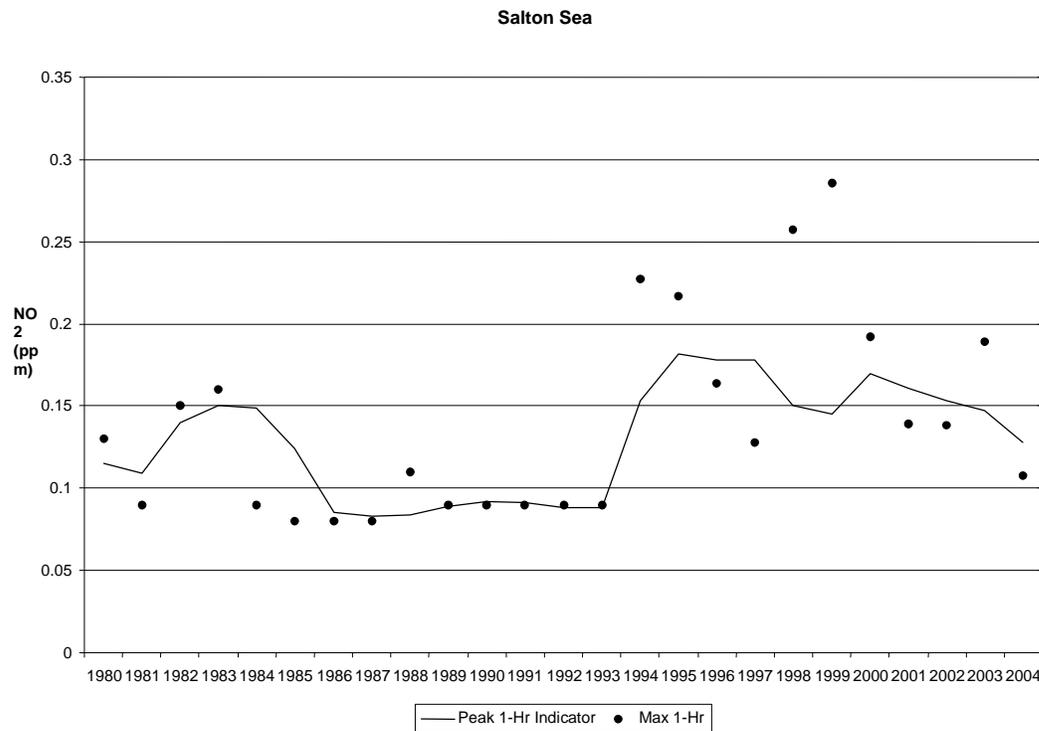


5.4.7.6 Salton Sea Air Basin

The Salton Sea Air Basin (SSAB) occupies the southeast corner of California and includes all of Imperial County, as well as the central portion of Riverside County. Less than 2 percent of the State's population lives in this air basin.

Figure 5.13 shows that the peak indicator and maximum concentration in the SSAB were well below the State 1-hr standard until about 1994. After 1994, there is a noticeable jump in both NO₂ metrics. This marked increase is attributed to the addition of two new NO₂ monitoring sites in the 1990s. Calexico-East and Calexico-Ethel Street have consistently measured the highest NO₂ maximum concentrations in the SSAB since their installation. In spite of their elevated readings, the peak indicator has been gradually decreasing from 0.17 ppm to 0.15 ppm since 1999. The maximum concentration has shown a lot more variability, but has not exceeded the State 1-hr standard since 1999. From 2000 through 2004, the maximum concentration did not exceed 0.2 ppm.

Figure 5.13 Salton Sea

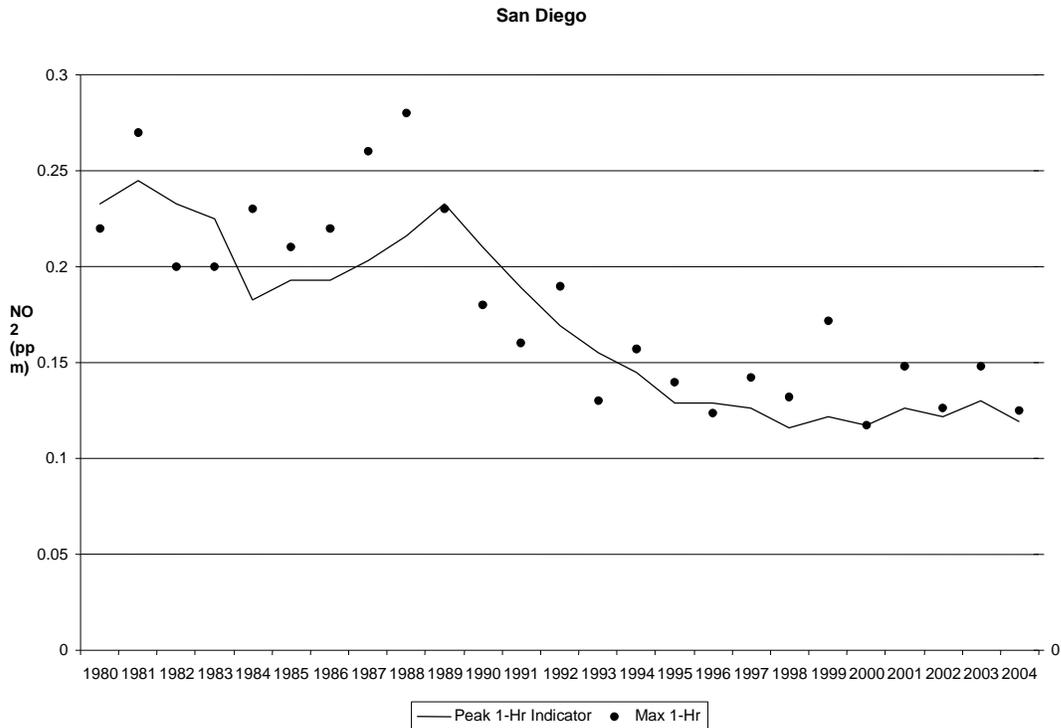


5.4.7.7 San Diego Air Basin

The San Diego Air Basin (SDAB) lies in the southwest corner of California and includes all of San Diego County. About 8 percent of California's population lives in the SDAB. However, the population and emissions are concentrated mainly in the western portion of the air basin. Because of its southerly location and proximity to the ocean, much of the SDAB has a relatively mild climate, without marked seasonality.

The 1-hour NO₂ statistics for the SDAB indicate a substantial improvement in NO₂ air quality since the late 1980s. The peak 1-hour indicator shows about a 50% decline from 1989 to 2004 (Figure 5.14). The measured maximum 1-hour concentration also shows about a 50% decline from 1989 to 2004. The SDAB had its last State 1-hr exceedance in 1989 and the observed values for the past ten years have ranged between 0.157 ppm and 0.126 ppm.

Figure 5.14 San Diego

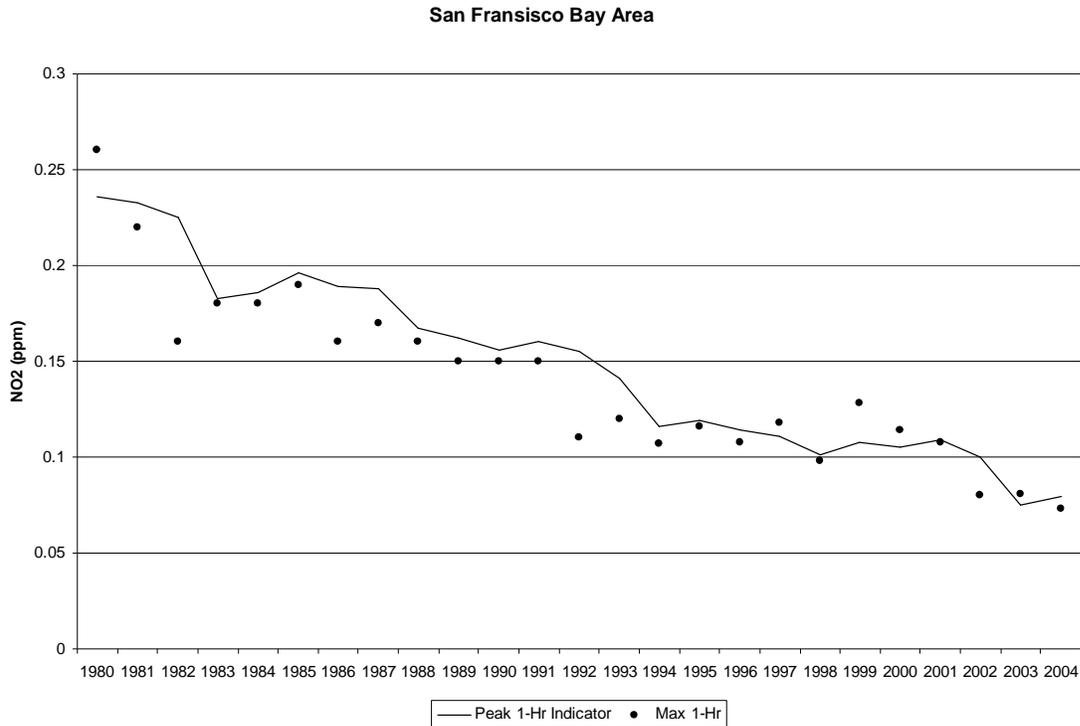


5.4.7.8 San Francisco Bay Area Air Basin

The nine-county San Francisco Bay Area Air Basin (SFBAAB) includes all of Alameda, Contra Costa, Marin, Napa, San Francisco, San Mateo, and Santa Clara counties, the southern half of Sonoma County, and the southwestern portion of Solano County. The SFBAAB is California's second largest urban area and is home to about 19 percent of California's citizens. Because of the SFBAAB's more favorable climate, with cooler temperatures and better ventilation, air quality is usually much better here than in the South Coast, San Joaquin Valley, and Sacramento Air Basins.

NO₂ air quality trends for the SFBAAB do show a consistent downward trend from 1980 to 2004. The peak 1-hour indicator declined about 65 percent from 1982 to 2004, and the maximum 1-hour concentration declined about 70 percent (Figure 5.15). The other noticeable thing from Figure 5.15 is that the peak 1-hr indicator and the maximum 1-hr concentration track each other very closely. Unlike some of the other air basins, the maximum 1-hr concentration does not have a high degree of variability. Maximum 1-hr concentrations have been less than half the State 1-hr standard since 1993.

Figure 5.15 San Francisco Bay Area

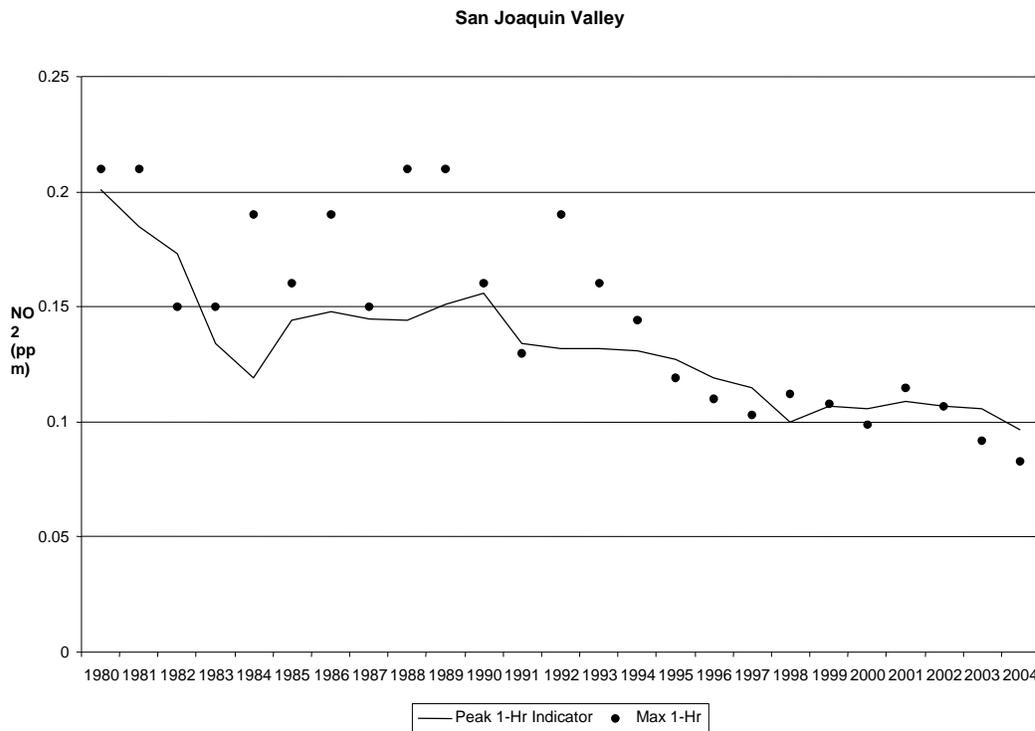


5.4.7.9 San Joaquin Valley Air Basin

The San Joaquin Valley Air Basin (SJVAB) occupies the southern two-thirds of California's Central Valley. The eight-county area includes Fresno, Kings, Madera, Merced, San Joaquin, Stanislaus, and Tulare counties, as well as the western portion of Kern County. Close to 10 percent of the State's population resides in the SJVAB.

Since the SJVAB lies in the southern portion of the Central Valley, its geography poses a significant challenge to clean air. Despite this, the SVJAB has been in attainment of the State 1-hr standard for NO₂. Between 1980 and 2004, the peak 1-hr indicator declined about 50% and the maximum 1-hr concentration declined 57% (Figure 5.16). As shown in Figure 5.16, the SJVAB has not had an exceedance of the State 1-hr standard since 1980. Also, maximum 1-hr concentrations have been less than half the State 1-hr standard since 1995.

Figure 5.16 San Joaquin Valley

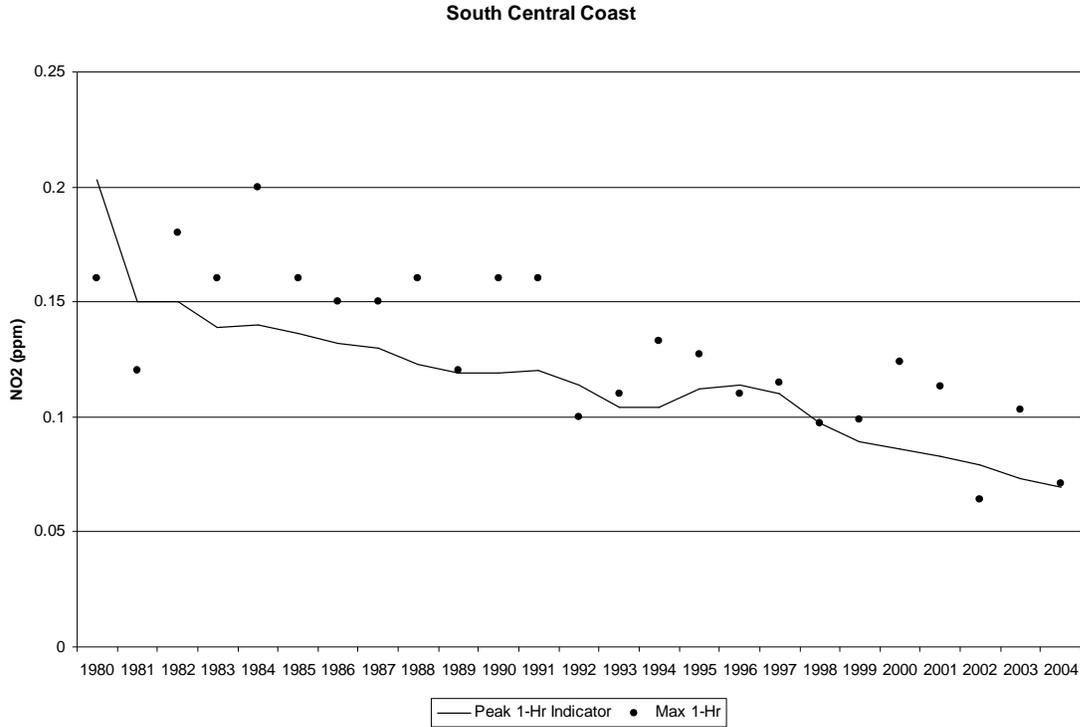


5.4.7.10 South Central Coast Air Basin

The South Central Coast Air Basin (SCCAB) includes all of San Luis Obispo, Santa Barbara, and Ventura counties. About 4 percent of the State's total population lives in the SCCAB.

As with the San Joaquin Valley, the SCCAB did not exceed the State 1-hr standard between 1980 and 2004 (Figure 5.17). As with most of the air basins, it too has seen a decline in its peak 1-hr indicator and maximum 1-hr concentrations. The peak 1-hr indicator declined 66% between 1980 and 2004. The maximum 1-hr concentration declined 55% during the same time.

Figure 5.17 South Central Coast

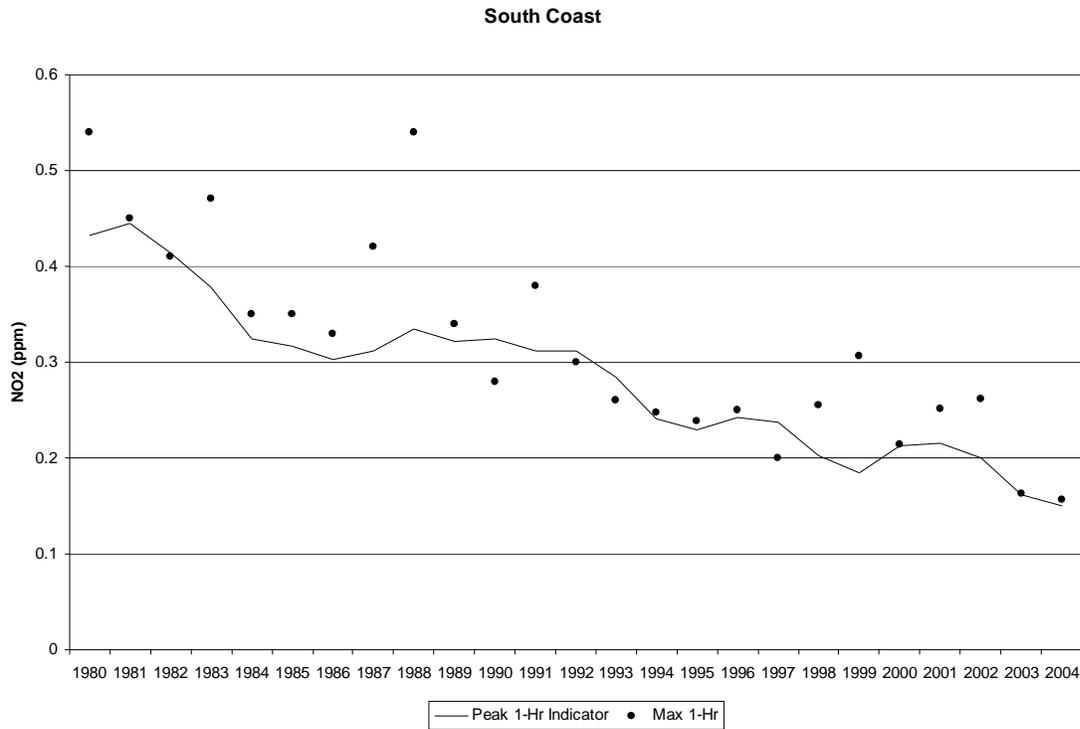


5.4.7.11 South Coast Air Basin

The South Coast Air Basin (SoCAB) includes California's largest metropolitan region, and 43 percent of the State's population lives within its boundaries. The area includes the southern two-thirds of Los Angeles County, all of Orange County, and the western urbanized portions of Riverside and San Bernardino counties. The area's air quality is aggravated by surrounding mountains, frequent low inversion heights, and stagnant air conditions. These factors all act together to trap pollutants within the SoCAB.

In January of 2004, the SoCAB was the last area designated as attainment of the NO₂ standard. From 1980 to 2004, the peak 1-hr indicator declined 63% and the maximum 1-hr concentration declined 70% (Figure 5.18). This area has come a long way in reducing peak NO₂ levels. In 1988, the maximum 1-hr concentration was 0.54, more than double the State 1-hr standard. In 2004, it had declined steadily to 0.157 ppm.

Figure 5.18 South Coast



5.5 Analysis of Peak Nitrogen Dioxide Exposure in California

5.5.1 Introduction

This section provides information on the ranges of peak outdoor 1-hour NO₂ concentrations to which people in different parts of California are potentially exposed. We use the term “potentially” because daily activity patterns influence a person’s exposure. For example, being inside a building will decrease a person’s exposure to outdoor NO₂ concentrations. However, any person who is outdoors during the peak concentration period will be exposed to the peak concentration. Furthermore, the exposures presented here provide an integrated regional perspective rather than an indication of exposure at any individual location.

This exposure analysis is based solely on “outdoor” NO₂ data, as measured by the statewide network of ambient NO₂ monitoring sites. The following tables and graphs present information on the population that could be exposed to different peak NO₂ concentrations within each air basin or planning area in California, as well as for the State as a whole.

5.5.2 Calculation of Peak Outdoor Nitrogen Dioxide Exposures

This analysis is based on the Inverse Distance Weighting method from the Geostatistical Analyst 8.2 software. For this discussion, NO₂ peak indicator values and population counts were assembled at the census tract level and merged to provide highly resolved exposures across a range of peak NO₂ concentrations.

Concentrations of many air pollutants, including NO₂, may change substantially from place to place. Accordingly, population exposure estimates tend to be more accurate when the population data and air quality data on which they are based are highly resolved, geographically. Population counts by census tract provide a convenient source of highly resolved population data. A typical census tract contains several thousand people. As a result, densely populated areas have many census tracts, while sparsely populated areas have very few.

Air quality data from the statewide network of monitors were also resolved to each census tract. The concentration assigned to each census tract was a weighted-average of the concentrations measured at the monitors. The weight assigned to each monitor is a function of its distance from the centroid of the census tract. In this way, close monitors are more influential than are distant monitors. Geographical barriers, such as mountain ranges that may impede the movement of emissions and pollutants, were not considered in the calculations, but this omission had little impact on the results since monitors typically collect data in populated areas on both sides of such barriers.

Ambient NO₂ data used in this exposure analysis were extracted from ARB’s historical database, ADAM and represent the annual 1-hour peak indicator values for each site during the 2002 through 2004 time period. The peak indicator, sometimes referred to as the Expected Peak Day Concentration (EPDC), is a statistical estimate of the highest concentration expected to occur once per year, on average. It is an estimate of the 99.73 percentile (364/365 percentile) of the 1-hour NO₂ concentrations measured at a monitoring site. An exponential-tail model is used to calculate the peak indicator, making use of the highest twenty percent of all daily maximum values during a 3-year period. Exposure calculations based on the EPDC provide an assessment of the highest expected 1-hour exposure for each monitor.

Spatial distributions of peak exposures were prepared for a 1-hour averaging time, using year 2000 census data. On a statewide basis, almost all of California’s population is exposed to peak 1-hour values below 0.15 ppm.

Complete results of the exposure analysis for the 1-hour averaging time are given in the following tables and figures. Results are presented for each of the air basins or planning areas, as well as for the State as a whole. For each area, the population is grouped by peak NO₂ concentrations in 0.01 ppm ranges. Exposure was only assigned to a census tract if a monitor was nearby, to avoid using non-representative data from monitors too far away. These instances are indicated in the Tables as “Representative Data Not Available”. For example, in the Salton Sea Air Basin, 3.7 percent of the population had no nearby monitor and thus no assigned exposure.

Table 5.7 and Figures 5.19 through 5.30 show the distribution of exposure estimates based on the peak 1-hour indicator. No areas of the State show any percentage of their population exposed to peak 1-hour NO₂ levels above the State 1-hr standard of 0.25 ppm. Furthermore, ten areas (Lake County, Lake Tahoe, Mojave Desert, Mountain Counties, North Central Coast, North Coast, Sacramento Valley, San Francisco Bay Area, San Joaquin Valley, and South Central Coast) have 100 percent of their population exposed to peak 1-hour NO₂ levels below 0.12 ppm, one half the state standard.

The highest 1-hour exposures occur in the South Coast portion of the state. In this area, about 50 percent of the population was exposed to peak 1-hour NO₂ levels above 0.12 ppm, and the maximum exposures were between 0.14 ppm and 0.15 ppm. In comparison, the maximum exposure levels were somewhat lower in San Diego County (0.12 ppm to 0.13 ppm) and the Salton Sea Air Basin (0.12 ppm to 0.13 ppm). The peak exposure values for all three of these areas were still well below the level of the current State 1-hour NO₂ standard.

Figures 5.19-5.30 show the frequency of population exposure to specific levels of the peak 1-hour NO₂ concentration, and the cumulative percent of the population exposed as the concentrations increase. Some areas do not have enough monitors to provide representative exposure data for 100 percent of the population. In these cases, data are only provided for the portion of the population which had nearby monitors. As a result, an area may not have data for 100 percent of the population displayed in the graph. For the areas with monitored data, no part of the population was exposed to NO₂ levels above the State 1-hr standard.

Table 5.7 Summary of Nitrogen Dioxide Peak 1-Hour Indicator Population-Weighted Exposure

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed
Great Basin Valleys	Representative Data Not Available		27558	86.1%
	0.04	0.05	2657	8.3%
	0.05	0.06	583	1.8%
	0.06	0.07	1208	3.8%
	GBV Total:		32006	
Lake County	Representative Data Not Available		13703	23.5%
	0.03	0.04	32271	55.3%
	0.04	0.05	5030	8.6%
	0.05	0.06	7305	12.5%
	LC Total:		58309	
Lake Tahoe	0.02	0.03	522	1.1%
	0.03	0.04	11636	25.2%
	0.04	0.05	892	1.9%
	0.05	0.06	32176	69.6%
	0.06	0.07	974	2.1%
	LT Total:		46200	
Mojave Desert	Representative Data Not Available		86883	10.6%
	0.05	0.06	67230	8.2%
	0.06	0.07	55469	6.8%
	0.07	0.08	183724	22.5%
	0.08	0.09	251514	30.8%
	0.09	0.1	146494	17.9%
	0.1	0.11	18192	2.2%
	0.11	0.12	7027	0.9%
	MD Total:		816533	
Mountain Counties	Representative Data Not Available		135038	33.1%
	0.02	0.03	19885	4.9%
	0.03	0.04	1305	0.3%
	0.04	0.05	14140	3.5%
	0.05	0.06	41720	10.2%
	0.06	0.07	125757	30.8%
	0.07	0.08	58550	14.3%
	0.08	0.09	11644	2.9%
	MC Total:		408039	
North Coast Central	Representative Data Not Available		33375	4.7%
	0.03	0.04	6940	1.0%
	0.04	0.05	370218	52.1%
	0.05	0.06	299187	42.1%
	0.06	0.07	878	0.1%
NCC Total:		710598		
North Coast	Representative Data Not Available		166204	54.0%
	0.03	0.04	37926	12.3%
	0.04	0.05	57835	18.8%
	0.05	0.06	46054	15.0%
	NC Total:		308019	
Northeast Plateau	Representative Data Not Available		87578	100.0%
	NEP Total:		87578	

Table 5.7 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed
Sacramento Valley	Representative Data Not Available		201945	8.7%
	0.05	0.06	294880	12.6%
	0.06	0.07	374550	16.0%
	0.07	0.08	1408587	60.3%
	0.08	0.09	54315	2.3%
	SV Total:		2334277	
Salton Sea	Representative Data Not Available		17235	3.7%
	0.06	0.07	233212	50.1%
	0.07	0.08	73389	15.8%
	0.08	0.09	12744	2.7%
	0.09	0.1	60857	13.1%
	0.1	0.11	33046	7.1%
	0.11	0.12	22361	4.8%
	0.12	0.13	13042	2.8%
	SS Total:		465886	
San Diego County	Representative Data Not Available		2324	0.1%
	0.06	0.07	3276	0.1%
	0.07	0.08	44897	1.6%
	0.08	0.09	1455813	51.9%
	0.09	0.1	1084220	38.7%
	0.1	0.11	133525	4.8%
	0.11	0.12	68416	2.4%
	0.12	0.13	10242	0.4%
SDC Total:		2802713		
San Francisco Bay Area	0.04	0.05	1043	0.0%
	0.05	0.06	2762793	41.9%
	0.06	0.07	2501269	37.9%
	0.07	0.08	1326996	20.1%
	SFBA Total:		6592101	
San Joaquin Valley	Representative Data Not Available		41826	1.3%
	0.04	0.05	14424	0.5%
	0.05	0.06	41029	1.3%
	0.06	0.07	585078	18.3%
	0.07	0.08	1684975	52.8%
	0.08	0.09	571171	17.9%
	0.09	0.1	250882	7.9%
SJV Total:		3189385		
South Coast Central	Representative Data Not Available		960	0.1%
	0.02	0.03	929	0.1%
	0.03	0.04	234730	16.8%
	0.04	0.05	393724	28.2%
	0.05	0.06	452388	32.4%
	0.06	0.07	141046	10.1%
	0.07	0.08	150354	10.8%
	0.08	0.09	23692	1.7%
SCC Total:		1397823		

Table 5.7 (continued)

Air Basin	Lower Conc Limit (ppm)	Upper Conc Limit (ppm)	Census 2000 Pop Affected	% of Pop Exposed
South Coast	Representative Data Not Available		3025	0.0%
	0.07	0.08	28757	0.2%
	0.08	0.09	488585	3.3%
	0.09	0.1	1297257	8.9%
	0.1	0.11	2015877	13.8%
	0.11	0.12	3459709	23.7%
	0.12	0.13	3914005	26.8%
	0.13	0.14	2586559	17.7%
	0.14	0.15	794960	5.4%
	SC Total:		14588734	
California	Representative Data Not Available		786458	2.3%
	0.02	0.03	21336	0.1%
	0.03	0.04	324808	1.0%
	0.04	0.05	860643	2.5%
	0.05	0.06	4060230	12.0%
	0.06	0.07	4023611	11.9%
	0.07	0.08	4960229	14.7%
	0.08	0.09	2869478	8.5%
	0.09	0.10	2850830	8.4%
	0.10	0.11	2201239	6.5%
	0.11	0.12	3557513	10.5%
	0.12	0.13	3940307	11.6%
	0.13	0.14	2586559	7.6%
	0.14	0.15	794960	2.3%
	State Total:		33838201	

Figure 5.19 Lake Tahoe

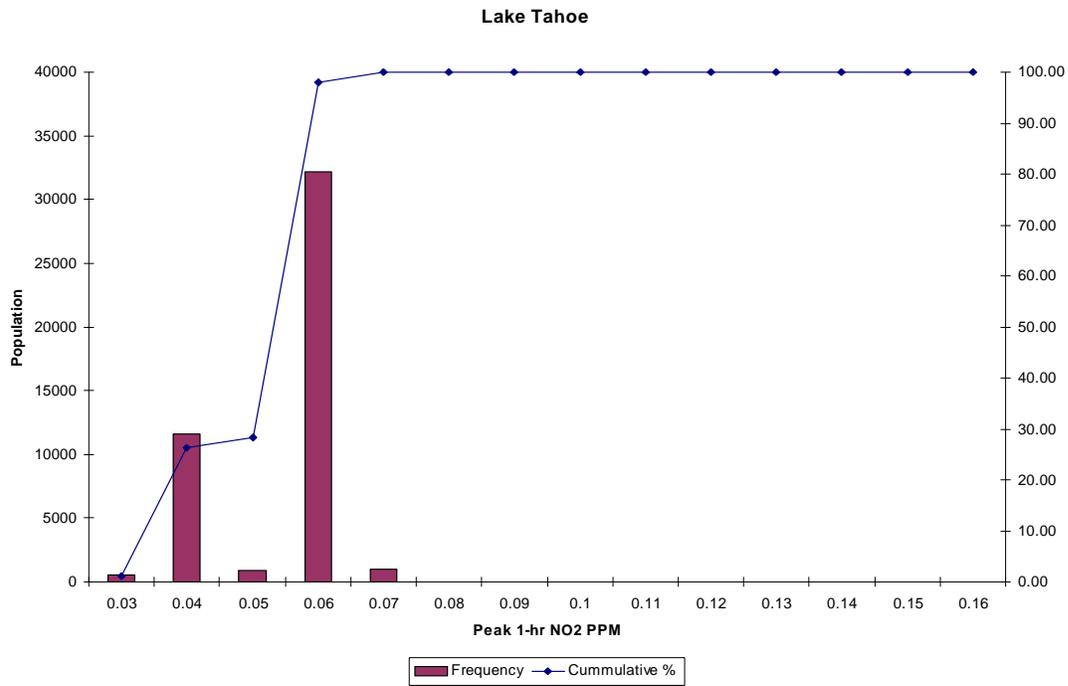


Figure 5.20 Mojave Desert

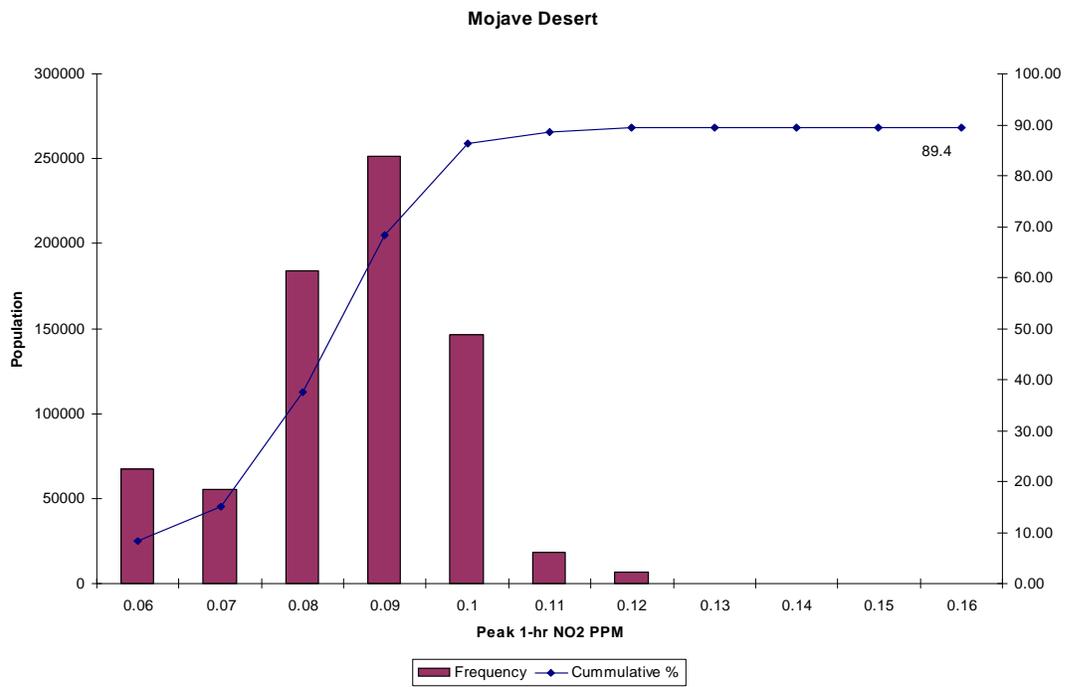


Figure 5.21 North Central Coast

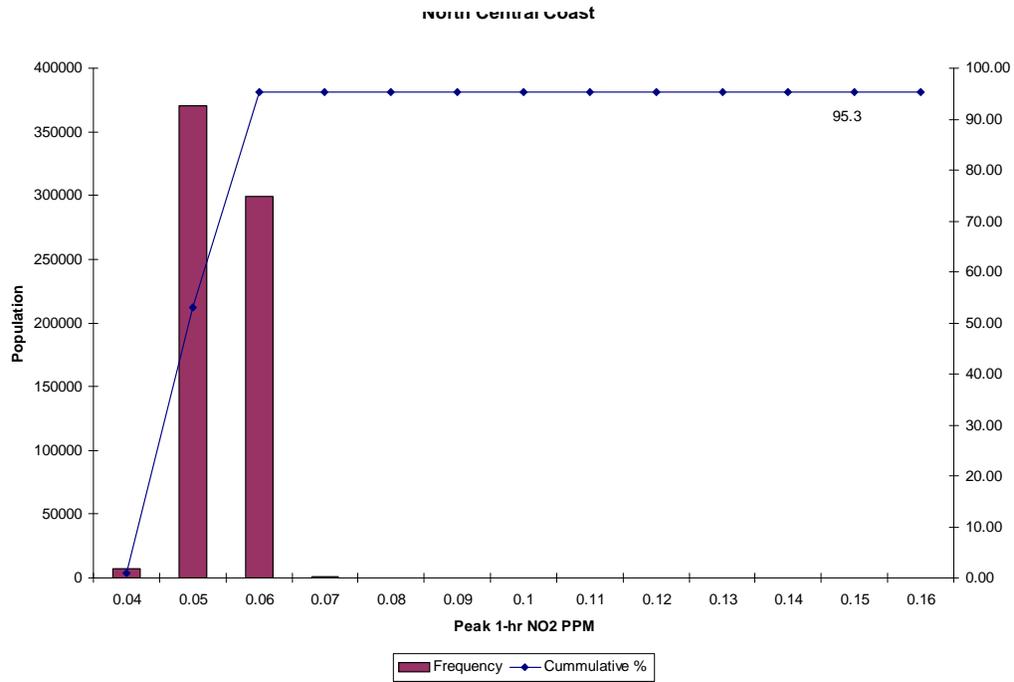


Figure 5.22 North Coast

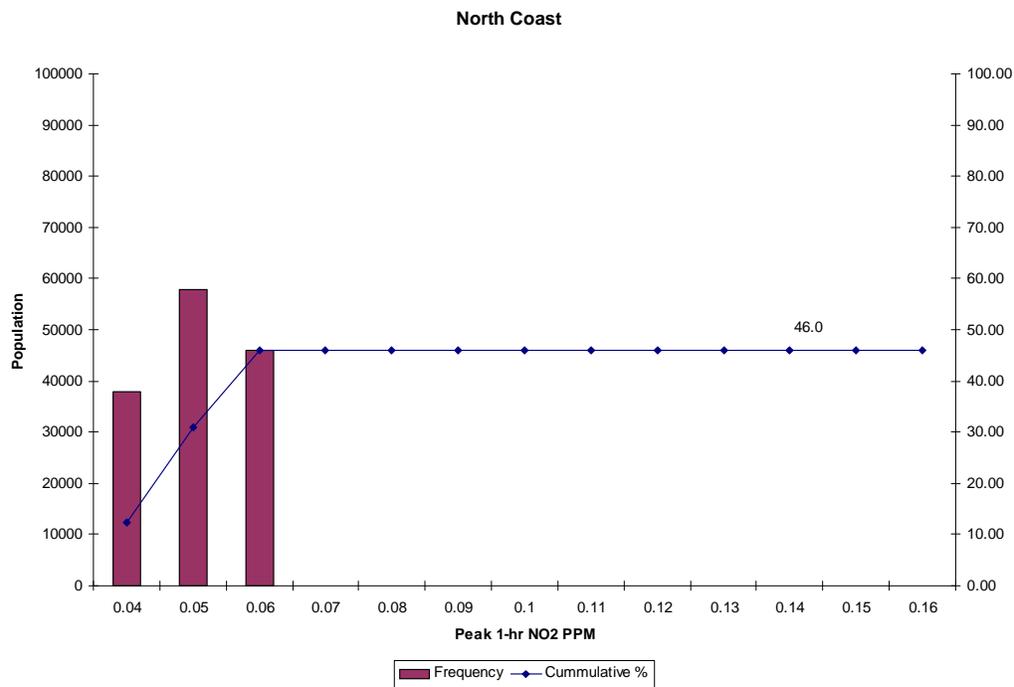


Figure 5.23 Sacramento Valley

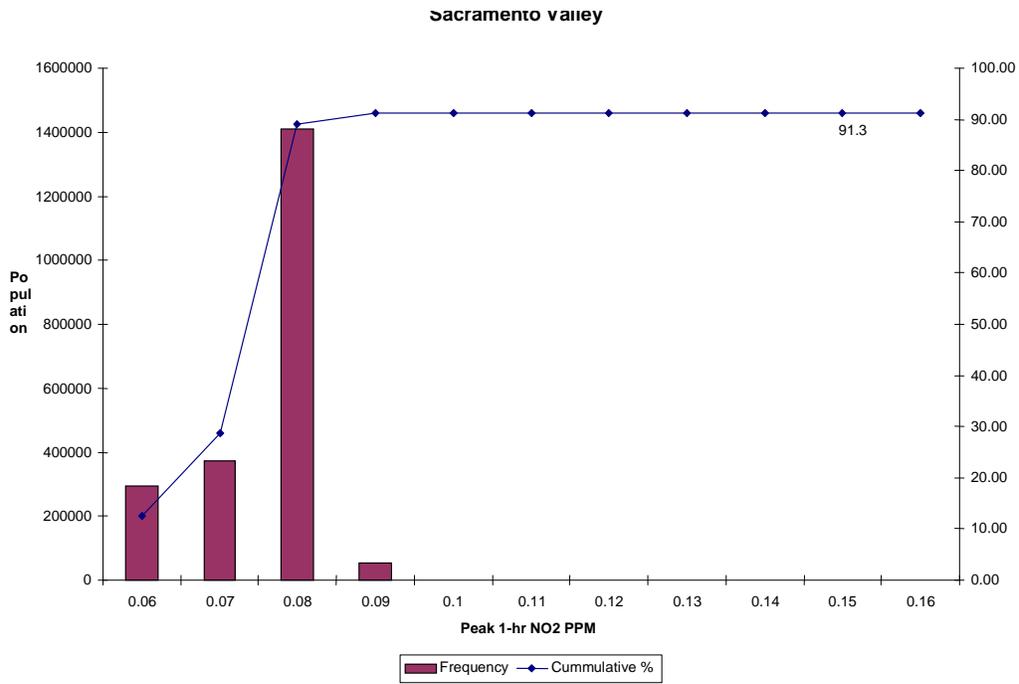


Figure 5.24 Salton Sea

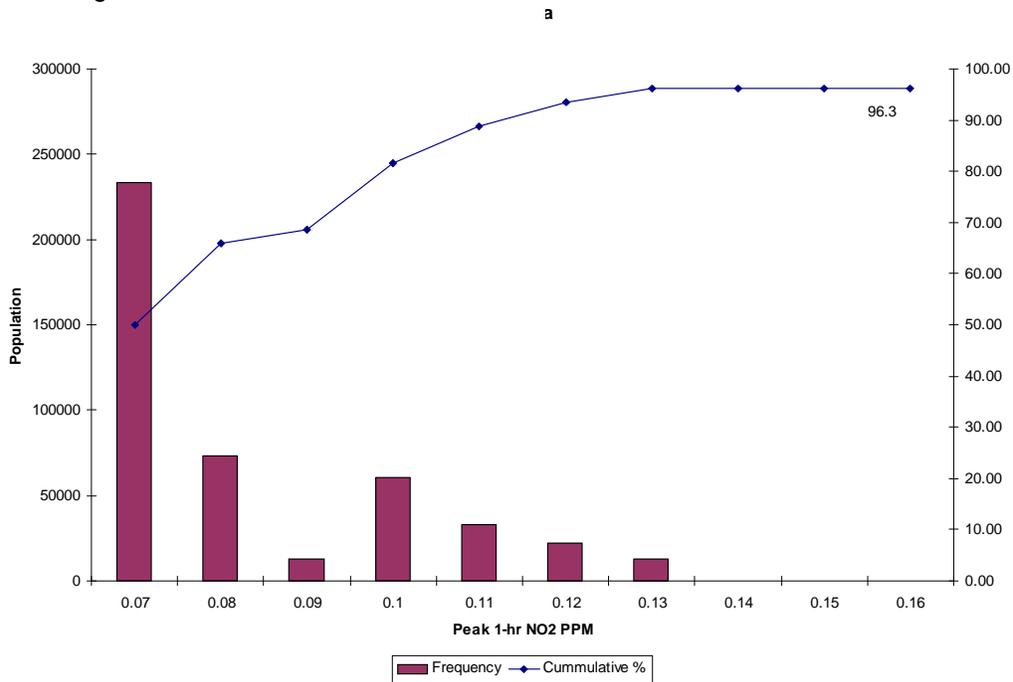


Figure 5.25 San Diego

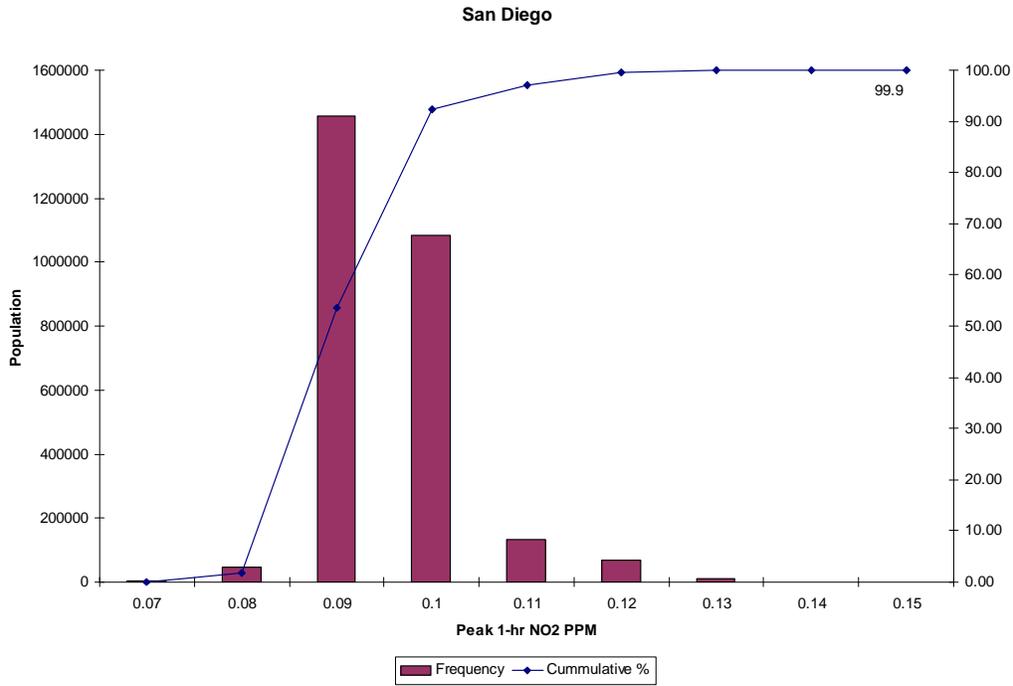


Figure 5.26 San Francisco

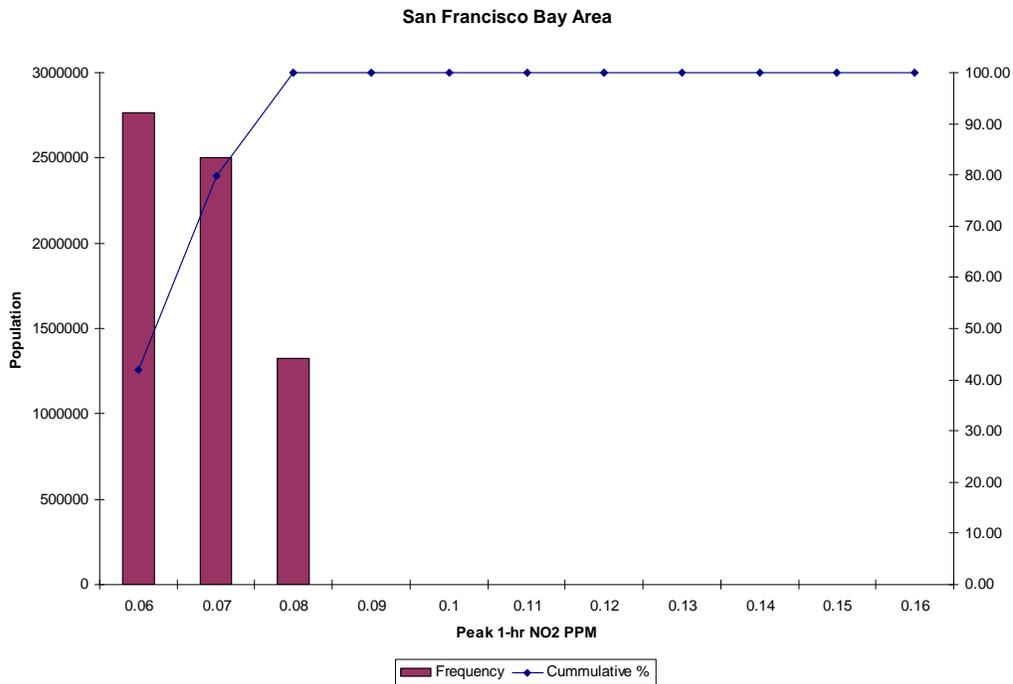


Figure 5.27 San Joaquin Valley

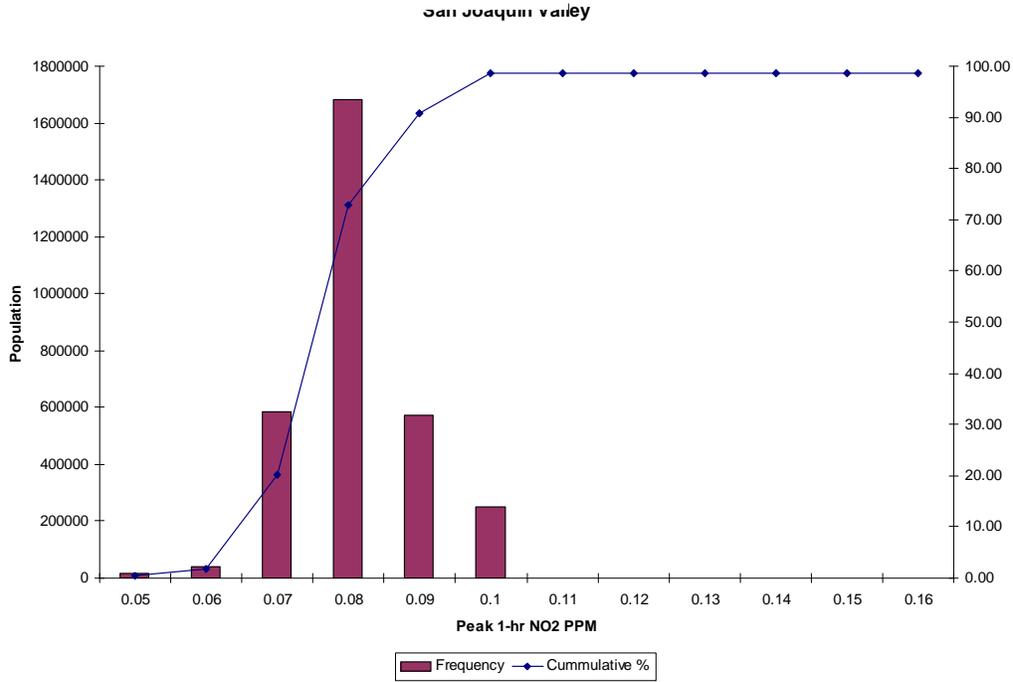


Figure 5.28 South Central Coast

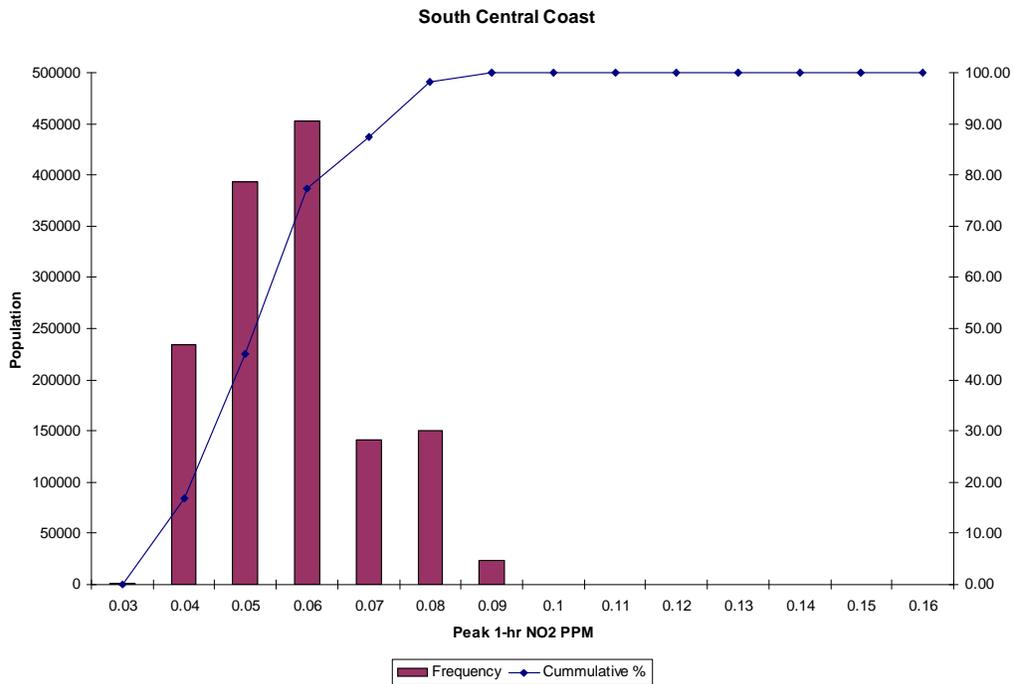


Figure 5.29 South Coast

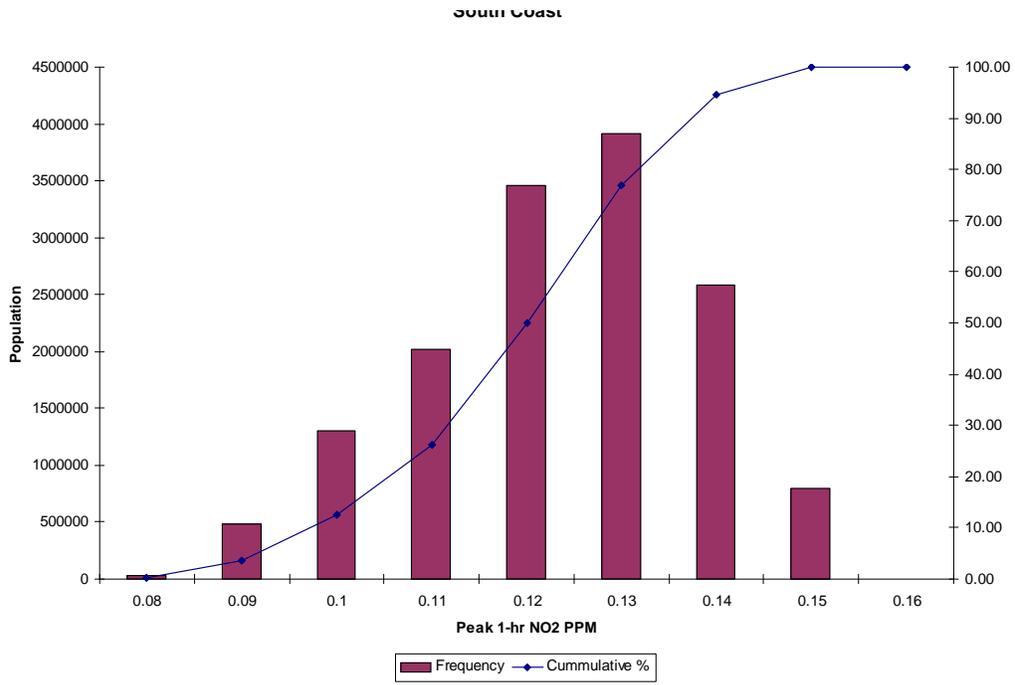
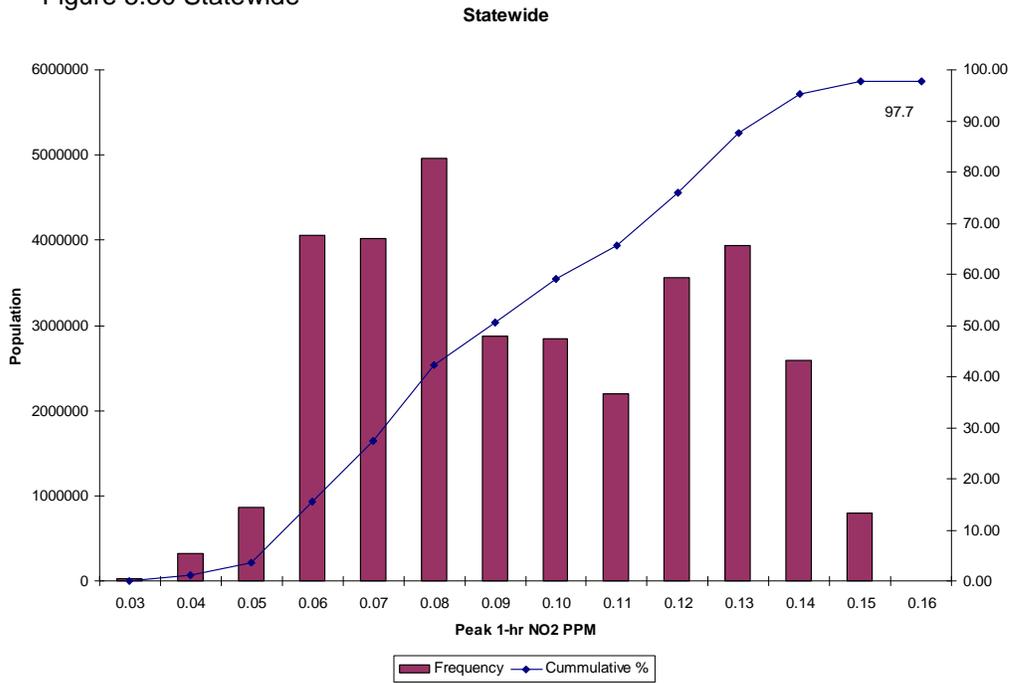


Figure 5.30 Statewide



5.6 Indoor and Personal Exposure to Nitrogen Dioxide

5.6.1 Introduction

Californians' indoor exposures to NO₂ are determined by the presence and use of indoor sources, particularly gas appliances, and by outdoor NO₂ concentrations. The main factors influencing indoor NO₂ concentrations are gas stoves, indoor-outdoor air exchange rates, and the seasonal effects. Winter levels are typically higher than summer levels, and gas appliances are used more in winter (Schwab et al. 1994; Spengler et al. 1994; Monn 2001). Californians who spend a substantial amount of time in a kitchen near an operating gas stove or range may experience a substantial exposure to NO₂.

This section discusses indoor NO₂ concentrations and sources, and Californians' personal exposures to NO₂. Information is also presented on some of the related nitrogen compounds known to occur in indoor environments. This section focuses on California-specific literature as well as some information from other parts of the United States. The scientific literature contains many excellent exposure studies conducted outside of the United States and some studies that deal specifically with occupational exposures. Generally, those studies have not been included in this review because exposure patterns may not be similar to those in California; for example, regulations on motor vehicle exhaust, climatic conditions, and housing structures in other countries may differ from those in California. However, some of these types of studies are mentioned peripherally if they support major findings of California-specific studies.

5.6.2 Nitrogen Dioxide Concentrations

During the 1970s and 1980s, governmental agencies and the academic community began raising concerns about indoor air quality, partially in response to tighter, more energy efficient building practices. Indoor NO₂ became a particular concern associated with the use of gas appliances.

Since then, investigators have conducted several large studies to measure indoor NO₂ concentrations in California. However, the data from these studies do not provide a complete characterization of indoor NO₂ concentrations because data were primarily obtained from homes in the Los Angeles basin, where outdoor NO₂ levels were particularly high. Also, measurements were collected over extended times ranging from two days to one week. Such measurements are insufficient to fully characterize indoor NO₂ concentrations because indoor sources are used intermittently, and indoor levels can fluctuate greatly over a period of a few minutes or hours. Samples spanning such lengthy times do not reflect the variation of actual concentrations that fluctuate during the day, nor do they capture peak exposure levels. Moreover, results from these averaging times do not permit direct comparison to the California ambient standard of 250 ppb, which is based on a one-hour averaging time. Nonetheless, these California studies provide useful information regarding the relative contributions of different sources to indoor NO₂ concentrations, and Californians' long-term average exposure levels.

5.6.2.1 California Studies

Investigators have conducted several studies to measure NO₂ concentrations inside homes located in Southern California. Homes in this region predominantly use gas appliances, and the ambient NO₂ level is often elevated relative to other parts of the state. The gas industries in the area have sponsored several residential studies to measure indoor NO₂ levels.

5.6.2.1.1 Concentrations in Homes

In a recent study, Lee et al. (2002) measured indoor NO₂ and nitrous acid (HONO) levels in 119 residences in Southern California using a six-day integrated sample. Data were collected in April and May of 1996. The average indoor (living room) and outdoor concentrations of NO₂ were 28.0 and 20.1 ppb, respectively. Three factors were found to be positively associated with indoor NO₂ levels: community (outdoor) levels, the presence of a gas range, and the presence of an air conditioner. The authors suggest that the association with air conditioners might be indicative of a lower air exchange rate. The average indoor HONO concentration was 4-6 ppb, compared to 0.9 ppb for outdoor HONO. Indoor HONO levels were correlated with indoor NO₂ levels and were about 17% of the magnitude of indoor NO₂ levels.

In another large study, Spengler et al. (1994) conducted a large study of personal exposure to NO₂ in the Los Angeles basin during 1987 and 1988. The study provides a valuable database for understanding exposure to NO₂ with a population representative sample of 482 households and 682 individuals in the

Los Angeles basin. Passive badges were used to collect samples in bedrooms and outdoors for a 48-hour time integrated sample. Indoor results, represented by bedroom data, indicate overall 48-hour concentrations had a mean of 27.2 ppb and a median of 24.6 ppb. The distribution is represented by 25th, 75th, and 90th percentile values of 16.7, 35.0, and 44.8 ppb, respectively. Thus, 10 percent of the homes had bedroom concentrations greater than 44.8 ppb.

Spengler et al. (1994) analyzed data according to the fuel used by the kitchen range. The contribution of gas appliances with pilot lights to total exposure was evident. Homes containing a gas range without a pilot light averaged 4 ppb more NO₂ in the bedroom than homes with electric ranges. Homes containing gas ranges with pilot lights averaged 15 ppb more NO₂ in the bedroom than homes with electric ranges. These differences were statistically significant at $p < 0.01$. The authors thus hypothesized that the pilot light may influence the indoor NO₂ concentration. Additionally, the presence of a pilot light may be associated with other factors that affect the indoor NO₂ concentration such as the age of the house, tendency to heat with the range, and the location of the unit within more congested parts of the city. The effect of season on bedroom concentrations was not statistically significant.

A California Residential Indoor Air Quality Study was conducted in the winter of 1991/1992, sponsored by members of the gas industry (Wilson et al. 1993). Samples were obtained in 293 homes throughout the state. Samples were collected with a passive Ogawa badge for a 48-hour period. Average concentrations were 25 and 23 ppb for indoor and outdoor measurements, respectively. Maximum NO₂ concentrations were 177 and 80 ppb for indoor and outdoor, respectively. At these elevated 48-hour levels, it is very likely that the California one-hour standard of 250 ppb was exceeded for at least some portion of the time.

Petreas et al. (1988) measured NO₂ levels inside more than 250 mobile homes throughout California during the summer of 1984 and winter of 1985. Mobile homes were selected for study based on their tight energy efficient construction and their typical small volume. Week-long, integrated NO₂ measurements were obtained with passive monitors. Mobile homes using gas for cooking had significantly higher indoor NO₂ levels than those using electricity. During the summer, mean NO₂ levels in kitchens and bedrooms of gas homes were 22.89 and 15.99 ppb, respectively. During the same time period, mean levels in kitchens and bedrooms of electric homes were 8.98 and 7.77 ppb, respectively. During the winter phase of the study, gas homes displayed higher concentrations than they did during the summer. Winter kitchen and bedroom concentrations averaged 28.68 and 19.98 ppb, respectively. The electric homes had lower NO₂ concentrations in winter than in summer with mean winter kitchen and bedroom levels at 6.29 and 6.17 ppb, respectively. The authors concluded that the most important variables in predicting indoor NO₂ levels were the type of cooking fuel, the inverse of the house volume, and the geographic location. Homes in the Los Angeles basin, as a group, had significantly higher indoor NO₂ levels than mobile homes in the rest of the state.

In an early NO₂ study, the Southern California Gas Company (SoCalGas) sponsored a study to measure 1-week average NO₂ levels in 600 homes during the spring, summer, and winter of 1984 and 1985 (Wilson et al. 1986). Samples were collected at three locations: kitchen, bedroom, and outside with passive diffusion tubes. Median levels in the kitchen for spring, summer, and winter were 73, 89, and 101 $\mu\text{g}/\text{m}^3$ (39, 47, and 56 ppb), respectively. In the spring and summer, median concentrations in the kitchen were greater than those outside, which were in turn greater than bedroom concentrations. For measurements collected during the winter, the median concentrations outside and in the kitchen were approximately the same, and greater than those in the bedroom.

Table 5.8 presents mean indoor and outdoor NO₂ concentrations from studies discussed in this section.

Table 5.8 – Summary of California Indoor Residential NO₂ Studies

Mean Indoor Concentration (ppb)	Mean Outdoor Concentration (ppb)	Averaging Time	Region	Reference
28 (living room)	20.1	6 day	Southern California	Lee et al. 2002
27.23 (bedroom) (90 th =44.8)	38.26 (90 th =62.9)	48 hour	Los Angeles basin	Spengler et al. 1994
25 (max=177)	33 (max=80)	48 hour	All of California	Wilson et al. 1993
Summer 22.89 (kitchen, gas home) 15.99 (bedroom, gas home) 8.98 (kitchen, electric home) 7.77 (bedroom, electric home) Winter 28.68 (kitchen, gas home) 19.98 (bedroom, gas home) 6.29 (kitchen, electric home) 6.07 (bedroom, electric home)	11.20 13.78 22.41 23.74	1 week	All of California (mobile homes)	Petreas et al. 1988
39 (kitchen, spring)* 47 (kitchen, summer)* 54 (kitchen, winter)*	28 (spring)* 41 (summer)* 57 (winter)*	1 week	Southern California	Wilson et al. 1986

*median value

5.6.2.1.2 Schools

Linn et al. (1996) conducted a limited study of NO₂ levels inside classrooms in three communities of southern California: Rubidoux, Upland, and Torrance. Linn et al. used passive badge samplers inside the school to collect 24-hour integrated samples. The mean of 108 indoor samples was 16 ppb NO₂, with the range extending from 1 to 77 ppb. Indoor concentrations averaged about half of outdoor concentrations.

5.6.2.1.3 Other California Environments

Concern for the physical damage of sensitive equipment and museum collections from air pollution precipitated studies in unique environments. A telephone switching office in Burbank, CA was monitored for levels of criteria pollutants because photochemical pollutants and their by-products can damage telecommunication networks (Bellcore, 1993). Indoor concentrations of pollutants at this location were dependent on the outdoor concentrations, the building's air exchange rate, and removal or production rates based on indoor chemistry. Indoor NO₂ levels ranged from 80 to 100% of outdoor levels. Median

monthly levels ranged from 30 to 50 ppb. At the 95th percentile level, monthly values ranged from 40 to 86 ppb.

Hisham and Grosjean (1991) measured indoor air concentrations of several air pollutants at nine Southern California museums. The maximum 24-hour time integrated indoor NO₂ concentration was 123 ppb. Twenty-four-hour time integrated NO₂ concentrations for the museums ranged from 0-62 ppb, corrected for interference from peroxyacetyl nitrate (PAN). Changes in indoor concentrations closely tracked changes in outdoor concentrations. For NO₂, the median indoor to outdoor ratio ranged from 0.25 to 1.36. The authors provided no explanation for a ratio greater than one, but indicate the indoor/outdoor ratio for NO₂ varied substantially with sub-locations and air exchange rates.

5.6.2.2 *Studies Outside of California*

Studies conducted outside of California are illustrative of indoor concentrations that might also occur in some California indoor environments. Some of these studies also confirm the clear link of elevated indoor NO₂ levels with gas-fueled appliances.

5.6.2.2.1 Short-Averaging-Time Studies

Results from studies with shorter averaging times have peak concentrations that are considerably higher than those with longer averaging times. The peak concentrations can be obscured when only long-averaging-time concentrations are reported.

Brauer et al. (1990) measured NO₂ concentrations with a continuous monitor in two research houses in Illinois and Maryland. Combustion sources included a gas range and space heaters. Peak NO₂ levels in houses when a convective space heater was used for a 4-hour period were 890 and 1,020 ppb. Corresponding 24-hour average NO₂ concentrations in these homes were 187 and 231 ppb. Operation of the gas range and one burner led to peak concentrations ranging from 115 to 198 ppb. For the same experiments, 24-hour averages were between 27 and 45 ppb NO₂.

Results from Harlos et al. (1987) indicate that daytime concentrations were consistently higher than the week-long integrated concentrations. Investigators measured NO₂ concentrations in about 150 homes with infants in Albuquerque, NM in the fall and winter of 1984-1985. Samples were collected in the kitchen, living room, infants' bedroom, and outdoors, for 1-week and 1-day periods. Three types of samplers were used: an active pump, Yanagisawa badges, and Palmes tubes. The authors reported that homes with NO₂ concentrations in the upper quartile used gas cooking appliances, those in the lowest quartile used electric appliances; however, distributions were not provided. The mean 1-day NO₂ concentrations by location for gas homes, in order of descending concentration were: kitchen, 65.5 ppb; living room, 50.2 ppb; bedrooms, 42.9 ppb; outdoors, 12.2 ppb. Mean concentrations averaged over one week for the same locations were 37, 35, 22, and 10 ppb, respectively.

Another study of particular interest due to the use of samples with short averaging times was conducted in the United Kingdom (Dennekamp et al. 2001). Investigators measured 5-minute peaks up to 1000 ppb NO₂ when cooking with a 4-burner gas stove (measured at face level in front of the cook). Elevated exposure occurs during these episodic events of high NO₂ concentrations, particularly during cooking because the individual remains near the source.

5.6.2.2.2 Long-Averaging-Time Studies

As discussed above, studies using samples with long averaging times (days to weeks) typically show lower concentrations than studies reporting shorter averaging times.

Triche et al. (2005) collected 2-week passive NO₂ samples with Palmes tubes in homes to determine if use of indoor heating sources were correlated with respiratory symptoms in nonsmoking women. Indoor NO₂ concentration data were collected for 888 women. Median indoor NO₂ levels in living rooms ranged from 9.3 to 17.7 ppb NO₂, with one exception – the median level in homes that used a gas space heater was 54.8 ppb. NO₂ concentrations at the 90th percentile level were considerably higher: 40.6 ppb for homes that did not use a combustion source, 80.2 ppb for those who used a fireplace, 83.8 ppb for those that used a kerosene heater, 147.2 ppb for those that used a gas space heater, and 51.5 ppb for those that used a wood stove. Because these results are for 2-week sampling periods, it is likely that peak concentrations were considerably higher during use of the combustion appliance.

Members of the research team (Triche et al. 2005) mentioned above conducted a similar study in Connecticut and Massachusetts (van Strien et al. 2004), with the objective of defining a relationship between exposure to NO₂ and HONO and respiratory symptoms in infants under one year old. Passive Palmes tubes were used to collect a 2-week NO₂ sample for 768 homes. The quartile cut points for residential NO₂ concentrations were 5.1, 9.9, and 17.4 ppb. As with the study discussed above, it is likely that peak concentrations were higher than the reported 2-week integrated samples.

In a Boston study of 24-hour time-integrated passive samples collected in homes of asthmatic children in public housing, Brugge et al. (2003) found mean indoor NO₂ levels were either close to or exceeded the NAAQS annual level of 53 ppb. Zipprich et al. (2002) collected 48-hour passive NO₂ samples in homes in Richmond, VA. Mean concentrations in the bedrooms, living rooms, and outdoors were 18, 19, and 15 ppb, respectively. Ninety-eight percent of the homes had gas stoves.

Lambert et al. (1992) measured NO₂ concentrations in the bedrooms of 1,109 homes with gas cooking ranges in Albuquerque, NM during the winter from 1988-1990. Mean bedroom concentrations, measured with passive diffusion samples over two-week periods, ranged from 18 to 28 ppb. However, the distribution was quite wide with many of the concentrations falling between 50 and 150 ppb.

Investigators monitored NO₂ levels inside and outside 137 homes in Portage, WI during 1980 and 1981 (Spengler et al. 1983). One-week samples were collected with passive Palmes tubes. In this rural community, ambient NO₂ concentrations averaged 10 to 15 µg/m³ (5-8 ppb). Kitchen levels in homes with gas stoves averaged about 50 µg/m³ (27 ppb) higher, and bedroom levels were about 30 µg/m³ (17 ppb) higher than outdoor levels. Ten percent of the homes which used gas as the cooking fuel had annual average kitchen NO₂ levels higher than the NAAQS of 100 µg/m³ (53 ppb). Indoor NO₂ concentrations for homes using electric cooking averaged 8 µg/m³ (4 ppb), lower than the outdoor level.

Residential NO₂ and HONO concentrations are also available from a study conducted in the United Kingdom to examine relationships between exposure and respiratory symptoms in adults (Jarvis et al. 2005). Once again, passive samplers were deployed for 14-day samples, this time in the kitchen and outdoors. Median kitchen values were 3.10, 12.76, and 13.83 ppb for indoor HONO, indoor NO₂ and outdoor NO₂, respectively. The maximum values were 20.55, 59.12, and 36.09 ppb for indoor HONO, indoor NO₂ and outdoor NO₂, respectively.

5.6.2.2.3 *Unique Environments*

A unique environment is created at indoor ice-skating rinks. The ice resurfacing machines (fuel-powered, not electric) emit NO₂ that remains in the rink, potentially leading to elevated exposure for the occupants. Levy et al. (1998a) measured NO₂ levels in 19 enclosed ice skating rinks in Boston over 3 winters. The first year they used Palmes tubes with a one-week sampling time, in subsequent years they used Yanagisawa badges with a one-day sample time. The overall mean indoor NO₂ concentration in Year 1 in rinks using propane powered resurfacers was 248 ppb, compared with 54 ppb for gasoline powered, and 30 ppb for electric resurfacers. In Years 2 and 3, the mean working-hour indoor NO₂ concentration was 206 ppb for propane-fueled ice resurfacers, compared with 132 ppb for gasoline-fueled and 37 ppb for electric-powered resurfacers. In a Swedish study, Berglund et al. (1994) found that the most important source of personal NO₂ exposure among schoolchildren was indoor ice-skating arenas.

Colbeck et al. (1998) measured NO₂ concentrations in car parks in the United Kingdom, as well as nearby shops and car park toll booths. In one location, the NO₂ concentration in an adjacent shop was 24.5 µg/m³ (13 ppb), in the car park ranged from 58-90 µg/m³ (31-48 ppb), and in the car park toll booth ranged from 43-60 µg/m³ (23-32 ppb). In a different location, these values were 25.6 µg/m³ (13.6 ppb), 54-78 µg/m³ (29-41 ppb), and 42-56 µg/m³ (22-30 ppb), respectively.

5.6.3 Factors that Influence Indoor Nitrogen Dioxide Concentrations

Where indoor combustion sources such as wall furnaces, floor furnaces, gas stoves, and unvented gas logs (not permitted in California) are present, they have a large influence on indoor NO₂ concentrations (Spengler et al. 1994; Pitts et al. 1989; Wilson et al. 1986; Wilson et al. 1993). In the absence of indoor sources, indoor NO₂ levels are largely determined by outdoor levels due to the infiltration of outdoor air (Spengler et al. 1994; Weschler and Shields 1994; Levy et al. 1998b).

5.6.3.1 Indoor NO₂ Sources

Many studies have been designed to determine associations between environmental factors and health effects, rather than directly quantify the emissions from appliance use. One such study found that indoor NO₂ levels were associated with gas cooking, cigarette smoking, reported traffic levels, and presence of a kerosene heater, while the use of a kitchen vent was associated with reduced indoor NO₂ levels (Farrow et al. 1997). Other investigators used multiple regression models and determined that indoor NO₂ concentrations are influenced by outdoor concentrations, use of a gas stove, particularly one with pilot lights, presence of a gas dryer in the living area, and use of a floor or wall furnace (Marbury et al. 1988).

5.6.3.1.1 Combustion Sources

Gas stoves and ranges used for cooking are a source of indoor NO₂, particularly when the stove is not vented to the outdoors. The stove emits NO₂ directly into the kitchen, and the NO₂ then mixes with air in the rest of the house.

With the use of shorter-averaging-time monitors, investigators have been able to measure peak NO₂ concentrations while a gas appliance is in operation. In an ARB-funded cooking study, Fortmann et al. (2001) measured indoor NO₂ during various cooking protocols. Measurement periods with a continuous monitor varied from approximately one to five hours, and included food preparation, cooking, and clean-up times. NO₂ levels increased when a gas stove was used for cooking. For example, while making a fried chicken dinner, NO₂ levels in the kitchen, living room, and bedroom, averaged over the entire cooking event were 191, 195, and 184 ppb, respectively. However, the average did not characterize the peak concentrations of 375, 401, and 421 ppb measured in the kitchen, living room, and bedroom, respectively. During other cooking tasks such as broiling fish, baking lasagna, frying tortillas, and stir-frying produce, indoor NO₂ levels ranged from 30 to 170 ppb, averaged over the entire cooking event. During a cycle of automatic oven cleaning with a gas stove, indoor NO₂ levels in the kitchen, living room, and bedroom exceeded 400 ppb (averaged over the entire event). For the oven cleaning protocol, the maximum NO₂ level was 673 ppb, measured in the bedroom. In contrast, NO₂ concentrations remained below 45 ppb during similar cooking protocols performed with an electric stove and range.

Levy et al. (1998b) identified the use of a gas stove in a home as the most significant contributor to personal NO₂ exposure. Investigations conducted in 15 countries found that mean personal (2-day average) NO₂ exposure was 34.8 ppb in homes with a gas stove used during the sampling period, compared to 20.5 ppb in homes without gas stove use.

In another large study, Lee et al. (1998) measured two-week average indoor NO₂ concentrations in 517 Boston homes and found that concentrations within gas stove residences ranged from 27.8 - 30.7 ppb, but only ranged from 6.4 - 10.9 ppb for electric stove residences. For those residences with gas stoves, concentrations ranged from 30.0 - 33.1 ppb for stoves with pilot lights and 22.1 - 22.5 ppb for those without pilot lights.

In the Wilson et al. (1986) gas industry-sponsored study conducted in Southern California, the contribution of combustion sources to indoor NO₂ concentrations was identified and quantified. Gas ranges with pilot lights contributed to higher kitchen NO₂ levels than gas ranges with electronic ignition devices. During the winter, homes with pilot lights had average NO₂ concentrations 17 to 46 µg/m³ (9 to 24 ppb) greater than homes with electric ranges. Homes without pilot lights on the gas range had an average NO₂ concentration that was 17 µg/m³ (9 ppb) greater than homes with electric ranges.

The same study confirmed that vented appliances can contribute to increased indoor NO₂ concentrations. Twenty percent of wall and floor furnaces sampled were suspected of significantly contributing to indoor NO₂ levels due to cracked fireboxes and poor venting. Data from the winter sampling period indicate that homes with wall furnaces had an average winter NO₂ concentration that was 26 to 41 µg/m³ (14 to 22 ppb) greater than homes with a forced air heater. Homes with gas floor furnaces had an average NO₂ concentration that was 44 to 61 µg/m³ (24 to 32 ppb) greater than homes with a forced air furnace. Detailed inspection of the 42 homes with the highest NO₂ levels provided information about the contribution of gas appliances to indoor NO₂ concentrations. Factors that contributed to the high NO₂ levels included improper gas stove burner alignment, improper use of the range to heat the home, exhaust from wall and floor furnaces and gas water heaters spilling into the home, and illegal use of unvented space heaters (Wilson et al. 1986).

Girman et al. (1982) studied the emissions from gas cooking stoves and unvented space heaters in a room-sized environmental chamber. When a kerosene heater was operated for 46 minutes, the room reached an NO₂ concentration of approximately 1 ppm. The level quickly declined when the heater was turned off (air exchange per hour was 1.90). A gas fueled space heater operated for approximately 10 minutes led to a room concentration of about 1500 ppb NO₂. This level declined more slowly with an air exchange rate of 0.5 air changes per hour (ach). The authors calculated steady-state pollutant concentrations from specific unvented gas-fired space heaters operating continuously in a 1400 square foot house with well-mixed air (1.0 ach). Their calculations indicate that steady-state NO₂ levels would range from 180 to 650 ppb.

Gas fireplaces can also emit NO₂, particularly those that are unvented. Dutton et al. (2001) studied emissions from an unvented natural gas fireplace in a home in Boulder, CO. Two-hour time weighted average room concentrations from this study were found to be 90, 350, and 360 ppb NO₂. The authors concluded that prolonged operation of this fireplace without improved ventilation could emit sufficient NO₂ to affect sensitive populations.

Table 5.9 on the following page summarizes indoor NO₂ concentrations that have been reported near strong indoor sources.

5.6.3.2 Outdoor NO₂

For buildings without sources of NO₂, indoor concentrations are closely related to outdoor concentrations. In one study conducted in the Los Angeles Basin, 40 percent of the variation in bedroom concentrations was explained by variations in outdoor concentrations (Spengler et al. 1994). Wilson et al. (1986) stated that on average, outdoor NO₂ levels contribute more NO₂ indoors than does the average gas appliance. Many studies have focused on the concentrations of NO₂ in the outdoor environment; however, this section only discusses studies relating these data to indoor environments.

Table 5.9 NO₂ Concentrations Near Indoor Sources

Average Concentration (ppb)	Peak Concentration (ppb)	Comment	Reference
191 kitchen 195 living room 184 bedroom	375 kitchen 401 living room 421 bedroom	Cooked full meal with use of gas stove and range for 2 hrs, 20 min; average conc. is time-weighted over 7 hrs.	Fortman <i>et al.</i> , 2001
90 (low setting) 350 (med setting) 360 (high setting)	N/R	Natural gas unvented fireplace, 2-hr-time-weighted average in main living area of house (177 m ³). (Not permitted in California homes, but may occur).	Dutton <i>et al.</i> , 2001
N/R	1000	Room concentration with kerosene heater operating for 46 min.	Girman <i>et al.</i> , 1982
N/R	1500	Room concentration with gas heater operating for 10 min.	Girman <i>et al.</i> , 1982
180 to 650	N/R	Calculated steady-state concentration from specific unvented gas space heaters operating in a 1400 ft ² house, 1.0 ach	Girman <i>et al.</i> , 1982

N/R = Not Reported

5.6.3.2.1 *Indoor/Outdoor Ratios*

Indoor/outdoor NO₂ ratios vary significantly based on location, the presence of a gas range, and occupant activities. Lee et al. (2002) calculated indoor/outdoor ratios for two communities in southern California: Upland - at a valley site with high ambient NO₂, and towns in San Bernardino County - at a mountain site with low ambient levels. In all cases, the NO₂ concentrations were greater indoors than outdoors. The ratios of indoor to outdoor NO₂ levels were significantly higher in the mountain site (mean = 3.24) compared to the valley site (mean = 1.07). When a gas range was present the mean indoor/outdoor ratio for all homes was 2.27, in the absence of a gas range it was 1.22. In the presence of an air conditioner, the indoor to outdoor ratio was 1.07, while it was 3.03 without an air conditioner. Air conditioners may serve as a surrogate for the community, since 93% of the valley homes had an air conditioner while only one mountain home had one. The authors did not provide further discussion on the results of indoor NO₂ and presence of an air conditioner. In an earlier study, Lee et al. (1998) measured NO₂ indoor/outdoor ratios from 0.97 – 1.54 in 517 Boston homes.

In the mobile home study conducted by Petreas et al. (1988) during the summer, the mean indoor/outdoor ratio was 1.3 to 1.8. During the winter it was 0.8 to 1.2. Only 10% of the mobile homes had collocated outdoor monitors, thereby limiting the data set. The authors acknowledge that the assignment of outdoor values to some homes may have distorted the ratios. However, the indoor/outdoor ratio for gas homes was three to four times that of the electric homes.

The mean indoor/outdoor ratio at three schools in southern California was 0.5 (Linn et al. 1996). In a study of southern California museums, median indoor/outdoor ratios were less than one, ranging from 0.25 to 0.50, except for one which was 1.36 (Hisham and Grosjean, 1991).

A number of international studies have also reported NO₂ indoor/outdoor ratios. Yang et al. (2004) measured indoor/outdoor ratios of 0.82 in Australian residences and 0.88 in Korean references. Indoor/outdoor ratios of 0.80 and 0.79 were measured in residences and non-smoking offices, respectively, in Hong Kong (Chao et al. 2000, 2001). In Switzerland, the reported indoor/outdoor ratio for NO₂ concentrations in homes was about 0.4 to 0.8 for homes without indoor sources, and about three times higher in homes with gas appliances (Monn, 2001). Heal et al. (1999) measured an indoor/outdoor ratio of 0.91 in the UK. Finally, in a study across many countries, Levy et al. (1998b) found that the use of a gas stove in a home was the dominant activity influencing NO₂ concentrations, with an increase in indoor/outdoor ratios from 0.7 to 1.2 for individuals using a gas stove.

5.6.3.3 *Microenvironments and Other Factors*

NO₂ exposure in different microenvironments was measured for a portion of the participants in the Los Angeles Personal Exposure study (Colome et al. 1992). Forty-eight participants wore NO₂ passive sampling badges for each of two consecutive days, up to eight times over a year. They also wore an additional badge while at home and exchanged it for a different badge while they were away from home, thus gathering 'at home' and 'away from home' exposure measurements. Seasonal variation was observed, with NO₂ concentrations higher in the winter for outdoor, indoor, and personal exposures (although not discussed in detail). Results indicate that badges worn while away from home had the highest mean NO₂ exposure, with means ranging from 34 to 75 ppb. In descending order, the next highest measurement was the outdoor badge (30-48 ppb), followed by personal – at home (26-45 ppb), and bedroom badge (23-39 ppb). Outdoor NO₂ and bedroom NO₂ concentrations were the principal independent variables explaining personal exposures. Data were not collected for kitchen NO₂ concentrations. Subjects in houses using gas as a cooking fuel had significantly higher NO₂ exposures than those who did not use natural gas (significant at p<0.05). Other variables appearing as significant, but not as consistently were pilot lights, gas furnace, travel, time in the house, and range use.

An inspection of the data indicates that although means in given microenvironments were at or below 75 ppb, many maximum levels were greater than 200 ppb. It is likely that individuals with very high exposures were experiencing short-term exposures greater than the ambient air quality standard of 250 ppb for 1 hour-averaging time. The daily personal exposure, 'at home', and 'away from home' exposure measurements all had multiple maxima above 200 ppb: for 'away from home' exposure, four reported maxima were greater than 300 ppb.

Factors such as geographic location and house volume can be correlated with indoor NO₂ levels. Investigators in Boston identified housing characteristics associated with NO₂ levels in each of three seasons (1984 to 1986; Lee et al. 1996). Lower airflow, smaller volume homes, and higher outdoor NO₂ concentrations were associated with higher indoor NO₂ concentrations in apartments and condominiums. Mean indoor concentrations ranged from 15.9 to 29.1 ppb NO₂. In this study, Palmes tubes were used to measure indoor NO₂ levels for two-week periods. In the mobile home study discussed previously, Petreas et al. (1988) identified gas cooking, the inverse of the house volume and geographic location (due to elevated ambient NO₂ levels in southern California) as the most important variables related to indoor concentration. Partti-Pellinen et al. (2000) indicates that a ventilation system with chemical filtration of incoming air can reduce nitrogen oxide levels to about 35% of outdoor levels when outdoor levels are high.

5.6.3.3.1 Seasons

In most studies, indoor NO₂ levels were found to be higher in the winter than in the summer (Spengler et al. 1983; Wilson et al. 1986; Ryan et al. 1988). Schwab et al. (1994) hypothesize this is due to the

increased use of gas heaters and longer cooking times during the cooler months, increased ambient levels during the winter, and seasonal patterns in air exchange rates. These authors studied concentrations in bedrooms over a three year period in Albuquerque, NM. They noted variations from year to year: the period of elevated NO₂ varied slightly, the winter indoor levels varied year to year, and there were variations in the size of the difference between range-type groups (gas with pilot light, gas without pilot light, and electric).

Spengler et al. (1994) reported seasonal variation in personal exposures measured in the Los Angeles basin. Personal exposure during winter months averaged 8 ppb higher than other months. For all range types the difference in personal exposures between the high and low ambient NO₂ zones was equivalent to the difference in the outdoor levels (18 ppb). Sarnat et al. (2000) also found personal NO₂ exposures in Baltimore to be higher in the winter (15.8 – 16.3 ppm) than in the summer (7.9 - 8.7 ppb).

5.6.4 Personal Exposures to NO₂

Information on personal NO₂ exposures comes from the use of passive samples. People's personal exposures to NO₂ vary directly by their personal locations and activities throughout the day. Activity data is particularly useful for understanding the potential for exposure to NO₂ indoors. As shown in Table 5.10, California adults spend about 87 percent of their time indoors, on average, about 6 percent of their time outdoors, and 7 percent of their time inside vehicles (Jenkins et al., 1992). Young California children (age 0-2) spend an average of 85% of their time indoors while children age 3 to 5 spend 76% of their time indoors (Phillips et al., 1991). Because these pre-school aged children spend the greatest amount of time inside the home, they have the greatest potential for exposure to elevated indoor NO₂ concentrations. Additionally, 70% of the population under age 12 spends an average of 66 minutes per day in the kitchen, a location that can have elevated NO₂ concentrations (Phillips et al. 1991). Seventy-five percent of the adult population spends time in the kitchen, for an average of 98 minutes per day (Jenkins et al. 1992).

Spengler et al. (1994) measured personal exposures for about 700 individuals in the Los Angeles basin. Passive personal samples were collected over two consecutive 24-hour periods. The median personal and outdoor levels were 35 ppb while the median indoor level was 24.6 ppb. The distribution for personal exposure samples follows: 25th percentile, 25.4 ppb; 75th percentile, 47.2 ppb; and 90th percentile, 59.8 ppb; 99th percentile, 90.2 ppb. Personal exposures were always higher than indoor bedroom concentrations, and were correlated with the gas range appliance fuel, indicating that exposures were experienced near operating gas appliances or possibly at elevated outdoor locations. Personal exposure for individuals in homes with gas ranges without pilot lights exhibited a mean increase of 5 ppb over individuals in homes with electric ranges. Those in homes with gas ranges and pilot lights averaged 15 ppb more than those with electric ranges. The authors concluded:

“Indoor concentrations are strong predictors of personal exposure: 59 percent of the variation in personal exposure is explained by variations in bedroom concentrations. Ambient concentrations exhibit an important influence on personal exposure to NO₂ in the Los Angeles Basin: 48 percent of the variation in personal exposure is explained by variations in concentrations recorded by the outdoor monitor.”

Table 5.10 Average Percent of Time Californians Spend in Major Locations

AGE	AVERAGE PERCENT OT TIME ¹			
	Inside the Home	Other Indoors	Outdoors	Inside a Vehicle
Children				
0-2	85	4	7	4
3-5	76	9	10	5
6-11	71	12	13	4
All Children (0-11)	76	10	10	4
Adults and Teens	62	25	6	7

¹ From: Wiley et al., 1991a, ARB Contract no. A733-149; Phillips et al.(1991). and Wiley et al., 1991b, ARB Contract no. A6-177-33; Jenkins et al. (1992)

In a study of elementary school children, limited personal monitoring was conducted in three communities of southern California. Over two school years, 107 sampling days (24-hour integrated measurements) of data were collected with passive badges. The mean of personal air concentrations was 22 ppb, with the range extending from 1 to 84 ppb NO₂. The mean personal measurement fell between the mean inside school measurement of 16 ppb and the mean outside school measurement of 33 ppb (Linn et al. 1996).

In another study, personal exposure to NO₂ was measured for 26 Los Angeles adults aged 47 and older with a history of heavy smoking and chronic symptoms related to smoking (Hackney et al. 1992). Investigators used 24-hour time integrated badges to monitor personal exposure to NO₂ during the high-NO₂ season (fall and winter), daily for 2 weeks. For each individual the average of his or her personal daily NO₂ exposure ranged from 58 to 235 µg/m³ (32-130 ppb). The group-mean personal exposure was 101 µg/m³ (56 ppb). The authors noted that for most subjects the correlations between 24-hour average personal NO₂ concentrations and monitoring station concentrations were positive and statistically significant, even though the participants were indoors 80% to 90% of the time. The study was conducted during a period of very high ambient NO₂ concentrations (overall average ambient NO₂ was about 60 ppb); one-hour average ambient NO₂ concentrations during the study were slightly below 300 ppb. Due to the strong influence from outdoor concentrations, the authors comment that “the implications of our results for typical North Americans with COPD are uncertain”. The authors concluded that for this study, personal exposures were not substantially affected by smoking.

5.6.5 Indoor Chemistry of Nitrogen Dioxide and Related Compounds

NO₂ concentrations decline relatively quickly in the indoor environment when indoor sources are not in use. For example, concentrations rise while a gas burner is operating, then when it is turned off, the concentration is at a peak, and rapidly declines (Spicer et al. 1993). This observation led researchers to hypothesize that NO₂ is removed by processes other than just exchange with outside air (Spicer et al. 1989). Spicer et al. (1989) calculated a mean NO₂ removal rate of 1.6 per hour for nine occupied residences. This rate represents removal due to all processes including the effects of air exchange between the indoor and outdoor environments. When the decline attributable to outdoor air was subtracted, the removal rate due to reactive processes was 0.8 per hour. In this example, the mean half-life for reactive removal of NO₂ was 52 minutes, exclusive of air exchange processes.

Ekberg (1995) documented the removal of NO₂ from indoor air in offices by measuring the supply air and exhaust air of office buildings in urban environments with significant traffic. Data indicated that NO₂ concentrations of the air entering and leaving a building were reduced by 5 to 10 ppb while in the building, presumably due to the reactive processes. This phenomenon was not observed for NO.

Several oxidized nitrogen compounds in addition to NO₂ are emitted during combustion by gas appliances and/or are formed through chemical reactions. The most notable additional species, are nitric oxide (NO), nitrous acid (HONO), and nitric acid (HNO₃); Pitts et al. 1989, Spicer et al. 1993, Brauer et al. 1990). Pitts et al. (1985) first observed formation of HONO from the reaction of ppm levels of NO₂ with water vapor in an indoor environment. The rate of formation of HONO exhibited first order kinetics with respect to NO₂ at a rate of 0.25 ppb per minute per ppm of NO₂. Pitts et al. (1989) later reported direct emission of HONO during the operation of two burners on a gas stove. During stove operation, in the absence of ventilation, pollutants reached steady state levels in about an hour; the HONO concentration was more than 50 ppb and the NO₂ concentration was 500 ppb. Investigators again observed the formation of gaseous HONO from the reaction of NO₂ (at ppm levels) with water vapor in indoor environments with no combustion source operating. Spicer et al. (1993) reported steady state nitric acid (HNO₃) concentrations of 0.8 ppb during operation of an unvented convective space heater. Brauer et al. (1990) noted an absence of HNO₃ during combustion, likely due to the high reactivity of HNO₃ with surfaces. Based on removal reactions, indoor NO₂ had a lifetime of about one hour, while the lifetime for NO and HONO was several hours, and that for HNO₃ was 30 minutes or less (Spicer et al., 1993).

Spicer et al. (1993) calculated production rates of HONO from reactions involving NO₂. In a test house, 55 ppb of HONO was produced per hour per ppm of NO₂. This rate is considerably higher than that calculated by Pitts et al. (1985), presumably due to the use of fans to mix the air, consequently accelerating the reactions. Spicer et al. (1993) injected NO₂ into the test house until it reached a maximum concentration of 320 ppb. This level rapidly decayed while HONO was produced, reaching a maximum HONO concentration of 18 ppb, which subsequently had a relatively slow decay rate.

Experiments indicated that HONO was adsorbed to surfaces and released at a later time when equilibrium conditions changed to favor its release (Spicer et al. 1993, Febo and Perrino 1991). HONO concentrations in homes with gas appliances were typically greater indoors than outdoors and range from 10 - 20 ppb (Febo and Perrino 1991; Spengler et al. 1993). HONO is present in indoor air as an acidic aerosol and is likely to be a respiratory irritant, though its respiratory toxicity has not been thoroughly investigated.

5.6.6 Summary

Indoor NO₂ concentrations are highly variable: mean values measured with averaging times of days to a week typically range from 8 to 56 ppb, with maximum levels averaged over a similar time period as high as 100 to 400 ppb or greater. Indoor measurements are generally made using passive monitors that have a long averaging time and do not capture peak exposure levels as concentrations fluctuate throughout the day. Most of the multi-day averaging time data do not permit direct comparison with the 1-hour California ambient air quality standard of 250 ppb.

When indoor combustion sources such as gas stoves, wall furnaces, or space heaters were present, they had a large influence on indoor NO₂ concentrations. Continuous samplers indicated indoor levels reached more than 400 ppb during routine cooking with a gas stove, well above the State ambient standard. Indoor/outdoor NO₂ ratios vary greatly. They range from less than one for homes without an indoor source to values greater than 3 for homes with sources. Outdoor NO₂ levels influence indoor levels due to the infiltration of outdoor air.

Personal exposure to NO₂ is largely influenced by the type of range fuel used for cooking and the outdoor NO₂ concentration. The median personal exposure measured using a 48-hour passive badge in a large Los Angeles basin study was 35 ppb, with a 99th percentile at 90.2 ppb.

In the absence of continually-emitting indoor sources, indoor NO₂ levels can decline quickly due to infiltration of outdoor air and reactive processes. Indoor NO₂ reacts on indoor surfaces to produce HONO and has a lifetime of about one hour. HONO is more persistent in the indoor environment, with a lifetime of several hours. It can be present in the indoor environment at concentrations greater than those outdoors.

5.6.7 Conclusions

In indoor environments, Californians can be exposed to NO₂ at levels that exceed the current NO₂ ambient air quality standard. In a residential setting, indoor sources such as gas stoves and ranges, as well as gas wall and floor furnaces can produce peak concentrations that exceed the current standard of 250 ppb for 1 hour. People's proximity to these sources during cooking and other activities presents a public health concern. Additional research would provide more data on short-term peak indoor concentrations and personal exposures to NO₂, and on other nitrogenous species such as HONO, NO, and HNO₃.

5.7 Summary of Micro-Environmental In-Vehicle Nitrogen Dioxide Measurements

A number of studies regarding in-vehicle micro-environmental measurements have been conducted. The first involved on-road measurements using an electric powered vehicle that traveled the Los Angeles freeway corridors. The second involved measurements taken in some instrumented school buses.

5.7.1 Mobile Monitoring of NO/NO₂ in Los Angeles

5.7.1.1 On-Road NO₂ Measurements

From February through April, 2003, the ARB conducted roadway measurements using an instrumented RAV4 electric vehicle, driven on freeways, arterial roads, and residential streets in Los Angeles (Westerdahl et al. 2005). NO_x, NO and NO₂ were among a number of pollutants measured. The oxides of nitrogen were measured using real-time with an API 220E unit (chemiluminescence method), with a 10 second averaging time. Other analytes included black carbon, particle-bound PAHs, ultrafine particle (UFP) number concentrations, UFP size distributions, PM_{2.5} mass (some runs), CO, and CO₂. (Westerdahl et al. 2005).

The freeway driving was centered around a loop having a large contrast in diesel truck volumes. The loop began near the University of Southern California (USC), went east on the I-10 freeway, and connected via short lengths on the SR 60 and I-5 freeways to the I-710 south to Long Beach. A short time was spent in a residential area of Long Beach, then the route was re-traced, with an additional segment taken on the I-110 north to Pasadena and back to USC. Truck volumes ranged from about 14% trucks on the 710 to about 1% on the I-110. Overall traffic volumes were heavy on all freeways except the northernmost portion of the I-110 approaching Pasadena. Four freeway days were analyzed: Feb. 14 and 20, and April 7 and 16. Average NO and NO₂ concentrations for these days are summarized in Table 5.11. From this table, it appears the fraction of NO_x that was NO₂ was consistently in the 15 to 20% range, although NO₂ averaged from 40 to 70 ppb.

Table 5.11 On-road measurements of nitrogen oxides

Date	Time of Day	NO (ppb)	NO ₂ (ppb)	% of Total NOX that is NO ₂
(2003)		Mean±SD	Mean±SD	
Feb 14	13:45-17:45	190 ± 130	47 ± 50	20
Feb 20	11:00-12:50	210 ± 140	40 ± 50	16
April 7	9:35-11:25	380 ± 130	54 ± 10	12
April 16	11:30-13:40	280 ± 190	68 ± 50	19

NO/NO₂ concentrations varied widely by location. Table 5.12 shows NO and NO₂ concentrations by road segment for the same four days. The numbers presented are the average and standard deviation of the daily averages.

NO concentrations appeared to be a strong function of traffic volume, particularly truck traffic volumes (listed as average trucks per day, TrPD), while NO₂ concentrations showed a less pronounced relationship. The average daily vehicle counts for these freeway segments from Caltrans were 210, 270, and 180 thousand per day, for the 110N, 10E, and 710S, respectively, so vehicle volume showed much less variation than the truck volumes. The fraction of NO_x that is NO₂ appeared to have an inverse relationship with total NO concentrations, ranging from nearly two-thirds of the total in residential locations to only 15% on I-710 with its heavy diesel truck traffic. This is likely due to the reaction of traffic-generated NO with ozone.

Table 5.12 NO and NO₂ concentrations by road segment

Location	NO (ppb)	NO ₂ (ppb)	% of Total NO _x that is NO ₂	Number of Days
	mean ±SD	mean ±SD		
Long Beach Residential	19 ± 7	26 ± 7	57 ± 10	4
Pasadena Residential	16 ± 7	23 ± 16	57 ± 7	3
USC Start	60 ± 34	39 ± 12	41 ± 11	4
USC Finish	34 ± 19	38 ± 14	54 ± 17	3
Arterial Roads North of USC	82 ± 47	36 ± 20	31 ± 12	3
110N freeway (approx 3,500 TrPD)	182 ± 63	54 ± 8.3	24 ± 4.3	4
10e freeway (approx 10,000 TrPD)	272 ± 44	49 ± 13	16 ± 5.5	4
710S freeway (approx 25,000 TrPD)	406 ± 128	66 ± 26	15 ± 0.6	4

TrPD = average number of diesel-powered trucks per day.

5.7.1.2 Bus Study Overview with NO₂ Results

To investigate measurement approaches and future exposures of children who commute to school by diesel school buses, a study was carried out in spring 2002 (Fitz et al. 2003). The study measured pollutant concentrations inside five conventional diesel school buses, model years 1975 to 1993, over actual school bus routes in Los Angeles. For comparison, a 1998 diesel bus outfitted with a particulate trap and a 2002 bus powered by natural gas (with no catalyst) were also included. Buses were outfitted with dual sets of real-time instruments to measure black carbon (BC), particle-bound PAHs, PM_{2.5}, fine particle counts, CO, and NO₂, which allowed front versus back and inside versus outside comparisons. Also included were integrated measures of VOCs, aldehydes, and 1,3-butadiene. SF₆ was introduced into each bus's exhaust as a tracer gas to distinguish the bus's own exhaust from that of other diesel vehicles and help quantify the extent of self-pollution (the re-entrainment of the bus's own exhaust). In keeping with observed operating practices, windows were kept closed in the morning, due to cool temperatures, and were kept partially opened in the afternoon. The full report is available at <http://www.arb.ca.gov/research/schoolbus/schoolbus.htm>. Measurements indicated that for the conventional, uncontrolled, diesel-powered buses, self-pollution was a significant contributor to on-board concentrations when windows were closed and ventilation was reduced. For example, concentrations of BC, PAHs, and the tracer gas were several times higher on conventional diesel buses when windows were closed compared to when windows were open. The trap-equipped bus and the CNG-powered bus exhibited much less of an increase in these concentrations when windows were closed. Self-pollution also appeared to increase with the age of the bus. The high concentrations of pollutants already present on roadways, especially if traffic was heavy, and the direct influence of other vehicles being followed also contributed to high pollutant concentrations on board the buses. For example, concentrations were several times higher on urban routes compared to the rural/suburban route.

5.7.1.3 NO₂ Measurements

NO₂ was measured with two in-house instruments based on the reaction with Luminol, converted to concentrations via photomultiplier tube. NO₂ and PAN were measured in one-minute intervals. A third instrument, a TEI Model 42, measured NO/ NO₂/NO_x with the federal method (chemiluminescence) to calibrate the other two instruments. Measurements were made in the back of the bus at breathing height, and, depending on the run, either in the front of the bus or outside the front door of the bus. NO was not measured. The following table presents the average NO₂ concentrations on the buses, by bus type, for closed window and open window conditions, along with concurrent ambient concentrations taken from the West Los Angeles and Central Los Angeles AQMD monitoring stations.

Table 5.13 Average NO₂ concentrations for open and closed window configurations. Ambient concentrations are from the West Los Angeles and Central Los Angeles AQMD.

	Bus Average windows closed (ppb)	Ambient Air Average, morning (ppb)	Bus Average windows open (ppb)	Ambient Air Average afternoon (ppb)
Conventional Diesel Bus	76 (n=7)	27	77 (n=7)	20
CNG Bus	34 (n=1)	28	39 (n=1)	18
Trap Bus	42 (n=2)	34	86 (n=2)	30

n = number of runs

For conventional, uncontrolled diesel buses, NO₂ concentrations were 2 to 3 times higher on-board buses compared to ambient air.

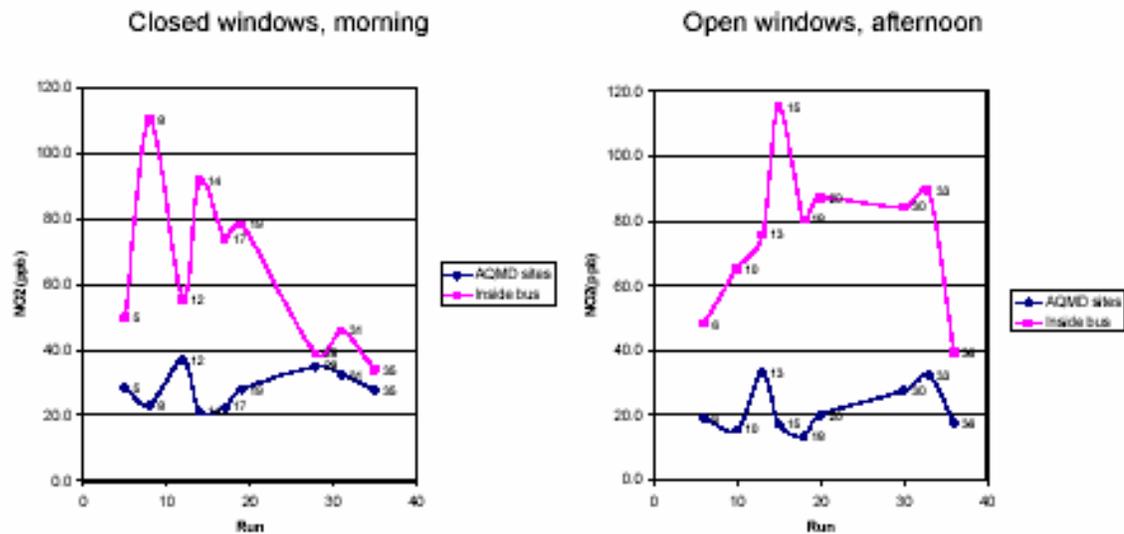


Figure 5.31. NO₂ closed and open window bus measurements.

These results are also presented in figure 5.31, taken from p. 159 of the final report (Fitz et al. 2003). These graphs show the relatively high bus-to-bus and day-to-day variability in the NO₂ results. In spite of this variability, these graphs show no consistent increase in on-board NO₂ concentrations due to closed windows, unlike BC or PAH concentrations. The higher NO₂ concentrations on board (top lines) were therefore probably due to higher roadway NO₂ concentrations rather than self-pollution and closed windows. However, the CNG bus (runs 35 and 36) and runs 28 and 31 for the trap-equipped bus (out of runs 28, 30, 31, and 33) appeared to have on-board NO₂ concentrations closer to ambient concentrations than conventional diesel buses typically did. The exceptions were afternoon runs 30 and 33 of the trap bus, which appeared similar to the conventional diesel buses.

Overall, it appeared that the buses, including the trap-equipped bus, were not producing large amounts of NO₂, although there is some unresolved uncertainty as to whether the trap was functioning as intended. If trap-equipped buses were to generate large amounts of NO₂, in-vehicle NO₂ concentrations might be high when windows are closed.

5.7.1.4 NO₂ and Diesel Emission Controls

An analysis of roadside concentrations of NO_x and NO₂ measured in London during 1997-2003 has been reported by Carslaw (2005). He reported that there has been a downward trend in NO_x, but a steady or increasing level of NO₂ resulting in an increase in the NO₂ to NO_x ratio. The investigator noted that the increased use of diesel particulate filters fitted to buses was likely an important contributing factor to this trend. As part of their design, diesel particulate filters make NO₂ to help oxidize and burn the trapped PM. They therefore emit higher levels of NO₂ and have been used in London for a number of years. The author also noted that new light- and heavy-duty engine technologies and management approaches may also be contributing to this trend.

5.8 Spatial Variability of NO₂ Concentrations

Spatial variability of NO₂ concentrations, or the concentration distributions from primary sources, has been studied by a number of investigators. One of the main focuses has been on the study of NO₂ concentrations in relation to the distance and location from freeways or roadways. For example, Singer et al. (2004) reported that typically, NO₂ concentrations are the highest next to the roadway, and decrease with the distance downwind of the roadway. The authors studied weeklong integrated NO₂ and NO_x concentrations with passive samplers placed outside of 10 elementary schools (sampling for 14 weeks in the spring, 8 weeks sampling in the fall). The authors reported that schools upwind or far

downwind of freeways were similar to regional pollution levels. For schools located within 350 m downwind of a freeway, concentrations of NO₂ and NO_x increased closer to the freeway, with the highest concentrations measured at a school located directly adjacent to a major freeway and shopping center (60% and 100% higher than regional concentrations, respectively).

In another study, Roorda-Knape et al., (1998) measured traffic related pollutants such as PM 2.5, benzene, and NO₂ indoors and outdoors of schools at a number of sites from roadways in the Netherlands. The authors reported that there was no gradient found for PM10, PM2.5, or benzene; however, the outdoor concentrations of NO₂ and black smoke decreased with distance from the roadside. The report showed that NO₂ concentrations in classrooms were significantly correlated with traffic intensity, the percentage time downwind, and distance of the school from the road.

Jerrett et al., (2005) reviewed a number of air pollution concentration models with a focus on NO₂. One relevant finding is that the inverse distance weighting (IDW) model used for the population exposure estimates in the current report may result in over-smoothing actual concentrations and therefore may not provide a representative spatial patterns of exposure. This shortcoming is addressed by recent advances in modeling NO₂ concentration. For example, Wu and colleagues (2005). as part of the Southern California Children's Health Study (CHS) developed an Individual Exposure Model to better evaluate children's long term exposure to air pollutants. The values were derived from the regional monitoring stations used for the CHS. The authors reported that overall within-community variability of personal exposures was highest for NO₂ (+/- 20-40%) and suggested that proper siting of air monitoring stations relative to emission sources is important to better estimate community mean exposures. Also, Ross et al. (2006) reported on modeling intra-urban distribution of NO₂ in San Diego using passive diffusion tubes to sample NO₂ concentrations at 39 sites. The model could predict about 79% of the variability in NO₂ concentrations with four variables: traffic density within 40-300 m of sampling location, traffic density within 300-1000 m, length of the road within 40 m, and the distance to the Pacific coast.

As noted above, there is considerable spatial variability in exposures to NO₂ and current monitoring locations may not capture the areas with higher concentrations within the region. Children living in census tracts with high traffic density and attending CA public schools located near busy roads are disproportionately minority and economically disadvantaged (Gunier et al., 2003, Green et al., 2004).

5.9 References

ARB 2005. 2005 Air Quality Data CD <http://www.arb.ca.gov/aqd/aqcd/aqcdld.htm>

Bellcore, 1993. Fourteen months of ozone and oxides of nitrogen measurements at a telecommunications office in southern California. Special Report SR-ARH-002873, November 1993.

Berglund M, Braback L, Bylin G, Johnson J, Vahter M, 1994. Personal NO₂ exposure monitoring shows high exposure among ice-skating schoolchildren. *Archives of Environmental Health*, 49, (1): 17-24.

Brauer M, Ryan PB, Suh HH, Koutrakis P, Spengler JD, Leslie NP, and Billck IH, 1990. Measurements of nitrous acid inside two research houses. *Environ Sci Technol*, 24 (10): 1521-1527.

Brugge D, Vallarino J, Ascolillo L, Osgood ND, Steinbach S and Spengler J, 2003. Comparison of multiple environmental factors for asthmatic children in public housing. *Indoor Air* 13: 18-27.

Carlaw. 2005. Evidence of an increasing NO₂/NO_x emissions ratio from road traffic emissions . *Atmos. Environ.* 39 (26): 4793-4802.

Chao CYH and Law A, 2000. A study of personal exposure to nitrogen dioxide using passive samplers. *Building and Environment* 35(6): 545-553.

Chao CYH, 2001. Comparison between indoor and outdoor air contaminant levels in residential buildings from passive sampler study. *Building and Environment* 36(9): 999-1007.

- Colbeck I, 1998. Nitrogen dioxide in the workplace environment. *Environmental Monitoring and Assessment* 52(1-2): 123-130.
- Colome SD, Wilson AL, Spengler JD, 1992. Nitrogen dioxide exposure studies–Volume 5. Personal exposure to nitrogen dioxide in the Los Angeles basin: a microenvironmental approach. Topical Report. Gas Research Institute, GRI-92/0427.
- Dennekamp M, Howarth S, Dick CAJ, Cherrie JW, Donaldson K Seaton A, 2001. Ultrafine particles and nitrogen oxides generated by gas and electric cooking. *Occup Environ Med.* 58: 511-516.
- Dutton SJ, Hannigan MP, Miller SL, 2001. Indoor pollutant levels from the use of unvented natural gas fireplaces in Boulder, Colorado. *J. Air & Waste Manage Assoc.* 51: 1654-1661.
- Ekberg LE, 1995. Concentrations of NO₂ and other traffic related contaminants in office buildings located in urban environments. *Building and Environment*, 30, (2): 293-298.
- Farrow A, Greenwood R, Preece S, Golding J, 1997. Nitrogen dioxide, the oxides of nitrogen, and infants' health symptoms. *Archives of Environmental Health*, 52, (3): 189-194.
- Febo A, Perrino C, 1991. Prediction and experimental evidence for high air concentration of nitrous acid in indoor environments. *Atmospheric Environment*, 25A, (5/6): 1055-1061.
- Fitz DR, Winer AM, Colome SD, Behrentz E, Sabin LD, Lee SJ, Wong K, Kozawa K, Pankratz DV, Bumiller K, Gemmill D, Smith M. 2003. Characterizing the range of children's pollutant exposure during school bus commutes. Final Report, Contract No. 00-322. California Air Resources Board, Research Division, Sacramento, CA.
- Fortmann R, Kariher P, Clayton R. 2001. Indoor air quality: residential cooking exposures. Final Report to ARB, Contract No. 97-330.
- Girman JR, Apte MG, Traynor GW, Allen JR, Hollowell CD, 1982. Pollutant emission rates from indoor combustion appliances and sidestream cigarette smoke. *Environment International*, 8: 213-221.
- Green RS, Smorodinsky S, Kim JJ, McLaughlin R, Ostro B. 2004. Proximity of California public schools to busy roads. *Environ Health Perspect* 112:61-6.
- Gunier RB, Hertz A, Von Behren J, Reynolds P 2003. Traffic density in California: Socioeconomic and ethnic differences among potentially exposed children. *J Expo Anal Environ Epidemiol*; 13:240-6.
- Hackney JD, Linn WS, Avol EL, Shamoo DA, Anderson KR, Solomon JC, Little DE, Peng R-C, 1992. Exposures of older adults with chronic respiratory illness to nitrogen dioxide. *Am Rev Respir Dis* 146: 1480-1486.
- Harlos DP, Marbury M, Samet J, Spengler JD, 1987. Relating indoor NO₂ levels to infant personal exposures. *Atmospheric Environment* 21, (2): 369-376.
- Heal MR, O'Donoghue MA, Agius RM, Beverland IJ, 1999. Application of passive diffusion tubes to short-term indoor and personal exposure assessment of NO₂. *Environment International* 25(1): 3-8.
- Hisham MWM and Grosjean D, 1991. Air pollution in southern California museums: indoor and outdoor levels of nitrogen dioxide, peroxyacetyl nitrate, nitric acid, and chlorinated hydrocarbons. *Environ Sci Technol* 25(5): 857-862.
- Jarvis DL, Leaderer BP, Chinn S, Burney PG, 2005. Indoor nitrous acid and respiratory symptoms and lung function in adults. *Thorax* 60: 474-479.
- Jenkins PL, Phillips TJ, Mulberg EJ, Hui SP, 1992. Activity patterns of Californians: use of and proximity to indoor pollutant sources. *Atmospheric Environment*, 26A(12): 2141-2148.
- Jerrett, M., Arain, A., Kanaroglou, P., Bekerman, B., Potoglou, D., Sahuvaroglu, T.S., Morrison, J., and Glovis, C. (2005). A review and evaluation of intraurban air pollution exposure models. *J. Exp anal and Environ Epidemiol.* 15:185-204.
- Lambert WE, Samet JM, Stidley CA, 1992. Classification of residential exposure to nitrogen dioxide. *Atmospheric Environment*, 26A (12): 2185-2192.

- Lee K, Yanagisawa Y, Spengler JD, Fukumura Y, Billick IH, 1996. Classification of house characteristics in a Boston residential nitrogen dioxide characterization study. *Indoor Air*, 6: 211-216.
- Lee K, Levy JI, Yanagisawa Y, Spengler JD, Billik IH, 1998. The Boston residential nitrogen dioxide characterization study: classification and prediction of indoor NO₂ exposure. *J. Air and Waste Management Assoc.* 48(8): 736-742.
- Lee K, Xue J, Geyh AS, Ozkaynak H, Leaderer BP, Weschler CJ, Spengler JD, 2002. Nitrous acid, nitrogen dioxide, and ozone concentrations in residential environments. *Environmental Health Perspectives*, 110 (2): 145-149.
- Levy JI, Lee K, Yanagisawa Y, Hutchinson P, Spengler JD, 1998a. Determinants of nitrogen dioxide concentrations in indoor ice skating rinks. *Amer J Public Health*, 88:(12) 1781-1786.
- Levy JI, Lee K, Spengler, JD, Yanigasawa, Y. 1998b. Impact of residential nitrogen dioxide exposure on personal exposure: an international study. *J. Air & Waste Manage Assoc*, 48: 553-560.
- Linn WS, Shamoo DA, Anderson KR, Peng R-C, Avol EL, Hackney JD, Gong H, 1996. Short-term air pollution exposures and responses in Los Angeles area school children. *J. Exposure Analysis and Environmental Epidemiology*, 6:(4) 449-472.
- Marbury MC, Harlos DP, Samet JM, Spengler JD, 1988. Indoor residential NO₂ concentrations in Albuquerque, New Mexico. *JAPCA*, 38: 392-398.
- Monn C, 2001. Exposure assessment of air pollutants: a review on spatial heterogeneity and indoor/outdoor/personal exposure to suspended particulate matter, nitrogen dioxide and ozone. *Atmospheric Environment*, 35: 1-32.
- Partti-Pellinen KR, Marttila O, Ahonen A, Suominen O, Haahtela T, 2000. Penetration of nitrogen oxides and particles from outdoor into indoor air and removal of the pollutants through filtration of incoming air. *Indoor Air* 10: 126-132.
- Petreas M, Liu K-S, Chang B-H, Hayward SB, Sexton K, 1988. A survey of nitrogen dioxide levels measured inside mobile homes. *JAPCA*, 38(5): 647-651.
- Phillips TJ, Jenkins PL, and Mulberg EJ, 1991. Children in California: activity patterns and presence of pollutant sources. Proceedings of the 84th Annual Meeting and Exhibition, Air & Waste Management Association, (17) Paper no. 91-172.5, June 1991.
- Pitts JN, Biermann HW, Tuazon EC, Green M, Long WD, Winer AM, 1989. Time-resolved identification and measurement of indoor air pollutants by spectroscopic techniques: gaseous nitrous acid, methanol, formaldehyde and formic acid. *JAPCA*, 39(10): 1344-1347.
- Pitts JN, Wallington TJ, Biermann HW, Winer AM, 1985. Identification and measurement of nitrous acid in an indoor environment. *Atmospheric Environment*, 19(5): 763-767.
- Roorda-knape MC, Janssen NAH, De Hartog JJ, Van Vliet NV, Harssema H, Brunekreef B. Air pollution from traffic in city districts near major motorways. (1998). *Atmosph. environ.* 32:1921-1930.
- Ross, Z., English, P.B., Scalf, R., Gunier, R., Smorodinsky, S, Wall, S., and Jerrett, M. (2006) Nitrogen dioxide prediction in Southern California using land use regression modeling: potential for environmental health analyses. *J. Exp. Sci and Environ. Epidem.* 16:106-114.
- Ryan PB, Soczek, ML, Treitman RD, Spengler JD, 1988. The Boston residential NO₂ characterization study II. Survey methodology and population concentration estimates. *Atmospheric Environment* 22(10): 2115-2125.
- Sarnat JA, Koutrakis P, Suh HH, 2000. Assessing the relationship between personal particulate and gaseous exposures of senior citizens living in Baltimore, MD. *J Air Waste Manag Assoc.* 50(7):1184-98.
- Schwab M, McDermott A, Spengler JD, Samet JM, Lambert WE, 1994. Seasonal and yearly patterns of indoor nitrogen dioxide levels: data from Albuquerque, New Mexico. *Indoor Air-International Journal of Indoor Air Quality and Climate*, 4(1) 8-22.

- Singer, BC, Hodgson, AT, Hotchi, T, Kim, JJ. 2004. Passive measurement of nitrogen oxides to assess traffic-related pollutant exposure for the East Bay children's respiratory health study. *Atmosph Environ.* 38:293-403.
- Spengler J, Schwab M, Ryan PB, Colome S, Wilson AL, Billick I, Becker E, 1994. Personal exposure to nitrogen dioxide in the Los Angeles basin. *J. Air & Waste Manage Assoc*, 44: 39-47.
- Spengler JD, and Brauer M, 1993. Nitrous acid in Albuquerque, New Mexico, homes. *Environ. Sci. Technol.* 27: 841-845.
- Spengler JD, Duffy CP, Letz R, Tibbitts TW, Ferris BG, 1983. Nitrogen dioxide inside and outside 137 homes and implications for ambient air quality standards and health effects research. *Environ Sci Technol*, 17(3): 164-168.
- Spicer CS, Kenny DV, Ward, GH, Billick IH, 1993. Transformations, lifetimes, and sources of NO₂, HONO, and HNO₃ in indoor environments. *J. Air Waste Manage. Assoc.* 43: 1479-1485.
- Spicer CW, Coutant RW, Ward GF, Joseph DW, Gaynor AJ, Billick IH, 1989. Rates and mechanisms of NO₂ removal from indoor air by residential materials. *Environment International*, 15: 643-654.
- Triche EW, Belanger K, Bracken MB, Beckett WS, Holford TR, Gent JF, McSharry JE, Leaderer BP, 2005. Indoor heating sources and respiratory symptoms in nonsmoking women. *Epidemiology* 16(3): 377-384.
- Van Strien RT, Gent JF, Belanger K, Triche E, Bracken MB and Leaderer BP, 2004. Exposure to NO₂ and nitrous acid and respiratory symptoms in the first year of life. *Epidemiology* 15(4): 471-478.
- Weschler CJ, and Shields HC, 1994. Indoor chemistry involving O₃, NO, and NO₂ as evidenced by 14 months of measurements at a site in southern California. *Environ Sci Technol.* 28: 2120-2132.
- Westerdahl D, Fruin SA, Sax T, Fine PM, Sioutas C. 2005. Mobile platform measurements of ultrafine particles and associated pollutant concentrations on freeways and residential streets in Los Angeles. *Atmosph. Environ.* 39:3597-3610.
- Wiley JA, Robinson JP, Cheng YT, Piazza T, Stork L, Pladsen K, 1991a. Study of children's activity patterns. University of California, Berkeley. Final report, ARB Contract No. A733-149.
- Wiley JA, Robinson JP, Piazza T, Garrett K, Cirksena K, Martin G, 1991b. Activity patterns of California residents. University of California, Berkeley. Final report, ARB Contract No. A67-177-33.
- Wilson AL, Colome SD, Baker PE, Becker EW, 1986. Residential indoor air quality characterization study of nitrogen dioxide, Phase I, Volume 1: Prepared for Southern California Gas Company.
- Wilson AL, Colome SD, Tian Y, 1993. California Residential Indoor Air Quality Study: Volume 1, Methodology and descriptive statistics. Sponsored by Gas Research Institute, Pacific Gas and Electric Company, San Diego Gas and Electric Company, and Southern California Gas Company, May 1993.
- Wu, J., Lurmann, F., Winer, A., Lu R., Turco, R., and Funk, T. (2005) Development of an individual exposure model for application to Southern California children's health study. *Atmosph. Environ.* 39:259-273
- Yang W, Lee K, Chung M, 2004. Characterization of indoor air quality using multiple measurements of carbon dioxide. *Indoor Air* 14: 105-111.
- Zipprich JL, Harris SA, Fox JC, Borzelleca JF, 2002. An analysis of factors that influence personal exposure to nitrogen oxides in residents of Richmond, Virginia, *Journal of Exposure Analysis and Environmental Epidemiology* 12: 273-285.

6 Controlled Human Exposure Studies

6.1 Summary

NO₂ is the most abundant of the nitrogen oxides (NO_x) in the atmosphere and is the NO_x species that represents the greatest risk to human health. This review focuses on human clinical studies of exposure to NO₂. Human clinical studies utilize controlled laboratory conditions to measure health-related effects of exposure. These studies help in characterizing exposure-response relationships, and are highly relevant to assessing health risks in humans. However, human clinical studies are limited to exposures of short duration, to mild and transient responses, and to small sample sizes of relatively healthy subjects.

In most human clinical studies of healthy individuals, exposures to NO₂ at concentrations less than 4.0 ppm do not cause symptoms or alter pulmonary function. In healthy individuals, exposures in the range of 1.5 to 2 ppm may increase airway responsiveness; similarly, exposures in the range of 1 to 2 ppm NO₂ induce mild airway inflammation. A number of studies have shown effects on lung lymphocytes, but there is a lack of consistency among these studies.

Some, but not all, clinical studies of asthmatics have found that subjects with asthma appear to be more sensitive to effects of NO₂ on airway responsiveness compared with healthy subjects. For a number of studies of asthmatics, short-term exposures to NO₂ at 0.2-0.3 ppm resulted in an increase in airway responsiveness (Kleinman et al. 1983 (0.2 ppm, 2 hr), Jorres et al. 1990 (0.25 ppm, 30 min), Bylin et al. 1988 (0.27 ppm, 30 min.), Bauer et al. 1986 (0.3 ppm, 30 min). Strand et al. (1996) observed no effect 30 min after exposures to NO₂ (0.25 ppm, 30 min.) but observed an increase in airway responsiveness 5 hr after exposure. However, the findings have not been consistent across other studies with similar (but not identical) protocols. The reasons may be due, in part, to differences in the subjects recruited for the various studies. Even in clinical studies where, on average, there are no differences between responses with filtered air vs. NO₂ exposures, the data on individual responses suggest that there is substantial inter-individual variability in response. Thus, the clinical studies of asthmatics suggest that some individuals experience increased airway responsiveness after exposures to NO₂ in the range of 0.2-0.3 ppm. Several studies found transient decreases in lung function in asthmatics at 0.3 ppm during the initial part of the exposure, but the findings were not consistent. The effects of NO₂ on airway inflammation in asthmatics have not been adequately studied.

Several clinical studies in subjects with asthma have shown that NO₂ exposure increases allergen responsiveness, with effects observed at concentrations as low as 0.26 ppm for 30 min. Studies found that exposures to NO₂ followed by inhaled allergen resulted in decrements in lung function (Strand et al. 1997, 1998). Subsequent studies using similar exposure protocols found evidence of an enhanced allergic response after NO₂ exposures compared with filtered air controls, including an increased inflammatory response in lung fluid (Barck et al. 2002), and evidence of activation of eosinophils in lung fluid and sputum (Barck et al. 2002, 2005). Decrements in lung function were not observed in these studies.

A small number of studies evaluated effects in individuals with chronic obstructive pulmonary disease; several found small decrements in FEV₁ at 0.3 ppm, but the findings were inconsistent. Older smokers may also be a subgroup at increased risk of lung function decrements at NO₂ levels slightly above the CA 1-hour standard for NO₂ of 0.25 ppm.

A limited number of studies explored the cardiac, vascular and systemic effects of NO₂ exposure, but these data were not conclusive. Finally, limited studies explored the effects of NO₂ on airway responsiveness to other pollutant challenges, with inconsistent results. However, several studies found that NO₂ at levels only slightly above the California standard may act synergistically with SO₂ in enhancing responses to allergen challenge.

Overall, the clinical studies suggest NO₂ exposures near the current ambient air quality standard for NO₂ (0.25 ppm, 1 hour average) may enhance the response to inhaled allergen in people with allergic asthma. For a subset of asthmatics, exposures to NO₂ at levels near the current ambient air quality standard may cause increased airway reactivity.

6.2 Introduction

NO₂ is the most abundant oxide of nitrogen in the atmosphere, and represents the greatest risk to human health. The U.S. Environmental Protection Agency (EPA) has established a National Ambient Air Quality Standard (NAAQS) for NO₂ of 0.053 ppm (100 µg/m³), measured as an annual arithmetic mean. The US EPA lists no U.S. counties that are currently in violation of the NAAQS for NO₂ (<http://www.epa.gov/oar/oaqps/greenbk/nindex.html>). California has established only a short-term (1 hour) standard of 0.25 ppm (470 µg/m³).

NO₂ is considered an important outdoor pollutant not only because of its potential health effects, but because it is an essential precursor in the formation of tropospheric ozone via photochemical reactions, and contributes to the formation of atmospheric acids and secondary particles. This review will focus on the evidence, provided by human clinical studies, for human health effects attributable to NO₂ exposure, and will not address the role of NO₂ in the photochemical generation of other pollutants.

This chapter will briefly address the strengths and weaknesses of human clinical studies, review the key human exposure studies of NO₂, assess the contribution of these studies toward understanding the health effects of ambient NO₂ exposure, and discuss their implications in establishing appropriate outdoor air quality standards.

Typically, the database for risk assessment arises from three separate investigative approaches: epidemiology, animal toxicology, and human inhalation studies. Each possesses advantages but also carries significant limitations. For example, the epidemiological investigation examines exposures in the “real world” but struggles with the realities of conducting research in the community, where exposure characterization is typically imprecise and cigarette smoking, socioeconomic status, occupational factors and concomitant exposure to other air pollutants (indoors and out) can be important confounders. Outcomes are most frequently evaluated by questionnaire, and sophisticated measures of physiological responses cannot be made routinely. In contrast, inhalation studies in animals allow precision in quantifying exposure duration and concentration, measurement of a wide variety of physiologic, biochemical, and histological endpoints, investigation of chronic health effects studies in animals, and examination of extremes of the exposure-response relationship. Often, however, interpretation of these studies is constrained by difficulty in extrapolating findings from animals to humans, and from relatively high doses to lower environmental exposures.

Controlled, quantitative studies of exposed humans offer a third approach. The next section discusses the advantages and limitations of human clinical studies in providing scientific information that can be used by policy makers when determining ambient air quality standards.

6.3 Human Clinical Studies of Air Pollution: An Overview

Experimental exposure of human volunteers to various pollutants under controlled conditions can provide useful data on pathophysiological changes of direct relevance to standard setting. These human clinical studies (often termed controlled human exposure studies) can utilize laboratory atmospheric conditions, which can be engineered to be relevant to ambient pollutant atmospheres, and can measure health-related effects that result from breathing the atmospheres. The carefully controlled environment allows investigators to identify responses to individual pollutants, to characterize exposure-response relationships, and to examine interactions among pollutants *per se* or with other variables such as exercise, humidity, or temperature. Susceptible populations can participate, such as those with acute and chronic respiratory and cardiovascular diseases, with appropriate limitations based on subject comfort and protection from risk. Endpoint assessment traditionally has included symptoms and pulmonary function, but more recently has been extended using a variety of markers of pulmonary, systemic, and cardiovascular effects.

Human clinical studies have limitations as well. For practical and ethical reasons, microbiological or biochemical examination of pollutant-induced tissue damage is more limited. Additionally, studies must be limited to relatively small groups, to short durations of exposure (i.e., minutes to hours), and to pollutant concentrations that are expected to produce only mild and transient responses. Because of the small sample size in clinical studies here is limited statistical power to observe a response. Also, studies of small samples may not be representative of the range of responses in the general population; and, there

is increasing scientific evidence that genetics and other individual host factors (e.g., smoking status, prior exposure to ambient pollutant, dietary factors) may be important determinants of an individual's susceptibility to a given pollutant. Additionally, for safety and ethical reasons, among those with chronic medical conditions such as asthma or cardiovascular disease, only those with mild or moderate disease are usually studied. The fetus, infants and young children, pregnant women, and immunocompromised individuals are not studied in this setting. This selection bias in recruiting volunteers reduces the generalizability of such studies. Finally, controlling the experimental conditions may result in failure to capture effects found in complex real-world exposures, such as ambient air containing NO₂ in combination with other pollutants. Additionally, the acute, transient responses seen in clinical studies cannot necessarily be used to predict health effects of chronic exposures at lower doses or short-term repeated exposures which may be the more relevant exposure scenarios encountered by the public, as discussed in Chapter 5.

It should be emphasized, however, that these limitations all tend to underestimate pollutant effects. Therefore, finding a response that can be related to specific exposure conditions constitutes a valuable component to the standard setting process. In contrast, given the potential limitations of human clinical studies, negative findings may in some cases reflect the constraints of study design more than biological reality.

Frampton and Utell (1999) reviewed specific issues of protocol design in human clinical studies. The basic design of controlled human exposure studies is as follows: Volunteers are exposed to one or more carefully measured pollutants or filtered air through a mouthpiece (oral breathing only) or in a chamber (oronasal breathing). Responses are compared to responses after exposures to filtered air. A few studies have used exposures to ambient air as a baseline for comparison. (The use of an ambient air control instead of a filtered air control could potentially decrease the ability to see an effect of experimental exposures to NO₂.) Subjects wait several weeks between the controlled exposures and are not aware of whether he/she is breathing filtered air or air pollutant during the study (single-blinded). Because potentially sensitive subpopulations are also of interest for purposes of standard setting, controlled exposures of pollutants often include people with pre-existing lung conditions (e.g., asthma), and may include adolescents and elderly adults. Subjects may be at rest or may exercise intermittently either on a treadmill or bicycle ergometer. Exercise can increase the quantity of pollutants reaching the deep lung, particularly for relatively insoluble compounds such as NO₂ by both increasing ventilation and causing a switch from nasal to oronasal breathing. However, exercise can also elicit other physiological changes that affect the response to pollutant exposures.

Data collected usually include graded respiratory symptoms and a variety of indices of pulmonary function, such as the amount of air one can exhale in one second after a deep inspiration (FEV₁) or the airway resistance (R_{aw} or SR_{aw}). Airway responsiveness (the tendency of the airways to constrict in response to a stimulus) can also be compared after exposure to filtered air vs. air pollutant. Methods used in the experimental setting to induce airway responsiveness include inhalation of an aerosolized pharmacologic agent (e.g., histamine, methacholine, or carbachol), sulfur dioxide (SO₂), aerosolized saline (dilute or concentrated), or hyperventilation with cold or dry air. Increased airway responsiveness (or bronchial hyper-reactivity) is a hallmark of asthmatics and is also observed in many persons with chronic obstructive pulmonary disease and in otherwise healthy individuals. Most of the clinical studies of the effects of NO₂ in asthmatics include an evaluation of airway reactivity. There is no generally accepted criterion regarding the magnitude of increased responsiveness that should be considered significant from a regulatory perspective. However, the clinical significance of increased airway reactivity is the potential for a flare-up or exacerbation of asthma or other underlying pulmonary disease following increased bronchial response to nonspecific airborne irritants.

Frampton and Utell (1999) reviewed the general techniques for measurements of pulmonary mechanics, airway responsiveness, direct sampling of airways, and other measures of respiratory effects. Other factors in specific studies or protocol design (such as subject selection criteria and characterization, dose and duration of exposure, timing of data or specimen collection) may differ from study to study. These differences may lead to inconsistencies in results across similar studies.

6.4 Human Clinical Studies of Exposure to NO₂

This review is not intended to be an exhaustive compilation of human studies of NO₂, but to present the spectrum of effects seen in human clinical studies, and to highlight published reports that are most relevant to assessing risks and establishing appropriate environmental standards. Studies selected for inclusion in this review involved human subjects exposed to defined atmospheres containing NO₂ under controlled, experimental conditions, with an emphasis on studies published since the last review of the California ambient air quality standard for NO₂ (CARB, 1992).

We will first review studies of healthy subjects exposed to NO₂ alone, and then consider subjects with pre-existing disease. Studies of NO₂ in combination with other pollutants will be considered separately. Of note, the exposure protocols for some chamber studies involve single exposures to NO₂ of varying duration (30 min. to up to six hours). Others have looked at the clinical response to short (15-30 min.), repeated exposures to NO₂. This intermittent exposure protocol might better reflect the short-episodic high exposures to NO₂ observed in real-life exposure. In addition, because personal exposure patterns to environmental NO₂ are characterized by long periods of exposure to low or moderate levels alternating with short periods of higher exposures, (e.g., in outdoor in heavy traffic, or in kitchens due to gas stove use), some studies were also done with short, repeated exposure patterns which might better reflect real-life NO₂ exposures.

Tables 6.1 to 6.6 summarize the studies that are particularly relevant to standard setting. The tables include those studies published since the last review of the California NO₂ standard and earlier studies that are particularly relevant to standard setting.

6.4.1 Effects of NO₂ in Healthy Individuals

6.4.1.1 Symptoms and Pulmonary Function

As detailed in the 1992 review, NO₂ studies examining responses of healthy volunteers to acute exposure to NO₂ as high as 4.0 ppm at exposures up to 5 hours have generally failed to show increased symptoms or alterations in lung mechanics as measured by pulmonary function testing or airway resistance (Hackney et al. 1978; Kim et al. 1991; Morrow et al. 1992; Vagaggini et al. 1996; Kerr et al. 1979; Frampton et al. 1991). Exposures ranging from 75 minutes to 5 hours at concentrations up to 4.0 ppm NO₂ did not alter pulmonary function (Linn et al. 1985b; Rasmussen et al. 1992; Mohsenin 1987b; Mohsenin 1988). One exception was a study conducted by Bylin et al. (1985). These investigators found increased airway resistance after a 20-minute exposure to 0.25 ppm NO₂ and decreased airway resistance after a 20-minute exposure to 0.5 ppm NO₂, but no change in airway responsiveness to aerosolized histamine challenge in the same subjects. The reasons for the observation of increased airway resistance at low concentrations of NO₂ but not at higher concentrations, are not entirely understood and have not been further investigated.

Since the 1992 CA review, several other clinical studies have studied the effect of NO₂ exposure on pulmonary function or symptoms in healthy individuals and found no effect on symptoms or pulmonary function at exposures up to 2 ppm for 4-6 hours. (Azadniv et al. 1998; Blomberg et al. 1999; Devlin et al. 1999). Vagginini et al. (1996) reported a mild increase in symptoms, but no effects on lung function after 0.3 ppm NO₂ for 1 hr with intermittent exercise. (Refer to Table 6.1 for further details on these studies)

Overall, most clinical studies indicate that acute exposures of young, healthy volunteers to NO₂ at levels as high as 4.0 ppm do not cause symptoms or alter lung function as measured by spirometry or flow resistance.

6.4.1.1.1 Effects on Pulmonary Function in Subpopulations of "Healthy Individuals"

Few human clinical studies of NO₂ have included elderly subjects. Morrow et al. (1992) studied the responses of 20 healthy older volunteers (13 smokers and 7 nonsmokers of mean age 61 years) following exposure to 0.3 ppm NO₂ for 4 hours with light exercise (see Table 6.1). There was no significant change in lung function related to NO₂ exposure for the group as a whole. However, the 13 smokers experienced a slight decrease in FEV₁ during exposure, and their responses were significantly different from the 7 nonsmokers (% change in FEV₁ at end of exposure: -2.25 vs. +1.25%, p = 0.01). In a recent study of six elderly individuals (mean age 68) with no smoking history, no change in spirometry was noted after

exposure to NO₂ (0.4 ppm for 2 hr with intermittent exercise) (Gong et al. 2005, Table 6.1). Thus, the limited data to date suggest that older smokers may be a subgroup at increased risk of lung function decrements at NO₂ levels slightly above the 1-hour standard for NO₂.

6.4.1.2 Airway Responsiveness

Although lung function appears to be minimally affected in healthy individuals at 4 ppm, several observations indicate that NO₂ exposures in the range of 1.5-2.0 ppm cause small but significant increases in airway responsiveness in healthy individuals, as detailed in the 1992 CA review. There have been no additional studies on airway responsiveness to NO₂ exposures in healthy individuals.

Several of the relevant studies in the 1992 review are summarized here: Mohsenin (1988) found that a 1-hour exposure to 2 ppm NO₂ increased responsiveness to methacholine, as measured by changes in specific airway conductance, without directly affecting lung function. Furthermore, pretreatment with oral ascorbic acid prevented the NO₂-induced increase in airway responsiveness (Mohsenin 1987b). Frampton et al. (1991) observed a mild increase in responsiveness to carbachol following a 3-hour exposure to 1.5 ppm NO₂. After carbachol challenge, the mean FEV₁ decrease with NO₂, relative to air exposure, was 2.71% (confidence interval 0.23 to 5.19). There was no significant difference with exposure to intermittent peaks of 2.0 ppm.

6.4.1.3 Airway Inflammation

NO₂ exposure elicits a neutrophilic inflammatory response in the airways, although the effect appears to be less intense than with ozone exposure at comparable concentrations. Studies by Sandstrom et al. (1990, 1991, 1992a, 1992b) found that short (20 min.) exposures to NO₂ at near-ambient levels (1.5-2 ppm) did not affect PMN concentrations in airways in healthy volunteers, as discussed in the 1992 CA review (CARB 1992). However, more recent studies have shown clear evidence for airway inflammation following prolonged exposure (four to six hours) to NO₂ at a concentration of 2.0 ppm, unaccompanied by symptoms or changes in lung function. These studies are summarized below (see also Table 6.1).

Healthy volunteers exposed to 2.0 ppm NO₂ for six hours with intermittent exercise (Azadniv et al. 1998) showed a slight increase in the percentage of PMN obtained in bronchoalveolar lavage (BAL) fluid 18 hours after exposure (air: 2.2±0.3%; NO₂: 3.1±0.4%). Blomberg et al. (1997) reported that 4-hour exposures to 2.0 ppm NO₂ resulted in a 2.8-fold increase in PMN in the bronchial wash (the first bronchial aliquot of BAL) of healthy subjects (after NO₂: 14%; after air: 5%) at six hours, and 1.5-fold increase in interleukin-8 at 1.5 hours; no differences were observed in bronchial biopsies. These findings suggest that airway inflammation may occur primarily either in the proximal airways or small airways. (The bronchial wash reflects changes in the proximal airways including the terminal bronchioles. No findings in the bronchial biopsy suggest that effects are not in the central airways.) Consistent with these findings, Devlin et al. (1999) exposed eight healthy nonsmokers to 2.0 ppm NO₂ for four hours, with intermittent exercise. BAL performed the following morning (16 hours after exposure) showed a 3.1-fold increase in PMN recovered in the bronchial wash fraction, indicating small-airway inflammation.

In a subsequent study, Blomberg et al. (1999) studied the effects of repeated exposures to NO₂ (4-hour exposures to 2 ppm NO₂) on four consecutive days, with BAL, bronchial biopsies, and BAL fluid antioxidant levels assessed 1.5 hours after the last exposure. The bronchial wash fraction of BAL fluid showed a two-fold increase in PMN and a 1.5-fold increase in myeloperoxidase, indicating persistent mild proximal airway inflammation with repeated NO₂ exposure. Solomon et al. (2000) also found increased PMNs in BAL after repeated exposures to NO₂ using a similar protocol.

Both animal studies and epidemiological studies suggest possible links between NO₂ exposures and susceptibility to respiratory infections (see chapters 8 and 9), and investigators have used clinical studies to explore possible effects of NO₂ exposure on cytokine production in airways and alveolar macrophage activity. Gavras et al. (1994) found no evidence that NO₂ exposure (2.0 ppm, 6 hr) affected alveolar macrophage phenotype or expression of the adhesion molecule CD11b or receptors for IgG when assessed in BAL fluid *immediately* after NO₂ exposure. However, 16 hours after exposure to NO₂ (2.0 ppm for four hours), Devlin et al. (1999) observed a reduction in alveolar macrophage phagocytosis and superoxide production in the bronchial wash (as well as an increase in PMNs, as noted above), indicating possible adverse effects on host defense. Repeated daily exposures to 2 ppm NO₂ (4 hours/day for four successive days) increased bronchial epithelial expression of epithelial cytokines (IL-5, IL-10, IL-13) and

ICAM-1 (Pathmanathan et al. 2003). The IL-5 and IL-13 cytokines, also produced by activated TH2 (helper T cells), are important contributors to the cascade of eosinophil activation and IgE synthesis. (Salvi et al. 1999; Zhu et al. 1999, Ngoc et al. 2005). These processes are important in the immunopathogenesis of asthma. Thus repeated exposure to NO₂ has the potential to exert a “pro-allergic” effect on the bronchial epithelium. I-CAM-1 is an important surface receptor for the common respiratory viruses, rhinovirus and respiratory syncytial virus.

In addition, recent studies on cytokine, macrophage, and epithelial responses also provide evidence for airway inflammatory effects at concentrations below 2.0 ppm. Frampton et al. (2002) examined NO₂ concentration responses in 21 healthy nonsmokers. Subjects were exposed to air, 0.6, and 1.5 ppm NO₂ for three hours, with intermittent exercise, with exposures separated by at least three weeks. BAL was performed 3.5 hours after exposure. PMN increased approximately 3-fold after exposure to 1.5 ppm NO₂ (but not 0.6 ppm), and lymphocytes increased in bronchial wash fluid and decreased in blood, suggesting an influx of lymphocytes from the blood to the airways in response to NO₂ exposure. Jörres et al. (1995) found that 3-hour exposures to 1 ppm NO₂ with intermittent exercise were associated with a small increase in levels of eicosanoids (thromboxane B2) but not inflammatory cells, in BAL fluid.

There is also evidence from both animal and human studies that exposure to NO₂ may alter lymphocyte subsets in the lung and possibly in the blood. Lymphocytes, particularly cytotoxic (CD8+) T cells and NK cells, play a key role in host defense against respiratory viruses by eliminating infected host cells. Richters and colleagues (Damji and Richters 1989; Richters and Damji 1988; Richters and Richters 1989; Kuraitis and Richters 1989) showed that mice exposed to NO₂ at levels as low as 4 ppm for eight hours demonstrated reductions in populations of CD8+ (T cytotoxic) lymphocytes in the spleen.

In humans, Sandstrom et al. (1990,1991) observed significant, dose-related increases in lymphocytes and mast cells recovered by BAL 24 hours after a single 20-minute exposure to NO₂ at 2.25 to 5.5 ppm during light exercise, whereas there was no change in PMNs (see above). There was no change in CD4+/CD8+ T-cell ratio. Repeated exposures to NO₂ may also cause different bronchoalveolar cellular reactions. Rubinstein et al. (1991) found that a series of four daily, 2-hour exposures to 0.60 ppm NO₂ resulted in a small (but not statistically significant) increase in Natural Killer (NK) cells recovered by BAL. In contrast, repeated exposures to 1.5 or 4 ppm NO₂ for 20 minutes every 2nd day on six occasions resulted in the reduction of the total number and proportion of (CD8+) T cells, changes in the balance of T lymphocyte subpopulations and a reduction in the number of NK cells in BAL fluid, 24 hours after the final exposure (Sandstrom et al. 1992a, 1992b). No effects were observed on PMN or total lymphocytes.

Lymphocyte changes were also seen at longer exposure times. Solomon et al. (2000) found a decrease in CD4+ (T helper) lymphocytes in BAL fluid 18 hours after four daily, 4-hour exposures to 2.0 ppm NO₂. As noted above, Azadniv et al. (1998) observed a small but statistically significant increase in PMN in BAL fluid 18 hr following single 6 hour exposures to 2.0 ppm NO₂; however there was no change in lymphocyte or alveolar macrophage counts in BAL. They also observed a small but significant reduction in CD8+ T lymphocytes in peripheral blood. In another study, exposures to 1.5 ppm NO₂ for three hours resulted in small increases in BAL lymphocytes (and BAL PMN) and decreased in blood lymphocytes with exposures to 0.6 and 1.5 ppm NO₂ (Frampton et al. 2002). This group also found gender differences in lymphocyte subset responses. Table 6.5 summarizes the findings of these studies on lymphocyte subset responses to NO₂ exposure.

In summary, single and repeated exposures to NO₂ (1.5-2 ppm for 3 -6 hours) in healthy individuals result in a neutrophilic inflammation in the proximal airways that can be detected in the bronchial wash of BAL. One study found evidence of an increase in an inflammatory marker after NO₂ exposure at 1 ppm without evidence of increase in neutrophils (Jörres et al. 1995). Taken together, these studies suggest that the threshold for airway inflammatory effects in young, healthy, exercising subjects is less than 1.0-1.5 ppm for 3 hours. These studies also indicate that separately analyzing the bronchial fraction (first bronchial wash) of BAL increases the sensitivity for detecting inflammatory effects of NO₂ exposure. The findings of increased neutrophils in the bronchial wash, but not in the BAL, suggest regional differences in the lung response to inhaled NO₂. This differential response may be due to differences in NO₂ exposure (delivery), absorbance, clearance, or antioxidant defenses in the proximal compared with the bronchoalveolar regions of the lung.

In general, however, the observed effects on lymphocyte subset responses have not been consistent among studies. Differences in the dose of NO₂ (concentration, duration, single vs. repeated exposure) and time of sampling among these studies, as well as the small numbers of subjects, may explain the varying findings. Furthermore, the clinical significance of transient, small changes in lymphocyte subsets is unclear. However, even small changes in susceptibility to respiratory viruses resulting from exposure to NO₂ may have a significant public health impact because of the large number of individuals exposed outdoors and in the home, both to NO₂ and to respiratory viruses.

6.4.1.4 *Host Defense in Healthy Individuals*

6.4.1.4.1 Susceptibility to Infections

Clinical studies have attempted to address the question of whether NO₂ exposure increases susceptibility to infection. Goings et al. (1989) exposed healthy volunteers to either 1-3 ppm NO₂ or to air for two hours per day for three consecutive days. A live, genetically engineered influenza A vaccine virus was administered intranasally to all subjects after exposure on day 2, and infection was determined by virus recovery from nasal washings, a 4-fold or greater increase in antibody titer, or both. Volunteers became infected more frequently in association with NO₂, but the effect was not statistically significant. The findings of this study were inconclusive, in part because the small number of subjects studied limited the statistical power to detect a significant effect. In addition, the attenuated, cold-adapted virus used in the study was incapable of infecting the lower respiratory tract, where NO₂ may have the most important impact on host defense.

Other approaches have been to evaluate the activity of alveolar macrophages obtained by bronchoalveolar lavage from NO₂-exposed individuals and to examine the susceptibility of respiratory epithelial cells to viral infection *in vitro*. Several NO₂ exposure scenarios, including continuous low-level exposure or intermittent peak exposures have been examined (Frampton et al. 1989). Alveolar macrophages obtained by BAL 3½ hours after a 3-hour continuous exposure to 0.60 ppm NO₂ tended to inactivate influenza *in vitro* less effectively than cells collected after air exposure, but the effect was not statistically significant (p<0.07). Frampton et al. (1989) observed the effect in cells from four of the nine subjects studied, suggesting individual variability in response. Two subsequent studies by other investigators involving 2.0 ppm NO₂ exposures for four or six hours, with intermittent exercise, found no effect on alveolar macrophage inactivation of influenza virus, either immediately or 18 hours after exposure (Azadniv et al. 1998; Devlin et al. 1999). The timing in these studies was different from the previous study by Frampton et al. (1989). In further studies, Frampton et al. (2002) examined NO₂ effects on viral infectivity of airway epithelial cells. Subjects were exposed to air, 0.6, and 1.5 ppm NO₂ for three hours, and bronchoscopy was performed 3.5 hours after exposure. Epithelial cells were harvested from the airways by brushing, and then challenged *in vitro* with influenza virus and respiratory syncytial virus. NO₂ exposure did not alter viral infectivity, but NO₂ exposure appeared to enhance epithelial cell injury in response to infection with RSV (p = 0.024). A similar non-significant trend was observed with influenza virus.

Since the 1992 review of the California NO₂ air quality standard, Helleday et al. (1995) found that 20 minute exposures to NO₂ at 1.5 to 3.5 ppm transiently reduced airway mucociliary activity, assessed by fiberoptic bronchoscopy 45 minutes after exposure. Mucociliary activity was increased 24 hours after NO₂ exposure at 3.5 ppm for 4 hours, suggesting the effects were transient. This effect would be expected to increase susceptibility to infection by reducing clearance of microorganisms from the airway. The control exposure in this study used ambient air as a baseline for comparison. (Note: this is unlikely to be a real limitation of the study since use of an ambient air control (instead of a filtered air control) might decrease the ability to detect an effect of experimental exposures to NO₂).

In summary, several studies in healthy individual provide evidence that prior exposure to NO₂ may increase the susceptibility to a viral infection through various mechanisms including decreased macrophage activity, decreased mucociliary action of respiratory epithelia, and increased susceptibility of respiratory epithelium to injury by subsequent viral challenge.

6.4.1.4.2 Anti-Oxidant Defense Mechanisms

Kelly 2003 and Brook et al. 2004 have implicated oxidative stress from ambient air pollution in the pathology of asthma and COPD, as well as cardiovascular disease. NO₂ is a potent oxidant, and several

studies support the hypothesis that oxidative stress may be an important mechanism underlying NO₂ toxicity. Pre-treatment with vitamin C decreased airway responsiveness to methacholine aerosol after NO₂ exposure (Mohsenin 1987b) and antioxidant supplementation may prevent reduced activity of an important lung protease inhibitor (Mohsenin 1991). (See next section.) Additionally, the respiratory tract lining fluid overlying the respiratory epithelium contains a wide spectrum of antioxidants, and several investigators have examined the effect of NO₂ on the protective antioxidant defense in the respiratory tract lining fluid. Kelly et al. (1996) examined the kinetics of NO₂-induced antioxidant reactions in healthy, nonsmoking adults exposed to NO₂ at 2 ppm for four hours with light, intermittent exercise. Bronchoscopy was performed at 1.5 hr, 6 hr, or 24 hr, on three separate groups. Ascorbic acid and uric acid concentrations were transiently decreased in bronchial wash and BAL fluids but returned to or exceeded control levels by 6 hours. In contrast, significant increases in reduced glutathione (GSH) concentration in bronchial wash were found at 1.5 and 6 hrs but returned to control levels by 24 hrs. There was no change in BAL levels of GSH. In a follow-up study by the same group, Blomberg et al. (1999) studied the effects of repeated exposures to NO₂ at 2 ppm for four hours on four consecutive days on healthy individuals. Repeated exposures to NO₂ resulted in a persistent neutrophilic inflammation in BAL, but the immediate anti-oxidant response observed after a single NO₂ exposure was attenuated. A small (2%) reduction in FEV₁ on day 1 was also attenuated after repeated exposures. Thus, repeated short NO₂ exposures appeared to induce mechanisms that attenuated the immediate anti-oxidant defense response observed after a single NO₂ exposure, despite the persistence of inflammatory cells in BAL. Further time-course studies are needed to investigate the effects of repeated NO₂ exposures on inflammation and resulting responses.

6.4.1.5 Induction of Emphysema

Emphysema is a lung disease characterized by the destruction of alveolar walls by a process that involves the release of elastases (enzymes that break down elastic tissues) by inflammatory cells. For instance, clinical emphysema in humans has been linked with deficient proteinase inhibitor activity in the lung, presumably due to inactivation by cigarette smoke. (Elastase is a specific proteinase; and deficient proteinase inhibitor activity would increase the ability of elastase to break down elastic tissue.) One mechanism by which chronic NO₂ exposure may result in structural lung injury is through the inactivation of lung proteinase inhibitors. Animal models involving prolonged exposure to relatively high levels of NO₂ have found pathological changes of emphysema (Evans et al. 1976; Lafuma et al. 1987). Mohsenin and Gee (1987) exposed healthy volunteers to 3 or 4 ppm NO₂ for 3 hours and observed a 45% decrease in the functional activity of α_1 -proteinase inhibitor in BAL fluid. As noted in the above section (anti-oxidant defense mechanisms), the increased elastase inhibitory capacity of the alveolar lining fluid after NO₂ exposures at 4.0 ppm was not observed if the subjects were pre-treated with oral Vitamin C and E supplements for four weeks (Mohsenin 1991). In contrast, Johnson et al. (1990) found no effect of exposure for three hours to continuous 1.5 ppm or intermittent peaks of 2.0 ppm NO₂ on either the concentration (immunoassay) or functional activity of α_1 -proteinase inhibitor in BAL fluid. The absence of an effect in the Johnson study may reflect the lower exposure levels used.

Frampton et al. (1989) observed a 47% increase in α_2 -macroglobulin, a metalloproteinase inhibitor released by alveolar macrophages, in BAL fluid 3.5 hours following 3-hour exposures to 0.60 ppm NO₂. This protein may have local immunoregulatory effects as well as providing protection against proteinases. Its increase following NO₂ exposure suggests a protective response. However, Frampton et al. (1989) observed no change in BAL fluid levels of α_2 -macroglobulin following similar exposures to 1.5 ppm NO₂.

Human clinical studies cannot be expected to provide insights into the health effects of chronic exposure to NO₂. Even these studies of BAL concentrations of protease inhibitors are somewhat inconsistent, and do not provide convincing evidence that exposure to NO₂, at concentrations well above current outdoor standards, causes airway remodeling or emphysema.

6.4.2 Effects of NO₂ on Subjects with Asthma

6.4.2.1 Symptoms, Lung Function, and Airway Responsiveness

Table 6.2 summarizes the clinical studies on asthmatics that investigate the effects of near ambient levels of NO₂ (< 1ppm) on airway reactivity, lung function, and respiratory symptoms. Most of these studies were included in the 1992 review (CARB 1992) and are included in the discussion below:

Overall, at exposure concentrations in the range of 0.1-0.5 ppm, there appeared to be little consistent effect on respiratory symptoms and lung function. Bauer et al. (1986) and Avol (1989) observed transient decrease in lung function (e.g., FEV₁) during the initial part of exposures to 0.3 ppm.

However, there is some evidence that NO₂ causes increased airway reactivity at these concentrations (Table 6.2). Additionally, although many studies between 0.1-0.5 ppm found no grouped effect on airway responsiveness, where individual data was presented, the data showed substantial inter-individual variability as discussed below (see Folinsbee 1992).

Several of the studies on airway reactivity near the current 1 hour California standard (0.25 ppm) are summarized here to illustrate the range of findings:

Orehek et al. (1976) were the first to report that relatively brief exposures of asthmatics to low-level NO₂ (0.1 ppm) might enhance subsequent responsiveness to challenge with a broncho-constricting drug. Airway resistance was also increased. Although NO₂ alone caused an increase in airway resistance in only 3 of 20 asthmatics, bronchial responsiveness to carbachol increased in 13 of these 20 subjects. This report was challenged because of the retrospective separation of responding from non-responding subjects; however, a re-analysis of data by Dawson and Schenker (1979) gave similar results. Orehek et al. (1976) illustrated the variability in responses between individuals. Hazucha et al. (1983) failed to confirm these results in a study of 15 asthmatic subjects (0.1 ppm).

Other investigators could not confirm effects of NO₂ at concentrations slightly above 0.1 ppm on lung function in either asthmatic adolescents (Koenig et al. 1985, 1988 (0.12 ppm)) or in mildly asthmatic adults (Koenig et al. 1985, 1987 (0.12 ppm)). Airway reactivity was not measured in these studies. Ahmed et al. (1992) reported (in an abstract) an increase in airway reactivity to carbachol in 13 of 20 subjects at 0.1 ppm for 1 hour, but the results were not reported in a peer-review journal (see CARB 1992 review). Thus, overall, there was little consistent evidence that NO₂ at about 0.1 ppm causes increased airway reactivity in asthmatics.

At NO₂ exposures in the range of 0.2-0.6 ppm, the results have been mixed. Several studies found no evidence of increased airway reactivity at 0.2-0.6 ppm (Jörres et al. 1991 (0.25 ppm), Morrow et al. 1989 (0.3 ppm), Roger et al. 1990 (0.15-0.6 ppm)). However, increased airway reactivity was associated with NO₂ exposures in this range in five other studies, indicating that this is not likely to be a spurious or chance finding (Kleinman et al. 1983 (0.2 ppm), Jörres et al. 1990 (0.25 ppm), Bauer et al. 1986 (0.3 ppm), Bylin et al. 1985 (0.48 ppm), Mohsenin 1987a (0.5 ppm)). Thus, three studies of asthmatics found evidence of increased airway reactivity at NO₂ levels in the range of 0.2-0.3 ppm. (Refer to the California 1992 review for additional discussion (CARB 1992).)

We identified two additional studies on airway reactivity in asthmatics published since the CA 1992 review: Strand et al. 1996, Folinsbee (1992). We also discuss here a study by Bylin et al. (1988) that had been omitted in our earlier review.

Bylin et al. (1988) evaluated NO₂ responses of asthmatics at 0.14, 0.27 and 0.53 ppm, and found statistically significant evidence of airway reactivity at 0.27 ppm. Strand et al. (1996) performed a series of studies in mild asthmatics in Sweden. They exposed the asthmatics to NO₂ at 0.26 ppm for 30 minutes during mild intermittent exercise, and found a late-phase increase in airway reactivity (histamine at 5 hours), whereas there was no statistically significant increase in reactivity at 30 minutes post-exposure. Previous studies measured airway reactivity within 60 minutes after exposures, and this delayed effect after NO₂ was not previously described.

Folinsbee (1992) conducted a pooled analysis of studies of airway hyper-responsiveness in asthmatics with NO₂ exposures; the analysis included all studies with individual data on airway-reactivity to NO₂ in the peer reviewed literature, project reports, and reports obtained by direct communications. Folinsbee combined studies into three groups of NO₂ exposures: less than 0.2 ppm (range 0.1-0.15 ppm; 0.20-0.30 ppm; and >0.3 ppm). For each group, Folinsbee compared the airway responsiveness after air and after NO₂ exposure and assigned a direction of change (e.g., increase or decrease) in airway responsiveness. He did not count individual data if responses were identical or indeterminate. Folinsbee found that, across studies, 73% of asthmatics with exposures at rest to NO₂ at concentrations near the ambient 1 hour California standard (NO₂ range: 0.20 - 0.30 ppm, n_{total} = 33) had an increase in airway responsiveness after exposures (p<0.01, sign test). A pooled analysis of asthmatics with NO₂ exposures during exercise

(NO₂ range: 0.20 - 0.30 ppm, n_{total} = 136) found no evidence of increase in airway responsiveness. Although the greater effect in the group at rest might seem counterintuitive, exercise can alter airway mechanics and decrease the relative uptake in the tracheobronchial region such that NO₂ effects on airway responsiveness might diminish with exercise (Follinsbee 1992). Follinsbee's (1992) analysis of studies with NO₂ exposures <0.2 ppm and >0.3 ppm found similar results (i.e., only those with exposures at rest had increased airway responsiveness after NO₂ exposures). A number of factors differed between studies, including methods used to determine nonspecific airway responsiveness, including protocols for testing airway responsiveness, challenge procedures, and timing of response. These differences limited the interpretation of the pooled analysis. Furthermore, the magnitude of the response could not be quantified in this type of analysis. Taken together, the results of the pooled qualitative analyses support the findings of individual studies on NO₂ effects on airway reactivity in asthmatics at 0.2-0.3 ppm, and provide some evidence for increased airway reactivity in asthmatics at levels below 0.2 ppm, but the data are not firm.

Overall, it is evident that NO₂ can increase airway reactivity responses among some asthmatics exposed for 30 min to 2 hours to NO₂ at 0.2-0.3 ppm. The data are more limited on effects of NO₂ on airway reactivity at concentrations less than 0.2 ppm.

The lack of findings in some studies may reflect, in part, lack of statistical power to observe an effect due to small sample size, differences in subjects (inter-individual variability) and exposure protocols. Differences in protocols include: mouthpiece vs. chamber, obstructed vs. non-obstructed asthmatics, sedentary vs. exercise, methods for airway sampling, and requirements for withholding medication. Identification of factors that predispose to NO₂ responsiveness requires further investigation. Also, these studies typically involved volunteers with mild asthma. Although it is generally not possible to obtain data in more severely affected asthmatics due to ethical and medical considerations, these individuals may be more susceptible to NO₂ exposure than the mild asthmatics used in chamber studies. Finally, there is increasing evidence that air pollution may affect asthmatics differently, depending on genetic susceptibility (Saxon et al. 2005); thus future studies are needed to investigate whether genetic factors predict which subgroups of asthmatics are more susceptible to NO₂.

6.4.2.2 Airway Inflammation

Studies have begun to focus on studies of NO₂-induced airway inflammation in asthmatics. Mild asthmatics exposed to NO₂ at 0.26 ppm for 30 minutes experienced an increase expression of an adhesion molecule (mac-1) on granulocytes in peripheral blood samples 30 minutes after NO₂ exposure, as well as a delayed increase in airway responsiveness (Strand et al. 1996). The increased expression of cell-surface adhesion molecules suggests an NO₂-induced priming of human granulocytes.

Jörres et al. (1995) found small reductions in FEV₁ (2.5% decrease with NO₂ vs. 1.3% for filtered air, p= 0.010) in 12 mild asthmatics following 1 ppm NO₂ exposure for 3 hours, with intermittent exercise. (The effect might be due to the responses for only 1-2 individuals.) In the BAL fluid of these asthmatics, they found no evidence of NO₂ effects on cellular numbers, but did find an increase in concentrations of inflammatory mediators associated with bronchoconstriction (PGD₂ and TxB₂), and a decrease in a mediator associated with bronchodilation (6-keto-PGF_{1α}). In contrast, healthy individuals (n=8) in the same study with the same exposure protocol had only a small increase in levels of eicosanoids (thromboxane B₂) in BAL fluid.

Vagaggini et al. (1996) found no significant differences in lung function, or percentages of inflammatory cells in nasal lavage and induced sputum in 8 mild asthmatics exposed to filtered air or 0.3 ppm NO₂ for 1 hour. Frampton and Utell (1999) found that both nasal lavage and induced sputum have relatively high between-subject variability and questionable correlation with BAL. Thus, it is difficult to extrapolate whether negative findings on nasal lavage or induced sputum would translate to negative findings on BAL.

In an ARB-sponsored study, Solomon et al. (2004) found that 3-hour exposures to 0.4 ppm NO₂, with intermittent exercise, did not alter pulmonary function. They also found no significant effect of NO₂ on inflammatory cells or proteins in sputum samples compared to filtered-air control, but they did not assess airway reactivity.

In summary, there is little evidence that short-term exposures to NO₂ at outdoor ambient concentrations (less than the current California one-hour standard of 0.25 ppm) has a direct effect on symptoms or alteration of lung function in people with mild asthma. Some studies of nonspecific airway responsiveness in asthmatics have seen an effect of NO₂ at 0.2-0.3 ppm, whereas others have not. An examination of the data on responses for individuals suggests substantial inter-individual variability in airway reactivity in response to NO₂ at levels near the current California standard of 0.25 ppm.

Given the clinical findings of increased airway reactivity in some mild asthmatics after exposures to NO₂ at 0.2-0.3 ppm, it is possible that more severe asthmatics, or individuals with particular sensitivity to NO₂ airway effects, would experience reductions in lung function and/or increased airway responsiveness at concentrations below 0.25 ppm. Furthermore, outdoor levels influence indoor concentrations, which may reach peak levels that are clinically important for some adults and children with asthma. Few studies have evaluated the effects of NO₂ on non-specific airway inflammation in asthmatics. Jörres et al. (1995) found evidence of airway inflammatory mediators in BAL of asthmatics exposed at 1 ppm; these changes were concomitant with small decrements in lung function. Healthy subjects in the same study showed a lower response to these markers of inflammation and no evidence of effect on lung function.

6.4.2.3 Effects on Allergen Responsiveness in Asthmatics

The potential for NO₂ exposure to enhance responsiveness to allergen challenge in asthmatics deserves special mention. Asthma is a disease of the airways characterized by chronic airway inflammation. In the case of allergic asthma, the lung's response to inhaled allergens (e.g. dust mite or pollen) leads to ongoing airway inflammation. The hallmarks of the inflammatory response include an influx of inflammatory cells (e.g., PMNs and eosinophils) that release cellular products that contribute and further enhance the on-going inflammatory response. NO₂, as an oxidant gas, could enhance allergen responsiveness by increasing the underlying injury and inflammation in the airway epithelium that is characteristic of asthma (Figure 6.1, from Krishna and Holgate, 1999). Epithelial cells damaged by NO₂ may release cellular products called cytokines (e.g., IL-1, TNK, RANTES, GM-CSF, sICAM) that could

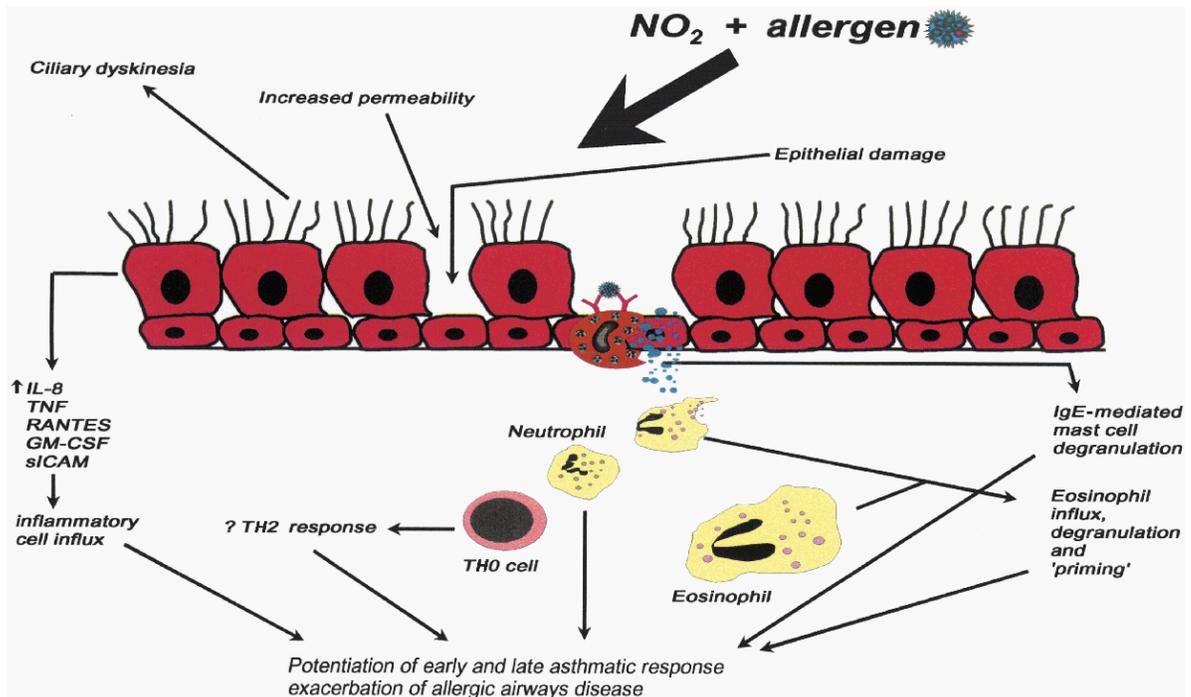


Figure 6.1. Possible inflammatory mechanisms by which NO₂ may enhance the aeroallergen response in allergic airway disease (from Krishna and Holgate 1999). Epithelial cells damaged by NO₂ may release cellular products called cytokines (e.g., IL-1, TNK, RANTES, GM-CSF, sICAM) that could play an important role in the recruitment and activation of eosinophils. It is postulated that some air pollutants may induce a T cell response that favors an allergic (TH2) or IgE response.

play an important role in the recruitment and activation of eosinophils. There is evidence that some air pollutants; e.g., diesel exhaust particles can induce a T cell response that favors an allergic (TH2) or IgE response. Although speculative, NO₂ could favor a TH2 response by similar mechanisms.

A number of human clinical studies support this proposed mechanism of NO₂ enhancement of the allergen response. As noted above, Helleday et al. (1995) found evidence that NO₂ exposures can promote ciliary dyskinesia in healthy individuals. Additionally, Strand et al. (1996) found evidence that asthmatics exposed to 0.26 ppm NO₂ for 30 minutes had activation of neutrophils in peripheral blood 30 minutes after exposure, and subsequent increased bronchial responsiveness five hours after exposure.

Additionally, Table 6.4 summarizes several recent studies that reported that low-level exposures to NO₂, both at rest and with exercise, enhance the response to specific allergen challenge in mild asthmatics.

Tunnicliffe et al. (1994) conducted exposures of eight subjects with asthma to 0.4 ppm NO₂ for only one hour at rest, and reported increased responsiveness to a fixed dose of allergen, both during the early and late phases of the response. For instance, after filtered-air exposure, the late phase response to allergen (mean FEV₁) decreased by 2.85% compared with a decrease of 8.1% after 0.4 ppm NO₂. They found no significant effect at 0.1 ppm, but the data suggested an exposure-response relationship for the late-phase response. Devalia et al. (1994) and Rusznak et al. (1996) described an effect of exposure to the combination of 0.4 ppm NO₂ and 0.2 ppm SO₂, but not either pollutant alone, on subsequent allergen challenge in mild asthmatics. (The 1-hour California standard for SO₂ is 0.25 ppm).

Strand et al. (1997) demonstrated increases in the late-phase allergen response, as measured by decreased peak flow three to nine hours after allergen challenge four hours after 30-minute exposures to 0.26 ppm NO₂. In further studies, Strand et al. (1998) investigated early and late phase responses to allergen following four daily repeated exposures to 0.26 ppm NO₂ for 30 minutes. All exposures took place with the subjects at rest. The investigators found small but statistically significant decrements in FEV₁ at 15 min (early phase) and 3-10 hr (late phase) after allergen challenge with previous NO₂ exposure compared with filtered air alone on four consecutive days. Thus, the enhancing effect of NO₂ on the asthmatic response remained unchanged after four days of repeated exposure. This contrasts with the inflammatory response to NO₂ in BAL studies in healthy subjects where the response changes from an increase after single exposure, to a decrease after repeated exposure to NO₂ (Sandstrom et al. 1991 and 1992, Table 6.1).

Jenkins et al. (1999) exposed asthmatic subjects to NO₂, ozone, and their combination followed by allergen challenge using two different protocols that varied time of exposure and gas concentration, but kept the total exposure constant (Table 6.4). In the studies using NO₂ alone, exposures at 0.4 ppm for 3 hr enhanced subsequent responsiveness to allergen, whereas exposures at 0.2 ppm for 6 hr did not. Thus, the peak concentration of exposure may be more important than the total dose. In further studies, Barck et al. (2002) used allergen challenge followed by bronchoalveolar lavage to show that 30 minute exposures to 0.26 ppm NO₂ at rest enhanced the airway inflammatory response to allergen challenge, when the challenge was done four hours after the NO₂ exposure. The bronchial wash and BAL fractions, collected 19 hours after allergen challenge, showed an increased percentage of neutrophils. They found an increase in eosinophilic cationic protein (ECP), but no change in eosinophil number, in the bronchial wash. Eosinophilic cationic protein is a product of eosinophils that contributes to airway injury in asthmatics. In contrast to previous studies (Strand et al. 1997, Strand et al. 1998), Barck et al. (2002) found no effect of NO₂ on symptoms or lung function immediately after allergen challenge (early-phase response). This might have been due, in part, to subject differences and statistical variability in ambient exposures to NO₂ on days of controlled NO₂ exposures compared with days with filtered air exposures. The authors reasoned that this might reduce the probability to detect the effect on lung function related to NO₂ exposure in the chamber. Additionally, the allergen dose (% allergen provocation) was different.

In a subsequent study, Barck et al. (2005) investigated the effect of a series of three, 15-minute exposures to 0.26 ppm NO₂, over 2 days (Day 1: 15 min, Day 2: 15 min x 2, 1 hour apart, followed by allergen challenge at 4 hours each day). Levels of ECP (blood and sputum) increased from Day 1 to Day 3. Again, Barck et al. (2005) found no concomitant subjective symptoms or pulmonary function changes or increased eosinophil or neutrophil counts in sputum or blood.

The findings of increased ECP but not eosinophil numbers in these experiments suggest that NO₂ may have an effect on degranulation of eosinophils but not on cell recruitment. As noted above, eosinophilic cationic protein is a product of eosinophils that contributes to airway injury in asthmatics. Serum ECP may reflect an enhanced eosinophil secretory activity, and increase adherence and transmigration (priming) (Venge et al. 1999). Serum ECP levels were increased in adults and older children with asthma and atopy, and correlated with disease activity and compliance with inhaled steroid therapy (Venge et al. 1999).

Solomon et al. (2004) recently found no effects on allergen responsiveness, as assessed by lung function measurements following 3-hour exposures to 0.4 ppm NO₂, with intermittent exercise. Except for a decrease in sputum eosinophils with NO₂ exposure compared with FA collected at 6 hours post-exposure, sputum samples at 6 hr and 24 hr showed no differences in inflammatory cells and inflammatory cytokines. They found no significant effect of NO₂ on other inflammatory cells. In this study, the allergen challenge was performed approximately one hour after the exposure; this and other methodologic differences from the Barck studies may account for the differing findings (Barck et al. 2002, 2005). Other studies suggest that NO₂ exposure (0.4 ppm for 6 hr) enhances allergen responsiveness (increased ECP) in the nasal mucosa in subjects with allergic rhinitis (Wang et al. 1995, 1999). However, there was no evidence in activation of inflammatory cells and mediators in the nasal airways after NO₂ exposure (0.26 ppm, 30 min) followed by allergen challenge at 4 h (Barck et al., 2005b).

Although Solomon et al. (2004) did not find an enhanced allergen response after NO₂ exposures, they pointed out that a subset (3/15) of asthmatics had substantially greater early airway narrowing with allergen challenge compared with non-responders or with the group as a whole. The three responders had lower airway reactivity to house dust mite allergen than did the non-responders, and hence received a greater dose of allergen (and experienced a higher macrophage response) after HDM allergen challenge followed by filtered air exposure. This observation that there were subsets of responders vs. non-responders is not unique to the Solomon study. Among studies that found enhanced group-mean bronchoconstrictor responses to allergen after NO₂, an examination of the data on individual responses indicates that not all asthmatic subjects demonstrate such responses (Tunnicliffe et al. 1994; Devalia et al. 1994; Ruznak et al. 1996; Strand et al. 1997, 1998; Jenkins et al. 1999). These data demonstrate that there is individual variability in susceptibility to enhancement of the response to allergen after NO₂ exposure among mild asthmatics.

Additional data from both animal exposure and *in vitro* exposure studies provide support for enhancement of allergen responsiveness by NO₂ exposure. Gilmour (1995) reviewed the evidence in animal models. Of particular interest is a rat model of house-dust-mite sensitivity in which a 3-hour exposure to 5 ppm NO₂, after a priming injection and pulmonary challenge with antigen, increased the specific immune response and immune-mediated pulmonary inflammation. NO₂ exposure also enhanced lymphocyte proliferation responses to allergen in both the spleen and mediastinal lymph nodes. Schierhorn et al. (1999) observed increased histamine release by cultured human nasal mucosa from surgical resections in response to exposure to NO₂ at 200 and 800 µg/m³ (0.106 and 0.424 ppm) for 24 hours. The magnitude of the effect was more pronounced than for ozone.

Several recent studies involving allergen challenge suggest effects at concentrations as low as 0.26 ppm, near the current California standard. They suggest that NO₂ may enhance the allergic response to an inhaled allergen, as evidenced by increased inflammatory response, eosinophil activation and modest, statistically significant decrements in lung function (Strand et al. 1997, 1998; Barck et al 2002, 2005). Only a few laboratories have studied the effects of NO₂ on enhancement of the allergen response.

Nonetheless, the rising incidence, prevalence, and mortality from asthma make these observations particularly important and timely. Using more sensitive endpoints investigators have found a biologically plausible coherent body of evidence that brief exposures to NO₂ (0.26 ppm for 30 min) enhances the allergic response in mild asthmatics. Additional work is needed to understand more completely the exposure-response characteristics, effects of exercise, relationship to severity of asthma, role of asthma medications, and other clinical factors. Additional animal and *in vitro* studies are needed to establish the precise mechanisms involved.

6.4.3 Effect of NO₂ on Subjects with Chronic Obstructive Pulmonary Disease

Table 6.3 summarizes the few studies that evaluated the responses to NO₂ in subjects with chronic obstructive pulmonary disease (COPD). In a study of 26 smokers with probable COPD (history of symptoms and reduced FEV₁), Hackney et al. (1992) found no lung function effects of exposure to 0.3 ppm NO₂ for four hours, with intermittent exercise. Similarly, in a group of 22 subjects with moderate COPD, Linn et al. (1985a) found no pulmonary effects of 1-hour exposures to 0.5, 1.0, and 2.0 ppm NO₂. Morrow et al. (1992), exposed 20 subjects with COPD for 4 hours to 0.3 ppm NO₂ in an environmental chamber, with intermittent exercise. Lung function was followed serially for the first four hours after exposure, and they observed significant reductions in lung function (forced vital capacity, FVC). Similarly, progressive decrements in FEV₁ occurred during the period following exposure; however, they did not find statistically significant decreases for FEV₁ until four hours after exposure.

Since the 1992 review, two other studies have examined NO₂ effects on COPD patients (see Table 6.3). Vagaggini et al. (1996) found a decrease in mean FEV₁ (3%) in COPD patients two hours after exposures to 0.3 ppm NO₂ for one hour with intermittent exercise, but no change in percentages of inflammatory cells in nasal lavage and induced sputum. In a study by Gong et al. (2005), elderly individuals with and without COPD exposed to NO₂ (0.4 ppm for 2 hours with intermittent exercise) had no change in lung function, oximetry, or cellular counts on induced sputum after exposure to NO₂. They noted a small decline in diastolic blood pressure and increase in heart rate in both groups.

In summary, two of five studies of COPD patients found small decrements in FEV₁ at 0.3 ppm NO₂ for 1 or 4 hours. The lack of findings in other studies may reflect the differences in study samples, durations of exposure, subjects' smoking status, and exercise protocol, timing of lung function testing, or small sample size. The studies on inflammatory changes with NO₂ in COPD patients were inadequate to draw any conclusions. Morrow et al. (1992) noted that changes in lung function were typical of the "restrictive" pattern observed with ozone, rather than the obstructive changes described by some with NO₂ exposure in asthmatics. This suggests the mechanism may involve stimulation of irritant nerve endings in the airway, with reflex inhibition of inspiration, rather than airway narrowing.

6.4.4 Effect of NO₂ on Subjects with Cardiovascular Disease

A very limited number of studies have tested the hypothesis that NO₂ exposure alters cardiovascular function. Nitric oxide (NO) serves as a key intercellular signaling molecule, with immunoregulatory functions and potent vasodilator properties. Inhalation of NO gas causes pulmonary vasodilatation, and is used therapeutically in patients with acute respiratory failure to lower pulmonary vascular pressures and improve the matching of ventilation and perfusion. It is possible that NO₂ exposure could result in

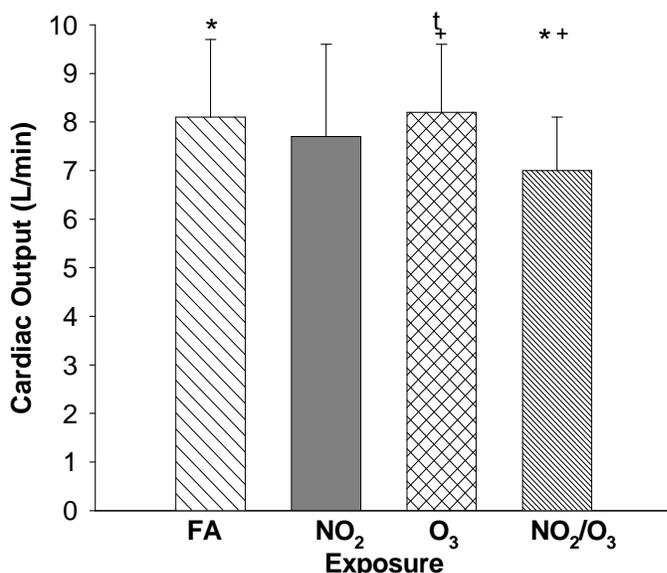


Figure 6.2. Mean ± SD cardiac output during exercise for each of the exposures. Matching symbols indicate a significant difference (P<0.05) between the marked exposures.

increased generation of NO and its vasoregulatory products, in the pulmonary vasculature.

Folinsbee et al. (1978) studied three groups of five healthy males exposed to 0.62 ppm NO₂ for two hours. The groups differed by duration of exercise during exposure: 15, 30, or 60 minutes. In addition to pulmonary function, outcome measures included indirect calorimetry, cardiac output using the CO₂ re-breathing technique, blood pressure, heart rate, and diffusing capacity of the lung for carbon monoxide (DLCO). They found no significant effects for the individual groups, nor for the 15 subjects analyzed together.

Drechsler-Parks (1995) used a different technique to assess changes in cardiac output: noninvasive impedance cardiography. Eight older adults (56 to 85

years of age) were exposed to 0.60 ppm NO₂, 0.45 ppm ozone (O₃), and the combination of 0.60 ppm NO₂ and 0.45 ppm O₃, for 2 hours, with intermittent exercise. The exercise-induced increase in cardiac output was smaller with the NO₂ + O₃ exposures than with the filtered air (FA) or O₃ exposures alone (Figure 6.2). There were no significant differences in minute ventilation, heart rate, or cardiac stroke volume, although the mean stroke volume was lower for NO₂ + O₃ than for air. Drechsler-Parks speculated that chemical interactions between O₃ and NO₂ at the level of the epithelial lining fluid led to the production of nitrite, leading to vasodilatation, with reduced cardiac preload and cardiac output.

This potentially important study has not been repeated, to our knowledge. One previous study (Linn et al. 1985b) reported small but statistically significant reductions in blood pressure after exposure to 4 ppm NO₂ for 75 minutes, a finding consistent with systemic vasodilatation in response to the exposure. However, many subsequent studies at concentrations generally less than 4 ppm have not reported changes in blood pressure in response to NO₂ exposure. As noted above, individuals with and without COPD (Gong et al. 2005) experienced a small decline in diastolic blood pressure at four hours and 22 hours after exposure to NO₂ (0.4 ppm for two hours with intermittent exercise).

There is also evidence that NO₂ exposure may affect circulating red blood cells. Posin et al. (1978) exposed 10 healthy males to 1 or 2 ppm NO₂ for 2.5 to 3.0 hours daily for two days. Blood obtained immediately after the second day of exposure showed reduced levels of hemoglobin and hematocrit (NO₂: 41.96 ± 2.75; sham exposure: 43.18 ± 2.83, p = 0.001), and reduced red blood cell acetylcholinesterase levels. Technically, the sham exposure (the environmental chamber with no NO₂ on the control day) was not equivalent to a filtered-air control exposure. This might bias towards no effect since the chamber air probably had more NO₂ than a filtered-air control.

In the study by Frampton et al. (2002) mentioned previously, healthy subjects were exposed to air, 0.6, and 1.5 ppm NO₂ for 3 hours, with intermittent exercise, and blood was obtained 3.5 hours after exposure. There was a significant, concentration-related reduction in hematocrit and hemoglobin levels in both males and females, confirming the findings of Posin et al. (1978). These studies suggest that NO₂ exposure in the range of 1 ppm for a few hours is sufficient to alter the red blood cell membrane. The reductions in blood hemoglobin were not sufficiently large to cause health effects for these healthy subjects. However, in the Frampton study, the reduction in hemoglobin represented the equivalent of about 200 ml of blood loss for a 70 kg male. This could conceivably have adverse cardiovascular consequences for someone with significant underlying lung disease, heart disease, or anemia.

6.5 Pollutant Concentration/Dose-Response Function

Some studies have investigated which of the following factors is most important in determining adverse health impacts: peak concentration of NO₂, duration of exposure, or total dose (concentration x duration). However, they provide somewhat conflicting results. Studies have shown evidence of airway inflammation in healthy individuals following prolonged exposure (four to six hours) to NO₂ at a concentration of 2.0 ppm (Azadniv et al. 1998, Blomberg et al. 1997, Devlin et al. 1999), whereas short (20 min.) exposures to NO₂ at 1.5-2 ppm did not (Sandstrom et al. 1990, 1992b). These results suggest that duration, not peak concentrations are more important. In contrast, Jenkins et al. (1999) studied a group of mild asthmatics exposed for 3 hours to NO₂ (0.4 ppm), and found that FEV₁ was decreased, whereas the same total dose but delivered at a lower concentration and over longer duration (6 hr at 0.2 ppm) had no effect on FEV₁. The latter set of results suggests that the threshold concentration rather than the total amount of pollutant inhaled over time was more important. No studies have been performed to investigate whether peak concentrations or total dose are more important at lower concentrations, which might be directly applicable to standard setting.

6.6 NO₂ and Other Pollutants

Environmental exposures to NO₂ do not occur alone, but rather as a complex mixture of pollutants, and failure to consider the presence of other pollutants may confuse interpretation of the observed effects. When considering mixtures of anthropogenic pollutants, it may be impossible to separate the effects of one component from those of others, particularly with the possibilities of synergistic or antagonistic interactions. In considering the health effects of mixtures, potential causal pathways should be carefully delineated. For example, Spengler et al. (1990) suggested that HONO may contribute to the health

effects attributed to indoor NO₂. Table 6.6 summarizes human clinical studies of NO₂-containing mixtures or sequential exposures that are most relevant to setting a NO₂ standard.

Several studies have investigated the effects of NO₂-ozone mixtures on pulmonary function. These studies have generally revealed no interactive effects; the observed pulmonary function decrements appear to reflect the ozone component of the mixtures. Drechsler-Parks et al. (1989) exposed young and older healthy nonsmokers for 2 hours, with intermittent exercise, to various combinations of 0.45 ppm ozone, 0.13 ppm peroxyacetyl nitrate, and 0.60 ppm NO₂, but found lung function effects only in the atmospheres containing ozone. Hazucha et al. (1994) found that pre-exposure of healthy women to 0.6 ppm NO₂ for 2 hours enhanced spirometric responses and methacholine airway responsiveness induced by a subsequent 2-hour exposure to 0.3 ppm ozone, with intermittent exercise. Koenig et al. (1994) found no pulmonary function effects of exposure to 0.3 ppm NO₂ in combination with 0.12 ppm O₃, with or without sulfuric acid or nitric acid, in 28 adolescents with mild asthma.

The effects of NO₂ exposure on SO₂-induced bronchoconstriction have been examined, but with inconsistent results. Jörres and Magnussen (1990) found an increase in airway responsiveness to SO₂ in asthmatic subjects following exposure to 0.25 ppm NO₂ for 30 minutes at rest, yet Rubinstein et al. (1990) found no change in responsiveness to SO₂ inhalation following exposure of asthmatics to 0.30 ppm NO₂ for 30 minutes, with 20 minutes of exercise.

Several human clinical studies have been conducted with diesel engine exhaust, which is a complex mixture of particles and gases, including NO₂. Diesel exhaust is beyond the scope of this review. However, one study (Rudell et al. 1999) has shown that reducing the number of particles ($2.6 \times 10^6 / \text{cm}^3$) by 50%, using a particle trap, did not significantly reduce the airway inflammatory effects found 24 hours after exposure, suggesting that gaseous components play a role in the airway effects of diesel exhaust. Alternatively, the inflammatory effect could have been "saturated", so that under these experimental conditions, no effect would be observed despite a 50% decrease in concentration.

In contrast, in a recent study, Gong et al. (2005) investigated the respiratory responses of elderly individuals with (n=6) and without (n=18) COPD to exposures to filtered air versus concentrated ambient air particles (CAP) at $\sim 200 \mu\text{g}/\text{m}^3$ and NO₂ (0.40 ppm), both independently and in combination (2 hours, mild intermittent exercise). Symptoms, spirometry, cellular counts on induced sputum, cardiovascular changes (electrocardiogram, blood pressure changes), and oximetry, were monitored 4 hours and 22 hours after exposure. They found no statistically significant increases in symptoms and forced expiratory spirometry measures after exposures to CAP or NO₂, either separately or combined. Exposures to CAP (but not NO₂) resulted in small, statistically significant decrements in maximal mid-expiratory flow (MMEF) and arterial O₂ saturation that were greater in healthy subjects compared with those with COPD. NO₂ did not modify the effect of CAP on MMEF and O₂ saturation. Gong et al. (2005) found small independent effects of both NO₂ and CAP exposures on diastolic blood pressure (decreased) and heart rate (increased) for both healthy individuals and those with COPD; combining pollutants had no added effect. NO₂ exposures had no effect on total white blood cell and columnar epithelial cell counts or percentages of cell types. NO₂ exposure did not modify the small CAP-induced decrease in the percentage of columnar epithelial cells.

Previous sections have discussed the studies of Rusznak et al. (1996) and Devalia et al. (1994), showing increased allergen responsiveness after exposure to NO₂ (0.40 ppm) and SO₂ (0.20 ppm), but not to either gaseous pollutant alone. As discussed in the section on Cardiovascular Disease, Drechsler-Parks (1995) found a significant decrease in cardiac output in elderly individuals for mixtures of NO₂ (0.60 ppm) and ozone (0.45 ppm) but not with the individual pollutants.

Overall, the data suggest that in asthmatics, NO₂ at levels only slightly above the California standard may enhance airway responsiveness to other pollutant challenges, and may act synergistically with SO₂ in enhancing responses to allergen challenge. The database on NO₂ as part of air pollution mixtures remains limited, in part because of the complexity of the experimental design and the difficulty in studying the most susceptible subjects, including those with significant chronic respiratory diseases such as moderate to severe asthmatics and those with COPD.

6.7 Clinical Exposure Studies in Children

Only a few NO₂ exposure studies have involved children. Koenig et al. (1985) exposed ten children with asthma (age 11-19) to 0.12 ppm of NO₂ for 60 minutes by mouthpiece, and found a non-significant trend of increased symptoms after NO₂ exposure and no effect on lung function. Airway reactivity was not tested. As part of the protocol, medications were not withheld. Thus, this group of asthmatics might have been better controlled, which might have contributed to the null findings. In Los Angeles, Avol et al. (1989) exposed 34 children with asthma (age 8-16) to 0.30 ppm of NO₂ for 3 hours. They found a transient decrease in FEV₁ during the first hour of exposure that improved by the third hour of exposure. There was no effect on airway reactivity. Lower respiratory symptoms increased during the week following NO₂ exposure. Overall, studies of children exposed to NO₂ in a controlled setting are very limited and provide little additional information that would help in establishing air quality standards for NO₂. Younger children with asthma, those with recent respiratory infections, and children with more severe asthma or other chronic lung diseases have not been studied in this setting.

6.8 Other Considerations

Controlled human studies suggest that NO₂ at 1-3 ppm may increase susceptibility to viral infections in healthy individuals. In asthmatics, NO₂ exposures may lead to increased airway responsiveness and an enhanced allergic response to inhaled allergen at levels near the current 1-hr standard of 0.25 ppm. As discussed above, the chamber studies have been conducted primarily in healthy adults and mild asthmatics, (both adults and older children).

Based on the current evidence from chamber studies, these findings may be relevant for other populations. For example, immunocompromised individuals, those with chronic lung diseases, and infants with immature immune systems might be at increased risk for respiratory infection with NO₂ exposures, although the chamber studies cannot provide data to characterize a dose-response in such individuals.

Increased airway responsiveness or airway hyper-reactivity (AHR) is more severe in children with asthma compared with adults with asthma, even after adjusting for airway size (Peat et al. 1994). Also AHR is more prevalent in children without asthma (10% of 7-9 year olds in compared with adolescent and adult asthmatics (Peat et al. 1996, Forastiere et al. 1996). Therefore, it is plausible that children with asthma, especially younger children may experience greater AHR at a given concentration of NO₂ or experience increased AHR after a lower dose compared to adolescent and adult asthmatics. Others with AHR, such as individuals (especially infants) post lower respiratory tract infections, or those with certain chronic lung diseases may experience increased AHR after NO₂ exposures.

Using more sensitive endpoints investigators have found a biologically plausible coherent body of evidence that brief exposures to NO₂ (0.26 ppm for 30 min) enhances the allergic response in mild asthmatics. There is no data available for exposures at lower concentrations using these sensitive endpoints for allergic inflammation. It is plausible that mild asthmatics after a recent respiratory infection or more severe asthmatics may experience a greater enhancement of the allergic response than those observed in the controlled human studies.

Whether NO₂ can, itself, cause inflammatory changes in the lung at ambient concentrations has not been adequately studied. However the late-phase increased AHR 5 hr after NO₂ exposures (0.26 ppm, 30 min) is likely a consequence of a release of inflammatory mediators and deserves further study (Strand et al. 1997). Most chamber studies use histamine or methacholine for measurements of AHR which may be a better measure of structural airway changes. However, the variable component of AHR is better measured by physical stimuli (e.g. cold air, exercise or hypertonic aerosols) or with AMP or mannitol. The variable component of AHR reflects airway inflammation, and future studies might consider measurements of AHR using AMP or mannitol might be better when studying the inflammatory effects of NO₂ and other air pollutants (Cockcroft and Davis, 2006).

As discussed in Chapter 5, ambient exposures to NO₂ may be episodic peak exposures to levels of 0.25 ppm or higher. Although there are no information on the effect of multiple brief (15-30 min peak exposures throughout the day which might be the most realistic scenarios for exposures, one study found that after four consecutive days of single daily exposures to NO₂ followed by daily inhaled allergen, there

was persistent decrements in lung function (i.e. there was no attenuation) (Strand et al. 1998), Additional studies on effects of repeated peak exposures are needed

6.9 Summary of Clinical Studies of NO₂

Evidence for human health effects of exposure to ambient NO₂ derives from epidemiological, animal exposure, and human clinical studies. This chapter has focused on human exposure studies; Tables 6.1 – 6.6 summarizes those studies that appear most relevant to the current re-evaluation of the California ambient air quality standard for NO₂.

As with human clinical studies of other ambient air pollutants, the majority of studies were limited to effects on symptoms, pulmonary function and airway responsiveness. Recently, investigators used techniques in the field of cellular and molecular biology to study the pathophysiological effects of NO₂ in biological specimens, such as sputum and bronchoalveolar lavage fluids. Table 6.7 summarizes the lowest NO₂ levels at which effects have been observed in clinical studies of healthy individuals and asthmatics.

The data in healthy subjects suggest an absence of acute effects on pulmonary function of breathing NO₂ concentrations less than 4 ppm for several hours, with or without exercise. However, it must be kept in mind that this finding is relevant only to young healthy subjects. Very few studies have examined responses in healthy elderly; one study suggests there may be significant spirometric effects of 0.3 ppm NO₂ in older smokers (Morrow et al. 1992).

Several studies have shown that NO₂ exposure induces airway inflammation as measured by bronchoscopy and bronchoalveolar lavage and may increase susceptibility to viral infection. It is clear that a mild degree of airway inflammation occurs in healthy subjects exposed in the range of 1.5 to 2 ppm NO₂ for several hours. A single study showed increases in eicosanoid levels in BAL fluid after exposure to 1 ppm (Jörres et al. 1995). Two studies have investigated the antioxidant activity over time in response to repeated daily exposures to NO₂ at 2 ppm. After four days, although there is a persistent increase in neutrophilic inflammation, the increase in antioxidant activity observed after a single exposure is attenuated. Taken together, these studies suggest that in healthy adults there may be a threshold for airway inflammatory effects of single, multi-hour NO₂ exposures at an approximate concentration of 1 ppm. Compensatory anti-inflammatory activity in the lung lining fluid may be diminished upon repeated NO₂ exposures. Several studies provide evidence that prior exposure to NO₂ may increase the susceptibility to a viral infection through various mechanisms including decreased macrophage activity, decreased mucociliary action of respiratory epithelia, and increased susceptibility of respiratory epithelium to injury by subsequent viral challenge.

A few studies have been conducted in elderly individuals with and without COPD, with somewhat inconsistent results. Morrow et al. (1992) found modest (4.8%) decreases in FEV₁ with COPD patients, after exposures at 0.3 ppm NO₂ for four hours with intermittent exercise, but no effect in healthy subjects. Vagaggini et al. (1996) found a small decrease (3%) in FEV₁ in COPD patients two hours after exposure to 0.3 ppm for one hour with intermittent exercise and no evidence of inflammatory changes in nasal lavage and induced sputum, whereas others found no changes in spirometry in COPD patients at equal or higher concentrations of NO₂ (Hackney et al. 1992; Linn et al. 1985a, Gong et al. 2005). These negative studies were conducted in Los Angeles where other ambient exposures to NO₂ and O₃ may have affected the chamber responses of the subjects. The observed differences may be due, in part, to differences in study sample, current smoking status, exposure duration, and study protocol. Additionally, Morrow et al. (1992) suggest that older smokers may be a subgroup at increased risk of lung function decrements at NO₂ levels slightly above the 1-hour standard for NO₂.

For subjects with asthma, there have been inconsistent findings on the direct effect of NO₂ on lung function effects at exposure concentrations less than 1 ppm. As discussed in the 1992 California NO₂ review, some studies found increased airway reactivity associated with low-level NO₂ exposures (0.1-0.5 ppm), whereas others did not. For a number of studies of asthmatics, short-term exposures to NO₂ at 0.2-0.3 ppm resulted in an increase in airway responsiveness (Kleinman et al. 1983 (0.2 ppm, 2 hr), Jorres et al. 1990 (0.25 ppm, 30 min), Bylin et al. 1988 (0.27 ppm, 30 min), Bauer et al. 1986 (0.3 ppm, 30 min).

Strand et al. (1996) observed no effect 30 min after exposures to NO₂ (0.25 ppm, 30 min.) but observed an increase in airway responsiveness 5 hr after exposure. A pooled analysis of data through 1992 (Follinsbee 1992) found evidence of airway responsiveness with NO₂ exposures of 0.3 ppm or less in asthmatics, primarily with exposures at rest. Several studies also found transiently altered lung function (e.g., decreased FEV₁) at 0.3 ppm, but the findings were not consistent. Since the 1992 California NO₂ review, Strand et al. (1996) found evidence of increased airway reactivity 5 hr after exposure to NO₂ at 0.26 ppm (30 min). The divergence of the findings from various studies suggest that some individuals with asthma might be particularly susceptible to the airway effects of NO₂ at or near 0.25 ppm. Clinical studies with larger numbers of subjects, and with careful subject characterization of asthmatic status, would be useful to further explore this issue. The results of a few controlled studies of lung function after NO₂ exposures in older children with mild asthma (age 8 years and above) did not differ from studies of adults with mild asthma. Asthmatics who have had a recent respiratory infection or exacerbation are usually excluded. For ethical reasons, no studies have been reported using young children or severe asthmatics.

Although non-specific airway responsiveness after NO₂ may be more varied, recent studies from the UK and Sweden suggest that, overall, subjects with asthma exposed to NO₂ at concentrations as low as 0.26 to 0.4 ppm, at rest, have an enhanced response to allergen challenge. Thresholds for the enhanced allergen response have not been determined.

Animal models of allergic asthma support the observation, and *in vitro* studies using human nasal epithelium suggest the mechanism may involve enhanced mast cell degranulation and histamine release. The studies with NO₂ followed by allergen challenge from the UK and Sweden, suggest that single and repeated NO₂ exposures at rest for short durations (30 minutes) enhanced allergen responsiveness in subjects with asthma at concentrations as low as 0.26 ppm. Compared with filtered air, NO₂ exposures enhanced the asthmatics' responses to allergen challenge. Enhanced responses included: a more pronounced late-phase decrement in lung function (peak expiratory flow) and evidence of increased cellular inflammation and eosinophil activity in lung lavage and/or sputum samples (Strand et al. 1997, 1998; Barck et al. 2002, 2005). Thus, recent studies suggest that people with allergic asthma breathing ambient air with NO₂ at 0.25 ppm might experience a more pronounced allergic response to an inhaled allergen. Using more sensitive endpoints, investigators have found a biologically plausible coherent body of evidence that brief exposures to NO₂ (0.26 ppm for 30 min) enhances the allergic response mild asthmatics.

Although the NO₂ exposures in these studies did not lead to a clinical asthma exacerbation in the laboratory setting, the response could be more pronounced and deleterious in those with more severe asthma. The observed enhancement in biological responses to inhaled allergen after NO₂ exposures is consistent with our current understanding of the inflammatory mechanisms that contribute to potentiation of responses to inhaled allergens. This proposed mechanism is not limited to NO₂. There is increasing evidence that air pollutants with strong oxidant properties (e.g., NO₂, ozone, and diesel exhaust particles) can potentiate the allergic response by similar mechanisms (Krishna et al. 1999)

The limitations of the controlled human exposure studies have been discussed. Study subjects were primarily healthy adults, and mild asthmatics. The elderly, those with chronic medical conditions, mild asthmatics after a recent respiratory tract infection, and more severe asthmatics are not usually studied in this setting. Also, other vulnerable populations, such as infants and young children, cannot be studied. Finally, there is little data on effects with repeated episodic peak exposures, and these repeated peak exposures might be the more relevant exposure scenario for populations.

In summary, current evidence from human clinical studies do not support statistically significant effects on lung function of exposures to current outdoor ambient concentrations in the U.S. However, there is evidence that NO₂, at ambient levels near 0.25 ppm, may enhance the allergic immune response in asthmatics. Some studies of asthmatics have found that NO₂ exposures at 0.2-0.3 ppm may increase airway responsiveness. Additionally, most human clinical studies of NO₂ have focused on pulmonary function changes, either direct effects or in combination with airway challenge. It remains possible that there are non-pulmonary effects of NO₂ exposure that contribute to the morbidity and mortality associations that have been reported in the epidemiology literature (see chapter 7). This has been true for PM exposure, where increasing experimental evidence points to mechanistic pathways involving systemic, vascular, and cardiac effects. NO₂ is an oxidant, with the potential for increasing the burden of

oxidative stress, a pathway that has been implicated for the health effects of PM exposure. It is plausible that NO₂ may enhance the effects of PM exposure.

Several clinical studies suggest there may be systemic and cardiovascular effects of NO₂ exposure. These data are insufficient to be conclusive, and do not provide adequate concentration-response data. However, there do appear to be effects on circulating red blood cells at concentrations as low as 0.6 ppm. Given the key role of another nitrogen oxide, nitric oxide, in cardiovascular function (Griffiths et al. 2005), additional studies are urgently needed to determine whether there are cardiovascular effects of NO₂ exposure, and, if so, the mechanisms involved.

6.10 References

Ahmed T, Marchette B, Danta I. Effect of 0.1 ppm NO₂ on bronchial reactivity in normals and subjects with bronchial asthma. *Am Rev Respir Dis* 1992, 125:152 (Abstr)

Avol EL, Linn WS, Peng RC, Valencia G, Little D, Hackney JD. Laboratory study of asthmatic volunteers exposed to nitrogen dioxide and to ambient air pollution. *Am Ind Hyg Assoc J*. 1988 Apr; 49(4):143-9.

Avol EL, Linn WS, Peng RC, Whynot JD, Shamoo DA, Little DE, Smith MN, Hackney JD. Experimental exposures of young asthmatic volunteers to 0.3 ppm nitrogen dioxide and to ambient air pollution. *Toxicol Ind Health*. 1989 Dec; 5(6):1025-34.

Azadniv M, Utell MJ, Morrow PE, Gibb FR, Nichols J, Roberts NJ Jr, Speers DM, Torres A, Tsai Y, Abraham MK, Voter KZ, Frampton MW. 1998. Effects of nitrogen dioxide exposure on human host defense. *Inhal Toxicol* 10(6):585-601.

Barck C, Sandstrom T, Lundahl J, Hallden G, Svartengren M, Strand V, Rak S, Bylin G. 2002. Ambient level of NO₂ augments the inflammatory response to inhaled allergen in asthmatics. *Respir Med* 96(11):907-917.

Barck C, Lundahl J, Hallden G, Bylin G. 2005. Brief exposures to NO₂ augment the allergic inflammation in asthmatics. *Environ Res* 95:58-66.

Barck, C.; Lundahl, J.; Holmstrom, M., and Bylin, G. Does nitrogen dioxide affect inflammatory markers after nasal allergen challenge? *Am J Rhinol*. 2005 Nov-2005 Dec 31; 19(6):560-6.

Bauer M A, Utell MJ, Morrow PE, Speers DM, Gibb FR. 1986. Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. *Am Rev Respir Dis* 134:1203-8.

Blomberg A, Krishna MT, Bocchino V, Biscione GL, Shute JK, Kelly FJ, Frew AJ, Holgate ST, Sandstrom T. 1997. The inflammatory effects of 2 ppm NO₂ on the airways of healthy subjects. *Am J Respir Crit Care Med* 156:418-24.

Blomberg A, Krishna MT, Helleday R, Soderberg M, Ledin MC, Kelly FJ, Frew AJ, Holgate ST, Sandstrom T. 1999. Persistent airway inflammation but accommodated antioxidant and lung function responses after repeated daily exposure to nitrogen dioxide. *Am J Respir Crit Care Med* 159:536-43.

Brook RD, Franklin B, Cascio W, Hong Y, Howard G, Lipsett M, Luepker R, Mittleman M, Samet J, Smith SC Jr, Tager I. 2004. Air pollution and cardiovascular disease: a statement for healthcare professionals from the Expert Panel on Population and Prevention Science of the American Heart Association. *Circulation* 109(21):2655-71.

Bylin G, Lindvall T, Rehn T, Sundin B. 1985. Effects of short-term exposure to ambient nitrogen dioxide concentrations on human bronchial reactivity and lung function. *Eur J Respir Dis* 66:205-17.

Bylin G, Hedenstierna G, Lindvall T, Sundin B. Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. *Eur Respir J*. 1988 Jul; 1(7):606-12.

California Air Resources Board (CARB). Review of the One-Hour Ambient Air Quality Standard for Nitrogen Dioxide: Technical Report and Staff Report, December 1992.

- Cockcroft DW, Davis BE. 2006. Mechanisms of airway hyperresponsiveness. *J Allergy Clin Immunol* 118(3):551-9
- Daigle CC, Chalupa DC, Gibb FR, Morrow PE, Oberdörster G, Utell MJ, Frampton MW. 2003. Ultrafine particle deposition in humans during rest and exercise. *Inhal Toxicol* 15:539-52.
- Damji KS, Richters A. 1989. Reduction in T lymphocyte subpopulations following acute exposure to 4 ppm nitrogen dioxide. *Environ Res* 49: 217-24.
- Dawson SV, Schenker MB. Health effects of inhalation of ambient concentrations of nitrogen dioxide. *Am Rev Respir Dis*. 1979 Aug; 120(2):281-92.
- Devalia JL, Rusznak C, Herdman MJ, Trigg CJ, Tarraf H, Davies RJ. 1994. Effect of nitrogen dioxide and sulfur dioxide on airway response of mild asthmatic patients to allergen inhalation. *Lancet* 344:1668-71.
- Devlin RB, Horstman DP, Gerrity TR, Becker S, Madden MC, Biscardi F, Hatch GE, Koren HS. 1999. Inflammatory response in humans exposed to 2.0 ppm nitrogen dioxide. *Inhal Toxicol* 11:89-109.
- Drechsler-Parks DM. 1995. Cardiac output effects of O₃ and NO₂ exposure in healthy older adults. *Toxicol Ind Health* 11:99-109.
- Drechsler-Parks DM, Bedi JF, Horvath SM. 1989. Pulmonary function responses of young and older adults to mixtures of O₃, NO₂, and PAN. *Toxicol Ind Health* 5:505-517.
- Evans MJ, Johnson LV, Stephens RJ, Freeman G. 1976. Renewal of the terminal bronchiolar epithelium in the rat following exposure to NO₂ or O₃. *Lab Invest* 35: 246-57.
- Folinsbee LJ, Horvath SM, Bedi JF, Delehunt JC. 1978. Effect of 0.62 ppm NO₂ on cardiopulmonary function in young male nonsmokers. *Environ Res* 15:199-205.
- Folinsbee LJ. 1992. Does nitrogen dioxide exposure increase airways responsiveness? *Toxicol Ind Health* 8(5):273-83.
- Forastiere, F.; Corbo, G. M.; Dell'Orco, V.; Pistelli, R.; Agabiti, N., and Kriebel, D. A longitudinal evaluation of bronchial responsiveness to methacholine in children: role of baseline lung function, gender, and change in atopic status. *Am J Respir Crit Care Med*. 1996 Mar; 153(3):1098-104.
- Frampton MW, Boscia J, Roberts NJJ, Azadniv M, Torres A, Cox C, Morrow PE, Nichols J, Chalupa D, Frasier LM, Gibb FR, Speers DM, Tsai Y, and Utell MJ. 2002. Nitrogen dioxide exposure: Effects on airway and blood cells. *Am J Physiol* 282:L155-L165.
- Frampton MW, Finkelstein JN, Roberts NJ Jr, Smeglin AM, Morrow PE, Utell MJ. 1989. Effects of nitrogen dioxide exposure on bronchoalveolar lavage proteins in humans. *Am J Respir Cell Mol Biol* 1:499-505.
- Frampton MW, Morrow PE, Gibb FR, Speers DM, Utell MJ. 1991. Effects of nitrogen dioxide exposure on pulmonary function and airway reactivity in normal humans. *Am Rev Respir Dis* 143:522-27.
- Frampton MW, Smeglin AM, Roberts NJ Jr, Finkelstein JN, Morrow PE, Utell MJ. 1989. Nitrogen dioxide exposure in vivo and human alveolar macrophage inactivation of influenza virus in vitro. *Environ Res* 48:179-92.
- Frampton MW, Utell MJ. 1999. Clinical studies of airborne pollutants. *Toxicology of the lung*. Third ed., Gardner EE, Crapo JD, McClellan RO, 455-81. Philadelphia, PA: Taylor & Francis.
- Gavras JB, Frampton MW, Ryan DH, Levy PC, Looney RJ, Cox C, Morrow PE, Utell MJ. 1994. Expression of membrane antigens on human alveolar macrophages after exposure to nitrogen dioxide. *Inhal Toxicol* 6:633-46.
- Gilmour MI. 1995. Interaction of air pollutants and pulmonary allergic responses in experimental animals. *Toxicology* 105:335-42.
- Goings SAJ, Kulle TJ, Bascom R, Sauder LR, Green DJ, Hebel JR, Clements ML. 1989. Effect of nitrogen dioxide exposure on susceptibility to influenza A virus infection in healthy adults. *Am Rev Respir Dis* 139: 1075-81.

- Gong H Jr, Linn WS, Clark KW, Anderson KR, Geller MD, Sioutas C. 2005. Respiratory responses to exposures with fine particulates and nitrogen dioxide in the elderly with and without COPD. *Inhal Toxicol* 17(3):123-32.
- Griffiths MJ, Evans TW. Inhaled nitric oxide therapy in adults. *N Engl J Med*. 2005 Dec 22; 353(25):2683-95.
- Hackney JD, Thiede FC, Linn WS, Pedersen EE, Spier CE, Law DC, Fischer DA. 1978. Experimental studies on human health effects of air pollutants. IV. Short-term physiological and clinical effects of nitrogen dioxide exposure. *Arch Environ Health* 33:176-81.
- Hackney JD, Linn WS, Avol EL, Shamoo DA, Anderson KR, Solomon JC, Little DE, Peng R-C. 1992. Exposures of older adults with chronic respiratory illness to nitrogen dioxide. *Am Rev Respir Dis*:146:1480-1486.
- Hazucha MJ, Folinsbee LJ, Seal E, Bromberg PA. 1994. Lung function response of healthy women after sequential exposures to NO₂ and O₃. *Am J Respir Crit Care Med* 150:642-47.
- Hazucha, MJ, Ginsberg JF, McDonnell WF, Haak ED Jr, Pimmel RL, Salaam SA, House DE, Bromberg PA. 1983. Effects of 0.1 ppm nitrogen dioxide on airways of normal and asthmatic subjects. *J Appl Physiol* 54:730-739.
- Helleday R, Blomberg A, Stjernberg N, Huberman D, Sandstrom T. 1995. Nitrogen dioxide exposure impairs the frequency of the mucociliary activity in healthy subjects. *Eur Respir J* 8:1664-88.
- Jenkins HS, Devalia JL, Mister RL, Bevan AM, Rusznak C, Davies RJ. 1999. The effect of exposure to ozone and nitrogen dioxide on the airway response of atopic asthmatics to inhaled allergen. *Am J Respir Crit Care Med* 160:33-39.
- Johnson DA, Frampton MW, Winters RS, Morrow PE, Utell MJ. 1990. Inhalation of nitrogen dioxide fails to reduce the activity of human lung alpha-1-proteinase inhibitor. *Am Rev Respir Dis* 142: 758-62.
- Jörres R, Magnussen H. 1990. Airways response of asthmatics after a 30 min exposure, at resting ventilation, to 0.25 ppm NO₂ or 0.5 ppm SO₂. *Eur Respir J* 3:132-37.
- Jörres R, Magnussen H. 1991. Effect of 0.25 ppm nitrogen dioxide on the airway response to methacholine in asymptomatic asthmatic patients. *Lung* 169:77-85.
- Jörres R, Nowak D, Grimminger F, Seeger W, Oldigs M, Magnussen H. 1995. The effect of 1 ppm nitrogen dioxide on bronchoalveolar lavage cells and inflammatory mediators in normal and asthmatic subjects. *Eur Respir J* 8:416-24.
- Kelly FJ. 2003. Oxidative stress: its role in air pollution and adverse health effects. *Occup Environ Med* 60(8):612-6.
- Kelly FJ, Blomberg A, Frew A, Holgate ST, Sandstrom T. 1996. Antioxidant kinetics in lung lavage fluid following exposure of humans to nitrogen dioxide. *Am J Respir Crit Care Med* 154(6 Pt 1):1700-5.
- Kerr HD, Kulle TJ, McIlhany ML, Swidersky P. 1979. Effects of nitrogen dioxide on pulmonary function in human subjects: an environmental chamber study. *Environ Res* 19: 392-404.
- Kim SU, Koenig JQ, Pierson WE, Hanley QS. 1991. Acute pulmonary effects of nitrogen dioxide exposure during exercise in competitive athletes. *Chest* 99:815-19.
- Kleinman MT, Bailey RM, Linn WS, Anderson KR, Whynot JD, Shamoo DA, Hackney JD. 1983. Effects of 0.2 ppm nitrogen dioxide on pulmonary function and response to bronchoprovocation in asthmatics. *J Toxicol Environ Health* 12: 815-26.
- Koenig JQ, Covert DS, Morgan MS, Horike M, Horike N, Marshall SG, Pierson WE. 1985. Acute effects of 0.12 ppm ozone or 0.12 ppm nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. *Am Rev Respir Dis* 132: 648-51.

- Koenig JQ, Covert DS, Marshall SG, Van Belle G, Pierson WE. The effects of ozone and nitrogen dioxide on pulmonary function in healthy and in asthmatic adolescents. *Am Rev Respir Dis*. 1987 Nov; 136(5):1152-7.
- Koenig JQ, Covert DS, Smith MS, van Belle G, Pierson WE. The pulmonary effects of ozone and nitrogen dioxide alone and combined in healthy and asthmatic adolescent subjects. *Toxicol Ind Health*. 1988 Dec; 4(4):521-32.
- Krishna MT, Holgate ST. 1999. Inflammatory mechanisms underlying potentiation of effects of inhaled aeroallergens in response to nitrogen dioxide in allergic airways disease. *Clin Exp Allergy* 29(2):150-4.
- Kuraitis KV, Richters A. 1989. Spleen cellularity shifts from the inhalation of 0.25-0.35 ppm nitrogen dioxide. *J Environ Pathol Toxicol* 9: 1-11
- Lafuma C, Harf A, Lange F, Bozzi L, Ponci JL, Bignon J. 1987. Effect of low-level NO₂ chronic exposure on elastase-induced emphysema. *Environ Res* 43: 75-84.
- Linn WS, Shamoo DA, Spier CE, Valencia LM, Anzar UT, Venet TG, Avol EL, Hackney JD. Controlled exposure of volunteers with chronic obstructive pulmonary disease to nitrogen dioxide. *Arch Environ Health*. 1985a Nov-1985 Dec 31; 40(6):313-7.
- Linn WS, Solomon JC, Trim SC, Spier CE, Shamoo DA, Venet TG, Avol EL, Hackney JD. 1985b. Effects of exposure to 4 ppm nitrogen dioxide in healthy and asthmatic volunteers. *Arch Environ Health* 40:234-39.
- Mohsenin V. 1987a. Airway responses to nitrogen dioxide in asthmatic subjects. *J Toxicol Environ Health* 22: 371-80.
- Mohsenin V. 1987b. Effect of vitamin C on NO₂-induced airway hyperresponsiveness in normal subjects. *Am Rev Respir Dis* 136: 1408-11.
- Mohsenin V. 1988. Airway responses to 2.0 ppm nitrogen dioxide in normal subjects. *Arch Environ Health* 43: 242-46.
- Mohsenin V. 1991. Lipid peroxidation and antielastase activity in the lung under oxidant stress: role of antioxidant defenses. *J Appl Physiol* 70: 1456-62.
- Mohsenin V, Gee JBL. 1987. Acute effect of nitrogen dioxide exposure on the functional activity of alpha-1-protease inhibitor in bronchoalveolar lavage fluid of normal subjects. *Am Rev Respir Dis* 136: 646-50.
- Morrow PE, Utell MJ. 1989. Responses of susceptible subpopulations to nitrogen dioxide, Capital City Press, Montpelier, VT.
- Morrow PE, Utell MJ, Bauer MA, Smeglin AM, Frampton MW, Cox C, Speers DM, Gibb FR. 1992. Pulmonary performance of elderly normal subjects and subjects with chronic obstructive pulmonary disease exposed to 0.3 ppm nitrogen dioxide. *Am Rev Respir Dis* 145:291-300.
- Ngoc PL, Gold DR, Tzianabos AO, Weiss ST, Celedon JC. Cytokines, allergy, and asthma. *Curr Opin Allergy Clin Immunol*. 2005 Apr; 5(2):161-6.
- Orehek J, Massari JP, Gayrard P, Grimaud C, Charpin J. 1976. Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. *J Clin Invest* 57: 301-7.
- Pathmanathan S, Krishna MT, Blomberg A, Helleday R, Kelly FJ, Sandstrom T, Holgate ST, Wilson SJ, Frew AJ. 2003. Repeated daily exposure to 2 ppm nitrogen dioxide upregulates the expression of IL-5, IL-10, IL-13, and iCAM-1 in the bronchial epithelium of healthy human airways. *Occup Environ Med* 60:892-96.
- Peat JK, Gray EJ, Mellis CM, Leeder SR, Woolcock AJ. 1994. Differences in airway responsiveness between children and adults living in the same environment: an epidemiological study in two regions of New South Wales. *Eur Respir J* 7(10):1805-13.
- Peat JK, Salome CM, Xuan W. 1996. On adjusting measurements of airway responsiveness for lung size and airway caliber. *Am J Respir Crit Care Med* 154(4 Pt 1):870-5.

- Peters A, Liu E, Verrier RL, Schwartz J, Gold DR, Mittleman M, Baliff J, Oh JA, Allen G, Monahan K, Dockery DW. 2000. Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 11:11-17.
- Posin C, Clark K, Jones MP, Patterson JV, Buckley RD, Hackney JD. 1978. Nitrogen dioxide inhalation and human blood biochemistry. *Arch Environ Health* 33:318-24.
- Rasmussen TR, Kjaergaard SK, Tarp U, Pedersen OF. 1992. Delayed effects of NO₂ exposure on alveolar permeability and glutathione peroxidase in healthy humans. *Am Rev Respir Dis* 146:654-59.
- Richters A, Damji KS. 1988. Changes in T-lymphocyte subpopulations and natural killer cells following exposure to ambient levels of nitrogen dioxide. *J Toxicol Environ Health* 25: 247-56.
- Richters A, Richters V. 1989. Nitrogen dioxide (NO₂) inhalation, formation of microthrombi in lungs and cancer metastasis. *J Environ Pathol Toxicol* 9: 45-51.
- Roger LJ, Horstman DH, McDonnell W, Kehrl H, Ives PJ, Seal E, Chapman R, Massaro E. 1990. Pulmonary function, airway responsiveness, and respiratory symptoms in asthmatics following exercise in NO₂. *Toxicol Ind Health* 6: 155-71.
- Rubinstein I, Bigby BG, Reiss TF, Boushey HA Jr. 1990. Short-term exposure to 0.3 ppm nitrogen dioxide does not potentiate airway responsiveness to sulfur dioxide in asthmatic subjects. *Am Rev Respir Dis* 141: 381-85.
- Rubinstein I, Reiss TF, Bigby BG, Stites DP, Boushey HA Jr. 1991. Effects of 0.60 PPM nitrogen dioxide on circulating and bronchoalveolar lavage lymphocyte phenotypes in healthy subjects. *Environ Res* 55: 18-30.
- Rudell B, Blomberg A, Helleday R, Ledin MC, Lundback B, Stjernberg N, Horstedt P, Sandstrom T. 1999. Bronchoalveolar inflammation after exposure to diesel exhaust: comparison between unfiltered and particle trap filtered exhaust. *Occup Environ Med* 56:527-34.
- Rusznak C, Devalia JL, Davies RJ. 1996. Airway response of asthmatic subjects to inhaled allergen after exposure to pollutants. *Thorax* 51:1105-8.
- Salvi S, Semper A, Blomberg A, Holloway J, Jaffar Z, Papi A, Teran L, Polosa R, Kelly F, Sandstrom T, Holgate S, Frew A. 1999. Interleukin-5 production by human airway epithelial cells. *Am J Respir Cell Mol Biol* 20(5):984-91.
- Sandstrom T, Andersson MC, Kolmodin-Hedman B, Stjernberg N, Angstrom T. 1990. Bronchoalveolar mastocytosis and lymphocytosis after nitrogen dioxide exposure in man: a time-kinetic study. *Eur Respir J* 3:138-43.
- Sandstrom T, Helleday R, Bjermer L, Stjernberg N. 1992a. Effects of repeated exposure to 4 ppm nitrogen dioxide on bronchoalveolar lymphocyte subsets and macrophages in healthy men. *Eur Respir J* 5:1092-96.
- Sandstrom T, Ledin MC, Thomasson L, Helleday R, Stjernberg N. 1992b. Reductions in lymphocyte subpopulations after repeated exposure to 1.5 ppm nitrogen dioxide. *Br J Ind Med* 49:850-854.
- Sandstrom T, Stjernberg N, Eklund A, Ledin MC, Kolmodin-Hedman B, Lindstrom K, Rosenhall L, Angstrom T. 1991. Inflammatory cell response in bronchoalveolar lavage fluid after nitrogen dioxide exposure of healthy subjects: a dose-response study. *Eur Respir J* 4:332-39.
- Saxon A, Diaz-Sanchez D. Air pollution and allergy: you are what you breathe. *Nat Immunol*. 2005 Mar; 6(3):223-6.
- Schierhorn K, Zhang M, Matthias C, Kunkel G. 1999. Influence of ozone and nitrogen dioxide on histamine and interleukin formation in a human nasal mucosa culture system. *Am J Respir Cell Mol Biol* 20:1013-19.

- Solomon C, Christian DL, Chen LL, Welch BS, Kleinman MT, Dunham E, Erle DJ, Balmes JR. 2000. Effect of serial-day exposure to nitrogen dioxide on airway and blood leukocytes and lymphocyte subsets. *Eur Respir J* 15:922-28.
- Solomon C, Balmes JR, Kleinman M. 2004. Effects of nitrogen dioxide on airway inflammation in allergic asthmatic subjects. Final Report 00-337, May 28, 2004, California Air Resources Board, Sacramento, CA.
- Spengler JD, Brauer M, Koutrakis P. 1990. Acid air and health. *Environmental Science and Technology* 24: 946-56.
- Strand V, Rak S, Svartengren M, Bylin G. 1997. Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. *Am J Respir Crit Care Med* 155:881-87.
- Strand V, Salomonsson P, Lundahl J, Bylin G. 1996. Immediate and delayed effects of nitrogen dioxide exposure at an ambient level on bronchial responsiveness to histamine in subjects with asthma. *Eur Respir J* 9:733-40.
- Strand V, Svartengren M, Rak S, Barck C, Bylin G. 1998. Repeated exposure to an ambient level of NO₂ enhances asthmatic response to a nonsymptomatic allergen dose. *Eur Respir J* 12:6-12.
- Tunncliffe WS, Burge PS, Ayres JG. 1994. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet* 344:1733-36.
- Vagaggini B, Paggiaro PL, Giannini D, Franco AD, Cianchetti S, Carnevali S, Taccola M, Bacci E, Bancalari L, Dente FL, Giuntini C. 1996. Effect of short-term NO₂ exposure on induced sputum in normal, asthmatic and COPD subjects. *Eur Respir J* 9:1852-57.
- Venge P, Bystrom J, Carlson M, Hakansson L, Karawaczyk M, Peterson C, Seveus L, Trulsson A. 1999. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. *Clin Exp Allergy* 29(9):1172-86.
- Wang JH, Duddle J, Devalia JL, Davies RJ. 1995a. Nitrogen dioxide increases eosinophil activation in the early-phase response to nasal allergen provocation. *Int Arch Allergy Immunol* 107:103-5.
- Wang JH, Devalia JL, Duddle JM, Hamilton SA, Davies RJ. 1995b. Effect of six-hour exposure to nitrogen dioxide on early-phase nasal response to allergen challenge in patients with a history of seasonal allergic rhinitis. *J Allergy Clin Immunol* 96:669-76.
- Wang JH, Devalia JL, Rusznak C, Bagnall A, Sapsford RJ, Davies RJ. 1999. Effect of fluticasone propionate aqueous nasal spray on allergen-induced inflammatory changes in the nasal airways of allergic rhinitis following exposure to nitrogen dioxide. *Clin Exp Allergy* 29:234-40.
- Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA. 1999. Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J Clin Invest* 103(6):779-88.

6.11 Tables

Table 6.1. Clinical studies of NO₂ exposure in healthy subjects*

Reference	Location	Participants	Approach & Methods	Findings
Azadniv et al. 1998	Rochester, NY, USA	2 studies, 12 healthy nonsmokers in each	Air vs 2 ppm NO ₂ for 6 h with intermittent exercise. Phase 1: BAL 18 h after exposure; Phase 2: BAL immediately after exposure.	Increased BAL neutrophils, decreased blood CD8+ and null T lymphocytes 18 hours after exposure. No effects on symptoms or pulmonary function.
Blomberg et al. 1997	Sweden	30 healthy nonsmokers	Air vs 2 ppm NO ₂ for 4 h, with intermittent exercise	Increased neutrophils and interleukin-8 in bronchial wash. Increases in specific lymphocyte subsets in BAL fluid. Symptoms, pulmonary function not reported.
Blomberg et al. 1999	Sweden	12 healthy nonsmokers	Air vs 2 ppm NO ₂ for 4 h on 4 days, with intermittent exercise.	After 4 days of NO ₂ , increased neutrophils in bronchial wash but decreased neutrophils in bronchial biopsy. 2% decrease in FEV ₁ after first exposure to NO ₂ , attenuated with repeated exposure. Symptoms not reported.
Devlin et al. 1999	Chapel Hill, North Carolina, USA	8 healthy nonsmokers	Air and 2.0 ppm NO ₂ for 4 h with intermittent exercise.	Increased bronchial lavage neutrophils, IL-6, IL-8, alpha ₁ -antitrypsin, and tissue plasminogen activator. Decreased alveolar macrophage phagocytosis and superoxide production. No effects on pulmonary function. Symptoms not reported.
Drechsler-Parks 1995	Santa Barbara, CA, USA	8 older healthy nonsmokers	4 2-h exposures with intermittent exercise: air, 0.60 ppm NO ₂ , 0.45 ppm O ₃ , and 0.60 ppm NO ₂ + 0.45 ppm O ₃ .	Significant reduction in cardiac output during exercise, estimated using noninvasive impedance cardiography, with NO ₂ + O ₃ . Symptoms, pulmonary function not reported.

Table 6.1 continued

Reference	Location	Participants	Approach & Methods	Findings
Frampton et al. 1991	Rochester, NY, USA	39 healthy nonsmokers	3 protocols, all for 3 h with control air exposure: 1) continuous 0.06 ppm NO ₂ ; 2) baseline 0.05 ppm NO ₂ with peaks of 2.0 ppm, and 3) continuous 1.5 ppm NO ₂ .	No symptoms or direct effects on pulmonary function. Increased airways responsiveness to carbachol after 1.5 ppm NO ₂ .
Frampton et al. 2002	Rochester, NY, USA	21 healthy nonsmokers	Exposure to air, 0.6, 1.5 ppm NO ₂ for 3 h with intermittent exercise.	Dose-related decrease in hematocrit, hemoglobin, blood lymphocytes, and T lymphocytes. Mild increase in neutrophils recovered in bronchoalveolar lavage. In vitro viral challenge of bronchial epithelial cells showed increased cytotoxicity after 1.5 ppm NO ₂ . No effects on symptoms or pulmonary function.
Gong et al. 2005	Los Angeles, CA, USA	6 elderly healthy nonsmokers (compared also with 18 elderly COPD, former smokers, Table 3)	2 h exposures to air, 0.4 ppm NO ₂ , and concentrated ambient particles (CAP) separately and in combination, intermittent exercise. Measured spirometry, electrocardiogram, blood pressure, oximetry, and cellular composition of induced sputum	NO ₂ : No effects on symptoms and pulmonary function. No change in induced sputum samples. Small decrease in diastolic blood pressure and increase in heart rate 4h and 22 h after exposure (NO ₂ and CAP separately). CAP also increased MMEF, decreased O ₂ saturation, and decrease in % columnar epithelial cells. (These CAP effects were more in healthy compared with COPD). NO ₂ and CAP in combination had no added effects.
Helleday et al. 1995	Sweden	8 healthy smokers, 8 healthy nonsmokers	3.5 ppm NO ₂ for 20 min. BAL 24 h after exposure compared with BAL after ambient air exposure.	Different inflammatory cell increases in smokers and nonsmokers. No effects on symptoms. Pulmonary function not reported. Control was with ambient air exposure with exercise.

Table 6.1 continued

Reference	Location	Participants	Approach & Methods	Findings
Helleday et al. 1995	Sweden	24 healthy nonsmokers, 8 in each of 3 groups	Bronchoscopic assessment of mucociliary activity: 1) 45 min after 1.5 ppm NO ₂ for 20 min; 2) 45 min after 3.5 ppm NO ₂ for 20 min; and 3) 24 h after 3.5 ppm NO ₂ for 4 h.	Complete abolition of mucociliary activity 45 min after NO ₂ ; increased activity 24 h after NO ₂ . Symptoms, pulmonary function not reported. Note: control exposure was with ambient air (instead of filtered air used in many studies). Order of procedures not randomized, subjects not blinded. However, these design issues are unlikely to bias BAL results.
Jörres et al. 1995	Germany	8 healthy nonsmokers	Air or 1 ppm NO ₂ exposure for 3 h with intermittent exercise.	Changes in eicosanoids, but not inflammatory cells, in BAL fluid. No change in lung function. Symptoms not reported. Results diminished compared with results of 12 asthmatics in same study.
Kelly et al. 1996	United Kingdom, Sweden	44 healthy nonsmokers, divided into 3 groups	Air vs 2 ppm NO ₂ for 4 h, with intermittent exercise. BAL at 1.5 h (group 1), 6 h (group 2) or 24 h (group 3)	Kinetic study of antioxidant levels in lung fluid: Bronchial wash and BAL with decreased ascorbic acid and uric in initial phases, return to control levels by 6 h and 24 h, respectively. GSH levels increased in bronchial wash at 1.5 and 6 h, returned to control levels at 24 h. Symptoms, pulmonary function not reported.
Kim et al. 1991	Seattle, Washington, USA	9 healthy athletes	Air, 0.18, and 0.30 ppm NO ₂ for 30 min with exercise	No effects on pulmonary function. Symptoms not reported.
Morrow et al. 1992	Rochester, NY, USA	20 elderly healthy smokers and nonsmokers	Air vs 0.3 ppm NO ₂ for 4 h with intermittent exercise.	No symptoms or pulmonary function effects for group as a whole. Smokers showed a 2.3% decline in FEV ₁ with NO ₂ that was statistically different from nonsmokers. Study also included COPD (see Table 3)

Table 6.1 continued

Reference	Location	Participants	Approach & Methods	Findings
Pathmanathan et al. 2003	United Kingdom, Sweden	12 healthy nonsmokers	Air vs 2 ppm NO ₂ for 4 h on 4 days, with intermittent exercise	Epithelial expression of IL-5, IL-10, IL-13, and ICAM-1 increased following NO ₂ exposure. Symptoms, pulmonary function not reported.
Posin et al. 1978	Downey, CA, USA	10 healthy nonsmokers	3 daily exposures for 2.5 h. 1 st day: air; 2 nd and 3 rd days: 1 or 2 ppm NO ₂ . Intermittent exercise. Subsequent control series of 3 daily air exposures.	Reduced hemoglobin and hematocrit, and red blood cell acetyl cholinesterase. Symptoms, pulmonary function not reported.
Rasmussen et al. 1992	Denmark	14 healthy nonsmokers	Air vs 2.3 ppm NO ₂ for 5 h	Small <i>increases</i> in FVC and FEV ₁ . Symptoms not reported. Reduced lung permeability and blood glutathione peroxidase after exposure. Only 1 week between exposures may have confounded results.
Sandstrom et al. 1990	Sweden	32 healthy nonsmokers, 4 groups of 8 subjects	4 ppm NO ₂ for 20 min with 15 min exercise. BAL 4, 8, 24, 72 h after exposure, compared with non-exposure control BAL	Increase in BAL mast cells and lymphocytes 4-24 h after exposure. No change in neutrophil counts. Odor and mild upper airway symptoms. No effects on pulmonary function.
Sandstrom et al. 1991	Sweden	18 healthy nonsmokers	2.25, 4.0, 5.5 ppm NO ₂ for 20 min with light exercise. BAL 24 h after exposure, compared with non-exposure control BAL	Increase in BAL mast cells (all concentrations) and lymphocytes (4.0 and 5.5 ppm). No change in neutrophil counts. Odor and throat irritation. No effects on pulmonary function.
Sandstrom et al. 1992a	Sweden	10 healthy nonsmoking men	4 daily exposures to 4 ppm NO ₂ for 20 min with 15 min exercise. BAL 24 h after exposure, compared with non-exposure control BAL.	Reduction in alveolar macrophages, NK cells, and CD8 lymphocytes in BAL; reduction in total lymphocytes in blood. No change in neutrophil counts. Lack of control air exposure with exercise is problematic. Symptoms, pulmonary function not reported.

Table 6.1 continued

Reference	Location	Participants	Approach & Methods	Findings
Sandstrom et al. 1992b	Sweden	8 healthy nonsmokers	1.5 ppm NO ₂ for 20 min with 15 min exercise, every 2 nd day x 6. BAL 24 h after exposure compared with non-exposure control BAL.	Reduced CD8 ⁺ T lymphocytes and NK cells in BAL fluid. No change in neutrophil count. Lack of control air exposure with exercise is problematic. Symptoms, pulmonary function not reported.
Solomon et al. 2000	San Francisco, California, USA	15 healthy nonsmokers	Air or 2.0 ppm NO ₂ with intermittent exercise, for 4 h daily x 4. BAL 18 hours after exposure.	Increased neutrophils in bronchial lavage, decreased CD4 ⁺ T lymphocytes in BAL. No changes in blood. Symptoms, pulmonary function not reported.
Vagaggini et al. 1996	Italy	7 healthy nonsmokers	Air vs 0.3 ppm NO ₂ for 1 h with intermittent exercise.	Mild increase in symptoms. No effects on lung function, nasal lavage, or induced sputum. Study also included individuals with asthma and COPD (see Table 2, 3).

* Includes those studies published since the 1992 CA review (CARB 1992) or were not included in the previous review. Earlier studies of airway inflammation also included.

Table 6.2. Effects of NO₂ exposure in subjects with asthma*

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Studies since the CARB 1992 review						
Folinsbee 1992		<p>Pooled analysis of studies with individual data (n=355)</p> <p>Direction of airway reactivity determined post-NO₂ vs. air (increased or decreased). Analysis using sign test.</p>	<p>Pooled analysis of studies of airway reactivity.</p> <p>Three groups analyzed: 0.1-0.2 ppm (n=105); 0.2-0.3 ppm (n=169); and >0.3-1.0 ppm (n=81).</p>	<p>Evidence of increased airway reactivity in separate analysis for three pooled groups</p> <p>0.1-0.2 ppm</p> <p>0.2-0.3 ppm</p> <p>>0.3-1.0 ppm</p>		<p>Includes reports not peer-reviewed. Analysis using sign test: For each individual, if greater physiologic response to the same of a lesser concentration of agent, effect of NO₂ on airway reactivity was (+). If response was decreased for a given inhalation challenge or dose level for the same response was increased, effect of NO₂ on airway reactivity was (-)</p> <p>Effect primarily with exposures at rest</p> <p>Differences in study methods and protocol limit interpretation of analysis. Magnitude of response cannot be quantified.</p>

Table 6.2 continued

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Jörres et al. 1995	Germany	12 mild asthmatics	1 ppm NO ₂ exposure for 3 h with intermittent exercise. BAL at x hours	Not done	2.5% decrease in FEV ₁ with NO ₂ , 1.3% with air. BAL: no increased in inflammatory cells but changes in several eicosanoids.	Results may have been primarily due to responses of 1-2 individuals. No effect on symptoms. Compared with results of 8 healthy individuals in same study, asthmatics had evidence of a wider inflammatory response
Solomon et al. 2004	USA (San Francisco)	10 mild asthmatics	0.4 ppm NO ₂ for 3 h with intermittent exercise	Not done	No effects on pulmonary function	No effect on sputum inflammatory cells
Strand et al. 1996	Sweden	19 mild asthmatics	Air vs 0.26 ppm NO ₂ for 30 min with intermittent exercise	Increased at 5 hr (histamine) Slight increased reactivity at 30 min, not significant (p=0.08)	No effects on pulmonary function.	Mild symptoms of sensitivity to irritants or exercise in week after NO ₂ exposure. Small reduction in lung volume after NO ₂ . Increased expression of an adhesion molecule in peripheral blood granulocytes 30 min. after NO ₂ exposure
Vagaggini et al. 1996	Italy	8 mild asthmatics	Air vs 0.3 ppm NO ₂ for 1 h with intermittent exercise.	Not done	No effect on pulmonary function	Asthmatics: No symptoms, no effects on nasal lavage or induced sputum. (Study also included healthy and COPD subjects. See Tables 1, 3)

Table 6.2 continued

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Earlier studies (see CARB 1992) ordered by increasing concentrations of NO ₂						
Orehek et al. 1976	France	20	0.1 ppm for 1 hr at rest. Chamber	Increased (carbachol) in 13/20	Increased airway resistance	Questionable statistical analysis. However, reanalysis by Dawson and Schenker gave similar results.
Ahmed et al. 1992		20	0.1 ppm for 1 hr at rest Chamber	Increased (carbachol) in 13/20	No effect	Results not published in peer-reviewed journal
Hazucha et al. 1983	USA (Chapel Hill, NC)	15	0.1 ppm for 1 hr at rest Mouthpiece.	No effect (Methacholine)	Slight increased airway resistance (NS)	Mild asthmatics. No effects on symptoms.
Koenig et al. 1985	USA (Seattle, WA)	10	0.12 ppm for 40 min at rest. Mouthpiece	Not tested	No effect	Subject age range 11-18. Medications not withheld. NS trend of increased symptoms after NO ₂ exposure
Koenig et al. 1987	USA (Seattle, WA)	10	0.12 ppm for 40 min (exercise 10 min) 0.18 ppm for 40 min (exercise 10 min)	Not tested	No effect	Subject age range 11-18. Medications only withheld for 4 hr preceding testing. "No significant reports of subjective symptoms" Exposure 40 min (30 min at rest, 10 min exercise)

Table 6.2 continued

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Bylin et al. 1988*		20	a) 0.14 ppm for 30 min b) 0.27 ppm for 30 min c) 0.53 ppm for 30 min	Equivocal at 0.14 ppm Increased (histamine) at 0.27 ppm. Equivocal at 0.53 ppm		Exposures to varying doses on 4 separate days. Tendency to increased reactivity after 0.14 and 0.53 ppm, but changes not statistically significant
Kleinman et al. 1983	USA (Los Angeles, CA)	31	0.2 ppm for 2 hr with intermittent exercise Chamber	Increased in 2/3 of subjects (methacholine)	No effect	Parametric and nonparametric statistical tests were not uniformly consistent
Jorres et al. 1990	Germany	14	0.25 ppm for 30 min at rest. Mouthpiece	Increased (SO ₂ challenge)	No effect	No effect on symptoms
Jörres and Magnussen 1991	Germany	11 mild asthmatics	0.25 ppm NO ₂ for 30 min with 10 min exercise Mouthpiece	No effect (methacholine)	No effects on pulmonary function	Symptoms not reported. Mild stable asthmatics
Bauer et al. 1986	USA (Rochester, NY)	15	0.3 ppm for 30 min with exercise Mouthpiece	Increased to hyperventilation with cold air	Transient decrease in FEV ₁	

Table 6.2 continued

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Rubinstein et al. 1990	USA (San Francisco, CA)	9	0.30 ppm for 30 min (10 min exercise) Chamber	No effect (sulfur dioxide)	No effect	No effect on symptoms. Also included test of small airways (single breath nitrogen)
Koenig et al. 1988	USA (San Francisco, CA)	12	0.30 ppm for 60 min with intermittent exercise Mouthpiece	Not tested	Slight transient decrease in FVC	No change found when combined with 0.12 ppm ozone
Avol et al. 1988	USA (Los Angeles, CA)	36 59	0.086 (in ambient air) 0.3 ppm for 2 hr with intermittent exercise 0.6 ppm Chamber	No effect (cold air)	No effect	No effect on symptoms. Analysis of results from subset of 20 more severe asthmatics showed similar lack of effect on symptoms and lung function. Incomplete data on airway reactivity in this subset
Avol et al. 1989	USA (Los Angeles, CA)	34	0.30 ppm for 3 hr with intermittent exercise Chamber	No effect (cold dry air)	Decreased after 1 hr, but improved by 3 rd hr of exposure	Subject age range 8-16. Lower respiratory symptoms increased during week following NO ₂ exposure

Table 6.2 continued

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Roger et al. 1990	USA (Chapel Hill, NC)	13	(a) 0.3 ppm for 110 min with intermittent exercise Chamber (b) 0.15 ppm for 80 min 0.30 ppm for 80 min 0.60 ppm for 80 min with intermittent exercise Chamber	Not tested No effect	Decreased FEV, FVC, Increased SR _{aw} No grouped effect	Also showed slight increase in upper respiratory symptoms. Baseline asthma severity slightly less than in single dose experiment. No significant changes in symptoms.
Bylin et al. 1985	Sweden	8	0.12 ppm for 20 min 0.24 ppm for 20 min 0.48 ppm for 20 min at rest Chamber	Increased at 0.48 ppm (histamine)	No effect on SR _{aw}	Reactivity tested only at 0 and 0.48 ppm NO ₂ . Non-significant trend for increased SR _{aw} at lower NO ₂ and decreased SR _{aw} at 0.48 ppm

Table 6.2 continued

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Mohsenin 1987a	USA (New Haven, CT)	10	0.5 ppm for 1 hr at rest Chamber	Increased	No effect on various lung function indices	No effect on symptoms. Unlike most studies, included tests of airflow at low lung volume
Kerr et al. 1979	USA (Baltimore, MD)	13	0.5 ppm for 1 hr exercise, 1 hr rest Chamber	Not tested	No effect	Increased symptoms in 7/13

* Not included in CARB 1992 review

Table 6.3. Effects of NO₂ exposure in subjects with Chronic Obstructive Pulmonary Disease (COPD) or other underlying diseases

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Studies since the CARB 1992 review						
Gong et al. 2005	Los Angeles, CA, USA	18 elderly COPD, former smokers (Study also included 6 elderly healthy nonsmokers, see Table 6.1)	2 h exposures to air, 0.4 ppm NO ₂ , and concentrated ambient particles (CAP) separately and in combination, intermittent exercise. and cellular composition of induced sputum	Not tested	No effects No change in induced sputum samples. Small decrease in diastolic blood pressure and increase in heart rate 4h and 22 h after exposure (NO ₂ and CAP separately).	No effects on symptoms Measured spirometry, electrocardiogram, blood pressure, oximetry, and cellular composition of induced sputum NO ₂ and CAP in combination had no added effects. CAP also increased MMEF, decreased O ₂ saturation, and decrease in % columnar epithelial cells. (These CAP effects were more in healthy compared with COPD).

Table 6.3 continued

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Vagaggini et al, 1996	Italy	7 COPD	0.3 ppm NO ₂ for 1 h with intermittent exercise.	Not tested	3% decrease in FEV ₁ in COPD subjects after NO ₂ , 5% increase with air. No effects on nasal lavage or induced sputum.	Increased symptom score in COPD subjects Study also included healthy and asthmatic subjects (see Table 1, 2)
Earlier studies in the 1992 CARB review						
Morrow et al. 1992	Rochester, NY, USA	20 COPD (Study also included 20 healthy elderly, Table 1)	0.3 ppm NO ₂ for 4 h with intermittent exercise	Not tested	4.8% decrease in FEV ₁ with COPD patients, (marginally significant).	No effect on symptoms Oral bronchodilator medication not withheld in majority of participants. Lung function decrements reversed by bronchodilator administration. Greater effect on lung function among subjects with milder disease Study also included 20 healthy elderly (See Table 1)
Hackney et al. 1992	USA (Los Angeles, CA)	26	0.3 ppm for 4 hr with intermittent exercise Chamber	Not tested	Slight decrease in peak flow in first two hr of exposure. Otherwise no effect	No effect on symptoms. Study also involved personal NO ₂ monitoring for 2 weeks combined with home lung function measurements. No relationship was found between personal NO ₂ exposure and lung function or symptoms.

Table 6.3 continued

Reference	Location	Participants	Approach & Methods	Results		Comments
				Airway Reactivity	Lung Function	
Linn et al. 1985	USA (Los Angeles, CA)	22	0.5 ppm for 1 hr for 1 hr ppm for 1 hr with intermittent exercise Chamber	Not tested	No effect	Minimal to mild increase in respiratory symptoms, regardless of NO ₂ concentration. Oral bronchodilator medication not withheld in a majority of participants. No NO ₂ effect on arterial oxygen saturation
Kerr et al. 1979	USA (Baltimore, MD)	7	0.5 ppm for 2 hr (1 hr exercise, 1 hr rest) Chamber	Not tested	No effect	No effect on symptoms. Very small study group

Table 6.4. Effects of NO₂ exposure on response to inhaled allergen in asthmatics.

Reference	Location	Participants	Approach & Methods	Findings
Barck et al. 2002	Sweden	13 mild asthmatics	Air vs 0.26 ppm NO ₂ for 30 min at rest, allergen challenge 4 h later, BAL 19 h later.	NO ₂ enhanced the airway inflammatory response to allergen challenge (inc. % neutrophils, inc. eosinophil activity). No effects on symptoms, pulmonary function.
Barck et al. 2005	Sweden	18 mild asthmatics	Air vs 0.26 ppm NO ₂ for 15 min on day 1, 15 min X 2 on day 2, followed by allergen challenge and sputum induction.	NO ₂ enhanced levels of eosinophilic cationic protein in sputum and blood. No effects on sputum cell numbers, symptoms, pulmonary function.
Barck et al. 2005b	Sweden	16 subjects with rhinitis and mild asthma	Air vs 0.26 ppm NO ₂ for 30 min followed by nasal allergen challenge at 4 hr. Nasal lavage before, 1,4,and 18 hr post challenge	No increase in % eosinophils, neutrophils, eosinophilic cationic protein, and myeloperoxidase. Tendency to increased sneezing the day after exposure to NO ₂ – allergen.
Devalia et al. 1994	United Kingdom	8 mild asthmatics	6 h exposures to combination of 0.4 ppm NO ₂ and 0.2 ppm SO ₂ followed by allergen challenge	Increased allergen responsiveness 10 min after exposure to combination of NO ₂ and SO ₂ (i.e., lower allergen dose needed to produce a 15% drop in FEV ₁), but not to individual gases. Symptoms not reported. No direct effects on pulmonary function.
Jenkins et al. 1999	United Kingdom	11 mild asthmatics	1) 6-h exposures to air, 0.1 ppm ozone, 0.2 ppm NO ₂ , and combination followed by allergen challenge; 2) 3-h exposures to air, 0.2 ppm ozone, 0.4 ppm NO ₂ , and combination; all with intermittent exercise.	All of the second exposure scenarios (ozone, NO ₂ , and combination), but none of the first exposure scenarios, resulted in enhanced responsiveness to allergen as measured by allergen dose to obtain 20% decrease in FEV ₁ . Authors conclude that response may have a concentration threshold. Symptoms not reported. No direct effects of NO ₂ on pulmonary function. (combination - see Table 5)

Table 6.4 continued

Reference	Location	Participants	Approach & Methods	Findings
Rusznak et al. 1996	United Kingdom	13 mild asthmatics	6 h exposures to combination of 0.4 ppm NO ₂ and 0.2 ppm SO ₂ followed by allergen challenge	Increased allergen responsiveness to combination of NO ₂ and SO ₂ , 10 min, 24, and 48 h after exposure as measured by allergen dose to obtain 20% decrease in FEV ₁ . Symptoms not reported. No direct effects of NO ₂ on pulmonary function.
Solomon et al. 2004	USA	15 mild asthmatics	Air vs 0.4 ppm NO ₂ with intermittent exercise for 3 h, followed by allergen challenge Sputum induction at 6 h.	No effects on pulmonary function. Decrease in sputum eosinophils 6 hr after NO ₂ + allergen exposure. No other effect on other inflammatory cells or biochemical markers of inflammation. Small subset (n=3) had substantial early airway narrowing and increased sputum cells and markers of inflammation after NO ₂ + allergen.
Strand et al. 1997	Sweden	18 patients with mild asthma, age 18-50 yrs	Exposure to 0.26 ppm NO ₂ for 30 min at rest followed by allergen challenge at 4 h.	Late phase (decreased peak flow at 3-9 hr), but not early phase (0.5-3 hr), response to allergen enhanced by NO ₂ . Mild odor. No direct effect of NO ₂ on pulmonary function.
Strand et al. 1998	Sweden	16 patients with mild to moderate asthma, age 21-52 yrs	4 daily repeated exposures to 0.26 ppm NO ₂ for 30 min at rest followed by allergen challenge at 4 h after each daily NO ₂ exposure.	Significant increase in both early (15 min) and late phase (3-10 h) response to allergen after exposure each day (as measured by decrease in FEV ₁). No evidence of attenuation of NO ₂ response during repeated exposures. No direct effects of NO ₂ on symptoms or pulmonary function.

Table 6.4 continued

Reference	Location	Participants	Approach & Methods	Findings
Tunnicliffe et al. 1994	United Kingdom	10 nonsmoking mild asthmatics age 16-60 yrs. 8 subjects completed.	Exposure to air, 0.1 ppm, and 0.4 ppm NO ₂ for 1 hr at rest, separated by at least 1 week, followed by allergen challenge. Fixed dose allergen challenge.	The allergen challenge led to a greater reduction in FEV ₁ after 0.4 ppm NO ₂ compared with allergen challenge after air, for both the early (p<0.009) and late (p<0.02) responses. No difference in nonspecific airway responsiveness. Symptoms not reported. No direct effects of NO ₂ on pulmonary function.
Wang et al. 1995 a, b	United Kingdom	2 groups of 8 subjects with allergic rhinitis	Exposure to 0.4 ppm NO ₂ for 6 h followed by nasal allergen challenge and nasal lavage	Increase in myeloperoxidase and eosinophil cationic protein in nasal lavage fluid following allergen challenge. Symptoms/pulmonary function not reported. (1995a may be an abbreviated report of 1995b)
Wang et al. 1999	United Kingdom	16 subjects with allergic rhinitis	Treatment with nasal fluticasone or placebo for 4 weeks followed by exposure to 0.4 ppm NO ₂ for 6 h, allergen challenge, and nasal lavage.	Fluticasone suppressed the NO ₂ and allergen-induced increase in eosinophil cationic protein in nasal lavage fluid. Symptoms/pulmonary function not reported.

Table 6.5. NO₂ effects on BAL lymphocyte subsets and activation in normal subjects.*

Reference	Exposure	CD3 ⁺	CD4 ⁺	CD8 ⁺	CD4 ⁺ /CD8 ⁺	NK Cells	Activation	B Cells
BLOOD								
Frampton et al. 2002	0.6, 1.5 ppm, 3 hr	↓	NC	NC	↑ males ↓ females	NC	NC	NT
Azadniv et al. 1998	2.0 ppm, 6 hr	NC	NC	↓	NC	NC	NC	NT
Rubinstein et al. 1991	0.60 ppm, 2 hr x 4 days	NC	NC	NC	NC	NC	NT	NC
Solomon et al. 2000	2.0 ppm, 4 hr x 3 days	NC	NC	NC	NC	NC	NC	NC
BAL								
Frampton et al. 2002	0.6, 1.5 ppm, 3 hr	NC	↑	NC	NC	NC	NC	NT
Azadniv et al. 1998	2.0 ppm, 6 hr	NC	NC	NC	NC	NC	NC	NT
Blomberg et al. 1997	2.0 ppm, 4 hr	NC	NC	NC	NC	↑	↑ (CD69)	↑
Blomberg et al. 1999	2.0 ppm, 4 hr x 4 days	NC	NC	NC	NC	NC	↑ (CD25)	NC
Helleday et al. 1994	3.5 ppm, 20 min	↑	NC	NC	NC	↑	NT	NC
Rubinstein et al. 1991	0.60 ppm, 2 hr x 4 days	NC	NC	NC	NC	↑	NT	NC
Sandstrom et al. 1992a	1.5 ppm, 20 min every 2nd day x 6	NC	NC	↓	↑	↓	NT	NC
Sandstrom et al. 1992b	4.0 ppm, 20 min every 2nd day x 6	NC	NC	↓	↑	↓	NT	↓
Solomon et al. 2000	2.0 ppm, 4 hr x 3 days	NC	↓	NC	NC	NC	NC	NC

*NC: No change. NT: Not tested. ↑: Increased. ↓: Decreased.

Table 6.6. Effects of exposure to NO₂ with other pollutants.

Reference	Location	Participants	Approach & Methods	Findings
Devalia et al. 1994	United Kingdom	8 mild asthmatics	6 h exposures to combination of 0.4 ppm NO ₂ and 0.2 ppm SO ₂ .	Increased allergen responsiveness 10 min after exposure to combination of NO ₂ and SO ₂ , but not to individual gases. Symptoms not reported. No direct effects on pulmonary function.
Drechsler-Parks et al. 1989	Santa Barbara, CA, USA	32 healthy nonsmokers	2 h exposures with intermittent exercise to 1) air, 2) 0.45 ppm O ₃ , 3) 0.13 ppm peroxyacetyl nitrate (PAN) + 0.45 ppm O ₃ , 4) 0.60 ppm NO ₂ + 0.45 ppm O ₃ , 5) 0.13 ppm PAN + 0.60 ppm NO ₂ + 0.45 ppm O ₃ .	Decrements in lung function attributable to O ₃ only, not to NO ₂ .
Drechsler-Parks 1995	Santa Barbara, CA, USA	8 healthy nonsmokers	4 2-h exposures with intermittent exercise: air, 0.60 ppm NO ₂ , 0.45 ppm O ₃ , and 0.60 ppm NO ₂ + 0.45 ppm O ₃ .	Significant reduction in cardiac output during exercise, estimated using noninvasive impedance cardiography, with NO ₂ + O ₃ . Symptoms/pulmonary function not reported.
Drechsler-Parks et al. 1995	Santa Barbara, CA, USA	32 healthy nonsmokers, 18-26 years and 51-76 years	2-h exposures with intermittent exercise to 1) air, 2) 0.45 ppm O ₃ , 3) 0.13 ppm peroxyacetyl nitrate (PAN)+0.45 ppm O ₃ , 4) 0.6 ppm NO ₂ +0.45 ppm O ₃ , 5) 0.13 ppm PAN+0.60 ppm NO ₂ +0.45 ppm O ₃	Pulmonary function changes driven by ozone; no evidence for NO ₂ effects on symptoms or pulmonary function.

Reference	Location	Participants	Approach & Methods	Findings
Gong et al. 2005	Los Angeles, CA, USA	18 elderly COPD, former smokers, no smoking in past year) (compared with 6 elderly healthy non-smokers)	2 h exposures to air, 0.4 ppm NO ₂ , and concentrated ambient particles (CAP) separately and in combination, intermittent exercise. Measured spirometry, electrocardiogram, blood pressure, oximetry, and cellular composition of induced sputum	NO ₂ : No effects on symptoms and pulmonary function. No change in induced sputum samples. Small decrease in diastolic blood pressure and increase in heart rate 4h and 22 h after exposure (NO ₂ and CAP separately). CAP also increased MMEF, decreased O ₂ saturation, and decrease in % columnar epithelial cells. (These CAP effects were more in healthy compared with COPD). NO ₂ and CAP in combination had no added effects.
Hazucha et al. 1994	Chapel Hill, North Carolina, USA	21 healthy female nonsmokers	2 h exposure to air or 0.6 ppm NO ₂ followed 3 h later by exposure to 0.3 ppm O ₃ , with intermittent exercise.	NO ₂ enhanced spirometric responses and airway responsiveness following subsequent O ₃ exposure. No effects on symptoms, and no direct effects of NO ₂ on pulmonary function.
Jörres & Magnussen 1990	Germany	14 mild asthmatics	30 exposures to air, 0.25 ppm NO ₂ , or 0.5 ppm SO ₂ followed 15 min later by SO ₂ hyperventilation challenge.	NO ₂ , but not SO ₂ , enhanced airway responsiveness to SO ₂ challenge. No effects on symptoms or direct effects on airway resistance.
Koenig et al. 1994	Seattle, Washington, USA	28 asthmatic adolescents; 6 subjects did not complete.	Exposure for 90 min with intermittent exercise to: 1) 0.12 ppm ozone + 0.3 ppm NO ₂ ; 2) 0.12 ppm ozone + 0.3 ppm NO ₂ + 68 µg/m ³ H ₂ SO ₄ ; or 3) 0.12 ppm ozone + 0.3 ppm NO ₂ + 0.05 ppm nitric acid.	No effects on symptoms or pulmonary function.

Reference	Location	Participants	Approach & Methods	Findings
Rubinstein et al. 1990	San Francisco, California, USA	9 stable asthmatics	30 min exposures to air or 0.3 ppm NO ₂ with 20 min exercise, followed 1 h later by SO ₂ inhalation challenge.	No effects on symptoms, pulmonary function, or SO ₂ responsiveness.
Rudell et al. 1999	Sweden	10 healthy nonsmokers	Air and diesel exhaust with and without particle trap for 1 h. BAL 24 h after exposures.	Increased neutrophils in BAL fluid, no significant reduction in effect with particle trap. Symptoms/pulmonary function not reported.
Rusznak et al. 1996	United Kingdom	13 mild asthmatics	6 h exposures to combination of 0.4 ppm NO ₂ and 0.2 ppm SO ₂ .	Increased allergen responsiveness to combination of NO ₂ and SO ₂ , 10 min, 24, and 48 h after exposure. Symptoms not reported. No effects on pulmonary function.

Table 6.7. Summary of Human Chamber Studies on Nitrogen Dioxide: Healthy Individuals vs. Asthmatics *

	Healthy Individuals	Asthmatics	Comment
Symptoms	No effect as high as 4 ppm ¹	Most studies showed no effect at 0.1 ppm-0.5 ppm	
Lung function	No effects at ≥ 4 ppm	Most studies showed no effect at 0.1-0.5 ppm ²	
Airway responsiveness	Increased at 1.5-2 ppm	Increased at 0.2-0.3 ppm in some studies. ³	Asthmatics: substantial between subject variability in response
Airway Inflammation	Exposures at 1.5-2 ppm (3-6 hours), increased neutrophils and epithelial cytokines in BAL. Brief (20 min.) exposures at 1.5-2 ppm-no effect. At 1 ppm: (Jorres et al. 1995) BAL -increase in one airway inflammatory mediator (eicosanoid); no change in cell numbers	At 1 ppm: (Jorres et al.1995) BAL -increase in several airway inflammatory mediators along with decrease FEV ₁ ; no change in cell numbers	Note: In the one study that compared both groups, asthmatics appear more responsive. (Jorres et al. 1995)

	Healthy Individuals	Asthmatics	
Host Defenses	Limited studies: Mucociliary dyskinesia at 1.5 ppm Increased anti-oxidant activity at 2 ppm Enhanced airway epithelial susceptibility to viral infection (<i>in vitro</i>) at 1.5 ppm	No studies	
Response to NO ₂ + allergen compared with filtered air + allergen	Not applicable	NO ₂ (0.26 ppm)+allergen compared with filtered air + allergen larger decrement in lung function(FEV ₁ or peak flow) increased neutrophils (BAL) evidence of eosinophil activation (BAL, blood, sputum).	Limited studies below 0.26 ppm ⁴

* Lowest level at which effects observed. Unless indicated, data not available on threshold level (i.e., level where no effect observed).

¹ Mild increase in symptoms in one study at 0.3 ppm (Vagganini et al. 1996)

² Transient decrease in FEV₁ at 0.3 ppm seen in some studies (Bauer et al. 1986, Avol 1989). Orehek et al. 1976 found increased airway resistance at 0.1 ppm, other studies failed to confirm. See CARB review 1992.

³ Kleinman et al. 1983 (0.2 ppm/2 hr); Jörres et al. 1990 (0.25 ppm/30 min); Bylin 1988 (0.27 ppm/30 min); Bauer et al. 1986 (0.3 ppm/30 min); Strand et al. 1996 (0.26 ppm/30 min). Orehek et al. 1976, increased in airway resistance and increased airway reactivity in 13/20 at 0.1 ppm but questionable statistical analyses, other studies at 0.1 ppm failed to confirm (see text and CARB review, 1992)

⁴ Decrements in lung function (Strand et al. 1997, 1998) Increased inflammatory response in lung fluid (Barck et al. 2002), Evidence of activation of eosinophils in lung fluid and sputum (Barck et al. 2002, 2005). Decrements in lung function were not observed in the latter studies.

Tunncliffe et al. 1994 found decreased FEV₁ after 0.4 ppm NO₂ but not at 0.1 ppm compared with filtered air. Jenkins et al. 1999 found decreased allergen dose to achieve given decrement in FEV₁ at 0.4 ppm x 6 hr, but not 0.2 ppm for 6 hr.

7 Epidemiological Studies

Experimental studies, such as the chamber studies reported in this document, provide valuable information about the acute effects of NO₂ exposure in humans in controlled environments. Epidemiologic studies, which are observational in nature, add to the experimental evidence by evaluating both short and long-term (i.e., a year or more) effects of outdoor and indoor NO₂ in free-living populations. Epidemiologic studies have been conducted to examine the associations between NO₂ exposure and outcomes such as lung function, respiratory symptoms, emergency department visits, hospitalizations and premature mortality. As such, they provide additional evidence of adverse effects from NO₂, subject to some limitations.

Epidemiologic studies are able to examine diverse populations, with a wide range of behaviors and exposures. However, there are several disadvantages to epidemiologic studies, including some that are specific to the study of NO₂. Epidemiologic studies of NO₂ may be subject to measurement error. Most outdoor epidemiologic studies rely on ecologic measures from background air pollution monitors, which may not reflect the actual exposures experienced by study participants. Although most studies of indoor NO₂ use personal monitors, these monitors typically average exposures over a one or two week period and do not pick up peaks in exposure which may be important for indoor sources. For NO₂ and other gases, exposure misclassification could tend to bias the association toward the null, making it more difficult to find an association, if one existed.

For example, Sarnat et al. (2001) demonstrated a very low and statistically non-significant association between personal exposure to NO₂ and ambient NO₂ in Baltimore and a moderate association in Boston (slope=0.19; 95% CI: 0.08-0.30) only in the summer (Sarnat et al. 2005). This city-specific discrepancy may be partly attributed to differences in air conditioning use, since air conditioning is used more in Baltimore than Boston. Ambient gas measurements in both cities were more strongly associated with personal exposure to PM_{2.5} than with their respective personal exposures. Thus, ambient measurements of NO₂ may not reflect personal exposures in individual microenvironments, which vary based on personal behaviors, such as time spent outdoors, exercise, opening of doors and windows, and use of air conditioning. In contrast, several studies of indoor NO₂ exposure were conducted that measured NO₂ exposure indoors using passive samplers or personal exposure badges worn by study participants. These studies measured NO₂ levels over a much smaller area than the background monitors, which reduces exposure misclassification.

Epidemiologic studies may also be subject to biases resulting from residual confounding from uncontrolled or poorly controlled variables such as seasonality, weather and co-pollutants. Time-series studies that examine the association between air pollution and health outcomes at a given location over a designated period of time have employed sophisticated modeling techniques to attempt to control for potential confounders. However, differentiating the effects of NO₂ from other air pollutants has been challenging, since they are often very highly correlated. Because NO₂ outdoor concentrations are directly related to motor vehicle emissions and to traffic density, greater exposures may occur near busy streets and to commuters in heavy traffic. Katsouyanni et al. (2001) and Atkinson et al. (2001) noted a high correlation between NO₂ levels and particulate matter (PM) generated from the same combustion sources. At a given site, a high correlation exists between NO₂ and organic and elemental carbon, inorganic acids, PM_{2.5}, and ultrafine particles (Gaudermann et al. 2000, 2002; McConnell et al. 2003; Seaton and Dennekamp 2003; Zhu et al. 2002a, 2002b). Therefore, NO₂ may be considered a good indicator of the complex gas-particle mixture originating from vehicular traffic. Many studies conducted worldwide over the last decade have found that fine particulate matter is associated with mortality and morbidity (Borja-Aburto et al. 1998; Morgan et al. 1998; Samet et al. 2000). Since NO₂ is strongly related to particles, as both come from the same combustion sources -- and, as indicated above, NO₂ is converted to nitrates and contributes to fine particle mass -- it is very difficult to differentiate between the independent effects of NO₂ and other pollutants. The association between NO₂ and health outcomes, therefore, may be confounded by other pollutants, such as particulate matter (PM₁₀ or PM_{2.5}) or by unmeasured pollutants, such as ultrafines. Given these limitations, outdoor studies may be more informative when direct or indirect adjustment for measured particles concentration (e.g., PM₁₀, PM_{2.5}, black smoke) was possible, when the studies were conducted in areas where the variability of NO₂ was larger than that of fine particles, or when modification of the effect of PM by NO₂ was evaluated, indicating consequences of exposure to traffic-derived particles. However, the role of pollutants that are typically correlated with NO₂ and/or unmeasured remains unknown.

Varying averaging times of NO₂ exposure were used in the epidemiologic studies in this review. Many researchers used a 24-hour maximum, while others reported results for 1-hour or 8-hour average levels. Since these metrics tend to be highly correlated, a positive association between NO₂ and a given health effect may be difficult to attribute to a precise averaging time.

Since many of these limitations are specific to individual study designs, they are reviewed more closely in the following sections. Despite the limitations, a large number of epidemiological studies published in the last several years have been informative. Moreover, several have shown positive associations between NO₂ levels and several health effects.

This review includes all relevant epidemiological studies of the short-term and long-term effects of both indoor and outdoor NO₂, including meta-analyses, published since 1989. This review excludes reviews, studies of low quality or poor statistical methods, and studies that did not provide numerical estimates for the effects of NO₂.

The following tables report relevant elements, including the mean or median values of NO₂, for each study. In the review sections, we reported results in the terms and units that the authors used. In each table, we standardized the effect estimates to a single NO₂ level (24 ppb) using the original estimates and units reported by the investigators. For the time-series studies excess risk was calculated as (Relative Risk – 1) X 100. Although there was no *a priori* restriction on the study locations, most investigations were conducted in the US (especially California) and Europe.

7.1 Outdoor Community-Based Studies: Short-Term Exposure

Several studies have reported associations between daily changes in NO₂ and subsequent changes in daily counts of mortality or morbidity, such as hospital admissions. These time-series studies have been conducted at the population level, including large cities in US or Europe, or among subjects with pre-existing chronic diseases like asthma. Outcomes including daily deaths or events such as hospital admissions were the dependent variables, while exposures including daily measurements of air pollutants and potential confounders were the independent variables. Since deaths and hospital admissions are rare events with a non-parametric distribution, a Poisson regression model was usually employed. To reduce confounding, time-series studies generally include adjustment for seasonal and other temporal trends as well as adjustment for weather effects and co-pollutants using smoothing functions (e.g., LOESS or Spline). A technical issue in time series studies is the choice of degrees of freedom (df) for the smoothing function of time. There appears to be no consensus as to what number of degrees of freedom is appropriate in adjusting for seasonal trends. Statistical diagnostics such as the Akaike information criterion (AIC) or residual autocorrelation or percent explained deviation of the regression model were often used to choose the df for temporal trends, but these diagnostics do not provide epidemiological justification for, or interpretation of, the fitted model. Df are often determined *a priori* based on the previous examination of several models. Using more df may result in a better fit to the observed data, but if too many df are used, they could also negate the effect of pollution and/or weather effects. Therefore, it is not surprising that we often observed smaller pollution effect estimates when more df were used to adjust for temporal trends. In addition, many studies included terms for co-pollutants that may potentially confound the associations, if they are associated with both NO₂ and the outcome under study. If the correlations between NO₂ and co-pollutants such as PM_{2.5} are very high, it is difficult to distinguish the independent effect of NO₂.

7.1.1 Time-Series Studies on Mortality

The acute effects of air pollutants on daily mortality have been evaluated in several cities or counties using time-series analyses. Table 1 presents the results of the available studies for percent change associated with 24 ppb increase in NO₂. Single-pollutant associations have been considered as well as the results of multi-pollutant models that included adjustment for particulate matter or other pollutants, as indicated in the comments column.

Several multi-city analyses and meta-analyses have been conducted for NO₂. Meta-analyses were useful since they increased statistical power and provided more stable effect estimates by considering all relevant studies. For example, a recent meta-analysis of time series investigations on daily mortality, which incorporated 109 studies from Canada, the U.S., Europe, South America, Mexico, Asia, Australia, and New Zealand published between 1982 and 2000 (Stieb et al. 2002), reported 32 effect estimates for NO₂ from single pollutant models and 15 from multi-pollutant models. Over a 24-hour range of mean NO₂ exposure (20.4-103.3 µg/m³), the overall effect estimate from the single pollutant model for all-cause

mortality was 2.8% (95%CI= 2.1-3.5) per 24 ppb increase, which decreased to 0.9% (-0.1-2.0) in multi-pollutant models including particles, CO, O₃, and SO₂. In single pollutant models, the effect estimate for NO₂ on respiratory deaths was higher than for all-cause mortality at 6.6±1.6%, whereas the estimate for cardiovascular deaths (3.2±0.5%) was similar to deaths from all-causes. No cause-specific results were shown for multi-pollutant models. The conclusions of this meta-analysis did not change after the analyses were repeated considering the issues related to the use of Generalized Additive Models (GAMs) (Stieb et al. 2003), including the inappropriate default convergence criteria and an underestimation of the standard errors.

The National Morbidity and Mortality Air Pollution Study (NMMAPS) most recently reported a time-series study using data from 90 U.S. metropolitan areas to examine the association between air pollution and daily deaths. Mortality data were obtained from records of the National Center for Health Statistics, air pollution data were obtained from the US Environmental Protection Agency, and corresponding weather data were abstracted from the National Climatic Data Center from 1987 to 1994. Dominici et al. (2003) reported a positive association between PM₁₀ and daily mortality. They found a statistically significant increase between NO₂ and daily mortality (approximately 0.3 percent per 10 ppb, or 0.7% per 24 ppb), with a one-day lag. In multi-pollutant models containing PM₁₀, PM₁₀ with ozone, PM₁₀ with SO₂, and PM₁₀ with CO, the NO₂ effect remained the same or was slightly higher (0.3 to 0.4 percent), but lost statistical significance.

The APHEA (Air Pollution and Health - A European Approach) initiative, a coordinated study of the short-term effects of air pollution on mortality using data from 15 European cities, found a statistically significant effect of NO₂ with daily mortality. Specifically, Touloumi et al. (1997) reported a 1.3% increase in the daily number of deaths (95%CI, 0.9-1.8) per 50 ug/m³ NO₂ (1-hour maximum). The effect remained statistically significant (0.6%, 95%CI=0 - 1.2%) after adjusting for black smoke. The APHEA-2 study on daily mortality including 29 cities (Katsouyanni et al. 2001) found that NO₂ was both a confounder and an effect modifier of PM₁₀, and the effects on daily mortality were stronger in areas with higher levels of NO₂. The overall estimated increase in the daily number of deaths for all ages for a 10 ug/m³ increase in daily PM₁₀ was 0.6% (95%CI = 0.4-0.8%), which was reduced to 0.4% (0.2-0.7%) with NO₂ in the model. This finding suggests that NO₂ may have confounded the PM₁₀-mortality association. In cities with lower average NO₂, the estimated increase in daily mortality for an increase of 10 ug/m³ in PM₁₀ was 0.19 (95% CI = 0.00-0.41), whereas in a city with higher average NO₂, the estimate was 0.80% (95% CI = 0.67-0.93%). The correlation coefficients between PM₁₀ and NO₂ ranged from 0.12 to 0.75. This positive interaction indicates that NO₂ may enhanced the effect of PM₁₀; or that in areas with higher NO₂, and therefore, more vehicle exhaust, PM likely contains more toxic substances than in areas with lower NO₂ (WHO, 2003). The conclusions about the effect modification remained the same when the data was re-analyzed using natural spline and penalized spline models (Katsouyanni et al. 2001).

A recent analysis of the APHEA-2 study (Samoli et al. 2006) focused on the association of NO₂ and mortality in the 29 cities included in the Katsouyanni et al. (2001) analysis and one additional city. In single pollutant models the percent increase in mortality for a 10 ug/m³ increase in 1-hour maximum NO₂ was 0.30 (95% CI = 0.22-0.38) for total mortality, 0.40 (95% CI = 0.29-0.52) for cardiovascular mortality (CVD) and 0.38 (95% CI = 0.17-0.58) for respiratory mortality. In two-pollutant models, adjusting in turn for the confounding effects of black smoke, PM₁₀, SO₂, and O₃, NO₂ associations with total and cardiovascular mortality were not confounded by any of those pollutants. The association with respiratory mortality was substantially confounded by black smoke and SO₂ levels. The effect of NO₂ on total and CVD mortality was observed mainly in western and southern European cities and was larger when smoking prevalence was lower and household gas consumption was higher. The effect on NO₂ on respiratory mortality was higher in cities with larger proportions of elderly persons and higher levels of PM₁₀.

Several collaborative studies on air pollution and daily mortality were conducted in Europe. An Italian study included eight cities (Biggeri et al. 2001), a French study included nine cities (Le Tertre et al. 2002), and a Spanish study included seven cities (Saez et al. 2002). All three investigations found a positive and statistically significant effect of NO₂ on daily mortality, using single pollutant models. No multi-pollutant models or correlations among the pollutants were reported in the Italian or French studies. The Spanish study found an increase in all-cause and cardiovascular mortality from NO₂, after taking into account the effects of all other pollutants (particles, O₃, SO₂, CO). However, they presented no correlations among the pollutants.

Recently, Simpson et al. (2005a) conducted a mortality study in four Australian cities (Brisbane, Sydney, Melbourne and Perth). One-hour maximum NO₂ was significantly associated with total mortality (pooled RR=1.0011 (1.0004-1.0019) per ppb), respiratory mortality (pooled RR=1.0036) and cardiovascular

mortality (pooled RR=1.0014). In a two-pollutant model with average 24-hour fine particles, the effect of NO₂ on total mortality remained about the same (1.0010) and was statistically significant, whereas the effect of fine particles was diminished when NO₂ was added to the model. Thus, NO₂ was more strongly associated with mortality than PM in this study.

A few single city studies have been conducted in the United States. Kelsall et al. (1997) studied deaths in Philadelphia from 1974-1978 and failed to find a significant effect of NO₂ in either single or multi-pollutant models. Fairley (1999) also did not find a significant effect of NO₂ either alone or after controlling for PM_{2.5} in a study of all-cause mortality from 1989 to 1996 in Santa Clara County, California. Similarly, Gwynn et al. (2000) did not find a significant effect of NO₂ on all-cause, cardiovascular or respiratory mortality in Buffalo, New York. On the other hand, Mar et al. (2000) found that NO₂ was associated with significant increases of about 7% per 20 ppb for total mortality and 10% for cardiovascular mortality in Phoenix, Arizona. This study found that NO₂ and PM_{2.5} were highly correlated (r=0.77). In Phoenix, the primary sources of NO₂ and CO are motor vehicles, and the correlation between these pollutants was also high (r=0.87). Lippmann et al. (2000) found a significant increase of 4% per 24 ppb NO₂ in total and cardiovascular mortality from 1985 to 1990 in the Detroit, Michigan area. However, data from 1992 to 1994 did not show any significant association between mortality and NO₂ levels. No multi-pollutant models were presented. In two-pollutant models with PM₁₀ and with PM_{2.5} Moolgavkar et al. (2003) found statistically significant increases in all-cause-mortality associated with NO₂ exposure in Los Angeles County (1.4% and 1.7% increase per 10 ppb, respectively).

A time-series analysis of infant mortality and air pollution in Mexico City (Loomis et al. 1999) found a 5.54% increase in infant mortality (95% CI = 1.00 – 10.10) for a 10 ppb increase in NO₂ with a 3-day lag period. However, when the effects of PM_{2.5}, O₃ and NO₂ were all considered together, the NO₂ effect was reduced to 0.4% per 10 ppb, while the PM_{2.5} effect remained unchanged.

7.1.1.1 Summary

Daily NO₂ concentrations were significantly associated with increased all-cause, cardiovascular and respiratory mortality. The effect estimate for all-cause mortality derived from a meta-analysis of data from several countries is 2.8% increase per 24 ppb. Adjusting for PM reduced the effect estimate to 0.9% which was no longer statistically significant. The 90 cities U.S. study found a 0.7% (0.3-1.2) increase in all-cause mortality per 24 ppb NO₂, but this estimate lost statistical significance after controlling for PM₁₀ and O₃. Thus, the independent effect of NO₂ on mortality is uncertain, since in many other multi-city and single city studies the NO₂ effect was attenuated after PM was added to the model. None of the studies reviewed in this section had low correlations between PM and NO₂, which makes it difficult to separate the effects of these two pollutants, both of which are associated with combustion sources. Also, there is concern for covariation with other mobile source related emissions. For example, in the Mar study in Phoenix, which showed a significant effect of NO₂, the correlation with CO, another mobile source pollutant, was very high. Other evidence indicates high correlations with ultrafine particles as well as other pollutants. On the basis of these findings, there is suggestive evidence that short-term variations of NO₂ *per se* affect daily mortality but further research is needed to determine if NO₂ has an independent effect.

7.1.2 Time-Series Studies on Morbidity among Adults (Hospital Admission, Emergency Room Visits)

Table 2 presents the results of the available studies on hospital admissions and emergency room visits.

There have been several time-series studies published on the effects of NO₂ on daily hospital admissions for cardiovascular and respiratory conditions. Mann et al. (2002) examined whether ischemic heart disease (IHD) hospital admissions were associated with air pollutants in those with and without secondary diagnoses of arrhythmia (ARR) or congestive heart failure (CHF) using a large data set of members of a large health maintenance organization residing in the South Coast Air Basin of California from 1988 to 1995. Daily CO and NO₂ (mean 37.2 ppb, IQR = 3.7-138) were both associated with an increased number of hospital admissions, with the greatest effects for CO. PM₁₀ was not associated with hospital admissions, although data were only available every 6 days and statistical power may be an issue. A 10-ppb increase in 24-hr average NO₂ was associated with a 2.32% (0.69-3.98%) increase in same-day IHD admissions in persons with a secondary diagnosis of CHF, a 1.81% (0.78-2.85%) increase in persons with a secondary diagnosis of ARR, and a 1.30% (0.51-2.10%) increase in IHD admissions in persons without either secondary diagnosis. CO and NO₂ were not evaluated in a multi-pollutant model because of collinearity; correlations between NO₂ and CO ranged from 0.64 to 0.86 in the seven regions, while NO₂ was modestly correlated with PM₁₀ (r=0.36-0.60). Air pollution was most strongly associated with hospital admissions for myocardial infarction. This study suggests that people with IHD and accompanying CHF and/or ARR constitute a sensitive subgroup in relation to the effects of

criteria ambient air pollutants associated with motor vehicle combustion, but given the high correlation between NO and CO and the modest correlation between NO₂ and PM₁₀, the independent role of NO₂ is unclear. In two-pollutant models with PM₁₀ and with PM_{2.5}. Moolgavkar (2003) found significant associations between NO₂ and hospital admissions for COPD in Los Angeles County (percent increase per 10 ppb=1.72 and 2.86, respectively).

Metzger et al. (2004) examined the relation between ambient air pollution and cardiovascular conditions using ambient air quality data and emergency department visit data in Atlanta, Georgia (1993-2000). For the entire study period, they collected data on over 4 million emergency department visits, as well as measurements of criteria pollutants. Moreover, measurements of fine and coarse particles and several physical and chemical characteristics of PM for the final 25 months of the study were available. The median 1-hour level of NO₂ during the study period was 44 ppb (10-90% range= 25.0-68.0). Using an *a priori* 3-day moving average in single-pollutant models, CVD visits were associated with NO₂, CO, PM_{2.5}, organic carbon, elemental carbon, and oxygenated hydrocarbons. However, the effects tended to be strongest with same-day pollution levels. The effect estimate for 20 ppb NO₂ (3 day average) on all CVD ER visits was 2.5% (1.2-3.9). There was a strong CO effect in this study (correlation coefficient between CO and NO₂=0.68), whereas PM₁₀ had a non-significant effect (correlation coefficient between PM₁₀ and NO₂=0.49). Adjustment for CO in a two-pollutant model reduced the effect estimate for NO₂, but statistical significance remained. When adjustment was made for PM_{2.5} for the shorter time period of data availability (2 years), both the NO₂ and the PM_{2.5} effects were attenuated and became statistically non-significant.

Using the same air pollution measurements as Metzger (2004), Peel et al. (2005) evaluated the associations between air pollution and respiratory emergency department visits in Atlanta, Georgia. Daily measurements of five pollutants, including PM₁₀, O₃, NO₂, CO, and SO₂ were available from January 1993 to August 2000. The mean daily 1-hour NO₂ value was 45.9 ppb. Detailed measurements of particulate matter were available for 24 months. They evaluated visits for asthma, COPD, upper respiratory infections and pneumonia. Considering an *a priori* 3-day cumulative lag (lag 0-2), PM₁₀, O₃, NO₂, and CO were associated with all respiratory admissions, and with upper respiratory infection (URI). The effect for 20 ppb 1-h NO₂ was 1.6% (95%CI=0.6-2.7) for all respiratory conditions and 1.9% (95%CI=0.6-3.1) for URI. COPD visits were associated only with 1-h NO₂ (3.5% per 20 ppb, 95%CI=0.6-6.5%) and CO. For asthma, a stronger NO₂ effect (4.7% for 20 ppb, 95%CI=1.1-8.5%) was detected using distributed lag models (lags 0 to 13 days). Associations with NO₂ for pediatric asthma visits were stronger than those for adults. The effect of NO₂ on emergency visits for asthma was not attenuated in multi-pollutant models, while the estimates for the other pollutants suggested weaker or no associations.

Wellenius et al. (2005) evaluated the association between air pollutants and hospitalization rates for congestive heart failure (CHF) among Medicare recipients in Allegheny County, Pennsylvania, during 1987-1999. Daily mean level of NO₂ (26.5 ppb) was highly correlated with PM₁₀ (0.64) and CO (0.70). After adjusting for PM₁₀, a strong effect was found for NO₂ (4.1% per 11 ppb 24-hour NO₂, 95%CI= 1.8-6.3%) and CO. After NO₂ was adjusted for CO, the effect of NO₂ was null. The authors concluded that the elevation of a combination of ambient particles, CO, and NO₂ -- all derived from motor vehicle emissions -- were responsible for increased hospitalization rates of CHF.

In a study of hospitalization for CHF in seven large U.S. cities, Morris et al. (1995) found an effect of NO₂ in Los Angeles, Chicago and New York, although these associations did not persist in multi-pollutant models with CO, SO₂, and ozone, except in New York. However, the effects of CO were more consistent, with 5 of 7 cities showing a significant effect in the multi-pollutant models. In Tucson, Arizona, Schwartz (1997) failed to find an NO₂ effect on hospital admissions for cardiovascular disease, although significant associations were found for both PM₁₀ and CO.

Three quantitative summaries of the first APHEA city-specific results have evaluated the relationship between various pollutants and several outcomes including: total respiratory hospital admissions in five cities (Spix et al. 1998), admissions for chronic obstructive pulmonary disease (COPD) in six cities (Anderson et al. 1997), and emergency admissions for asthma in four cities (Sunyer et al. 1997). NO₂ was associated with COPD but not with total respiratory conditions. An interaction was present between black smoke and high levels of NO₂, a finding that was in agreement with the APHEA-2 results on daily mortality. The results for asthma were less consistent since positive associations with NO₂ were found, but not in all age groups and not across all cities (Sunyer et al. 1997; Anderson et al. 1998). In the Anderson et al. (1998) study, the effect of NO₂ on asthma admissions in London remained unchanged when adjusted for black smoke, ozone, or pollen. In the over age 65 group the effect of NO₂ increased with ozone in the model. More recently, Galan et al. (2003) found that the effect of NO₂ on asthma

admissions in Madrid was independent from the effect of other pollutants as well as pollen exposure. The adjusted effect estimate was 2.4% (0.5-4.5%) per 5.3 ppb.

Observations are also available on NO₂ and hospital admissions for cardiovascular diseases. In the studies by Poloniecki et al. (1997) (myocardial infarction in London), Burnett et al. (1997) (all CVD admissions in Toronto), Burnett et al. (1999) (ischemic heart diseases and heart failure in Toronto), Atkinson et al. (1999a) (all CVD in London), Wong et al. (1999) (all CVD and heart failure), D'Ippoliti et al. (2003) (myocardial infarction in Rome), statistically significant effects were found for NO₂. In some of these studies, however, the effect estimates were reduced (Atkinson et al. 1999a; D'Ippoliti 2003) and sometimes became non-significant (Poloniecki et al. 1997) when the investigators controlled for particle concentrations.

Finally, a study of hospital admissions in four Australian cities (Brisbane, Melbourne, Perth and Sydney) (Simpson et al. 2005b) found that one-hour NO₂ was significantly related to both cardiac admissions (RR=1.0023 per ppb (1.0016-1.0030)) and elderly respiratory admissions (RR=1.0027 (1.0015-1.0039)). For cardiac admissions, the effects of NO₂ were reduced, but remained statistically significant when particles were added to the model. The same was true for the reciprocal impact of NO₂ on the particle effect. For respiratory admissions, the impact of particles was no longer significant with NO₂ in the model, although the impact of NO₂ was not affected by the inclusion of particles.

7.1.2.1 Summary

Several time-series studies on NO₂ and hospital admissions/emergency room visits for respiratory and cardiovascular diseases suggest an effect of NO₂ or associated combustion-source pollutants since the effect of NO₂ remained, even after adjusted for other pollutants. For example, Mann et al. (2002) found an effect of NO₂ and CO on hospital admissions for ischemic heart disease and CVD ER visits in California, while no PM effect was detected. However, since PM10 in this study was available only every 6 days, insufficient power may have limited the PM analysis. On the other hand, a study of CVD ER visits conducted in Atlanta found a significant effect for NO₂ for all CVD and IHD, and the effect for PM10 was not statistically significant. The correlation between PM10 and NO₂ was 0.49 in this study. Some investigators fail to show an independent effect of NO₂ and others have reported reduced or non-significant estimates for NO₂, after controlling for other pollutants, thus making it difficult to draw a definitive conclusion about the effects of NO₂ on adult morbidity and mortality.

7.1.3 Time-Series Studies on Asthma Morbidity among Children (Hospital Admissions and Urgent Care Visits)

Time-series analyses have been used to report associations between daily air pollutants and hospitalization or emergency room visits for asthma among children (Table 3).

Peel et al. (2005), described above, found an increase of 3.5% (0.6%-6.5%) per 20 ppb NO₂ in emergency department visits for all ages combined for COPD in Atlanta. This risk estimate for all ages was attenuated in a two-pollutant model with CO (data not shown), but the risk for pediatric asthma visits (2.7% per 20 ppb) was not attenuated in multi-pollutant models including PM10, ozone, and CO.

Emergency department visits in Seattle for childhood asthma during 15 months in 1995-1996 were evaluated in relation to PM, NO₂, and other pollutants (Norris et al. 1999). Using a nephelometer, these investigators found a small but statistically significant effect of PM10 and fine particles. One hour maximum NO₂ also had an effect, although it was not significant.

A comprehensive study was conducted in Canada with a specific evaluation of the role of fine and coarse particles and gaseous pollutants. Lin et al. (2003) applied a time-series analysis, as well as a case-cross over analysis to study asthma hospitalizations in children 6-12 years old living in Toronto between 1981 and 1993. NO₂ (annual average 25.2 ppb [IQR=19-30 ppb]) was positively associated with asthma hospitalization both in boys and girls, although the lag time associated with a significant effect was longer for girls (7 days) than for boys (3-6 days). The effect estimates at 6-day cumulative lag were 12% increase (1-23%) in boys and 16% (2-31%) in girls per 11 ppb increase in NO₂. The effect for NO₂ was robust to adjustment for coarse PM, as the effect estimates did not change.

European time-series analyses, conducted mostly within the first APHEA initiative (Katsouyanni et al. 1996), have suggested that gaseous air pollutants and PM were important determinants of hospitalization for respiratory conditions. Sunyer et al. (1997) examined three cities (Helsinki, London, Paris) with asthma emergency room visits for children 0-14 years from 1987-1992. The strongest and most statistically significant effect was found for NO₂, with a combined estimate of 3.7% increase (0.4-6.7%) per 50 µg/m³, at a cumulative lag of three days. Adjusting NO₂ with black smoke or SO₂ resulted in a

different pattern in London compared to Paris for children. In London, the association with NO₂ increased, while the associations with black smoke or SO₂ levels decreased; in Paris, the pattern was the opposite.

Before the publication of the APHEA-1 results, Buchdahl et al. (1996) analyzed the associations between NO₂, O₃ and SO₂ and emergency room visits for acute wheezing episodes (age 0-16 years) at a large hospital in London from 1992-1993. After adjusting for seasonal trend and temperature, O₃ was the only pollutant that increased the risk of emergency visits.

Medina et al. (1997) evaluated doctors' house calls for asthma in Paris between 1991 and 1995. Although they found a statistically significant effect of NO₂ in this study, 24-hour NO₂ was correlated with black smoke (r=0.69), PM10 (r=0.49), and SO₂ (r=0.54). In two-pollutant models, the effect of NO₂ disappeared with black smoke (a surrogate measure for particulate matter in this study), but the effect of black smoke remained statistically significant.

More recent investigations have highlighted the importance of air pollutants on hospital admissions or emergency visits for asthma, particularly in children. Anderson et al. (1998), for example, examined hospital admission data in London from 1987 to 1992. In a single pollutant model, only NO₂ and SO₂ were significantly related to children's hospital admissions. Associations with black smoke (BS) and O₃ were lower and not statistically significant. In two-pollutant models, the effect of NO₂ increased when black smoke was in the model, but decreased slightly with 8-hour ozone. Atkinson et al. (1999a) analyzed hospital admissions for asthma from 1992 to 1994 in London, considering PM10 together with other pollutants for the first time in a European study. All pollutants, except O₃, were associated positively with asthma admissions, although none of the associations were statistically significant. The analysis of the association between outdoor pollutants and visits to emergency departments for respiratory complaints in London during 1992-94 (Atkinson et al. 1999b) revealed a strong relationship between NO₂ levels and asthma visits in children, especially during the warm season. Adjustment for PM and ozone slightly reduced the effect of NO₂, but it remained statistically significant. For NO₂ the correlations with other pollutants, excluding ozone, were between 0.6 and 0.7. A parallel analysis of daily consultations for asthma and other lower respiratory conditions in London showed strong effects for NO₂ in children, again particularly during the summer, whereas no effect was found in adults (Hajat et al. 1999). In this study, an effect of PM10 and BS was also found, although lower than the one found for NO₂.

The results found in the time-series analysis of respiratory admissions in Rome from 1995 to 1997 were similar to what has been suggested by the London studies with regards to children's asthma admissions: NO₂ was strongly related to total respiratory admissions, and in particular, to acute respiratory infections and asthma among children (Fusco et al. 2001).

The APHEA-2 study evaluated the association between PM and BS, with gaseous pollutants only as confounders, and subsequent respiratory hospital admissions in eight cities (Atkinson et al. 2001). Among children 0-14 years old, a 1.2% increase in admissions for PM10 and a 1.3% increase for BS was reported per 10 µ/m³ increase in NO₂ levels. In multi-pollutant models, O₃ did not substantially alter the effect estimates for PM10 and BS, but the inclusion of NO₂ in the models dramatically reduced the effects of both PM10 and BS. This confounding with NO₂ indicates that the effects of the particles may be derived from traffic-related sources.

Anderson et al. (2001) evaluated a range of air pollutant measures considering hospital admissions in the West Midlands conurbation of the UK from 1994 to 1996. The effect of NO₂ reached borderline significance, whereas strong effects were found for PM10, BS and SO₂. Thompson et al. (2001) considered the association between PM10, as well as other traffic-related pollutants, and emergency department visits at the main Belfast hospital. They found statistically significant associations for PM10, NO₂, NOx, NO, and benzene, and reported a non-significant protective effect for O₃.

In San Paulo, Brazil, Gouveia and Fletcher (2000) studied respiratory admissions to hospitals among children under 5 years of age. They found non-statistically significant associations for PM10, NO₂, and O₃ for asthma admissions.

Morgan et al. (1998) examined NO₂, O₃ and PM and daily hospital admissions from 1990 to 1994 in Sydney, Australia, and found that 1-hour maximum NO₂ was the single pollutant related to childhood asthma admissions. Furthermore, the effect of NO₂ remained large and robust in sensitivity analyses, while both O₃ and particulates were not associated with childhood asthma admissions. In a study of hospital admissions in Brisbane from 1987 to 1994, however, Petroeschevsky et al. (2001) found that O₃ was the only pollutant associated with asthma admissions in the 0-14 year group. Particles (measured by nephelometer) and NO₂ were not associated with childhood asthma admissions.

In Seoul, South Korea, Lee et al. (2002) found that PM₁₀, NO₂, and O₃ were all related to asthma admissions, with the strongest and most robust effects for NO₂ and O₃. In multi-pollutant models, the magnitude of the effect of NO₂ did not vary, while those for PM₁₀, SO₂ and CO were reduced and became non-significant, suggesting that the effect of NO₂ on hospital admissions was not confounded by the other pollutants. Daily air pollution levels and daily respiratory hospital admissions in children for five cities in Australia and two cities in New Zealand were analyzed using city-specific case-crossover methods and pooling the results in a meta-analysis (Barnett et al. 2005). Mean 24-h NO₂ concentrations ranged from 7.0 ppb to 11.7 ppb and the correlation between NO₂ and PM_{2.5} ranged from 0.34 to 0.68. In the 1-4 year age-group, both PM_{2.5} and NO₂ were associated with total respiratory admissions, with the largest effect found for NO₂ (2.8% increase per 9.0 ppb 1-h NO₂, 95%CI=0.7-4.9%); in the 5-14 year old, associations were found for PM₁₀ and NO₂, again with the strongest effect for NO₂ (5.8% per 5.1ppb 24-hr NO₂, 95%CI=1.7-10.1%). In general, the largest association found was a 6% (95%CI=0.2-12.1%) increase in asthma admissions in relation to a 5.1 ppb increase in 24-hour NO₂. In the 1-4 year age group, control for PM₁₀ attenuated the effect of NO₂ to a non-significant value. In the 5-14 year age group, control for PM₁₀ did not affect the results for NO₂, whereas the effect of PM₁₀ was sensitive to the effect of NO₂. The impact of NO₂ was stronger in warmer cities and during the summer.

Finally, a recent study by Lee et al. (2006) found an association between hospital admission for asthma in children and NO₂ levels in Hong Kong, which remained statistically significant after controlling for PM, SO₂ and ozone. In the adjusted model a 14.4 ppb change in lag 3 NO₂ resulted in a 5.6% (95% CI=3.21-8.14) increase in hospital admission rate.

7.1.3.1 Summary

Epidemiologic studies have demonstrated associations between air pollution and hospital admissions, emergency room visits, and calls to doctors for asthma in children. Most of the studies indicated an effect of particulate matter and ozone. In many studies, however, NO₂ was strongly related to hospital admissions or emergency room visits for asthma, particularly in European studies. Moreover, the effects of NO₂ were stronger for cumulative lags of up to 6 days than for same-day levels. Indications of an effect were also derived from studies conducted outside of Europe, and in several instances, the effect of NO₂ remained after adjustment for other pollutants. In general, the effects of NO₂ on asthma outcomes appeared to be more robust than either the time-series mortality or cardiovascular hospitalization studies reviewed above.

7.2 Panel Studies on Asthmatic Children and Adults with Cardiac Arrhythmias

7.2.1 Asthma

Numerous field studies carried out over the past decade have tested for, and in many cases observed, acute associations between measures of daily respiratory ill-health and NO₂ concentrations in small groups of subjects. These studies have often focused on subjects with asthma. Field studies recruit and collect data from individual human subjects, as opposed to gathering administrative data on aggregate health outcomes, as in studies of daily mortality, hospital admissions, or ED visits. Because of the logistical burden associated with direct data collection from individual subjects, field studies tend to be small in both numbers of subjects and in duration of follow-up time. As a result, statistical power often limits the conclusions that can be drawn from these studies. Epidemiologic studies of asthma in relation to air pollution must also contend with the known peak in asthma exacerbations and admissions which occurs in the fall in many locations. This peak occurs independently of air pollution, but may interfere with ongoing panel studies by obscuring an association with air pollution during other periods of the year. The peak in asthma during the fall season would be expected, on average, to add noise to epidemiologic studies, but could also bias results if by chance it correlated with a peak or trough in NO₂ concentrations.

Although personal monitoring using passive diffusion NO₂ monitors has been used in epidemiologic literature, most studies still rely on fixed-site ambient monitoring for characterizing exposures of the population under study. As noted elsewhere, NO₂ penetrates indoors incompletely, especially where air conditioning is used for indoor temperature control in hot climates, such as inland areas of California. It is important to keep in mind that individual exposures to NO₂ may not be correlated highly with ambient measurements for subjects who spend most of their time indoors. This issue is relevant not only to acute effect studies (including field studies and hospital usage studies) but also to studies of long-term exposures.

Panel studies among asthmatics and children with chronic respiratory symptoms combine individuals with different levels of asthma severity and medication use, or combine asthmatics and non-asthmatics, and follow this panel of subjects who record daily health outcomes over several months. Air pollution is measured concurrently along with potential confounders that also change on a daily basis (e.g. weather factors, days of the week). There are various symptoms (see Table 4) considered as outcomes, including cough (unspecified, cough in combination with wheeze and tight chest, nocturnal cough), wheeze, shortness of breath with wheeze, and asthma attacks. We have considered lower respiratory symptoms when there was no information about wheezing or cough (e.g., Boezen et al. 1999). For studies on medication use (Table 5), bronchodilator or β -agonist use was considered. In the same panel studies, lung function changes (Table 6) (e.g., peak expiratory flow rate (PEF), forced expiratory volume (FEV), and forced vital capacity (FVC)) were also evaluated, requiring subjects to perform unsupervised daily PEF maneuvers in the morning or evening, or to attend repeated supervised spirometry tests.

After examining a panel of 22 asthmatic children, aged 9 to 19 years, living in a semi-rural area of Southern California with high levels of summer smog, Delfino et al. (2002) confirmed a previous association between PM₁₀ and O₃ with asthma symptoms, and also found NO₂ to be a relevant pollutant, after adjusting for outdoor pollens and fungal spores. However, PM₁₀ was moderately correlated with NO₂ ($r=0.49$). The effects of NO₂ were stronger in children with respiratory infections and in children who were not taking anti-inflammatory medications (odds ratio (OR)=1.9). In the latter group, the effects of both maximum 8 hour NO₂ and 8 hour PM₁₀ decreased when both pollutants were in the model (OR=1.50 and 1.19, respectively). However, the interaction between these pollutants was significant. Delfino et al. (2003) evaluated asthma symptoms among Hispanic children living in an area of Los Angeles County with major freeways and trucking routes. A total of 22 asthmatics were followed with a diary to monitor symptoms. Significant associations were found for PM₁₀ (OR per 37 $\mu\text{g}/\text{m}^3=1.45$) and NO₂ (OR per 1.4 ppb 8-hr=1.27). Also, elemental carbon (EC) and organic carbon (OC) were measured in this study and both had an effect on symptoms. NO₂ was more highly correlated with EC ($r=0.54$) and OC ($r=0.62$) than with PM₁₀ ($r=0.38$). In two-pollutant models of NO₂ and volatile organic compounds (VOCs), odds ratios for VOCs were generally reduced more than for NO₂. There were no two-pollutant models containing NO₂ and PM₁₀. The authors concluded that criteria pollutant gases, including NO₂, function at least partly as surrogate indicators for a causal mixture of toxic air pollutants.

Delfino et al (2004) followed a panel of 19 children with asthma for two weeks with personal PM nephelometers. They found central-site 5-day average 8-hr maximum NO₂ was inversely associated with percent predicted FEV₁ (per IQR increase in NO₂ of 10.5 ppb, -1.16% ; 95% CI, -2.4 to 0.1), and associations were similar for the 3- and 4-day average and for 1-hr maximum NO₂. However, NO₂ was confounded by personal PM with parameter estimates falling near zero. Associations of FEV₁ with personal PM were largely independent of NO₂.

Ostro et al. (2001) conducted a panel study of 138 African-American asthmatic children aged 8 to 13 years in central Los Angeles and Pasadena from August to October of 1993. They found that new episodes of cough were associated with exposures to NO₂ (OR=1.12, 95% CI=1.00-1.24 for a 50 ppb change in the 1 hour maximum) and with 24- hour PM₁₀ (OR=1.25, 95% CI=1.12-1.39 for a 17 $\mu\text{g}/\text{m}^3$ change). The effect estimate was much lower for 1-hour maximum PM₁₀ (OR=1.07). NO₂ was also associated with incidence of wheeze (OR=1.13). In Los Angeles, NO₂ was correlated with PM₁₀, PM_{2.5} and ozone ($r=0.63$, 0.34 , 0.48 , respectively). Although no multi-pollutant models were presented, the effect of 1-hour NO₂ was stronger than that of the 1-hour PM₁₀.

Mortimer et al. (2002) reported the results of the National Cooperative Inner-City Asthma Study (an investigation conducted in eight U.S. urban areas). A total of 846 children were followed for two weeks during the summer with a diary and PEF measurements. All of the pollutants investigated (PM₁₀, NO₂, O₃ and SO₂) were related to increases in morning symptoms. In single pollutant models, the odds ratio per 20 ppb for the average of lag 1-6 4-hour (6:00-10:00 hr) NO₂ was 1.48 (1.02-2.16), while the odds ratio per 15 ppb for the average of lag 1-5 ozone was 1.12 (0.97-1.30). Including both O₃ and NO₂ in the model reduced the odds ratio for NO₂ only slightly to 1.4 (0.93-2.09). There were no two-pollutant models with NO₂ and PM₁₀. Only O₃ was related to a decline in morning peak expiratory flow (PEF).

A recent study examined the effects of NO₂ on symptoms and rescue inhaler use in 990 asthmatic children who were followed for a median of nearly two months from 1993 to 1995 in 6 U.S. cities and Toronto, Ontario, Canada (Schildcrout et al. 2006). They found that a 20 ppb change in NO₂ was associated with a symptoms odds ratio of 1.09 (95% CI=1.03 – 1.15) and a rate ratio for rescue inhaler use of 1.05 (95% CI=1.01 – 1.09). The effects for symptoms and rescue inhaler use remained about the same when NO₂ was summed with other pollutants one at a time. PM₁₀ and ozone were not related to symptoms or rescue inhaler use.

Panel studies of asthmatic children have been conducted in European countries. Boezen et al. (1999) studied children in urban and rural areas of the Netherlands, and categorized them according to their bronchial hyperresponsiveness (BHR) and serum concentration of IgE. Based on data from three winters, there was a strong association between occurrence of lower respiratory tract symptoms, including wheeze, and both PM₁₀ and NO₂ among subjects with increased BHR and high IgE levels. No associations were found among children who did not have both of these factors. Evening PEF was also negatively influenced by PM₁₀ and NO₂. However, there were no multi-pollutant models in this study, and the correlations among the pollutants were not presented. Therefore, it was not possible to separate the effects of NO₂ from those of PM₁₀. Van der Zee et al. (1999) examined PEF and respiratory symptoms among children in urban and rural areas with and without asthma, chronic cough, or wheeze (classified as symptomatic). In urban areas, associations were found between PM₁₀ and lower respiratory symptoms, medication use, and a decrease in PEF among the symptomatic children. The effects of NO₂ were limited to an increased frequency of bronchodilator use. However, only minimal effects were observed in non-urban areas. Among the non-symptomatic children, no associations were found. Two pollutant models including NO₂ were not available. However, in models that included PM₁₀ and either black smoke, SO₂ or sulfate, the PM₁₀ effects remained but the effects of the other pollutants became non-significant. Finally, the European study PEACE, coordinated by researchers in the Netherlands and conducted in 14 centers, evaluated 2010 symptomatic children with a follow-up of two months (Roemer, 1998). There was no clear association of PM₁₀, BS, or NO₂ with various outcomes, including symptoms, medication use, and PEF measurements. This study was conducted during the winter of 1993 to 1994, when an influenza epidemic was particularly severe.

Since episodes of airflow obstruction and aggravation of symptoms in asthmatic subjects are often precipitated by viral infections, the study of Linaker et al. (2000) is relevant. They investigated 114 asthmatic children and followed them up for 13 months for respiratory infections and development of asthmatic symptoms, and measured personal NO₂ exposure. For a seven-day average NO₂ level of 28 µg/m³ vs. ≤ 8 µg/m³, the odds ratio was 1.9 (1.1-3.4) for risk of asthmatic exacerbations following respiratory infections.

Two studies have been conducted in Paris, France. Segala et al. (1998) studied children with mild (43) and moderate (41) asthma during a period of six months. Nocturnal cough was the symptom most strongly associated with air pollution in mild asthmatics, in particular PM₁₀, BS, and NO₂. No association between pollutants and PEF was found in the overall group, but when the analysis was restricted to 21 children taking no corticosteroids and no regularly scheduled beta-agonist, borderline statistically significant effects for PM₁₀ and NO₂ were observed. There were no multi-pollutant models presented, and NO₂ was significantly correlated with PM₁₀, black smoke and SO₂ (r=0.55, 0.61 and 0.54, respectively). A more recent study (Just et al. 2002) examined symptoms, medication use, and PEF among 82 children followed for three months (April-June). Again, nocturnal cough was associated with BS and NO₂, whereas no association was found for O₃. A larger association with NO₂ was seen for respiratory infections. NO₂ was correlated with PM, black smoke, and SO₂ (r=0.54, 0.92, and 0.69), but was not correlated with ozone (r=0.09). Therefore, it was not possible to separate the effects of BS and NO₂ on nocturnal cough. Both pollutants can be considered as indicators of diesel exhaust, which has been increasing in Paris since 1985.

A study of 11 adults with asthma in Rome, Italy (Lagorio et al. 2006) found that an increase of 5.3 ppb of ambient of NO₂ was associated with a 1.28% reduction in FEV₁, whereas PM_{2.5} was not associated with FEV₁ levels.

Finally, at an elementary school in Linz, Austria located next to an air monitoring station 163 children aged 7 to 10 years underwent repeated lung function examinations (11 to 12 tests per child) at the same time of day during one school year (Moshhammer et al. 2006). Most of the children in this study were healthy, although an undisclosed number did have asthma. In a two-pollutant model with PM_{2.5}, a 10 µg/m³ change in 8-hour mean (midnight to 8:00 a.m.) NO₂ reduced FEV₁ significantly by 1.01%, MEF50 by 1.99% and MEF25 by 1.96%. In the basic model the reduction of these parameters per 10 µg/m³ was highest for NO₂ followed by PM₁, PM_{2.5} and PM₁₀. The 24-hour mean NO₂ interquartile range in this study was 13.75 to 21.48 µg/m³.

7.2.2 Cardiovascular Effects

Peters et al. (2000) (Table 4) suggested that air pollution affects heart rhythm disorders among patients living in eastern Massachusetts with implanted defibrillators. In this study, PM_{2.5}, black smoke, NO₂ and CO were associated with increased risk of defibrillator discharges in 33 patients and the concentration-response relationship for NO₂ was the steepest. The previous day's NO₂ level was associated with an

OR of 1.8 (1.1-2.9) per 26 ppb. In two-pollutant models, the PM_{2.5}, CO, and black carbon effects were substantially attenuated but the NO₂ effect was unchanged. NO₂ was highly correlated with black carbon (r=0.75) and CO (r=0.71) and moderately correlated with PM₁₀ and PM_{2.5} (r=0.60 and 0.57, respectively). In a follow-up study of 203 patients from the same population, Dockery et al. (2005) found an odds ratio for ventricular arrhythmias of 1.2 (0.9-1.7) for a 24 ppb change in the mean of the same and previous day NO₂. In patients with a recent arrhythmia (past 3 days), the odds ratio for an interquartile change in NO₂ of 7.7 ppb was 1.34 (1.05-1.71).

Rich et al. (2005) evaluated the association between air pollution and severe arrhythmia among patients with implantable cardioverter defibrillators in Boston. They analyzed a total of 798 ventricular arrhythmias among 84 subjects using a case-crossover design. Hourly and daily levels of PM_{2.5}, black carbon, NO₂, SO₂, CO, and O₃ were available. The median daily NO₂ concentration was 22.4 ppb. PM_{2.5} and O₃ over the previous 24 hours were associated with arrhythmia, while similar effects for NO₂ and SO₂ were detected, but for the previous 48-hour moving averages. For NO₂, an interquartile range of 7.7 ppb over the last 48 hours increased the probability of severe arrhythmia by 18% (95%CI=4-35%). In two pollutant models (where the NO₂ in the previous 24 hours was considered), the effect of NO₂ was null when PM_{2.5} was in the model, whereas the effect of PM_{2.5} remained significantly elevated. Controlling for ozone did not attenuate the NO₂ effect.

Pekkanen et al. (2002) studied 45 adults with stable coronary artery disease and analyzed data from repeated biweekly in-clinic ECG measurements during submaximal exercise testing and outdoor ultrafine and fine particles measured at a central regional site of Helsinki, Finland. They found significant associations between risk of ST segment depression and ambient lag 2 day PM_{2.5} mass (OR 2.8, 95% CI: 1.42, 5.66). Similar magnitudes of association were found for ultrafine and accumulation mode particle number concentrations, and smaller but significant associations were also found for lag 2 day NO₂ (OR 2.02, 95% CI: 1.34, 3.04) and CO (OR 1.73, 95% CI: 1.26, 2.39), which were moderately correlated with the co-located particle measurements. Two pollutant models for PM and gases were not tested.

7.2.3 Summary

Overall, most panel studies evaluating aggravation of asthma noted an effect from NO₂. In many cases, the effect was stronger for NO₂ than for other pollutants. In studies where multi-pollutant models were presented, the effects of NO₂ were fairly robust to the inclusion of other pollutants such as PM₁₀, ozone, or VOCs. However, many of these studies were conducted in Europe where the concentrations and exposure patterns differ from those observed in California. In addition, there is evidence from several panel studies of a possible association between NO₂ and several cardiovascular outcomes.

7.3 Studies of Chronic Exposure

In addition to the growing literature examining the short-term effects of NO₂ on mortality risk, hospitalizations, symptoms, and lung function, long-term NO₂ exposures have also been the focus of epidemiologic investigations over the past decade. Controlled exposure studies have led the way in characterizing the acute effects of NO₂, including increased responsiveness to allergens in subjects with asthma. However, epidemiology addresses long-term impacts in humans, since it is impractical to study these effects using controlled human exposure studies. Much of the epidemiologic literature evaluating long-term NO₂ exposure focused on evidence for repeated exposures over several years on the persistent damage to the human lung, especially the small, terminal bronchiolar regions where vulnerability is greatest.

While this issue is critically important, the challenges to addressing it epidemiologically are formidable. Long-term NO₂ concentrations tend to correlate with long-term concentrations of other pollutants, making attribution to specific pollutants difficult. As mentioned above, many studies conducted in the last decade from all over the world have found that fine PM is associated with mortality and morbidity. Since NO₂ is strongly related to PM, as both come from the same combustion sources (and, as indicated above, NO₂ is converted to nitrates and contributes to fine PM mass), it is very difficult to differentiate the independent effects of NO₂ and other pollutants. This can lead to confounding of the relationship between NO₂ and health outcomes by other pollutants, such as PM. Given the problems outlined above, the available studies of chronic exposure were more informative when direct or indirect adjustment for measured PM concentration (PM₁₀, PM_{2.5}, black smoke) were possible, or when the studies were conducted in areas where the variability of NO₂ was larger than that of fine particles, or when modification of the effect of PM by NO₂ has been evaluated indicating consequences of exposure to traffic-derived PM.

Another problem is the mobility of population, which makes exposure assessment more difficult than for short-term studies. Furthermore, air pollution data has not been consistently collected over time, which makes long-term exposures difficult to construct. This has been a problem with respect to PM10 and PM2.5, which, in the past, have not been collected as extensively as NO₂, but which can confound the association between NO₂ and health effects. Studies conducted since 1999, in which PM2.5 was collected daily or every few days may, therefore, be more informative than some of the older studies.

Next, we review studies published primarily from 1997 onward in which health effects were tested in relation to NO₂ exposures extending from several weeks to many years (Tables 7-15). The available literature falls into five general categories: 1) studies addressing the incidence and prevalence of asthma, respiratory disorders, and atopy; 2) studies looking at lung function at one time period in several communities following long-term exposure (cross-sectional studies), or addressing growth or decline of lung function over many years in relation to long-term NO₂ exposures (cohort studies); 3) case-control and cohort studies looking at the long-term effects of NO₂ on cancer; 4) studies examining the effect of NO₂ on fetal development; and 5) studies examining long-term mortality risks; and 6) studies of indoor exposure. The most informative studies are briefly highlighted below.

7.3.1 Asthma, Respiratory Diseases, Lung Function

The Children's Health Study (CHS) was initiated in 1993 with a cohort of 3,676 school-aged children in grades 4, 7, and 10, from 12 demographically similar southern California communities representing a wide range in air quality (Peters et al. 1999b). To date, this study has reported associations between air pollution and several outcomes, including lung function, respiratory symptoms, and asthma incidence. In single-pollutant models, the prevalence rates of wheeze in boys were positively associated with NO₂ (adjusted OR=1.54, 95% CI=1.04-2.29), PM10, and PM2.5, although the associations with PM10 and PM2.5 were not statistically significant. Since NO₂ was positively correlated with PM10 ($r=0.74$), it is difficult to distinguish between the effects of NO₂ and PM10, since no multi-pollutant models were presented. Further analyses showed that children with asthma were particularly sensitive to the effects of air pollution (McConnell et al. 1999). Among these children, the prevalence of bronchitis and phlegm was positively associated with levels of NO₂, PM10, and PM2.5, but not O₃. The association with phlegm was strongest for NO₂ (OR=2.7 per 24 ppb, 95% CI=1.4-5.3) and of similar magnitude for PM10 and PM2.5. The association with bronchitis was strongest for PM10, but of similar magnitude for NO₂. However, these pollutants were highly correlated (see Peters et al. 1999b, above).

For the CHS, McConnell et al. (2003) examined the impact of air pollution on the incidence of bronchitic symptoms, by using yearly questionnaires (from 1996 to 1999) in children with asthma. There was a positive association between bronchitic symptoms and the 4-year average concentrations of NO₂, PM2.5, and elemental carbon. The effect of NO₂ was due entirely to the effect among children playing team sports at study entry, and was reduced and no longer significant after adjusting for O₃ or PM2.5, and was markedly reduced after adjusting for organic carbon.

Peters et al. (1999a) also studied children in Southern California from the CHS to assess lung function effects due to long-term exposure to four pollutants, including NO₂. By following up on these children after a period of 4 years, Gauderman et al. (2000) showed significant deficits in growth of lung function associated with exposure to PM10, PM2.5 and NO₂. These associations were statistically significant in the fourth-grade cohort and generally larger among children spending time outdoors. However, two-pollutant models suggested that no single pollutant measured was responsible for the observed deficits in lung function growth.

The second cohort of 1,678 children from the CHS, enrolled as fourth graders in 1996, was followed for 4 years to determine whether the growth in lung function of the children was associated with their exposure to ambient air pollutants (Gauderman et al. 2002). Significant deficits in lung function growth rate were associated with exposure to NO₂. However, in two-pollutant models, the effects of NO₂ on both FEV₁ and MMEF became positive and non-significant after controlling for acid vapor. Adjustment for PM10 and PM2.5 did not alter the effect of NO₂ on either FEV₁ or MMEF. NO₂ was highly correlated with acid vapor ($r=0.83$) as well as with PM10, PM2.5, elemental carbon and organic carbon ($r=0.64, 0.77, 0.93, \text{ and } 0.58$, respectively). Larger deficits in lung function growth rate were observed in children who reported spending more time outdoors.

Gauderman et al. (2004) recently confirmed these results, by following 1,759 4th graders enrolled in 1993 for 8 years until 2001. This study is particularly important since it followed lung development between the ages of 10 and 18 years of age. For girls, the lung typically stops developing at this age such significant decrements in lung function are likely to be permanent. For boys, lung development continues into the early 20s, but at a much lower rate. This study reported a strong inverse association between long-term

concentrations of NO₂ and lung growth, measured as the change in FEV1 over the eight-year study period. Besides NO₂, the strongest associations were observed for acid vapor and elemental carbon. An association was also reported between NO₂ and clinically important deficits in attained lung function, measured as being below 80% of the predicted value for FEV1. These deficits are strong risk factors for cardiovascular disease and mortality in adulthood. Again, the strongest associations were reported with NO₂, acid vapor and elemental carbon. For NO₂, effects appear to occur after long-term exposure in the 25–30 ppb range. A previous multi-city study in the United States, the American six-city study, investigated the effects of air pollution on respiratory health of pre-adolescent children living in several communities with varying levels of air pollution since the mid-1980s. Dockery et al. (1989) subsequently analyzed the respiratory health data from 5,422 of these children, who were 10 to 12 years old when they participated in a follow-up visit in 1980-1981. Exposure to NO₂ was negatively associated with prevalence of asthma and positively associated with chronic cough, although this association was not statistically significant (OR=1.6 per 16 ppb, 95% CI=0.3-10.5). In this study, PM15 (particulate matter less than 15 microns in diameter) was strongly associated with chronic cough and bronchitis. Except for ozone, the correlations between pairs of pollution measures varied between 0.53 and 0.98. Therefore, the effect of NO₂ may have been due to the correlation with PM15. There were no multi-pollutant models presented.

The long-term studies described above were based on NO₂ measurements taken at central site monitors. Recently, studies have also examined NO₂ exposure at the neighborhood level. As part of the CHS (Gauderman et al. 2005) examined the lifetime history of doctor-diagnosed asthma among a random sample of 208 children, each of whom had outdoor NO₂ measured outside his or her home. The overall average of the 2-week summer and 2-week winter measurements of NO₂ ranged from 12.9 ppb in Atascadero to 51.5 ppb in San Dimas. The OR for doctor-diagnosed asthma was 1.83 (1.04-3.22) per 5.7 ppb (interquartile range) of NO₂. Freeway exposure and measured NO₂ were also associated with wheezing and use of asthma medication. This study showed that measuring NO₂ was important for validation of the use of traffic measures for the population under study. However, it is possible that outdoor NO₂ was a marker of some other traffic-related pollutant responsible for increasing asthma risk.

Kim et al. (2004) conducted a neighborhood study of asthma and respiratory symptoms in school children in grades 3 to 5 living in the San Francisco Bay Area, a region with good air quality. Traffic-related air pollutants including NO₂ measured at neighborhood schools were increased near and downwind of major roads. They found associations between respiratory symptoms and traffic-related pollutants. Among those living at their current residence for at least 1 year, the adjusted odds ratios in relationship to an interquartile difference in NO₂ measured at the neighborhood school were 1.04 (95% CI = 0.98 – 1.10) for asthma in the past 12 months and 1.03 (95% CI = 1.00 – 1.06) for bronchitis.

Although the findings on asthma incidence, respiratory symptoms and lung function from studies in California are the most relevant for NO₂ standard setting, many other studies, most of which have been conducted in Europe, have found effects of either measured NO₂ or a mixture of traffic-related pollutants. In these studies, the levels of these pollutants are often estimated from car and truck traffic counts or distance to major roadways. The problem with the studies in terms of evaluating effects of a single pollutant is that the traffic-related pollutants are highly correlated and it is not possible to separate the effects of NO₂. Correlations between PM_{2.5} and NO₂ have been as high as 0.99 (Gehring et al. 2002) in Europe. In a study from the Netherlands (Janssen et al. 2003) which examined 2,071 children with respect to traffic exposure at school, associations between symptoms and traffic exposure were mainly seen in children with bronchial responsiveness (BHR) or atopic sensitization. For children sensitized to outdoor allergens, current wheeze was associated with NO₂ (OR=5.1 p=0.04). NO₂ was also associated with sensitization. A difference of 17.6 µg/m³ NO₂ was associated with elevated total IgE (OR: 3.12 (95% CI: 0.99 to 3.05)), skin prick test reactivity to any allergen (OR: 1.70 (95% CI: 1.03 to 2.81)), to indoor allergens (OR: 1.94 (95% CI: 1.13 to 3.33)) and to outdoor allergens (OR: 1.69 (95% CI: 0.91 to 3.14)).

German investigators looked at the association between traffic-related air pollution and parameters of atopy in 317 children 9 years of age living near major roads in two urban and one suburban areas of one city in West Germany (Kramer et al. 2000). Personal NO₂ exposure and NO₂ concentrations in front of the children's homes (outdoor NO₂) were measured. Outdoor NO₂ was correlated with traffic exposure but not with personal NO₂ exposure. Associations were dominated by the urban subgroup as follows: Outdoor home NO₂, but not personal NO₂, was significantly associated with reports of at least one week with symptoms of wheezing (OR for 10 µg/m³ increase, 14.9 (95% CI, 2.59, 86.4)), and with symptoms of allergic rhinitis (OR 9.08 (95% CI 2.06, 40.11)). An ever diagnosis of hay fever was associated with outdoor NO₂ (OR 4.24 (95% CI: 1.01, 17.8)), while asthma was not (OR 1.82 (95% CI : 0.36, 9.36)).

Atopic sensitization to pollen, house dust mite or cat, and milk or egg were each significantly associated with outdoor NO₂ (ORs ranged from 3.5 to 5.0), but not personal NO₂. The findings of this study suggest that NO₂ may have been important per se, or that it served as a marker for other traffic-related pollutants.

Although the vehicle mix in Europe is different from that in the United States, with many more diesel cars in Europe, the European studies are important in that they do provide additional evidence of an effect of traffic related pollutants on respiratory health.

7.3.1.1 Summary

The Southern California Cohort Studies (CHS), which are particularly relevant for NO₂ standard setting, found positive associations between outdoor NO₂ concentrations and several respiratory health outcomes in children. For example, the CHS showed a positive association between outdoor NO₂ concentrations and incidence of bronchitic symptoms in asthmatics that played team sports. In addition, the CHS found deficits in lung function growth associated with NO₂ exposure in children over an 8-year study period. This is a very serious effect that is a risk factor for chronic diseases and premature mortality later in life. Studies in Europe have also found associations between traffic related pollutants, including measured NO₂, and allergic sensitization in children. Most if not all of these studies are likely to have highly correlated co-pollutants, including NO₂, which are derived from vehicular traffic. However, there is reasonable support for the conclusion that NO₂ is at least one of the harmful constituents of this mix.

7.3.2 Cancer

Descriptions and results of the studies are presented in Table 13.

Nyberg et al. (2000) conducted a population-based case-control study with 1,042 lung cancer cases and 2,364 controls among stable male residents (40-75 years old) in Stockholm County 1950-1990. Local annual source-specific air pollution levels for NO_x/NO₂ and SO₂ were estimated using validated dispersion models and linked to residential addresses. Average traffic-related NO₂ exposure over 10 years was associated with lung cancer after adjustments for tobacco smoking, socioeconomic status, residential radon, and occupational exposures. In models incorporating both pollutants, the estimated effect of NO₂ was stronger. The 30-year averages of estimated NO₂ and SO₂ showed some correlation (Pearson's correlation=0.64).

Nafstad et al. (2004) reported an increased risk for lung cancer mortality (OR=1.11 (1.03-1.19) per 10 µg/m³) and a stronger increased risk for respiratory diseases other than lung cancer (OR=1.16 (1.06-1.26)) in association with NO_x concentrations based on data from a cohort of 16,209 men 40-49 years living in Oslo, Norway. No estimates were given for NO₂. PM was not measured in the study, and the association with NO_x might indicate that traffic-related pollution was important in this study.

In addition, several studies have looked at the effect of NO₂ on childhood cancer. Feychting et al. (1998) identified 142 cases of childhood cancer from a population of 127,000 children living within 300 meters of transmission lines in Sweden, were, including 39 cases of leukemia and 33 cases of central nervous system tumors. Approximately four referents per case were selected at random from the study base population. The 99th percentile of the NO₂ content of the outdoor air for one-hour averages over one year was used as an exposure metric. The study observed an increased risk of childhood cancer at high NO₂ concentrations (RR=3.8 (1.2-12.1) for ≥80 µg/m³ vs. ≤ 49 µg/m³). The number of cases was relatively small due to the rareness of the diseases. NO₂ may be a surrogate for traffic-related pollution, which contains many carcinogens.

Raaschou-Nielsen et al. (2001) conducted a study of 1,989 children diagnosed with cancer under age 15 years in Denmark and compared them to 5,506 children who were controls, and found an increased risk of Hodgkin's disease in the highest category of residential exposure to NO₂ (adjusted RR=6.7, 95% CI = 1.7 – 26) compared to the lowest category. Exposure of each child to NO₂ was analyzed prenatal and throughout childhood until 12 months before the diagnosis of cancer. Exposure to NO₂ was modeled from street configuration, traffic patterns, meteorological variables and background levels of air pollution. Hodgkin's disease was also related to benzene exposure, but no other air pollutants were measured. Therefore, it was not possible to determine whether the effects were due to NO₂ alone or whether NO₂ was a marker of other traffic-related pollutants, such as PM_{2.5}.

7.3.2.1 Summary

Despite the limitations in sample size of the studies, there seems to be some evidence for an association between NO₂ and lung cancer in Europe. Often the studies considered NO₂ concentrations as a proxy for

traffic-related pollution. In light of recent evidence of carcinogenic effects of diesel particles, these associations should be interpreted with caution and may well represent carcinogenicity of traffic-related air pollutants.

7.3.3 Fetal Effects – Reproductive and Birth Effects

A recent analysis evaluated 3,771 cases of term low birth weight, 3,509 cases of preterm low birth weight, 13,464 cases of preterm babies and 26,351 control births in Southern California (Wilhelm and Ritz 2003) (Table 14). In addition to the ambient air pollution concentrations this recent study also included a distance-weighted traffic density measure as exposure. NO₂ concentrations were associated with term low birth weight births and preterm birth. However, in multivariate models including NO₂ and quintiles of the distance weighted traffic density, the results for NO₂ were no longer statistically significant.

Liu et al. (2003) recently examined the association between pre-term birth, low birth weight, and intrauterine growth restriction, among singleton live births and ambient concentrations of SO₂, NO₂, CO, and O₃ in Vancouver, Canada from 1985 to 1998. Low birth weight was not associated with NO₂ exposure. Preterm birth was associated with exposure to NO₂ during the last month of pregnancy, while intrauterine growth retardation (IUGR) was associated with exposure to NO₂ during the first month of pregnancy. Elevated risks for IUGR associated with NO₂ persisted after adjustment for other co-pollutants. A more recent analysis of three Canadian cities (Calgary, Edmonton and Montreal) over the same time period (Liu et al. 2006) found significant effects of first, second and third trimester NO₂ levels on intrauterine growth restriction. However, these effects diminished to null when CO was included in the models.

Dales et al. (2004) reported an association between NO₂ concentrations and sudden infant death syndrome based on data from 12 Canadian cities. Estimates of NO₂ were reduced to zero if traffic density was considered in the same model.

A study of 276,763 live births between January 1996 and December 1997 in Seoul, South Korea (Ha et al. 2001) found an independent effect of first trimester NO₂ on low birth weight. The adjusted relative risk of low birth weight for each interquartile increase in NO₂ was 1.07 (95% CI=1.03-1.11). This effect remained when CO, SO₂, TSP and O₃ were all in the model. There was a reduction in birth weight of 8.41 grams for an interquartile increase of first trimester NO₂.

Lin et al. (2004) recently reported an association between low birth weight and NO₂ concentrations based on data from 92,288 full term live births in Taiwan between 1995 and 1997. No continuous estimates of the NO₂ effects were provided. NO₂ effects were smaller than those observed for SO₂ and were not statistically significant.

7.3.3.1 Summary

There seems to be some evidence for an association between traffic related air pollution and fetal effects. Data from California suggested that only preterm birth was associated with NO₂ itself, while Canadian studies found evidence for an association with intrauterine growth retardation and sudden infant death syndrome. The number of studies that evaluated NO₂ is small, and again, it is impossible to decipher whether these effects are due to NO₂ alone.

7.3.4 Infant Mortality

In a case-control study of infant mortality in the Czech Republic from 1989 to 1991, Bobak and Leon (1999) found that for a 50 µg/m³ increase in NO_x, the adjusted relative risk was 1.66 (95% CI = 0.98 – 2.81) for deaths from respiratory causes in the postneonatal (between 28 days and 1 year of age) period. However, this relative risk was reduced to 1.09 (95% CI = 0.55 – 2.09) after TSP and SO₂ were added to the model.

A study of mortality in post-neonates (1 month to 1 year old), persons aged 2 to 64 years and persons over 65 years found that the post-neonates were most susceptible to the effects of air pollution in Seoul, South Korea (Ha et al. 2003). Although there was a positive association between postneonatal mortality and PM₁₀, the authors failed to find an effect of NO₂ on either total or respiratory mortality in this age group. No multi-pollutant models were presented.

7.3.4.1 Summary

There is limited evidence linking longer-term exposure to NO₂ and other traffic-related pollutants to infant mortality. However, it is difficult to determine, from the existing evidence, the role that NO₂ itself plays.

7.3.5 Adult Mortality

Descriptions and results of the studies are presented in Table 15.

The Harvard Six Cities Study assessed all-cause mortality in 8,111 adults during a 14-16 year follow-up period (Dockery et al. 1993). Although NO₂ levels were recorded ranging from 6.1 ppb in Portage, Wisconsin to 21.9 ppb in Steubenville, Ohio, no effect estimates were provided. However, in a reanalysis of the data, Krewski et al. (2000) found that NO₂ was associated with an increased risk of both all-cause mortality (RR=1.25, 95% CI=1.07-1.46, per 15.8 ppb) and cardiopulmonary deaths (RR=1.28, 95% CI=1.04-1.59). Lung cancer was not associated with NO₂ levels. In this re-analysis, multi-pollutant models were not included, and NO₂ was highly correlated with PM2.5, TSP, PM15 and SO₂ (r=0.78, 0.82, 0.77 and 0.84, respectively). Therefore, it is not possible to know whether NO₂ alone was associated with increased mortality in this cohort.

The American Cancer Society Study II assessed the association between long-term exposure to air pollution and mortality in approximately 500,000 participants followed over a 16-year period (Pope, III et al. 2002). Mean NO₂ concentrations were 21.4 ppb with a standard deviation of 7.1 ppb for the time-period 1982 to 1998. No association was observed between NO₂ concentrations and all-cause mortality, cardiopulmonary disease mortality and all other cause mortality. The risk estimates for NO₂ and lung cancer deaths were negative, but not statistically significant.

Hoek et al. (2002) followed a sample of 5,000 adults aged 55 to 69 years over an 8-year period. An increase of 30 µg/m³ NO₂ estimated for background and local exposure was associated with cardiopulmonary deaths (adjusted RR=1.81; 95% CI= 0.98-3.34) (Table 15). The effect estimate was comparable to the one for black smoke, but had more uncertainty with wider confidence intervals.

Nafstad et al. (2004) reported data from a cohort of 16,209 men 40 to 49 years of age living in Oslo, Norway. The follow-up time was 16 years between 1972/73 and 1998. Controlling for a number of potential confounders, all cause mortality, respiratory disease mortality other than lung cancer and ischemic heart disease mortality was associated with NO_x exposure at the home address from 1974 to 1998.

Gehring et al. (2006) examined mortality in a cohort of about 4,800 women age 50-59 years living in North Rhine-Westphalia, Germany. The women were followed from 1985 and from 1990 to 2003. After adjustment for socioeconomic status and smoking, cardiopulmonary mortality was associated with living within 50 meters of a major road (adjusted RR=1.70; 95% CI=1.02 – 2.81) and with average NO₂ exposure during the first year of follow-up (adjusted RR per 8.5 ppb =1.57; 1.23 – 2.00). The effect was stronger when the 5-year average NO₂ was used as the exposure metric (adjusted RR=1.74; 95% CI= 1.29 – 2.33). Exposure to NO₂ was also associated with all-cause mortality.

7.3.5.1 Summary

The Harvard Six Cities study provides some evidence from the United States that there is an association between both all-cause and cardiopulmonary mortality and long-term NO₂ exposures measured at regional monitors. However, the ACS (American Cancer Society) study failed to find any NO₂ effect. Recent studies from Europe, which examined local long-term NO₂ concentrations, suggested an increased risk of all-cause mortality. It is very difficult to disentangle the relevance of NO₂ alone from NO₂ as a marker for traffic-related air pollution.

7.3.6 Indoor Studies

Indoor NO₂ levels in dwellings without indoor combustion sources are mainly due to outdoor concentrations (and distances from buildings to the roadways). However, indoor unventilated combustion sources (natural gas or propane stoves, kerosene heaters) can contribute to high indoor NO₂ levels. Exposure to high levels of NO₂ has also been noted in other specific environments, such as indoor ice skating arenas. Outbreaks of acute respiratory illness among ice hockey players and spectators have followed exposure to emissions from gas-fuelled ice-resurfacing machines (Pelham et al. 2002). NO₂ evolving from crops stored in closed silos can reach very high concentrations near the surface of the silage, thus producing the severe "silo-filler's" disease among exposed farmers (Leavey et al. 2004), although most farms currently use open silos, which do not put farmers at risk.

Several epidemiological investigations have been conducted in indoor settings, and the results are controversial (Table 16). Hasselblad et al. (1992) conducted a meta-analysis of indoor studies, and found an increased incidence of lower respiratory symptoms among children exposed to indoor NO₂. The investigators concluded that long-term exposure to NO₂ was associated with a higher prevalence of

respiratory symptoms in children younger than 12 years of age (OR=1.2, 95% CI: 1.1–1.3, for an increase in NO₂ exposure of 30 µg/m³). This meta-analysis, however, relied on a limited number of heterogeneous studies.

In a study of a birth cohort in Albuquerque, New Mexico that used passive samplers in the infants' bedrooms to measure indoor NO₂ exposure, indoor NO₂ measurements were not associated with respiratory illness incidence during the first 18 months of life (Samet et al. 1993). However, levels of NO₂ were very low (median around 10 ppb) in this cohort. A multicenter cohort study including newborns conducted in Europe failed to find an association between indoor NO₂ and cumulative rates of infection (Sunyer et al. 2004).

In contrast, some recent investigations have suggested specific effects of NO₂ among children with asthma or at risk for asthma because of family history. Chauhan et al. (2003) followed a cohort of asthmatic children in Great Britain and found that personal exposure to NO₂ was associated with more severe illness and an increased risk of virus-related asthma morbidity. In a birth cohort of newborns in New England having an asthmatic sibling, measured indoor NO₂ levels mainly from gas stoves were associated with an increased risk for wheeze and cough in the first year of life (Belanger et al. 2003). In the same cohort a strong association between indoor NO₂ measured with passive samplers and respiratory symptoms among the infants with an asthmatic sibling has been recently reported (van Strien et al. 2004). Infants living in homes with an NO₂ concentration exceeding 17.4 ppb (highest quartile) had a higher frequency of days with wheeze (RR =2.2; 95% CI =1.4 –3.4), persistent cough (1.8; 1.2–2.7), and shortness of breath (3.1; 1.8–5.6) when compared with infants in homes that had NO₂ concentrations lower than 5.1 ppb (lowest quartile), controlling for nitrous acid concentration. Nitrous acid exposure was not independently associated with respiratory symptoms in this study. No effect modification was observed by season, as the results were unchanged after stratifying by season. Investigators in the New England study also found associations between respiratory symptoms and NO₂ exposures dichotomized at or above 20 ppb in the older siblings with asthma (Belanger et al. (2006). Of note, the NO₂ measurements were made in the main living area, and interestingly, the effect was seen primarily in children living in multi-family dwellings. The authors theorized that there was less exposure measurement error for these housing units compared to those living in single-family dwellings.

In contrast to these findings, a recent British study (Jarvis et al. 2005) of 276 adults that measured indoor levels of both nitrous acid and NO₂ (correlation coefficient=0.77), found that an increase of 1 ppb in indoor nitrous acid was associated with a decrease in forced expiratory volume in one second (FEV₁) percentage, but no significant association of indoor NO₂ and lung function was observed. After adjusting for NO₂ measures, the association between nitrous acid and low lung function persisted. The cut-off point for the highest quartile of NO₂ exposure was 21.8 ppb.

Triche et al. (2005) studied non-smoking mothers of infants in Connecticut and Virginia. Continuous NO₂ concentrations, measured with Palmes tubes, were not associated with any respiratory symptoms in those women. However, NO₂ exposures of 80 ppb or greater (top quartile for gas heater users) were associated with chest tightness (RR=1.94; 95% CI=0.98-3.85) and wheezing (4.00; 1.45-11.0).

In Victoria, Australia, Garrett et al. (1998) measured indoor NO₂ levels in 80 homes using passive samplers in the bedrooms, living rooms, and kitchens of 148 children, 53 of whom were asthmatic. Respiratory symptoms were more common in children exposed to gas stoves (OR=2.3, 95% CI=1.0-5.2). A dose-response was evident between bedroom NO₂ levels and respiratory symptoms, with an OR equal to 3.62 (95% CI=1.08-12.08) for bedroom levels > 10.6 ppb compared to bedroom levels < 5.3 ppb. The risk of respiratory symptoms from gas stove exposure remained significant after adjusting for bedroom NO₂ concentrations.

Another Australian group (Pilotto et al. 2004) conducted a study of unflued gas heater replacement in a group of 118 school children with asthma. Eighteen schools using unflued gas heating in the winter were randomly allocated to either retain the heaters (10 control schools) or have replacement flued gas or electric heaters installed at the beginning of the winter (8 intervention schools). Difficulty breathing during the day (RR=0.41, 95% CI=0.07-0.98) and night (RR=0.32, 95% CI=0.14-0.69), chest tightness during the day (RR=0.45, 95% CI=0.25-0.81), and daytime asthma attacks (RR=0.39, 95% CI=0.17-0.93) were significantly reduced in the intervention group. Mean (standard deviation) NO₂ levels were 15.5 (6.6) ppb and 47.0 (26.8) ppb in the intervention and control schools, respectively.

Finally, researchers in Helsinki, Finland (Mukala et al. 2000) studied prevalence of cough in preschool children. They measured NO₂ exposure, as weekly averages, by placing personal passive-diffusion samplers (i.e., Palmes tubes) on the children's garments and inside and outside the day care centers. In addition, ambient air NO₂ concentrations were obtained from two nearby monitoring sites. The risk of

cough in the winter increased significantly (adjusted RR=3.63, 95% CI=1.41 – 9.30) in the highest personal NO₂ exposure category ($\geq 27.2 \mu\text{g}/\text{m}^3$ (14.4 ppb)) and in the middle category (16.2-27.2 $\mu\text{g}/\text{m}^3$) (RR=2.77 (1.19 – 6.42)) compared with the lowest category (<16.2 $\mu\text{g}/\text{m}^3$ (8.6 ppb)). Since the use of gas stoves and appliances is uncommon in Finland the personal measurements probably reflected exposure to ambient NO₂, which may have been measured most accurately by the Palmes tubes worn by the children. However, ambient NO₂ exposures most likely came from traffic-related emissions from motor vehicles. Since other pollutants were not measured, it was impossible to separate the effects of NO₂ from particulates or other gases.

The indoor air studies provide evidence that increased levels of NO₂ from gas stoves or other appliances are associated with respiratory symptoms. However, as discussed in Chapter 5 operations of indoor combustion sources tend to have very high peak exposures (up to 400-1000 ppb). Therefore, it is difficult to ascertain whether the effect is due to high peak exposures or the averaged concentrations. Thus, it is difficult to extrapolate the findings in indoor air studies to ambient outdoor situations, limiting the use of these data in determining a long-term average for standard setting.

7.3.6.1 Summary

Among infants with asthma or at risk for developing asthma, the frequency of reported respiratory symptoms in the first year of life is associated with NO₂ concentrations above 17.4 ppb. In general, the effect has not been detected among infants who were not at risk for asthma. Indoor studies of NO₂ exposure, however, may be potentially confounded by the effect of PM, since burning natural gas in gas stoves or cooking produces fine and ultrafine particles, in addition to NO₂. The effects of NO₂ on respiratory symptoms and lung function may also be partly explained by nitrous acid, which is produced by gas appliances and has been highly correlated with NO₂ levels.

7.4 Summary and Conclusions

The strongest epidemiological evidence of an effect of NO₂ on human health came from the studies of respiratory disease, including daily time-series studies measuring short-term exposure, panel studies and long-term exposure studies. The time series studies evaluating the relationship between hospital admission or ED visits and asthma in children were remarkably consistent and robust for NO₂. The associations with NO₂ often remained after the inclusion of measures of particulate matter in the regression models. Several asthma panel studies, including some from California, showed an effect of NO₂ on symptoms, medication use, and lung function. The effects of long-term exposure to NO₂ as an individual pollutant or as a marker of traffic related pollutants have been demonstrated in the Children's Health Study in Southern California. The finding of reduced lung function growth in children exposed to higher levels of NO₂ over an eight-year period is especially important, since it is a risk factor for chronic diseases and premature mortality later in life. These respiratory health effects have been observed in areas with average NO₂ levels currently experienced in some areas of California.

Although the findings for respiratory disease were the most robust, NO₂ exposure has also been associated with other diseases, particularly in susceptible subgroups such as infants, children, the elderly, and those with underlying cardiovascular disease. For example, there is growing evidence that NO₂ or other traffic-related pollutants are related to an exacerbation of symptoms in individuals with underlying cardiovascular disease and is a risk factor for adverse perinatal effects such as low birth weight and preterm birth. Thus, the NO₂ standard must be sufficiently stringent to protect these susceptible subgroups.

Health risks from nitrogen oxides may potentially result from NO₂ itself, from co-pollutants such as ultrafines or elemental carbon, or its reaction products, including ozone and secondary particles. Additionally, NO₂ concentrations often follow vehicle emissions so that NO₂ levels are generally a reasonable marker of exposure to traffic-related emissions. Finally, NO₂ may augment the effects of other pollutants. In some studies, especially those conducted in Europe, the strongest effects were found for NO₂, whereas PM had a weaker effect.

On the basis of exposure studies that measure traffic pollutants, it is likely that NO₂ is a better marker of local traffic than particulate matter, measured as either PM₁₀ or PM_{2.5}. However, NO₂ is highly correlated with several pollutants that traditionally have not been measured in epidemiologic studies, including black carbon, ultrafines, and PAHs. NO₂ may also be a better surrogate for ultrafine particles (Seaton 2003) in Europe than in the North America because of different combustion sources (e.g., more diesel vehicles in Europe). In many of the studies described in this chapter it was difficult to distinguish the effects of NO₂ from other traffic-related pollutants due to high correlations with measured pollutants or

unmeasured pollutants. Therefore, it is prudent to regulate the level of NO₂ in California, since many of the other traffic related pollutants are not currently regulated

7.5 References

- Anderson HR, Spix C, Medina S et al. Air pollution and daily admissions for chronic obstructive pulmonary disease in 6 European cities: results from the APHEA project. *Eur Respir J*. 1997; 10:1064-1071.
- Anderson HR, Ponce de Leon A, Bland JM, Bower JS, Emberlin J, Strachan DP. Air pollution, pollens, and daily admissions for asthma in London 1987-92. *Thorax* 1998; 53: 842-848.
- Anderson HR, Bremner SA, Atkinson RW, Harrison RM, Walters S. Particulate matter and daily mortality and hospital admissions in the west midlands conurbation of the United Kingdom: associations with fine and coarse particles, black smoke and sulphate. *Occup Environ Med* 2001; 58: 504-510
- Atkinson RW, Bremner SA, Anderson HR, Strachan DP, Bland JM, Ponce De Leon A. Short-term associations between emergency hospital admissions for respiratory and cardiovascular disease and outdoor air pollution in London. *Archives of environmental health* 1999a;54:398-411.
- Atkinson RW, Anderson HR, Strachan DP, Bland JM, Bremner SA, Ponce de Leon A. Short-term associations between outdoor air pollution and visits to accident and emergency departments in London for respiratory complaints. *Eur Respir J*. 1999b;13:257-265.
- Atkinson, R. W., Anderson, H. R., Sunyer, J., Ayres, J., Baccini, M., Vonk, J. M., Boumghar, A., Forastiere, F., Forsberg, B., Touloumi, G., Schwartz, J., and Katsouyanni, K. 2001. Acute effects of particulate air pollution on respiratory admissions: results from APHEA 2 project. *Air Pollution and Health: a European Approach. Am J Respir Crit Care Med* 164: 1860-1866.
- Avol EL, Gauderman WJ, Tan SM, London SJ, Peters JM. Respiratory effects of relocating to areas of differing air pollution levels. *Am J Respir Crit Care Med* 2001;164: 2067-2072.
- Barnett AG, Williams GM, Schwartz J, Neller AH, Best TL, Petroeschovsky AL, Simpson RW. Air pollution and child respiratory health: a case-crossover study in Australia and New Zealand. *Am J Respir Crit Care Med*. 2005 Jun 1;171(11):1272-8. Epub 2005 Mar 11.
- Belanger K, Beckett W, Triche E, Bracken MB, Holford T, Ren P et al. Symptoms of wheeze and persistent cough in the first year of life: associations with indoor allergens, air contaminants, and maternal history of asthma. *Am J Epidemiol* 2003;158:195-202.
- Belanger K, Gent JF, Triche EW, Bracken MB, Leaderer BP. Association of Indoor Nitrogen Dioxide Exposure with Respiratory Symptoms in Asthmatic Children. *Am J Respir Crit Care Med* 2006; 173:297-303.
- Biggeri A, Bellini P, Terracini B; Italian MISA Group. [Meta-analysis of the Italian studies on short-term effects of air pollution] *Epidemiol Prev*. 2001 Mar-Apr;25(2 Suppl):1-71.
- Bobak M, Leon DA. The effect of air pollution on infant mortality appears specific for respiratory causes in the postneonatal period. *Epidemiology* 1999;10:666-70.
- Bobak M, Leon DA. Pregnancy outcomes and outdoor air pollution: an ecological study in districts of the Czech Republic 1986-8. *Occup Environ Med* 1999;56:539-543.
- Boezen HM, van der Zee SC, Postma DS, Vonk JM, Gerritsen J, Hoek G, Brunekreef B, Rijcken B, Schouten JP. Effects of ambient air pollution on upper and lower respiratory symptoms and peak expiratory flow in children. *Lancet* 1999;353(9156):874-878.
- Borja-Aburto VH, Castillejos M, Gold DR, Bierzwinski S, Loomis D. Mortality and ambient fine particles in southwest Mexico City, 1993-1995. *Environ Health Perspect* 1998;106:849-855.
- Braga AL, Saldiva PH, Pereira LA, Menezes JJ, Conceicao GM, Lin CA, Zanobetti A, Schwartz J, Dockery DW. Health effects of air pollution exposure on children and adolescents in Sao Paulo, Brazil. *Pediatr Pulmonol* 2001;31:106-113

- Brauer M, Hoek G, van Vliet P, et al. Air pollution from traffic and the development of respiratory infections and asthmatic and allergic symptoms in children. *Am J Respir Crit Care Med*. 2002;166: 1092-1098.
- Braun-Fahrlander C, Vuille JC, Sennhauser FH, et al. Respiratory health and long-term exposure to air pollutants in Swiss schoolchildren. SCARPOL Team. Swiss Study on Childhood Allergy and Respiratory Symptoms with Respect to Air Pollution, Climate and Pollen. *Am J Respir Crit Care Med* 1997;155:1042-1049.
- Buchdahl R, Parker A, Stebbings T, Babiker A. Association between air pollution and acute childhood wheezy episodes: prospective observational study. *BMJ* 1996;312(7032):661-5.
- Burnett RT, Cakmak S, Brook JR, Krewski D. The role of particulate size and chemistry in the association between summertime ambient air pollution and hospitalization for cardiorespiratory diseases. *Environ Health Perspect* 1997;105:614-20.
- Burnett RT, Cakmak S, Brook JR. The effect of the urban ambient air pollution mix on daily mortality rates in 11 Canadian cities. *Can J Publ Health* 1998;89:152-156.
- Burnett RT, Smith-Doiron M, Stieb D, Cakmak S, Brook JR. Effects of particulate and gaseous air pollution on cardiorespiratory hospitalizations. *Arch Environ Health* 1999;54:130-9.
- Carr D, von Ehrenstein O, Weiland S et al. Modeling annual benzene, toluene, NO₂, and soot concentrations on the basis of road traffic characteristics. *Environ Res* 2002;90:111-118.
- Chauhan AJ, Inskip HM, Linaker CH, Smith S, Schreiber J, Johnston SL et al. Personal exposure to nitrogen dioxide (NO₂) and the severity of virus-induced asthma in children. *Lancet* 2003;61:1939-1944.
- Dales R, Burnett RT, Smith-Doiron M, Stieb DM, Brook JR. Air Pollution and Sudden Infant Death Syndrome. *Pediatrics* 2004;113:e628-e631.
- Delfino RJ, Zeiger RS, Seltzer JM, Street DH, McLaren CE. Association of asthma symptoms with peak particulate air pollution and effect modification by anti-inflammatory medication use. *Environ Health Perspect* 2002;110:A607-17.
- Delfino RJ, Gong H Jr, Linn WS, Pellizzari ED, Hu Y. Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. *Environ Health Perspect* 2003;111:647-656.
- Delfino RJ, Quintana PJE, Floro J, Gastañaga VM, Samimi BS, Kleinman MT, Liu L-JS, Bufalino C, Wu C-F, McLaren CE. Association of FEV₁ in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. *Environ Health Perspect*. 2004; 112: 932-41.
- D'Ippoliti D, Forastiere F, Ancona C, Agabiti N, Fusco D, Michelozzi P, Perucci CA. Air pollution and myocardial infarction in Rome: a case-crossover analysis. *Epidemiology* 2003;14:528-35.
- Dockery DW, Speizer FE, Stram DO, Ware JH, Spengler JD, Ferris BG. Effects of inhalable particles on respiratory health of children. *Am Rev Respir Dis* 1989;139:587-594.
- Dockery DW, Pope AC, Xu X, et al. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993;329:1753-1759.
- Dockery DW, Luttmann-Gibson H, Rich DQ, Link MS, Mittleman MA, Gold DR, Koutrakis P, Schwartz JD, Verrier RL. Association of air pollution with increased incidence of ventricular tachyarrhythmias recorded by implanted cardioverter defibrillators. *Environ. Health. Perspect*. 2005; 113: 670-674.
- Dominici F, McDermott A, Daniels M, Zeger SL, Samet JM. 2003. Mortality among residents of 90 cities. Health Effects Institute. Revised analyses of time-series studies of air pollution and health. Special Report. Capital City Press, Montpelier VT.
- Emenius G, Pershagen G, Berglind N et al. NO₂, as a marker of air pollution, and recurrent wheezing in children: a nested case-control study within the BAMSE birth cohort. *Occup Environ Med* 2003;60:876-881.
- Fairley D. Daily mortality and air pollution in Santa Clara County, California: 1989-1996. *Environ Health Perspect* 1999;107:637-641
- Feychting M, Svensson D, Ahlbom A. Exposure to motor vehicle exhaust and childhood cancer. *Scand J Work Environ Health* 1998;24:8-11.

Forastiere F, Forsberg B, Touloumi G, Schwartz J, Katsouyanni K. Acute effects of particulate air pollution on respiratory admissions: results from APHEA 2 project. *Air Pollution and Health: a European Approach. Am J Respir Crit Care Med* 2001;164:1860-1866.

Frischer T, Studnicka M, Gartner C., et al. Lung function growth and ambient ozone: a three-year population study in school children. *Am J Respir Crit Care Med* 1999;160:390-396.

Fusco D, Forastiere F, Michelozzi P, Spadea T, Ostro B, Arca M, Perucci CA. Air pollution and hospital admissions for respiratory conditions in Rome, Italy. *Eur Respir J.* 2001;17:1143-1150.

Galan I, Tobias A, Banegas JR, Aránguez E. Short-term effects of air pollution on daily asthma emergency room admissions. *Eur Respir J.* 2003; 22: 802-08.

Garrett MH, Hooper MA, Hooper BM, Abramson MJ. Respiratory symptoms in children and indoor exposure to nitrogen dioxide and gas stoves. *Am J Respir Crit Care Med* 1998; 158:891-5.

Gauderman WJ, McConnell R, Gilliland F, London S, Thomas D, Avol E, Vora H, Berhane K, Rappaport EB, Lurmann F, Margolis HG, Peters J. Association between air pollution and lung function growth in southern California children. *Am J Respir Crit Care Med* 2000; 162: 1383-1390.

Gauderman WJ, Gilliland F, Vora H, Avol E, Stram D, McConnell R, Thomas D, Lurmann F, Margolis HG, Rappaport EB, Berhane K, Peters J. Association between air pollution and lung function growth in southern California children. Results from a second cohort. *Am J Respir Crit Care Med* 2002; 166: 76-84.

Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters JM. The effect of air pollution on lung development from 10 to 18 years of age. *N.Engl.J Med.* 2004; 351 (11):1057-1067, 2004.

Gauderman WJ, Avol E, Lurmann F, Kuenzli N, Gilliland F, Peters J, McConnell R. Childhood asthma and exposure to traffic and nitrogen dioxide. *Epidemiology* 2005; 16:737-43.

Gehring U, Cyrys J, Sedlmeir G, et al. Traffic-related air pollution and respiratory health during the first 2 yrs of life. *Eur.Respir.J.* 2002; 19: 690-698.

Gehring U, Heinrich J, Kramer U, et al. Long-term exposure to ambient air pollution and cardiopulmonary mortality in women. *Epidemiology* 2006;17:545-51.

Gouveia N, Fletcher T. Respiratory diseases in children and outdoor air pollution in Sao Paulo, Brazil: a time series analysis. *Occup environ med* 2000 ; 57: 477- 483 .

Gwynn RC, Burnett RT, Thurston GD. A time-series analysis of acidic particulate matter and daily mortality and morbidity in the Buffalo, New York, Region. *Environ Health Perspect* 2000;108:125-133

Ha EH, Hong YC, Lee BE, Woo BH, Schwartz J, Christiani DC. Is air pollution a risk factor for low birth weight in Seoul? *Epidemiology* 2001; 12: 643-648.

Ha EH, Lee JT, Kim H, Hong YC, Lee BE, Park HS, Christiani DC. 2003. Infant susceptibility of mortality to air pollution in Seoul, South Korea. *Pediatrics* 2003; 111:284-90.

Hajat S, Haines A, Goubet SA, Atkinson RW, Anderson HR. Association of air pollution with daily GP consultations for asthma and other lower respiratory conditions in London. *Thorax.* 1999;54 (7):597-605.

Hasselblad V, Eddy DM, Kotchmar DJ. Synthesis of environmental evidence: nitrogen dioxide epidemiology studies. *J Air Waste Manage Assoc.* 1992; 42: 662-71. 9;54:597-605.

Hirsch T, Weiland SK, von Mutius E, et al. Inner city air pollution and respiratory health and atopy in children. *Eur.Respir.J.* 1999; 14: 669-677.

Hoek G, Brunekreef B, Verhoeff A, van Wijnen J, Fischer P. Daily mortality and air pollution in the Netherlands. *J Air Waste Manage Assoc* 2000; 50:1380-1389

Hoek G, Brunekreef B, Goldbohm S, Fischer P, van den Brandt PA. Association between mortality and indicators of traffic-related air pollution in the Netherlands: a cohort study. *Lancet* 2002; 360: 1203-1209.

Hong YC, Leem JH, Ha EH, Christiani DC. PM10 exposure, gaseous pollutants, and daily mortality in Incheon, South Korea. *Environ Health Perspect* 1999;107:873-878

Hong YC, Lee JT, Kim H, Ha EH, Schwartz J, Christiani DC. Effects of air pollutants on acute stroke mortality. *Environ Health Perspect* 2002;110:187-191.

- Jalaludin BB, Chey T, O'Toole BI, Smith WT, Capon AG, Leeder SR. Acute effects of low levels of ambient ozone on peak expiratory flow rate in a cohort of Australian children. *Int J Epidemiol*. 2000;29:549-57.
- Janssen NA, Brunekreef B, van Vliet P, et al. The relationship between air pollution from heavy traffic and allergic sensitization, bronchial hyperresponsiveness, and respiratory symptoms in Dutch schoolchildren. *Environ Health Perspect* 2003; 111: 1512-1518.
- Jarvis DL, Leaderer BP, Chinn S, Burney PG. Indoor nitrous acid and respiratory symptoms and lung function in adults. *Thorax* 2005; 60:474-9.
- Just J, Segala C, Sahraoui F, Priol G, Grimfeld A, Neukirch F. Short-term health effects of particulate and photochemical air pollution in asthmatic children. *Eur Respir J* 2002; 20:899-906.
- Katsouyanni K, Schwartz J, Spix C, Touloumi G, Zmirou D, Zanobetti A, Wojtyniak B, Vonk JM, Tobias A, Ponka A, Medina S, Bacharova L, Anderson HR. Short term effects of air pollution on health: a European approach using epidemiologic time series data: the APHEA protocol. *J Epidemiol Community Health*. 1996;50: S12-8.
- Katsouyanni K, Touloumi G, Samoli E, Gryparis A, Le Tertre A, Monopolis Y, Rossi G, Zmirou D, Ballester F, Boumghar A, Anderson HR, Wojtyniak B, Paldy A, Braunstein R, Pekkanen J, Schindler C, Schwartz J. Confounding and effect modification in the short-term effects of ambient particles on total mortality: results from 29 European cities within the APHEA2 project. *Epidemiology*. 2001; 12: 521-31.
- Kelsall JE, Samet JM, Zeger SL, Xu J. Air pollution and mortality in Philadelphia, 1974-1988. *Am J Epidemiol*. 1997;146:750-62.
- Koken PJ, Piver WT, Ye F, Elixhauser A, Olsen LM, Portier CJ. Temperature, air pollution, and hospitalization for cardiovascular diseases among elderly people in Denver. *Environ Health Perspect* 2003;111:1312-7.
- Kramer U, Koch T, Ranft U, Ring J, Behrendt H. Traffic-related air pollution is associated with atopy in children living in urban areas. *Epidemiology* 2000; 11: 64-70.
- Krewski D, Burnett RT, Goldberg MS, Hoover K, Siemiatycki J, Jerrett M, Abrahamowicz M, White WH, and others; Reanalysis of the Harvard Six Cities Study and the American Cancer Society study of particulate air pollution and mortality. Health Effects Institute. July 2000.
- Kwon HJ, Cho SH, Nyberg F, Pershagen G. Effects of ambient air pollution on daily mortality in a cohort of patients with congestive heart failure. *Epidemiology* 2001;12:413-419
- Lagorio S, Forastiere F, Pistelli R, et al. Air pollution and lung function among susceptible adult subjects: a panel study. *Environmental Health: A Global Access Science Source* 2006;5:<http://www.ehjournal.net/contents/5/1/11>.
- Leavey JF, Dubin RL, Singh N, Kaminsky DA. Silo-Filler's disease, the acute respiratory distress syndrome, and oxides of nitrogen. *Ann Intern Med* 2004; 141:410-1.
- Le Tertre A, Quenel P, Eilstein D, Medina S, Prouvost H, Pascal L, Boumghar A, Saviuc P, Zeghnoun A, Filleul L, Declercq C, Cassadou S, Le Goaster C. Short-term effects of air pollution on mortality in nine French cities: a quantitative summary. *Arch Environ Health*. 2002; 57: 311-9.
- Lee JT, Kim H, Song H, Hong YC, Cho YS, Shin SY, Hyun YJ, Kim YS. Air pollution and asthma among children in Seoul, Korea. *Epidemiology* 2002; 13: 481-4.
- Lee SL, Wong WH, Lau YL. Association between air pollution and asthma admission among children in Hong Kong. *Clin Exp Allergy* 2006;36:1138-46.
- Lin M, Chen Y, Burnett RT, Villeneuve PJ, Krewski D. The influence of ambient coarse particulate matter on asthma hospitalization in children: case-crossover and time-series analyses. *Environ Health Perspect* 2002; 110: 575-81.
- Lin M, Chen Y, Burnett RT, Villeneuve PJ, Krewski D. Effect of short-term exposure to gaseous pollution on asthma hospitalisation in children: a bi-directional case-crossover analysis. *J Epidemiol Community Health* 2003; 57(1): 50-5.
- Lin CM, Li CY, Yang GY, Mao IF. Association between maternal exposure to elevated ambient sulphur dioxide during pregnancy and term low birth weight. *Environ Res* 2004; 96: 41-50.

Linaker CH, Coggon D, Holgate ST, Clough J, Josephs L, Chauhan AJ, Inskip HM. Personal exposure to nitrogen dioxide and risk of airflow obstruction in asthmatic children with upper respiratory infection. *Thorax*. 2000; 55: 930-3.

Lippmann M, Ito K, Nadas A, Burnett RT. Association of particulate matter components with daily mortality and morbidity in urban populations. HEI Health Effects Institute, Cambridge Massachusetts 2000; 95: 1-86. <http://www.healtheffects.org>

Liu S, Krewski D, Shi Y, Chen Y, Burnett RT. Association between gaseous ambient air pollutants and adverse pregnancy outcomes in Vancouver, Canada. *Environ Health Perspect* 2003; 111: 1773-1778.

Liu S, Krewski D, Shi Y, et al. Association between maternal exposure to ambient air pollutants during pregnancy and fetal growth restriction. *J Expo Sci Environ Epidemiol* 2006.

Loomis D, Castillejos M, Gold DR, McDonnell W, Borja-Aburto VH. Air pollution and infant mortality in Mexico City. *Epidemiology* 1999; 10: 118-123.

Mann JK, Tager IB, Lurmann F, Segal M, Quesenberry CP Jr, Lugg MM, Shan J, Van Den Eeden SK. Air pollution and hospital admissions for ischemic heart disease in persons with congestive heart failure or arrhythmia. *Environ Health Perspect* 2002; 110: 1247-52.

Mar TF, Norris GA, Koenig JQ, Larson TV. Associations between air pollution and mortality in Phoenix, 1995-1997. *Environ Health Perspect* 2000;108:347-353

McConnell R, Berhane K, Gilliland F, et al. Air pollution and bronchitic symptoms in Southern California children with asthma. *Environ Health Perspect* 1999; 107: 757-760.

McConnell R, Berhane K, Gilliland F, et al. Prospective Study of Air Pollution and Bronchitic Symptoms in Children with Asthma. *Am. J. Respir. Crit. Care Med.* 2003; 168: 790-797.

Medina S, Le Tertre A, Quenel P, Le Moulllec Y, Lameloise P, Guzzo JC, Festy B, Ferry R, Dab W. Air pollution and doctors' house calls: results from the ERPURS system for monitoring the effects of air pollution on public health in Greater Paris, France, 1991-1995. *Evaluation des Risques de la Pollution Urbaine pour la Sante. Environ Res* 1997; 75: 73-84.

Metzger KB, Tolbert PE, Klein M, Peel JL, Flanders WD, Todd K, Mulholland JA, Ryan PB, Frumkin H. Ambient air pollution and cardiovascular emergency department visits. *Epidemiology* 2004; 15: 46-56.

Michelozzi P, Forastiere F, Fusco D, Perucci CA, Ostro B, Ancona C, Pallotti G. Air pollution and daily mortality in Rome, Italy. *J Occup Environ Med* 1998;55:605-610

Molfino NA, Wright SC, Katz I, et al. Effect of low concentrations of ozone on inhaled allergen responses in asthmatic subjects. *Lancet* 1991; 338: 199-203.

Moolgavkar SH. Air pollution and daily mortality in two U.S. counties: season-specific analyses and exposure-response relationships. *Inhal Toxicol* 2003; 15:877-907.

Moolgavkar SH. Air pollution and daily deaths and hospital admissions in Los Angeles and Cook counties. Health Effects Institute. Revised analyses of time-series studies of air pollution and health. Special Report. 2003. Capital City Press, Montpelier VT.

Morgan G, Corbett S, Wlodarczyk J, Lewis P. Air pollution and daily mortality in Sydney, Australia, 1989 through 1993. *Am J Public Health*. 1998a;88:759-64.

Morgan G, Corbett S, Wlodarczyk J. Air pollution and hospital admissions in Sidney, Australia, 1990 to 1994. *Am J Public Health* 1998b; 88:1761-6.

Morris RD, Naumova EN, Munasinghe RL. Ambient air pollution and hospitalization for congestive heart failure among elderly people in seven large US cities. *Am J Public Health* 1995; 85: 1361-5.

Mortimer KM, Neas LM, Dockery DW, Redline S, Tager IB. The effect of air pollution on inner-city children with asthma. *Eur Respir J* 2002; 19(4): 699-705.

Moshhammer H, Hutter HP, Hauck H, Neuberger M. Low levels of air pollution induce changes of lung function in a panel of school children. *European Respiratory Journal Express*. 2006; Published online February 2, 2006.

Mukala K, Alm S, Tiittanen P, Salonen RO, Jantunen M, Pekkanen J. Nitrogen dioxide exposure assessment and cough among preschool children. *Arch Environ Health* 2000; 55:431-8.

Nafstad P, Haheim LL, Wisloff T, et al. Urban air pollution and mortality in a cohort of Norwegian men. *Environ. Health Perspect.* 2004; 112: 610-615.

Nicolai T, Carr D, Weiland SK et al. Urban traffic and pollutant exposure related to respiratory outcomes and atopy in a large sample of children. *European Respiratory Journal* 2003; 21: 956-963.

Norris G, Young Pong SN, Koenig JQ, Larson TV, Sheppard L, Stout JW. An association between fine particles and asthma emergency department visits for children in Seattle. *Environ Health Perspect.* 1999; 107(6): 489-93.

Nyberg F, Gustavsson P, Jarup L, et al. Urban air pollution and lung cancer in Stockholm. *Epidemiology* 2000; 11: 487-495.

Oftedal B, Nafstad P, Magnus P, Bjorkly S, Skrondal A. Traffic related air pollution and acute hospital admission for respiratory diseases in Drammen, Norway 1995-2000. *Eur J Epidemiol.* 2003;18:671-5.

Ostro B, Sanchez JM, Aranda C, Eskeland GS. Air pollution and mortality: results from a study of Santiago, Chile. *J Expo Anal Environ Epidemiol.* 1996;6:97-114.

Ostro B, Lipsett M, Mann J, Braxton-Owens H, White M. Air pollution and exacerbation of asthma in African-American children in Los Angeles. *Epidemiology* 2001;12):200-8.

Peel JL, Tolbert PE, Klein M, Metzger KB, Flanders WD, Todd K, Mulholland JA,

Pekkanen J, Pearce N. Defining asthma in epidemiological studies. *Eur Respir J.* 1999; 14:951-7. Review.

Pekkanen J, Peters A, Hoek G, Tiittanen P, Brunekreef B, de Hartog J, et al. 2002. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the Exposure and Risk Assessment for Fine and Ultrafine Particles in Ambient Air (ULTRA) study. *Circulation* 106:933-938.

Pelham TW, Holt LE, Moss MA. Exposure to carbon monoxide and nitrogen dioxide in enclosed ice arenas. *Occup Environ Med* 2002; 59:224-33.

Peters A, Liu E, Verrier RL, et al. Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 2000; 11: 11-17.

Peters JM, Avol E, Gauderman WJ, et al. A study of twelve Southern California communities with differing levels and types of air pollution. II. Effects on pulmonary function. *Am J Respir Crit Care Med* 1999a; 159: 768-775.

Peters JM, Avol E, Navidi W, et al. A study of twelve Southern California communities with differing levels and types of air pollution. I. Prevalence of respiratory morbidity. *Am J Respir Crit Care Med* 1999b; 159: 760-767.

Petroeschovsky A, Simpson RW, Thalib L, Rutherford S. Associations between outdoor air pollution and hospital admissions in Brisbane, Australia. *Archives in environmental health* 2001; 56: 37 - 52.

Pilotto LS, Nitschke M, Smith BJ, Pisaniello D, Ruffin RE, McElroy HJ, Martin J, Hiller JE. Randomized controlled trial of unflued gas heater replacement on respiratory health of asthmatic schoolchildren. *Int J Epidemiol* 2004; 33:208-14.

Poloniecki JD, Atkinson RW, de Leon AP, Anderson HR. Daily time series for cardiovascular hospital admissions and previous day's air pollution in London, UK. *Occup Environ Med.* 1997 Aug; 54(8): 535-40.

Pope CA, III, Burnett RT, Thun MJ, et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 2002; 287: 1132-1141.

Prescott GJ, Cohen GR, Elton RA, Fowkes FG, Agius RM. Urban air pollution and cardiopulmonary ill health: a 14.5 year time series study. *Occup Environ Med* 1998;55:697-704.

Raaschou-Nielsen O, Hertel O, Thomsen BL, Olsen JH. Air pollution from traffic at the residence of children with cancer. *Am J Epidemiol* 2001; 153:433-43.

Rich DQ, Schwartz J, Mittleman MA, Link M, Luttmann-Gibson H, Catalano PJ, Speizer FE, Dockery DW. Association of short-term ambient air pollution concentrations and ventricular arrhythmias. *Am J Epidemiol.* 2005 Jun 15;161(12):1123-32.

- Roemer W, Hoek G, Brunekreef B, Haluszka J, Kalandidi A, Pekkanen J. Daily variations in air pollution and respiratory health in a multicentre study: the PEACE project. *Pollution Effects on Asthmatic Children in Europe. Eur Respir J* 1998;12:1354-61.
- Roemer WH, van Wijnen JH. Daily mortality and air pollution along busy streets in Amsterdam, 1987-1998. *Epidemiology* 2001;12:649-653
- Ryan PB, Frumkin H. Ambient air pollution and respiratory emergency department visits. *Epidemiology*. 2005; 16:164-74.
- Saez M, Ballester F, Barcelo MA, Perez-Hoyos S, Bellido J, Tenias JM, Ocana R, Fidueiras A, Arribas F, Aragonés N, Tobias A, Cirera L, Canada A., on behalf of the EMECAM group. A combined analysis of the short-term effects of photochemical air pollutants on mortality within the EMECAM project. *Environ Health Perspectives* 2002; 110:221-228
- Samet JM, Lambert E, Skipper BJ, et al. Nitrogen dioxide and respiratory illnesses in infants. *Am Rev Respir Dis* 1993; 148:1258-65.
- Samet JM, Dominici F, Curiero F, Coursac I, Zeger SL. Fine particulate air pollution and mortality in 20 U.S. cities 1987-1994. *N Engl J Med* 2000;343:1742-1749.
- Samoli E, Aga E, Touloumi G, Nisiotis K, Forsberg B, Lefranc A, Pekkanen J, Wojtyniak B, Schindler C, Niciu E, Brunstein R, Dodic Fikfak M, Schwartz J, Katsouyanni K. Short-term effects of nitrogen dioxide on mortality: an analysis within the APHEA project. *European Respiratory Journal Express*. 2006; Published online March 15, 2006.
- Sarnat JA, Schwartz J, Catalano PJ, Suh HH. Gaseous pollutants in particulate matter epidemiology: confounders or surrogates? *Environ Health Perspect* 2001; 109:1053-61.
- Sarnat JA, Brown KW, Schwartz J, Coull BA, Koutrakis P. 2005. Ambient gas concentrations and personal particulate matter exposures: implications for studying the health effects of particles. *Epidemiology* 2005; 16:385-95.
- Schildcrout JS, Sheppard L, Lumley T, Slaughter JC, Koenig JQ, Shapiro GG. Ambient air pollution and asthma exacerbations in children: an eight-city analysis. *Am J Epidemiol* 2006;164:505-17.
- Schindler C, Ackermann-Liebrich U, Leuenberger P, et al. Associations between lung function and estimated average exposure to NO₂ in eight areas of Switzerland. The SAPALDIA Team. Swiss Study of Air Pollution and Lung Diseases in Adults. *Epidemiology* 1998; 9: 405-411.
- Schwartz J. Air pollution and hospital admissions for cardiovascular disease in Tucson. *Epidemiology* 1997; 8: 371-7.
- Seaton A, Dennekamp M. Hypothesis: ill health associated with low concentrations of nitrogen dioxide--an effect of ultrafine particles? *Thorax*. 2003; 58: 1012-5.
- Segala C, Fauroux B, Just J, Pascual L, Grimfeld A, Neukirch F. Short-term effect of winter air pollution on respiratory health of asthmatic children in Paris. *Eur Respir J* 1998;11(3):677-85.
- Shima M, Adachi M. Effect of outdoor and indoor nitrogen dioxide on respiratory symptoms in schoolchildren. *Int J Epidemiol* 2000; 29: 862-870.
- Simpson RW, Williams G, Petroeschevsky A, Morgan G, Rutherford S. Associations between outdoor air pollution and daily mortality in Brisbane, Australia. *Arch Environ Health*. 1997;52:442-54.
- Simpson R, Williams G, Petroeschevsky A, Best T, Morgan G, Denison L, Hinwood A, Neville G, Neller A. The short-term effects of air pollution on daily mortality in four Australian cities. *Aust N Z J Public Health* 2005a; 29:205-12.
- Simpson R, Williams G, Petroeschevsky A, Best T, Morgan G, Denison L, Hinwood A, Neville G. The short-term effects of air pollution on hospital admissions in four Australian cities. *Aust N Z J Public Health* 2005b; 29:213-21.
- Spix C Anderson HR, Schwartz J, et al. Short-term effects of air pollution on hospital admissions of respiratory diseases in Europe: a quantitative summary of APHEA study results. *Arch Environ Health* 1998; 53:54-64.

- Stieb DM, Judek S, Burnett RT. Meta-analysis of time-series studies of air pollution and mortality: effects of gases and particles and the influence of cause of death, age, and season. *J Air Waste Manag Assoc.* 2002; 52: 470-84.
- Stieb DM, Judek S, Burnett RT. Meta-analysis of time-series studies of air pollution and mortality: update in relation to the use of generalized additive models. *J Air Waste Manag Assoc.* 2003; 53: 258-61.
- Studnicka M, Hackl E, Pischinger J, et al. Traffic-related NO₂ and the prevalence of asthma and respiratory symptoms in seven year olds. *Eur.Respir.J.* 1997; 10: 2275-2278.
- Sunyer J, Spix C, Quénel P, et al. Urban air pollution and emergency admissions for asthma in four European cities: the APHEA project. *Thorax* 1997; 52:760-765.
- Sunyer J, Basagana X. Particles, and not gases, are associated with the risk of death in patients with chronic obstructive pulmonary disease. *Int J Epidemiol* 2001;30:1138-1140
- Sunyer J, Puig C, Torrent M, Garcia-Algar O, Calico I, Munoz-Ortiz L, Barnes M, Cullinan P; Asthma Multicentre Infants Cohort Study. Nitrogen dioxide is not associated with respiratory infection during the first year of life. *Int J Epidemiol.* 2004 Feb;33(1):116-20.
- Tenias JM, Ballester F, Rivera ML. Association between hospital emergency visits for asthma and air pollution in Valencia, Spain. *Occup Environ Med* 1998;55:541-547.
- Thompson AJ, Shields MD, Patterson CC. Acute asthma exacerbations and air pollutants in children living in Belfast, Northern Ireland. *Arch Environ Health.* 2001; 56(3): 234-41.
- Timonen KL, Pekkanen J, Tiittanen P, Salonen RO. Effects of air pollution on changes in lung function induced by exercise in children with chronic respiratory symptoms. *Occup Environ Med* 2002;59:129-34
- Touloumi G, Katsouyanni K, Zmirou D, Schwartz J, Spix C, Ponka A, et al. Short-term effects of ambient oxidant exposure on mortality: a combined analysis within the APHEA project. *Air Pollution and Health: a European Approach.* *Am J Epidemiol.* 1997;146:177-85.
- Triche EW, Belanger K, Bracken MB, Beckett WS, Holford TR, Gent JF, McSharry JE, Leaderer BP. Indoor heating sources and respiratory symptoms in nonsmoking women. *Epidemiology* 2005; 16:377-84.
- Tsai SS, Goggins WB, Chiu HF, Yang CY. Evidence for an association between air pollution and daily stroke admissions in Kaohsiung, Taiwan. *Stroke.* 2003 Nov;34(11):2612-6. Epub 2003 Oct 09.
- van der Zee S, Hoek G, Boezen HM, Schouten JP, van Wijnen JH, Brunekreef B. Acute effects of urban air pollution on respiratory health of children with and without chronic respiratory symptoms. *Occup Environ Med* 1999; 56: 802-12.
- van Strien RT, Gent JF, Belanger K, Triche E, Bracken MB, Leaderer BP. Exposure to NO₂ and nitrous acid and respiratory symptoms in the first year of life. *Epidemiology.* 2004; 15: 471-8.
- Wellenius GA, Bateson TF, Mittleman MA, Schwartz J. Particulate air pollution and the rate of hospitalization for congestive heart failure among Medicare beneficiaries in Pittsburgh, Pennsylvania. *Am J Epidemiol.* 2005 Jun 1;161(11):1030-6
- WHO. "Health Aspects of Air Pollution with Particulate Matter, Ozone and Nitrogen Dioxide". Report from WHO Working Group Meeting, Bonn, 13 - 15 January 2003.
<http://www.euro.who.int/document/e79097.pdf>
- Wilhelm M, Ritz B. Residential proximity to traffic and adverse birth outcomes in Los Angeles county, California, 1994-1996. *Environ.Health Perspect.* 2003; 111: 207-216.
- Wong CM, Ma S, Hedley AJ, Lam TH. Effect of air pollution on daily mortality in Hong Kong. *Environ Health Perspect* 2001; 109: 335-340
- Wong TW, Lau TS, Yu TS, Neller A, Wong SL, Tam W, Pang SW. Air pollution and hospital admissions for respiratory and cardiovascular diseases in Hong Kong. *Occup Environ Med* 1999; 56:679-83.
- Wong GW, Ko FW, Lau TS, Li ST, Hui D, Pang SW, Leung R, Fok TF, Lai CK. Temporal relationship between air pollution and hospital admissions for asthmatic children in Hong Kong. *Clin Exp Allergy.* 2001; 31:565-9.
- Wong TW, Tam WS, Yu TS, Wong AHS. Associations between daily mortalities from respiratory and cardiovascular diseases and air pollution in Hong Kong, China. *Occup Environ Med* 2002; 59:30-35

Yang CY, Chen YS, Yang CH, Ho SC. Relationship between ambient air pollution and hospital admissions for cardiovascular diseases in Kaohsiung, Taiwan. *J Toxicol Environ Health A*. 2004;67:483-93.

Zhu Y, Hinds WC, Kim S, Shen S, Sioutas C.. Study of ultrafine particles near a major highway with heavy-duty diesel traffic. *Atmos Environ* 2002a; 36:4323-4335.

Zhu Y, Hinds WC, Kim S, Sioutas C. Concentration and size distribution of ultrafine particles near a major highway. *J Air Waste Manag Assoc* 2002b; 52:1032-42.

List of Tables

Table 1.	Time series studies of NO ₂ and daily mortality
Table 2.	Time series studies of NO ₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)
Table 3.	Time series studies of NO ₂ and hospital admissions (HA), emergency department admissions (ER), general practitioner visits (GP) for asthma in children
Table 4.	Panel studies: symptoms changes in relation to NO ₂ in asthmatic children and cardiac arrhythmias in adults
Table 5.	Panel studies: medication use changes in relation to NO ₂ in asthmatic children
Table 6.	Panel studies: lung function changes in relation to NO ₂ in asthmatic children
Table 7.	Outdoor community based studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies
Table 8.	Outdoor community based studies on long-term effects: asthma, respiratory disorders, and atopy in cohort studies
Table 9.	Outdoor within-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies
Table 10.	Outdoor within-community studies on long-term effects: asthma, respiratory disorders, and atopy in cohort studies
Table 11.	Outdoor community based studies on long-term effects: lung function in cross-sectional studies
Table 12.	Outdoor community based studies on long-term effects: lung function in cohort studies
Table 13.	Outdoor NO ₂ exposure and cancer risk
Table 14.	Outdoor NO ₂ exposure and fetal effects
Table 15.	Outdoor NO ₂ exposure and mortality risk
Table 16.	Indoor NO ₂ exposure and morbidity risk

7.6 Tables

Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Ostro (1996)	Santiago, Chile	1989-1991	All	0.9	(0.4, 1.7)	1	1h	55.6	Strong correlation with PM10 (0.73). No effect of NO ₂ after adjustment for PM10.
Kelsall (1997)	Philadelphia, PA, USA	1974-1988	All	0.3	(-0.7, 1.3)	0	24h	39.6	No NO ₂ effect in multipollutant models
Simpson (1997)	Brisbane, Australia	1987-1993	All	-11.5	(-47.3, 48.4)	0	24h	14.0	Nephelometry was used here for PM. Effect of particles and of ozone. NO ₂ 1-hr max correlation with bsp .508. NO ₂ 24-hr max correlation with bsp .515. No mention of effect of NO ₂ when bsp in model.
			CVD	-30.9	(-73.0, 77.0)	0			
			Resp.	-63.3	(-94.1, 129.6)	0			
			All	7.2	(-19.2, 42.2)	0	1h	28.4	
			CVD	-9.2	(-43.2, 45.4)	0			
			Resp.	27.1	(-48.0, 210.7)	0			

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Toulomi (1997)	Six APHEA cities, Europe	1977-1992	All	1.2	(0.8, 1.6)	n/a	1h	37.2-71.3	0.6% (0-1.2%) change for unit=26.5ppb when adjusting for BS
Borja Aburto (1998)	Mexico City, Mexico	1993-1995	All	2.8	(-1.1, 6.8)	1-5	24h	37.7	Strong correlation with PM _{2.5} (0.71). No effect of NO ₂ in two pollutant models
			CVD	3.4	(-3.7, 11.1)				
			Resp.	5.7	(-5.8, 18.6)				
Burnett (1998)	11 cities, Canada	1981-1991	All	4.2	(2.1, 6.4)	varied	24h	23.5	Small changes in multipollutant models. City specific estimates for NO ₂ were independent from PM _{2.5} city mean
Michelozzi (1998)	Rome, Italy	1992-1995	All	2.0	(0.5, 3.5)	2	24h	52.6	Larger effect in the centre of the city. Adjustment for PM reduced to non significance the NO ₂ effect estimate.

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Morgan (1998a)	Sidney, Australia	1989-1993	All	3.6	(0.1, 7.2)	0-1	24h	13	In three pollutant model: all causes 0.41(-2.47-3.38%) CVD 0.9% (-5.2-7.44%) Resp. 7.6% (-2.9-18.2%) for 9.5 ppb change
			CVD	3.1	(-1.1, 7.5)	1			
			Resp.	10.4	(-0.4, 22.4)	0-1			
			All	1.1	(-0.6, 2.9)	0-1	1h	26	
			CVD	0.7	(-1.2, 2.6)	1			
			Resp.	3.4	(-1.3, 8.4)	1			

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Fairley (1999)	Santa Clara County, USA	1989-1996	All	2.6	p>.05	1	24h	28	No NO ₂ effect when adjusting for PM. Main analyses for nitrate and PM2.5.
Hong (1999)	Inchon, Korea	1995-1996	All	6.4	(1.5, 11.6)	1	24h	24.1	No NO ₂ effect in this study; a combined air pollution indicator (including NO ₂) was a better predictor.
			CVD	2.9	(-8.1, 15.2)				
			Resp.	11.1	(-8.8, 35.4)				
Gwynn (2000)	Buffalo, NY, USA	1988-1990	All	2.6	(-0.4, 5.6)	3	24h	20.5	No effect of NO ₂ , main effect of acidic sulfate aerosols
			CVD	1.5	(-3.5, 6.8)	2			
			Resp.	7.8	(-3.0, 19.8)	1			
Hoek (2000)	The Netherlands	1986-1994	All	4.4	(3.0, 5.8)	1	24h	17.0 (median)	NO ₂ closely correlated with BS and CO. NO ₂ effect remained when adjusting for PM10
			All	4.7	(3.5, 6.1)	0-6			

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Lippmann (2000)	Wayne County, MI, USA	1985-1990	All	3.5	(1.4, 5.6)	2	24h	23.3	Analyses in Wayne County only for days with particle data, main analyses for particles
			CVD	4.5	(1.5, 7.7)	2			
			Resp.	3.9	(-3.6, 12.1)	3			
		1992-1994	All	1.3	(-1.4, 4.2)	1	24h	23.3	
			CVD	2.7	(-1.3, 7.0)	1			
			Resp.	-1.9	(-10.8, 7.8)	3			
Mar (2000)	Phoenix, AZ, USA	1995-1997	All	8.5	(2.4, 14.6)	1	24h	30	NO ₂ closely correlated with CO. Both NO ₂ and CO strong assoc. with mortality. The effect of PM was lower. Unit of NO ₂ (0.02 ppb) unclear
			CVD	12.1	(4.8, 20.7)	0			
Samet (2000), Dominici (2003)	NMMAAPS, 20-90 U.S. cities	1987-1994	All	0.7	(0.3, 1.2)	1	24h	11.0-39.4	Main analyses for particles and ozone. Size of NO ₂ effect at lag 1 remained similar but lost statistical significance after adjustment for PM10 and O3

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Katsouyanni (2001)	APHEA II, 29 European cities	1990-1996	All	n/a	n/a	n/a	24h	13.8-49.8 (medians)	NO ₂ effect modifier of the PM ₁₀ mortality association
Kwon (2001)	Seoul, Korea	1994-1998	All	3.5	(2.3, 4.8)	n/a	24h	31.7	2-poll.model with PM ₁₀ : similar effect estimate for NO ₂ , effect of PM ₁₀ disappeared.
			CHF*	10.9	(-0.8, 23.9)	n/a			
Anderson (2001)	West Midlands, UK	1994-1996	All	1.2	(-0.3, 2.7)	0+1	1h	37.2	High correlation with PM _{2.5} (0.61). Stronger effects than with PM, although not significant. Effects of NO ₂ stronger in the warm season
			CVD	2.2	(-0.1, 4.5)	0+1			
			Resp.	2.3	(-1.4, 6.2)	0+1			

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Biggeri (2001)	8 cities, Italy	1995-1999	All	5.6	(4.1, 7.0)	0-1	24h	30.6-45.8 (range)	Strong CO effect. No multipollutant models.
			CVD	7.0	(4.6, 9.4)				
			Resp.	9.4	(2.4, 16.9)				
Roemer (2001)	Amsterdam, Netherlands	1987-1998	All	4.5	(2.1, 7.0)	1	24h	24.4 (background)	BS had a very strong effect. No multipollutant models
				2.0	(0.5, 3.6)			34.5 (traffic)	
Sunyer (2001)	Barcelona, Spain	1990-1995	All.	9.3	(-4.5, 19.7)	n/a	24h	9.0 (24h IQR, only statistic provided)	Case-crossover analysis. Stronger effect for particles

* (CHF): congestive heart failure

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Wong (2001a)	Hong Kong, China	1995-1997	All (warm season)	2.0	(-1.0, 5.0)	1	24h	25.5 (warm period)	Stronger effect of NO ₂ and SO ₂ than PM ₁₀ or ozone. NO ₂ associated with CVD independently from SO ₂ . Part of same data analyses by other authors, see Wong 2002 below
			CVD (warm season)	0.0	(-6.0, 6.0)				
			Resp. (warm season)	5.0	(-1.0, 13.0)				
			All (cold season)	5.4	(2.2, 8.6)	1	24h	33.8 (cold period)	
			CVD (cold season)	10.8	(5.4, 17.3)				
			Resp (cold season)	9.7	(2.2, 17.3)				

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Saez (2002)	EMECAM, 7 Spanish city	1990-1996	All	3.1	(1.9, 4.3)	n/a	24h	17.4-37.6	Results for NO ₂ independent from ozone, PM10, SO ₂ , but influenced by CO. NO ₂ effect still significant in 5 poll. model.
			CVD	5.2	(-0.3, 156.7)	n/a			
			Resp.	8.5	(-3.9, 11.3)	n/a			
Stieb (2002)	Meta-analysis	1958-1999	All	2.8	(2.1, 3.5)	No mention	24hr	13.0-37.9	Effect for all-cause mortality was reduced to 0.9% in multi-pollutant models including particles CO, O ₃ , and SO ₂ . No multi-pollutant models for respiratory or CVD.
			Resp.	6.6	(3.5, 9.7)				
			Circ.	3.2	(2.2, 4.2)				
Le Tertre (2002)	9 cities, France	1990-1995	All	3.4	(1.8, 5.0)	0 or 1	24h	14.8-28.6 (medians)	No multipollutant models.
			Resp.	3.6	(-2.0, 9.6)				
			CVD	4.2	(1.4, 7.0)				
Hong (2002)	Seoul, Korea	1995-1998	Stroke	9.2	(3.3, 15.5)	2	24h	32.5	Association of NO ₂ stronger than for PM10

(...cont.) Table 1. Time series studies of NO₂ and daily mortality

Author (year)	Area, Country	Period	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Wong (2002)	Hong Kong, China	1995-1998	Resp.	6.0	(1.8, 10.4)	0-1	24h	29.9	Assoc with resp. mortality disappeared in model with SO ₂ and ozone; assoc. with CVD mortality remained sig. in 4-poll. model with SO ₂ , PM10 and ozone.
			CVD	3.7	(-0.5, 7.5)	0-2			
Samoli (2006)	APHEA II, 30 European cities	1990-1997	All	1.4	(1.0, 1.7)	0-1	1 hr	24.5 – 82.0	Total mortality and CVD mortality estimates robust to inclusion, alternately, of black smoke, PM ₁₀ , SO ₂ and O ₃ . NO ₂ effects on respiratory mortality higher in cities with more elderly. Effect on CVD higher with lower smoking prevalence.
			Resp	1.7	(0.8, 2.7)				
			CVD	1.8	(1.3, 2.4)				
Simpson (2005)	4 cities, Australia	1996-1999	All	2.9	(1.4, 4.4)	0-1	1 hr	16.3-23.7	BSP reduced effect in multi-pollutant model. O ₃ did not change effect estimate.
			Resp	9.5	(4.2, 14.9)				
			CVD	3.9	(1.4, 6.2)				

Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Morris (1995)	Los Angeles, CA, USA	1986-1989	65+	Heart failure	3.4	(2.3, 4.3)	0	24h max	77	Effect found in 3 of 7 cities. Of 3 with effect, the effect disappeared in 2 after controlling for other pollutants
	Chicago, IL, USA				3.8	(1.6, 5.9)			45	
	New York, NY, USA				1.6	(0.5, 3.0)			64	
Schwartz (1997)	Tucson, AZ, USA	1988-1990	65+	CVD	1.5	(-4.8, 8.2)	0	24h	19.3	
Burnett (1997)	Toronto, Canada	1992-1994 (summers)	All ages	Resp.	19.7	(10.6, 29.5)	0-4	1h	38.5	Effects reduced to non significance after adjustment for fine particles
				Cardiac	22.1	(7.7, 38.4)				

(cont.) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Poloniecki (1997)	London, UK	1987-1994	All ages	Combined circulatory disease	1.9	(0.4, 3.6)	1	24h	35 (median)	Results stronger for myocardial infarction but sensitive to BS adjustment
				AMI*	2.2	(0.7, 3.8)				
				ARR*	2.2	(0.5, 7.8)				
Anderson (1997)	APHEA 6 cities, Europe	1977-1989	All ages	COPD*	1.7	(0.2, 4.2)	up to 3	24h	22.3-35.5	Strong effects were noted for TSP and BS. No multipollutant model available
					1.2	(0.3, 2.0)		1h	33.9-51.9	
Sunyer (1997)	APHEA 4 cities, Europe	1987-1992	15-64	AD	3.4	(0.7, 6.1)	0-3	24h	18.6-36.6 (medians)	NO ₂ effect remained significant when adjusting for BS
Spix (1998)	APHEA 4 cities, Europe	Approx 1977-1991	15-64	RD	0.9	(-1.4, 3.3)	0	24h	18.6-28.1	Effect of BS stronger when NO ₂ was high
			65+	RD	1.7	(-1.6, 5.4)				

(cont.) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Morgan (1998b)	Sidney, Australia	1990-1994	65+	COPD*	6.1	(-1.1, 13.8)	1	24h	15	NO ₂ effect for COPD decreased whereas for CVD remained in multipollutant model
				CVD	12.0	(7.7, 16.6)	0			
				COPD*	3.8	(-0.1, 7.9)	1	1h	29	
				CVD	5.5	(7.7, 16.6)	0			

*(ARR): arrhythmia; (COPD): chronic obstructive pulmonary disease; (AMI): acute myocardial infarction

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Anderson (1998)	London, UK	1987-92	All ages	AD	3.0	(1.2, 4.9)	2	24h	37.2	Effect estimates unchanged when adjustment was made for BS and O ₃ . Also pollens considered.
Tenias (1998)	Valencia, Spain	1994-1995	14+	AD	39.3	(9.4, 76.7)	0	24h	30.6	The effect remained the same after adjustment for BS and O ₃ .
					17.9	(3.7, 33.6)	0	1h	53.1	
Prescott (1998)	Edinburgh, UK	1992-1995	>= 65	ER for CVD	-1.2	(-12.2, 11.1)	0-3	24h	26.4	
			< 65	ER for CVD	-2.1	(-18.6, 17.6)				

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Burnett (1999)	Toronto, Canada	1980-1994	All ages	Respiratory infection	6.6	(4.2, 9.0)	0	24h	25.2	4.04%, 6.68%, 7.76%, respectively (p value<0.01) after adjustment for PM2.5
				Heart failure	9.0	(6.1, 12.0)	0-1			
				IHD*	9.2	(7.0, 11.5)	0-2			
				Asthma	3.2	(0.5, 5.9)	0			
Atkinson (1999a)	London, UK	1992-1994	All ages	ER asthma	2.9	(0.9, 5.0)	0	1h	50.3	No multipollutant model presented.
Wong (1999)	Hong Kong	1994-1995	All ages	RD	9.4	(6.0, 13.3)	0-3	24h	27.2	Collinearity with other pollutants
				CVD	6.0	(3.2, 9.4)	0-1			

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Anderson (2001)	West Midlands, UK	1994-1996	All ages	RD	1.4	(-0.2, 3.1)	0+1	1h	37.2	High correlation with PM2.5 (0.61).
				CVD	0.2	(-1.2, 1.7)				
Fusco (2001)	Rome, Italy	1995-1997	All ages	RD	5.1	(1.7, 8.7)	0	24h	46	Effect reduced to 0.9% (ns) in a bipollutant model with CO Effect reduced to 1.4% (ns) in a bipollutant model with CO
				AD	9.6	(-0.3, 20.5)	2			

*(IHD): Ischemic heart disease

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Petroeschovsky (2001)	Brisbane, Australia	1987-1994	All ages	Resp.	-2.5	(-5.1, 0.5)	1	1h	28.2	No significant associations were found for NO ₂ over study period, although significantly positive seasonal interactions were found for asthma & RD in autumn, winter, & spring
				AD	-8.4	(-13.9, -2.5)	0-3			
				CVD	-2.9	(-5.4, -0.5)	3			

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Mann (2002)	South Coast Air Basin, CA, USA	1988-1995	Persons with ARR*	IHD*	4.4	(7.0, 1.9)	0	24h	37.2	No multipollutant models
			Persons with CHF*	IHD*	5.7	(1.7, 9.8)				
			Persons without secondary diagnosis	IHD*	3.1	(1.2, 5.1)				
Koken (2003)	Denver, CO, USA	1993-1997 (July-August)	>65	CVD	n/a	n/a	n/a	24h	32.7	No association found but effect estimate not reported
Oftedal (2003)	Drammen, Norway	1994-2000	All ages	RD	13.6	(3.7, 24.3)	0	24h	17.9	Benzene had the strongest association

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Galan (2003)	Madrid, Spain	1995-1998	All ages	ER for AD	15.8	(6.0, 26.9)	3	24h	35.6	Adjustment for other pollutants (PM and O ₃) and pollens did not alter the association: RR=1.024 (1.005-1.045)
D'Ippoliti (2003)	Rome, Italy	1995-1997	18+	AMI*	12.3	(0.9, 25.8)	0	24h	45.8	Case-crossover analysis. Strong effect of PM and CO in this study
Tsai (2003)	Kaohsiung, Taiwan	1997-2000	All ages	Stroke	86.8	(47.7, 135.6)	0	24h	28.2	Effect on warm days only. NO ₂ remained in two pollutant models.

*(CHF): congestive heart failure; (ARR): arrhythmia; (AMI): acute myocardial infarction; (IHD): ischemic heart disease

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Yang (2004)	Kaohsiung, Taiwan	1997-2000	All ages	CVD; temp >= 25 C	57.6***	(39.2, 78.6)	0	24h	28.2	Effect modification by temperature. NO ₂ had a very strong effect in this study, and remained in multipollutant models.
				CVD; temp < 25 C	207.3***	(168.7, 251.7)				
Metzger (2004)	Atlanta, GA, USA	1993-2000	All ages	CVD	3.0	(1.4, 4.7)	3 day moving avg.	1h	44 (median)	Strong CO effect, no PM10 effect. Adjusting for CO reduced NO ₂ the NO ₂ effect, but significance remained.
				IHD*	3.5	(0.6, 6.4)				

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Barnett (2005)	Australia and New Zealand	1998-2001	1-4 yrs	RD	7.6	(2.0, 13.6)	0-1	1h	15.7-23.2	Case-crossover analysis.
			5-14 yrs		13.0	(4.3, 22.5)				
			5-14 yrs		30.4	(8.1, 57.3)		24h	7.0-11.7	
Peel (2005) (same study as Metzger, 2004)	Atlanta, GA, USA	1993-2000	All ages	ER for RD	1.9	(0.7, 3.2)	0,1,2 moving avg.	1h	45.9	For pediatric asthma visits (ages 2-18) RR=1.027 (1.005-1.050). Estimates for asthma visits not attenuated in multipollutant models.
			All ages	ER for COPD*	4.2	(0.7, 7.8)				
Rich (2005)	Boston, MA, USA	1995-2002	All ages	Ventricular ARR	67.5***	(13.0, 154.8)	0-2	24h	22.4 (median)	Case-crossover analysis.

(... cont) Table 2. Time series studies of NO₂ and hospital admissions (HA)/ emergency department admissions (ER) for cardiovascular (CVD), respiratory disease (RD) and asthma (AD)

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Wellenius (2005)	Allegheny County, PA, USA	1987-1999	65+ yrs	Congestive heart failure	9.0	(4.1, 14.3)	0	24h	26.5	Case-crossover analysis; adjusted for PM10
Simpson (2005)	4 cities, Australia	1996-1999	All ages 65+	Cardiac	5.7	(3.9, 7.5)	0-1	1 hr	16.3-23.7	For multi-pollutant model with bsp, % increase for cardiac 3.4% (1.4, 5.4) and resp. 5.7% (2.2, 9.5)
				Resp.	6.7	(3.7, 9.8)				

* (COPD): chronic obstructive pulmonary disease; (IHD) ischemic heart disease ; (ARR) arrhythmia

***OR reported instead of RR

Table 3. Time series studies of NO₂ and hospital admissions (HA), emergency department admissions (ER), general practitioner visits (GP) for asthma in children

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Buchdahl (1996)	London, UK	1992-1993	0-16	ER	5.4	(-10.3, 25.8)	0	24h	31.8	This study showed an O ₃ effect, but PM was not measured.
Medina (1997)	Paris, France	1991-1995	0-14	GP calls	23.8	(10.0, 38.1)	0-3	24h	29.7	NO ₂ effect disappeared when controlling for BS
Sunyer (1997)	APHEA, 3 European cities	1987-1992	0-14	HA	2.4	(0.5, 4.4)	varied	24h	18.6-36.6 (median)	NO ₂ effect (p<0.05) remained when adjusting for BS
Morgan (1998b)	Sidney, Australia	1990-1994	1-14	HA	4.7	(-2.4, 12.3)	0	24h	15	NO ₂ effect remained (p<0.05) after adjusting for PM and O ₃
					4.4	(0.9, 7.9)		1h	29	
Anderson (1998)	London, UK	1987-1992	0-14	HA	4.3	(0.9, 7.8)	0-3	24h	57.2	Results unchanged when adjusting for BS; lower effect estimates when adjusting for O ₃ .

(... cont) Table 3. Time series studies of NO₂ and hospital admissions (HA), emergency department admissions (ER), general practitioner visits (GP) for asthma in children

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Atkinson (1999a)	London, UK	1992-1994	0-14	ER	5.9	(2.9, 9.0)	1	1h	50.3	Adjustment for PM and O ₃ slightly reduced the effect estimate, but it remained statistically significant.
Atkinson (1999b)	London, UK	1992-1994	0-14	HA	1.4	(-1.0, 3.8)	3	1h	50.3	
Hajat (1999)	London, UK	1992-1994	0-14	GP calls	6.9	(1.7, 12.4)	0-1	24h	33.6	NO ₂ was strongly related with the outcome in children, no effect of PM found.
Norris (1999)	Seattle, WA, USA	1995-1996	0-18	ER	10.3	(-2.0, 25.4)	0	1h	34.0	NO ₂ effect disappeared when controlling for other pollutants
					-2.6	(-24.5, 22.8)	2	24h	20.2	
Gouveia (2000)	São Paulo, Brazil	1992-1994	0-5	HA	1.5	(-0.9, 3.8)	2	24h	92.4	Admissions for total respiratory diseases reported. NO ₂ slightly lower with PM in the model.

(... cont) Table 3. Time series studies of NO₂ and hospital admissions (HA), emergency department admissions (ER), general practitioner visits (GP) for asthma in children

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Anderson (2001)	West Midlands, UK	1994-1996	0-14	HA	3.3	(-1.5, 8.4)	0-1	1h	37.2	
Fusco (2001)	Rome, Italy	1995-1997	0-14	HA	22.9	(6.1, 42.4)	1.0	24h	46.0	NO ₂ negative effect (p<0.05)
Petroeshevsky (2001)	Brisbane, Australia	1987-1994	0-14	ER	-5.9	(-12.3, 1.0)	0	1h	28.2	
Thompson (2001)	Belfast, Ireland	1993-1995	n.a.	ER	31.3	(7.4, 54.9)	0-3	24h	19.2 (warm season) 9 (cold season)	NO ₂ effect disappeared in multipollutant model
Wong (2001b)	Hong Kong	1993-1994	0-14	HA	41.7	p=.001	0	24h	22.9	Also strong effect of PM and SO ₂
Braga (2001)	São Paulo, Brazil	1993-1997	0-19	HA (RD)	3.6	(1.9, 5.3)	0	24h	74.9	NO ₂ effect became not significant in multipollutant models

(... cont) Table 3. Time series studies of NO₂ and hospital admissions (HA), emergency department admissions (ER), general practitioner visits (GP) for asthma in children

Author (year)	Area, Country	Period	Age (yrs)	Outcome	% excess risk for 24 ppb increase	95% CI for % excess risk for 24 ppb increase	Lag (days)	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Lee (2002)	Seoul, Korea	1997-1999	0-15	HA	25.8	(17.0, 34.9)	2-3	24h	31.5	NO ₂ effect remained when adjusting for other pollutants
Lin (2002 and 2003)	Ottawa, Canada	1981-1993	6-12 (boys)	HA	28.1	(2.2, 57.1)	6	24h	25.2	Case-cross over analysis. No PM2.5 effect in this study.
			6-12 (girls)	HA	38.2	(4.4, 80.2)	6	24h	25.2	
Lee (2006)	Hong Kong	1997-2002	0-18	HA	15.6	(12.4, 18.8)	3	24h	34.3	Effect remained significant (5.64; 95% CI=3.21, 8.14) when PM10, PM2.5, SO ₂ and ozone in model simultaneously.

Table 4. Panel studies: symptoms changes in relation to NO₂ in asthmatic children and cardiac arrhythmias in adults

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Lag (days)	OR for 24 ppb increase	95% CI for 24 ppb increase	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Segala (1998)	Paris, France	1992-1993, 6 months	43 mild asthmatics	7-15	Asthma attacks	0	2.2	(1.2, 4.0)	24h	30.2	SO ₂ also showed a strong effect. No multipollutant model
					Nocturnal cough	4	1.9	(1.3, 3.0)			
					Respiratory infection	3	2.1	(1.0, 4.3)			
Roemer (1998)	PEACE (14 European Centers)	1993-1994, 2 months	2010 symptomatic children	6-12	Cough	1	1.0	(0.96, 1.0)	24h	4.7-39.7	No effect of other pollutants

(... Cont) Table 4. Panel studies: symptoms changes in relation to NO₂ in asthmatic children and cardiac arrhythmias in adults

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Lag (days)	OR for 24 ppb increase	95% CI for 24 ppb increase	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
van der Zee (1999)	The Netherlands	1992-1995	320 symptomatic children	7-11	Cough	2	1.0	(0.9, 1.1)	24h	11.7-27.0 (median)	Main effect of PM. No multi-pollutant models that included NO ₂ . PM10 effect robust to inclusion of one other pollutant (SO ₂ , sulfate, black smoke).
					Lower respiratory symptoms	0	1.1	(0.9, 1.4)			
Boezen (1999)	The Netherlands	1992-1995, winters	121 subjects with BHR and high serum total IgE 3 months	7-11	Lower respiratory symptoms	0-4	1.9	(1.5, 2.6)	24h	12.6-28.7	PM effects of similar size. No multi-pollutant models. No correlations among pollutants given.
			67 subjects with BHR and low serum total IgE 3 months				1.2	(0.6, 2.6)			

(.... Cont) Table 4. Panel studies: symptoms changes in relation to NO₂ in asthmatic children and cardiac arrhythmias in adults

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Lag (days)	OR for 24 ppb increase	95% CI for 24 ppb increase	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Peters (2000)	Eastern Massachusetts, USA	Jan 1995- Dec 1997	33 patients with implanted cardioverter defibrillators for arrhythmias with at least 1 event	22-85	Defibrillator discharges precipitated by ventricular tachycardias or fibrillation	1	1.7	(1.1, 2.7)	24h	23	NO ₂ had the strongest effect. Also analyzed PM10, PM2.5, black carbon, CO, ozone, SO ₂ . In two-pollutant models, PM2.5, CO and black carbon effect reduced to almost 0 and NO ₂ unchanged
Linaker (2000)	Southampton, UK	Oct 1994- Dec 1995	Asthmatics: 63 boys, 51 girls	7-12	Episodes of reduced PEF* following respiratory infection	0-6	1.9**	(1.1, 3.4)**	n/a	n/a	** OR for >14.8 vs <4.2. Palmes tubes fitted with badge clip to be worn outdoors and kept in bedroom at night.

* (PEF): peak expiratory flow

(... Cont) Table 4. Panel studies: symptoms changes in relation to NO₂ in asthmatic children and cardiac arrhythmias in adults

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Lag (days)	OR for 24 ppb increase	95% CI for 24 ppb increase	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Ostro (2001)	Los Angeles-Pasadena, CA, USA	13 weeks (Aug-Oct 1993)	138 African-American children with physician-diagnosed asthma requiring medication in preceding year	8-13	Wheeze	3	1.1	(1.0, 1.1)	1h	68.1 (Pasadena) 79.5 (L.A.)	Strong PM10 effect
					Cough		1.1	(1.0, 1.1)			
					Shortness of breath		1.1	(1.00, 1.1)			
Mortimer (2002)	8 urban areas, USA	1993, summer	846 asthmatics up to 14 days	4-9	Any morning symptom consistent with asthma	1-6	1.6	(1.0, 2.5)	4h	Approx. 25	Effect estimate reduced to 1.4 (0.9-2.1) in model with ozone. No two-pollutant model with PM10.

(... Cont) Table 4. Panel studies: symptoms changes in relation to NO₂ in asthmatic children and cardiac arrhythmias in adults

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Lag (days)	OR for 24 ppb increase	95% CI for 24 ppb increase	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Delfino (2002)	Southern California, USA	Mar- Apr 1996, 61 days	22 asthmatics	9-19	Asthma attacks	0	1.6	(0.9, 2.8)	8h	15	Effect seen mostly among children not on medication
Just (2002)	Paris, France	Apr-Jun 1996, 3 months	82 asthmatics	7-15	Asthma attacks	0-2	2.0	(0.6, 4.7)	24h	28.5	NO ₂ highly correlated with black smoke (r=0.92), which was also related to nocturnal cough.
					Nocturnal cough	0	2.5	(1.2, 4.9)			
					Respiratory infections	0-2	11.4	(1.0, 124.1)			

(... Cont) Table 4. Panel studies: symptoms changes in relation to NO₂ in asthmatic children and cardiac arrhythmias in adults

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Lag (days)	OR for 24 ppb increase	95% CI for 24 ppb increase	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Pekkanen (2002)	Helsinki, Finland	Winter 1998-1999	45 adults with coronary heart disease	Mean 68.2	ST-segment depression during exercise	2	24.1	(3.8, 153.7)	24h	29.7 U _g /m ³ (median) 15.7 ppb	Correlation of NO ₂ with PM _{2.5} was 0.35 and with PM _{2.5-10} was 0.10.
Delfino (2003)	Los Angeles, CA, USA	Nov 1999-January 2000, up to 3 months	22 asthmatics	10-16	Asthma symptoms	0	60.2	(2.3, 1639.2)	8h	5.9	Strong effect for EC and OC

(... Cont) Table 4. Panel studies: symptoms changes in relation to NO₂ in asthmatic children and cardiac arrhythmias in adults

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Lag (days)	OR for 24 ppb increase	95% CI for 24 ppb increase	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Dockery (2005)	Boston, MA, USA	July 1995- July 2002	203 patients with implanted cardioverter defibrillators for arrhythmias	19-90 (avg. 64)	Defibrillator discharges precipitated by ventricular tachycardias or fibrillation	0-1	1.2	(0.9, 1.7)	48h	22.7 (median)	Interaction with prior ventricular arrhythmia within 3 days: OR=1.34 (1.05-1.71) for 7.7 ppb change (IQR)
Schildcrout (2006)	7 North American cities	November 1993- September 1995	990 asthmatics	5-12	Asthma symptoms	2	1.1	(1.0, 1.2)	24h	17.8-26.0 (medians)	Effect remained at about 1.09 when NO ₂ summed with other pollutants one at a time. PM ₁₀ and ozone not related to symptoms.

Table 5. Panel studies: medication use changes in relation to NO₂ in asthmatic children

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Lag (days)	OR for 24 ppb increase	95% CI for 24 ppb increase	Mean NO ₂ for study period in ppb (or range of means)	Comments
Roemer (1998)	PEACE (14 European Centres)	1993-1994, 2 months	2010 symptomatic children	6-12	Bronchodilators use	2	1.0	(0.9, 1.0)	4.7-39.7	No effect of other pollutants
Segala (1998)	Paris, France	1992-1993, 6 months	43 mild asthmatics	7-15	b ₂ -agonists use	3	1.3	(0.8, 2.3)	30.2	
van der Zee (1999)	The Netherlands	1992-1995	320 symptomatic children	7-11	Bronchodilators use	1	1.3	(1.1, 1.5)	11.7-27.0 (median)	Main effect of PM
Schildcrout (2006)	7 North American cities	November 1993-September 1995	990 asthmatics	5-12	Rescue inhaler uses	2	Rate ratio for number of inhaler uses: 1.1	(1.0, 1.1)	17.8-26.0 (medians)	Effect remained at about 1.05 when NO ₂ summed with other pollutants one at a time. PM ₁₀ , ozone and SO ₂ not related to rescue inhaler use.

Table 6. Panel studies: lung function changes in relation to NO₂ in asthmatic children

Author (year)	Area, Country	Period	Population	Age (yrs)	Lag	Outcome	Effect Estimate	Unit Change (in ppb)	Effect Estimate	95% CI for effect estimate	Mean NO ₂ for study in ppb (or range of means)	Comments
Roemer (1998)	PEACE (14 European Centers)	1993-1994, 2 months	2010 symptomatic children	6-12	0	PEF* am	Beta	53	B=0.7	(-.2,1.6)	4.7-39.7	No effect of other pollutants
					0-6	PEF* pm	Beta	53	B=.4	(-.05,1.3)		
Segala (1998)	Paris, France	1992-1993, 6 months	21 asthmatics	7-15	3	PEF* am	Beta from GEE linear model	1	B=.275	SE=.150	30.2	SO ₂ also showed a strong effect. No multipollutant model

(cont.) Table 6. Panel studies: lung function changes in relation to NO₂ in asthmatic children

Author (year)	Area, Country	Period	Population	Age (yrs)	Lag	Outcome	Effect Estimate	Unit Change (in ppb)	Effect Estimate	95% CI for effect estimate	Mean NO ₂ for study period in ppb (or range of means)	Comments
Boezen (1999)	The Netherlands (5 centres)	1992-1995	121 subjects with BHR and high serum total IgE 3 months	7-11	0-4	PEF* am	OR	24	1.1	(0.9, 1.3)	12.2-28.6	Larger effect for PM and BS
					1	PEF* pm	OR	24	1.0	(0.9, 1.1)		
			0		PEF* am	OR	24	0.8	(0.7, 0.9)			
			0-4		PEF* pm	OR	24	0.7	(0.6, 1.0)			
van der Zee (1999)	The Netherlands	1992-1995	320 symptomatic children	7-11	2	PEF* pm	OR	24	1.0	(0.8, 1.3)	11.7-27.0 (median)	Main effect of PM.
					0-4	PEF* pm	OR	24	0.7	(0.5, 1.1)		
Jalaludin (2000)	Sidney, Australia	1994	125 asthmatics	3-5	0	PEF*	Beta	10	B=.3646	SE=.3473	15	Main effect of ozone in this study

(cont.) Table 6. Panel studies: lung function changes in relation to NO₂ in asthmatic children

Author (year)	Area, Country	Period	Population	Age (yrs)	Lag	Outcome	Effect Estimate	Unit Change (in ppb)	Effect Estimate	95% CI for effect estimate	Mean NO ₂ for study period in ppb (or range of means)	Comments
Timonen (2002)	Kuopio, Finland	1994, 5 weeks	33 asthmatics	7-12	0-3	FVC*	Beta	5.8	B=-17.7	SE=6.57	14.8	Strong effect of BS and NO ₂ in this study
					2	FEV ₁ *	Beta	5.8	B=-11.4	SE=4.54		
					0-3	MMEF*	Beta	5.8	B=-23.7	SE=22.5		
Delfino (2004)	Alpine, California (inland from San Diego)	Fall 1999 Spring 2000	19 asthmatics	9-17	0-4	FEV ₁	Beta	10.5	B=-1.16	(-2.4 to 0.1)	19.6 (mean of 8 hr max)	NO ₂ effect on FEV ₁ drop to near zero when personal PM in the model.
Moshammer (2006)	Linz, Austria	2000-2001 school year	163 school children, most of whom healthy	7-10	1	FEV ₁	Beta	5.3	B=0.89	P<0.001	9.58	Effect estimates robust to inclusion of PM _{2.5} in the models, indicating an independent effect of NO ₂ .
					1	MEF ₂₅	Beta	5.3	B=2.17	P<0.01		
					1	MEF ₅₀	Beta	5.3	B=1.96	P<0.001		

(cont.) Table 6. Panel studies: lung function changes in relation to NO₂ in asthmatic children

Author (year)	Area, Country	Period	Population	Age (yrs)	Lag	Outcome	Effect Estimate	Unit Change (in ppb)	Effect Estimate	95% CI for effect estimate	Mean NO ₂ for study period in ppb (or range of means)	Comments
Lagorio (2006)	Rome, Italy	Spring, Winter 1999, one month each	Adults with asthma (11),	18-64	0-	FEV ₁	Beta	5.3	B=-1.28	P<0.001	37.4	PM2.5 also associated with decreased FEV1 for adults with COPD but not for asthmatics.
			Adults with COPD (11)	50-80	0-1	FEV ₁	Beta	5.3	B=-1.38	P<0.005		
			Adults with IHD (7)	(40-64)	0-1	FEV ₁	Beta	5.3	B=0.00	P=0.994		

* (PEF): peak expiratory flow; (FVC): forced vital capacity; (FEV₁): forced expiratory volume in 1 second; (MMEF): mid-maximal expiratory flow

Table 7. Outdoor between-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	Effect estimate for 24ppb change	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Dockery (1989)	Six cities, USA	1980-1981	5,422 white children	10-12	Chronic cough, adjusted	OR	2.0	(0.2, 33.3)	6.5-22.9	Strong associations observed for PM15 and chronic cough, No association between asthma or persistent wheeze and any of pollutants (TSP, PM2.5, sulphates, SO ₂ , NO ₂) besides ozone
					Persistent wheeze, adjusted	OR	0.7	(0.3, 2.0)		

(cont.) Table 7. Outdoor between-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	Effect estimate for 24ppb change	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Braun-Fahrlander (1997)	10 communities, Switzerland	1992-1993	4,470 children	6-7, 9-11, 13-14 yrs	Chronic cough, adjusted	OR	1.7	(1.2, 2.4)	6.6-27.7	Chronic cough and nocturnal dry cough without cold were also associated with PM ₁₀ , and SO ₂ . Correlation between PM ₁₀ and NO ₂ was high (0.94), no association found for asthma. For nocturnal dry cough and PM10 OR=2.88 (1.69-4.89)
					Nocturnal dry cough without cold, adjusted	OR	2.2	(1.6, 3.0)		
					Wheeze, adjusted	OR	0.7	(0.4, 1.1)		
					Conjunctivitis symptoms, adjusted	OR	1.6	(1.2, 2.1)		

(cont.) Table 7. Outdoor between-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	Effect estimate for 24ppb change	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Studnika (1997)	8 nonurban communities, Austria	1994	843 children	7-8	Bronchial asthma last 12 mos	POR*	8.78 for change from 6.0/7.0 to 14.7/17.0 ppb	p<.05	6.0-17.0	NO ₂ levels were considered to be primarily indicators of traffic related air pollution. Sample size is relatively low. No multipollutant models. Symptoms not associated with ozone or SO ₂
					Parent-reported "ever asthma"	POR*	5.81 for change from 6.0/7.0 to 14.7/17.0 ppb	p<.05		

(cont.) Table 7. Outdoor between-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	Effect estimate for 24ppb change	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Peters (1999a)	12 communities in Southern California, USA	1994	pupils (n=3676)	9-15	Wheeze, adjusted	OR	1.1	(0.9, 1.4)	2.7-42.6	Strongest associations were observed for acid vapor and wheeze. Associations were seen with NO ₂ measured 1986-1990 (shown in table) as well as with 1994 concentrations. No association found for asthma and bronchitis. NO ₂ correlated with PM10 and acid vapor (r=0.74 and 0.83, respectively,
					Wheeze, adjusted, boys	OR	1.5	(1.0, 2.2)		
McConnell (1999)	12 communities in Southern California, USA	1993	493 children	9-15	Bronchitis	POR*	1.3	(0.8, 2.2)	21.9	Associations of similar strength were seen for PM10, PM2.5 and acid vapor.
					Phlegm	POR*	2.7	(1.4, 5.3)		
					Cough	POR*	1.6	(0.9, 2.7)		

* (POR): prevalence odds ratio

Table 8. Outdoor between-community studies on long-term effects: asthma, respiratory disorders, and atopy in cohort studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	OR for 24 ppb increase	95% CI	NO ₂ time for mean and % change	Mean NO ₂ for study period in ppb (or range of means)	Comments
Shima (2000)	7 communities, Japan	1992-1994	Pupils, n=842	9-10	Wheeze, adjusted	3.9	(1.1, 16.7)	3-yr average	7.0-31.3	Incidence of asthma (range 0-3.6%) and wheeze (range 0-6.5%) was low; no absolute numbers are given.
					Asthma, adjusted	5.9	(1.3, 42.1)			
McConnell (2003)	12 communities in Southern California, USA	1993-1999	Pupils with asthma, n=475	9-13	Between community, adjusted	1.6	(1.0, 2.0)	4-yr average	19.4	Associations of similar strength were seen for PM _{2.5} and elemental carbon. The effect of NO ₂ was independent of adjustments PM ₁₀ , PM _{10-2.5} , acids, and elemental carbon. Co-linearity of the effect was observed for ozone, PM _{2.5} and OC. Effect of NO ₂ in children who played sports.
					Within community, adjusted	5.1	(1.6, 18.8)			
					Within community, children who played team sports	12.2	(0.1, 53.1)			

Table 9. Outdoor within-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	OR for 24 ppb increase	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Hirsch (1999)	Dresden, Germany	1995-1996	Children, n=5,421	5-7, 9-11	Wheeze, adjusted	POR*	1.7	(0.7, 4.2)	17.9	Based on annual mean concentration of pollutants from a 1 km ² grid (using GIS); estimates are given for exposure at home address, for the 9-11 year old children a combination of exposure at home and school address gave comparable results. Effects of similar magnitude were seen for SO ₂ , CO and benzene. Associations were mostly seen in non-atopic children.
					Morning cough, adjusted	POR*	2.5	(1.2, 5.2)		
					Asthma, adjusted	POR*	2.0	(0.8, 4.9)		
					Bronchitis, adjusted	POR*	2.6	(1.6, 4.3)		

(cont.) Table 9. Outdoor within-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	OR for 24 ppb increase	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Kramer (2000)	Düsseldorf, Germany	1996-1997	Children, n=317	9	Wheeze, adjusted	OR	11.1	(0.6, 191.1)	23.5-32.7	Sensitization to other allergen also positively associated. No association with personal NO ₂ . Participation (38%) and the number of participants (n=182 in the urban areas) were low.
					Bronchial asthma, adjusted	OR	0.6	(0.1, 7.7)		
Nicolai (2003)	Munich, Germany	1995-1996	Children, n=7,509	5-7, 9-11	Asthma, respiratory symptoms and atopy	Increased risk for current wheeze and cough comparing highest to lowest tertile.		No model with continuous data, no range reported	GIS model to estimate concentrations of air pollutants. No risk estimate which could be pooled with other studies. High traffic counts were associated with cough current wheeze and current asthma.	

(cont.) Table 9. Outdoor within-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	OR for 24 ppb increase	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Janssen (2003)	20 city districts in The Netherlands	1997-1998	All pupils (n=2,071) attending classes (4-8)	7-12	Recurrent wheeze, adjusted	OR	4.1	(0.97, 16.7)	18.4	Association with respiratory symptoms was seen mainly in children with BHR or atopic sensitization. Effects on wheeze and conjunctivitis also seen for truck traffic, effects on skin prick test reactivity strongest for NO ₂ ; these effects were present for indoor as well as outdoor allergens.
					Current conjunctivitis, adjusted	OR	11.2	(2.3, 55.4)		
					Hay fever even, adjusted	OR	5.6	(0.8, 40.5)		
					Elevated total IgE, adjusted	OR	17.7	(0.97, 16.7)		
					Skin prick test reactivity for any allergen, adjusted	OR	3.8	(1.1, 13.6)		

(cont.) Table 9. Outdoor within-community studies on long-term effects: asthma, respiratory disorders, and atopy in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	OR for 24 ppb increase	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Kim (2004)	Alameda County, California, USA	2001	Children n=1,111, residents for 1 or more years	Grades 3-5	Asthma in last 12 months	OR	1.3	(0.9, 1.9)	23	No multipollutant models were evaluated because of the high interpollutant correlations.
					Bronchitis in last 12 months	OR	1.2	(1.0, 1.5)		
Gauderman (2005)	12 communities in Southern California, USA	2000	Children, n=208	14,17	Lifetime history of asthma	OR	12.7	(1.2, 137.5)	12.9-51.5	Model-based estimates of NO ₂ , PM, and CO were very highly correlated (R>.90) indicating that the NO ₂ -based estimates should be considered an estimate of traffic-related pollution in general rather than simply exposure to this specific pollutant.
					Recent wheeze	OR	9.8	(1.3, 73.0)		

* (POR): prevalence odds ratio

Table 10. Outdoor within-community studies on long-term effects: asthma, respiratory disorders, and atopy in cohort studies

Author (year)	Area, Country	Period	Population	Outcome	OR for 24 ppb increase	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Brauer (2002)	The Netherlands	1996-2001	Prospective birth cohort, n=4,146	Wheeze, unadjusted at 2 yrs	1.6	(1.0, 2.6)	13.6	Outdoor levels at the home of subjects were estimated using a validated model. Reported associations refer to disease status at 2 yrs. Estimates of similar size obtained for highly correlated pollutants PM2.5 and soot. Strongest association seen for ear, nose and throat infections and air pollutants.
				Wheeze, adjusted	1.7	(0.96, 3.0)		
				Asthma, unadjusted	1.6	(0.7, 3.8)		
				Asthma, adjusted	2.1	(0.7, 6.0)		
Gehring (2002)	Munich, Germany	1996-2001	Prospective birth cohort, n=1,757	Wheeze, adjusted	0.7	(0.3, 1.8)	14.7	GIS based modeling of exposure at the birth address. Comparable effect estimates obtained for PM2.5 and soot. Stronger effect estimates obtained at age of 1 year (1.36 (1.07; 1.74) dry cough at night, adjusted), especially in males.
				Dry cough at night, adjusted	3.1	(1.1, 9.0)		

(cont.) Table 10. Outdoor within-community studies on long-term effects: asthma, respiratory disorders, and atopy in cohort studies

Author (year)	Area, Country	Period	Population	Outcome	OR for 24 ppb increase	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Emenius (2003)	Sweden	1994-1998	Nested case control study of children (n=540) from birth cohort	Recurrent wheeze	2.3	(0.6, 8.4)	7.3-16.7	Analyses used estimated winter time averages outside the children's homes, range of these exposures not presented. Stronger effects for children that did not fulfill the criterion for recurrent wheeze until their second year of life. Interaction between NO ₂ and ETS exposure.

Table 11. Outdoor community based studies on long-term effects: lung function in cross-sectional studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Unit change (ppb)	Effect Estimate	95% CI or SE	Mean NO ₂ for study period (or range)	Comments
Schindler (1998)	8 communities in Switzerland	1993	9,651 adults	18-60	% change in average FVC, adjusted	5.3	-0.59%	(-1.19%, .01%)	4.9-24.5 (annual)	Effects were stronger when personal monitoring of NO ₂ was considered in addition. Effects were given for zone differences which ranged between 9.9 – 62.4 µg/m ³ NO ₂
					% change in average FEV ₁ , adjusted	5.3	-0.15%	(-.83%, .54%)		
Peters (1999b)	12 communities in Southern California, USA	1986-1990	3,293 children	9-15	ml of FVC, adjusted	25	B: -42.6	SE: 13.5	2.7-42.6 (24h)	
					ml of FEV ₁ , adjusted	25	B: -23.2	SE: 12.5		
					ml of MMEF, adjusted	25	B: -27.5	SE: 21.7		

Table 12. Outdoor community based studies on long-term effects: lung function in cohort studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Unit change	Effect Estimate	95% CI for Effect Estimate	Mean NO ₂ for study period in ppb (or range of means)	Comments
Frischer (1999)	Austria	1994-1996	1,060 children	6-8	FVC, adjusted, % change	24	-30.2	(-31.2,-29.3)	2.7-15.1	Results are shown for winter time estimates, which is the season with high levels of NO ₂ .
					FEV1, adjusted, % change	24	-15.5	(-16.6,-14.3)		
					MMEF, adjusted, % change	24	-47.7	(-49.9,-45.4)		

(cont.) Table 12. Outdoor community based studies on long-term effects: lung function in cohort studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Unit change	Effect Estimate	95% CI for Effect Estimate	Mean NO ₂ for study period in ppb (or range of means)	Comments
Gauderman (2000)	12 communities in Southern California, USA	1993-1997	1,498 4th graders	9-15	FVC, adjusted, % change	24	-0.3	(-0.7, -0.03)	4-41	Decreases were observed in association with NO ₂ and all measures of lung function. Effects were most consistent with NO ₂ , PM10, and acid vapor.
					FEV1, adjusted, % change	24	-0.5	(-0.9, -0.08)	4-41	
					MMEF, adjusted, % change	24	-0.7	(-1.4, -0.05)	4-41	

(cont.) Table 12. Outdoor community based studies on long-term effects: lung function in cohort studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Unit change	Effect Estimate	95% CI for Effect Estimate	Mean NO ₂ for study period in ppb (or range of means)	Comments
Gauderman (2002)	12 communities in Southern California, USA	1996-2000	1,678 4th graders enrolled in 1996	9-11	FVC, adjusted, % change	24	-0.2	(-0.1, 0.21)	4-37	Decreases were observed in association with NO ₂ and all measures of lung function. Effects were most consistent with acid vapors, which are highly correlated with NO ₂ (0.83)
					FEV1, adjusted, % change	24	-0.4	(-0.8, 0.12)	4-37	
					MMEF, adjusted, % change	24	-0.8	(-1.5, -0.01)	4-37	

(cont.) Table 12. Outdoor community based studies on long-term effects: lung function in cohort studies

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Unit change	Effect Estimate	95% CI for Effect Estimate	Mean NO ₂ for study period in ppb (or range of means)	Comments
Gauderman (2004)	12 communities in Southern California, USA	1993-2001	1,759 4th graders enrolled in 1993	9-11	FVC, adjusted in ML	34.6	-95.0	(-189.4, -0.6)	4-39	Effects on FVC and FEV1 were largest for acid vapors, which was highly correlated to NO ₂ .
					FEV1, adjusted in ML	34.6	-101.0	(-164.4, -38.4)		
					MMEF, adjusted in ML	34.6	-211.0	(-377.6, -44.4)		

Table 13. Outdoor NO₂ exposure and cancer risk

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	RR for ppb increase	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Feychting (1998)	Sweden	n/a	142 cases of childhood cancer and 550 controls	n/a	All cancer	RR	1.6	(1.0, 5.9)	20.7 (median)	A relative risk estimate of 2.7 (95% CI: 0.9-8.5) was found for total cancer at NO ₂ concentrations of 50 µg/m ³ or higher compared to 39 µg/m ³ or lower. At 80 µg/m ³ , the relative risk was 3.8 (95% CI 1.2-12.1).
					Leukemia	RR	2.5	(0.4, 14.0)		
					Central nervous system tumors	RR	5.9	(1.0, 32.6)		
Nyberg (2000)	Stockholm County, Sweden	1950-1990	1,042 cases of lung cancer and 2,364 controls	40-75	Lung cancer	aRR*	1.5	(0.9, 2.6)	n/a	Full range of exposure not reported. Results were robust against consideration of SO ₂ concentrations. There might be a suggestion for a threshold around 30 µg/m ³ NO ₂ .
Hoek (2002)	The Netherlands	1986-1994	5,000 adults	55-69	Lung cancer mortality	aRR*	1.4	(0.3, 7.3)	19.4	Only 60 cases were identified. Estimates are for background and local concentrations combined.

(cont.) Table 13. Outdoor NO₂ exposure and cancer risk

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	RR for ppb increase	95% CI	Mean NO ₂ for study period in ppb (or range of means)	Comments
Nafstad (2004)	Oslo, Norway	1972-1998	16,209 men	40-49	Lung cancer mortality	aRR*	1.6	(1.1, 2.2)	5.7 (median)	No estimates are given for NO ₂ . Particles not measured. NO _x indicator of traffic related pollution.
					Respiratory disease other than lung cancer	aRR*	2.0	(1.3, 2.8)		

*(aRR): adjusted relative risk

Table 14. Outdoor NO₂ exposure and fetal effects

Author (year)	Area, Country	Period	Population	Outcome	Effect Estimate	Effect Estimate for 24 ppb increase	95% CI for 24 ppb increase	Mean NO ₂ for study period in ppb (or range of means)	Comments
Ha (2001)	Seoul, South Korea	1996-1997	276,763 births	Low birth weight	RR	1.4	(1.2, 1.7)	33.6	Effects for changes in the first trimester.
Liu (2003)	Vancouver, Canada	1986-1998	229,085 births	Preterm birth, exposure during last month	aOR*	1.2	(0.98, 1.5)	19.4 (24h)	Full range of exposure not reported. Similar results observed with other pollutants (SO ₂ and CO). Pollutants were highly correlated. NO ₂ with SO ₂ and CO (r=0.61, 0.72, respectively). Elevated risks for IUGR associated with NO ₂ persisted after adjustment for other copollutants.
				Low birth weight, exposure during first month	aOR*	1.0	(0.8, 1.2)		
				Intrauterine growth retardation, exposure during first month	aOR*	1.1	(1.0, 1.3)		

(cont.) Table 14. Outdoor NO₂ exposure and fetal effects

Author (year)	Area, Country	Period	Population	Outcome	Effect Estimate	Effect Estimate for 24 ppb increase	95% CI for 24 ppb increase	Mean NO ₂ for study period in ppb (or range of means)	Comments
Wilhelm (2003)	Los Angeles, CA, USA	1994-1996	13,464 pre-term births and 21,124 controls	Pre-term birth	RR	1.1	(1.0, 1.2)	n/a	NO ₂ concentrations were taken from the best station near to the home address. Estimates of NO ₂ were reduced to null, if traffic density was considered in the same model.
			3,771 term low birth weight births and 26,351 controls	Low birth weight	OR	1.2	(1.0, 1.3)		
Dales (2004)	12 Canadian cities	1984-1999	Daily number of sudden infant deaths (n=1,556)	Sudden infant death	RR	1.4	(1.2, 1.6)	20.3 (24h)	SO ₂ results also significant.

(cont.) Table 14. Outdoor NO₂ exposure and fetal effects

Author (year)	Area, Country	Period	Population	Outcome	Effect Estimate	Effect Estimate for 24 ppb increase	95% CI for 24 ppb increase	Mean NO ₂ for study period in ppb (or range of means)	Comments
Lin (2004)	Taipei and Kaoshiung, Taiwan	1995-1997	92,288 full-term births	Low birthweight	aOR*	1.1**	(0.9, 1.3)**	23.1 – 34.3 (range)	**OR for comparison between high (>32.9 ppb) and low (<26.1 ppb) NO ₂ concentrations during the entire pregnancy
Liu (2006)	3 Canadian cities	1986-2000	386,202 live births, 10.9% with IUGR	Intrauterine growth restriction (IUGR) during 1 st trimester	OR	1.2	(1.1, 1.3)	24 (24 hr)	Effect for NO ₂ no longer observed when CO and PM2.5 in the model.
				IUGR during 2 nd trimester	OR	1.2	(1.1, 1.3)		
				IUGR during 3 rd trimester	OR	1.2	(1.1, 1.3)		

*(aOR): adjusted odds ratio

Table 15. Outdoor NO₂ exposure and mortality risk

Author (year)	Area, Country	Period	Population	Age (yrs)	Outcome	Effect Estimate	RR for 24 ppb increase	95% CI for RR for 24 ppb increase	Mean NO ₂ for study period in ppb (or range of means)	Comments
Hoek (2002)	The Netherlands	1986-1994	5,000 adults	55-69	All-cause mortality	aRR*	1.6	(0.9, 2.8)	19.4	Estimates are for background and local concentrations combined. Only 185 cardio-pulmonary deaths and 489 all-cause deaths were identified.
					Cardio-pulmonary mortality	RR	2.4	(0.97, 6.2)		
Nafstad (2004) (NO _x not NO ₂)	Oslo, Norway	1972-1998	16,209 men	40-49	All-cause mortality	aRR*	1.4	(1.3, 1.5)	5.7 (5yr median average)	No estimates are given for NO ₂ . Particles not measured. NO _x indicator of traffic related pollution.
Gehring (2006)	North Rhine-Westphalia, Germany	1985-2003	4,752 women	50-59	All-cause mortality	aRR	1.6	(1.1, 2.3)	20.7 (1 year and 5 year mean)	
					Cardio-pulmonary mortality	aRR	3.6	(1.8, 7.1)		

*(aRR): adjusted relative risk

Table 16. Indoor NO₂ exposure and morbidity risk

Author (year)	Location	Study years	Study population	Age at assessment	Average NO ₂ in ppb	Outcome	Statistic	Unit difference	Effect estimate	95% CI for effect estimate	Comments
Samet (1993)	Albuquerque, NM, USA	1988-1992	Birth cohort of 1,205 healthy infants	Up to 1.5 yrs	n/a	Upper respiratory	aOR*	20-40 ppb vs. 0-20 ppb	1.06	(.97, 1.16)	ORs not significantly elevated for current or lagged NO ₂ exposures or stove type
Garrett (1998)	Victoria, Australia	1994-1995	148 children, 53 of whom asthmatic	7 to 14 years	Median 6 ppb, maximum 128 ppb.	Respiratory symptoms (at least one of cough, shortness of breath, wheeze, chest tightness, asthma attack)	aOR	>10.6 ppb vs < 5.3 ppb	3.62	(1.08 – 12.08)	
Mukala (2000)	Helsinki, Finland	1991	162 pre-school children	3 to 6 years	Medians ranged from 9.5 – 15.4	Cough	aRR	14.4 ppb vs. < 8.6 ppb	3.63	(1.41 – 9.30)	Use of gas stoves uncommon in Finland, so personal measurements reflect ambient levels, and may be correlated with other traffic related pollutants.

(cont.) Table 16. Indoor NO₂ exposure and morbidity risk

Author (year)	Location	Study years	Study population	Age at assessment	Average NO ₂ in ppb	Outcome	Statistic	Unit difference	Effect estimate	95% CI for effect estimate	Comments
Belanger (2003)	CT and MA, USA	1998-2000	Birth cohort of 593 infants with asthmatic sibling, mothers without asthma	Up to 1 yr	n/a	Persistent cough	aOR*	10 ppb	1.21	(1.05, 1.40)	
						Wheeze	aOR*	10 ppb	1.10	(.96, 1.25)	
			Birth cohort of 256 infants with asthmatic sibling, mothers with asthma			Persistent cough	aOR*	10 ppb	1.01	(.81, 1.26)	
						Wheeze	aOR*	10 ppb	1.10	(.87, 1.40)	
Chauhan (2003)	Southampton, UK	1994-1995	Cohort of 114 asthmatic children	7..9-11.6	5.2 - 5.4 (median)	Lower respiratory tract symptoms, on scale	Arbitrary low respiratory-tract score used, so only significant differences reported			Significant increases in severity of lower RT symptoms and reduction in PEF* of more than 12 L/min for picornavirus for high compared with low NO ₂ exposure before the start of the virus-induced exacerbation.	

(Cont.) Table 16. Indoor NO₂ exposure and morbidity risk

Author (year)	Location	Study years	Study population	Age at assessment	Average NO ₂ in ppb	Outcome	Statistic	Unit difference	Effect estimate	95% CI for effect estimate	Comments
Pilotto (2004)	Adelaide, Australia	2000	Primary school children (45 intervention and 73 control children)	8.6 (average)	13.7-14.6 (home) 12.8-12.9 (personal monitor)	Difficulty breathing during day	RR	Intervention vs. control	0.41	(.07, .98)	Control schools: unflued gas heaters. Intervention schools: unflued gas heaters replaced with flued gas or electric heaters installed at the beginning of winter.
						Difficulty breathing during night	RR		0.32	(.14, .69)	
						Chest tightness during day	RR		0.45	(.25, .81)	
						Daytime asthma attacks	RR		0.39	(.17, .93)	
van Strien (2004)	CT and MA, USA	1996-1998	Birth cohort of 768 infants with asthmatic sibling	Up to 1 yr	9.9 median	Wheeze	aRR*	4th quartile vs. 1st quartile	1.45	(.92, 2.27)	Effect found for NO ₂ but not nitrous acid. 1st quartile: < 5.1 ppb. 4th quartile: >17.4 ppb.
						Persistent cough	aRR*		1.52	(1.00, 2.31)	
						Shortness of breath	aRR*		2.38	(1.31, 4.34)	

*(aRR): adjusted rate ratio; (aOR): adjusted odds ratio; (PEF): peak expiratory flow

(Cont.) Table 16. Indoor NO₂ exposure and morbidity risk

Author (year)	Location	Study years	Study population	Age at assessment	Average NO ₂ in ppb	Outcome	Statistic	Unit difference	Effect estimate	95% CI for effect estimate	Comments
Sunyer (2004)	UK and Spain	1995-1997	Population-based birth cohort and hospital-based birth cohort (n=1,611)	Up to 1 yr	5.79-45.9 (range of medians)	LRTI*	aOR*	>30 ppb vs ≤ 5 ppb	1.31	(.75,2.26)	
						Rate of anti-biotics	ARR*		0.95	(.77,1.19)	
Jarvis (2005)	Great Britain	1999/2000	276 adults	Mean age 43.5 years	12.76 ppb (median)	Decrease in FEV ₁	Percent change	1 ppb	-0.10	-0.23 to 0.02	No association with symptoms of asthma for NO ₂ or for HONO. Decrease in FEV ₁ significantly association with HONO, but not with NO ₂ .
Triche (2005)	CT and VA, USA	1994-1996	888 women who had given birth within the last 3-5 months	Up to 1 yr	13.5 (median for no heating) 12.1 (median for heating)	Chest tightness	aRR*	>80 ppb vs. <80 ppb	1.94	(.98, 3.85)	
						Wheezing	aRR*		4.00	(1.45, 11.0)	

*(LRTI): lower respiratory tract infection; (aRR): adjusted rate ratio; (aOR): adjusted odds ratio

8 Toxicological Studies in Experimental Animals and In Vitro Test Systems

8.1 Introduction

This section presents toxicological data in animals relevant to the review of the 0.25 ppm one-hour ambient air quality standard for NO₂, and to the establishment of an annual average standard for NO₂. The review of the literature is not an exhaustive summary of all the information published regarding toxicological effects of NO₂ in animals. The review focuses on literature published after the CARB, 1992 review and studies with exposures at 1 ppm or less.

As reported elsewhere in this document, Californians' exposure to NO₂ has decreased considerably over the past 20 years with one-hour maximum levels remaining below 0.25 ppm in recent years. Based on maximum ambient outdoor NO₂ concentrations humans can be exposed to, emphasis is placed on those studies conducted with exposures of 1.0 ppm or less (or daily variable NO₂ concentrations that average <1.0 ppm). Concentrations higher than 1.0 ppm may not be relevant due to different mechanisms of toxic action that might occur at these high levels compared to ambient exposure conditions. In addition, emphasis on animal studies using exposures up to 1.0 ppm is indicated by pharmacokinetic modeling data that estimate humans receive a 2-4 times greater tissue dose of NO₂ at sensitive pulmonary sites relative to rodents (see Section 8.2). Assuming equivalent pharmacodynamic responses between species, this would suggest that exposures as low as 0.25 ppm NO₂ in humans could result in the same degree of injury as exposure to 1 ppm NO₂ in rodents.

A comprehensive summary of published reports regardless of the NO₂ exposure concentration used are presented here for carcinogenicity/mutagenicity studies, allergic interaction studies and pre- and post-natal developmental studies. While the carcinogenic effects of NO₂ are not the focus of the standard, animal studies in this area of research are summarized here to provide a thorough review of the toxicology literature. Most experimental animal data on the mutagenic and carcinogenic effects of NO₂ will be reviewed because the development of cancer by a carcinogenic substance is considered a non-threshold event, until scientifically proven otherwise. Thus, any exposure to a cancer-causing substance, no matter how small, will result in an incremental increase in the chance of causing cancer.

Animal studies investigating allergen interactions with NO₂ are included here even though concentrations exceeding 1.0 ppm were often used. Staff felt this was important considering that the NO₂ standards are partly based on the increased sensitization of people to allergens following NO₂ exposure. Finally, a summary of all NO₂ exposure studies during development is presented to provide a historical view of the database for what is known to be one of the most sensitive periods of exposure to oxidant gases in animals.

In the first review of the one-hour ambient air quality standard for NO₂ (CARB, 1992), the conclusion was that animal toxicological studies, controlled human studies and epidemiological reports provided evidence for adverse effects of NO₂ exposure but did not suggest a new or different one-hour standard was needed. This analysis was based on the limited evidence of adverse effects resulting from exposure to NO₂ at concentrations below the standard of 0.25 ppm. Specifically, for animal toxicology reports, the evidence for effects at or below the standard included a report that NO₂ exposure as low as 0.2 ppm for 3 hrs caused an increase in respiratory tract cells (e.g., mast cells) associated with allergic and inflammatory responses (Hayashi and Kohno, 1985). Also, Miller *et al.* (1980) reported an increase in pentobarbital-induced sleeping time in mice at exposures of 0.25 ppm NO₂ for 3 hrs, and Iqbal *et al.* (1981) reported biosynthesis of a carcinogenic compound associated with NO₂ exposures of 0.20 ppm for 4 hrs. In longer term studies, mice exposed to 0.25 ppm NO₂ for 12 weeks to 6 months resulted in decreased T-cell subpopulations that may affect systemic immune response (Richters and Damji, 1988; Richters and Damji, 1990).

At NO₂ exposure concentrations between 0.25 and 0.50 ppm, the CARB, 1992 Review summarized key studies that observed alterations in cells involved in allergic and asthmatic responses (e.g., mast cell

degranulation) after exposure to 0.50 ppm for 4 hrs (Thomas *et al.*, 1967); damage to the lung-blood barrier at 0.40 ppm after 1 week, and at 0.47 ppm after 10 days (Sherwin and Carlson, 1973; Sherwin and Layfield, 1976); decreased function of cells responsible for respiratory immune defense with short-term exposure to 0.30 ppm (Schlesinger, 1987); and changes in cell populations of the lung involved in respiration with 6 week exposure to 0.34-0.5 ppm (Sherwin and Richters, 1982; Chang *et al.*, 1986). Collectively, these animal studies supported the human studies indicating a role for NO₂ in increased bronchial reactivity in asthmatics, increased incidence of respiratory illness, and potential exacerbation of chronic obstructive lung disease.

To provide a more comprehensive and historical perspective of the NO₂ animal data, this review will also summarize key animal studies that were presented in the previous NO₂ review.

8.2 Dosimetry of Nitrogen Dioxide in the Respiratory Tract

The NO₂ dosimetry studies provide useful interspecies information regarding doses necessary to induce various toxic responses. The experimental and modeling dosimetry information was used here to compare animal-to-human mechanisms of action and to estimate exposure concentrations used in animal studies that would be relevant to human exposure.

For NO₂ and other inhaled oxidant gases, the term dosimetry refers to estimating or measuring the amount of the gas that reaches specific target sites in the lung after exposure to a given concentration. An understanding of the dosimetry of NO₂ can assist in estimating doses necessary to induce various toxic responses in mammalian species and reduce the uncertainty in animal-to-human extrapolation.

While oxidant gas dosimetry models were originally developed for ozone, the models can be applied to other oxidant gases such as NO₂ whose reactions with biochemical constituents are similar to ozone (Overton, 1984; Overton and Graham, 1990). Like ozone, NO₂ has low water solubility and can penetrate deep into lung airways before reacting with tissue or liquid lining constituents. Although NO₂ is less reactive than ozone, slightly more NO₂ is deposited in the airways because of its greater aqueous solubility relative to ozone (ATS, 1996). In addition, the less reactive nature of NO₂ may allow the gas to penetrate deeper into the submucosa and epithelium of more peripheral airways relative to ozone (Hazucha, 1999).

The uptake of NO₂ in the respiratory tract has been measured in animal species. About 50% to 60% of inspired radiolabeled ¹³NO₂ was retained in primates during quiet respiration (Goldstein *et al.*, 1977). In an isolated perfused rat lung model, the average percentage uptake was 65% (Postlethwait and Mustafa, 1989). In tracheostomized dogs, regional lung uptake was 90% during both rest and exercise while non-tracheostomized dogs had a total respiratory uptake of 78% at rest and 94% during exercise (Kleinman and Mautz, 1991). At rest, upper respiratory tract absorption of NO₂ in the dogs during nose breathing was about twice that found during mouth breathing. These findings are reasonably consistent with experimental dosimetry findings in humans, in which NO₂ respiratory tract deposition was observed to be about 72% at rest and 87% with exercise in asthmatic subjects (Bauer *et al.*, 1986).

The dosimetry models must account for a number of animal species-dependent parameters including, lower respiratory tract dimensions and morphology, ventilation, the transport of NO₂ in airspaces, the loss of NO₂ to airway and alveolar surfaces, and the chemical reactions of NO₂ with the constituents of lower respiratory tract tissues and fluids (Miller *et al.*, 1992; Miller, 1994). While data is limited for some of these parameters, enough data exists to provide reasonable estimates and qualitatively correct dosimetry results that agree with the morphological findings and experimental dosimetry data (Overton, 1984). Both dosimetry models and experimental animal studies identify the bronchiolar-alveolar duct junction, or centriacinar region, as the primary target site of lung damage due to NO₂ inhalation.

Dosimetric models in experimental animals and humans predict that net dose of NO₂ is essentially constant in the lower lung airways, but drops off by more than a factor of ten in going from the last conducting airway generation to the first pulmonary generation as a result of the dramatic increase in total lung volume (Overton and Graham, 1990). However, actual tissue dose is low in the trachea, rises to a maximum at the bronchiolar-alveolar duct junction, the primary site of lung injury, and then rapidly decreases distally (Miller *et al.*, 1992; Overton and Graham, 1995).

The net dose in any given airway generation is the total mass of NO₂ lost from the airway lumen, while the tissue dose represents the amount of NO₂ penetrating the liquid lining layer and absorbed by the underlying tissue. The sudden rise in tissue dose in the centriacinar region is a result of a large reduction in the liquid lining thickness going from the terminal bronchioles to the pulmonary region. The liquid lining provides protection to the underlying epithelium from oxidant injury (Miller *et al.*, 1992; Overton and Graham, 1995). Lung lining fluid thickness estimates of distal airways was based, in part, on actual measurements taken at various conducting airway generations and in alveoli.

Miller *et al.* (Miller *et al.*, 1982) predicted tissue dose curves of NO₂ in lungs of rats, guinea pigs, rabbits, and man, based on normal respiration and the limited model parameters of the time. The alveolar ducts were predicted to be the region of maximal tissue dose in rats, whereas the maximal dose region was the last generation of bronchioles in guinea pigs and rabbits. This shift is probably reflective of the model formulation concerning the transition from mucus-lined to surfactant-lined regions of the lung and of the lack of respiratory bronchioles in the rat. The region of maximal NO₂ tissue dose in man was the first generation respiratory bronchioles. For a tracheal NO₂ concentration of 0.44 ppm, both rabbit and man had the highest maximal tissue dose of approximately 1 x 10⁻⁵ ug NO₂/cm²-breath. The predicted maximal tissue dose for the guinea pig and rat was roughly one-half and one-fourth, respectively, of that of rabbits and man. The authors did not identify the species-dependent model parameters most responsible for the differences in tissue dose among species.

In a recent study, transport of inhaled NO₂ was mathematically simulated at various airway levels in rats, dogs and humans (T sujino *et al.*, 2005). Both real-time and mean NO₂ concentrations in the upper and lower airways were higher in humans when compared with rats and dogs. Specifically, the mean NO₂ concentrations in the 5th and 10th generation bronchi of humans was 12-fold and 8-fold higher, respectively, than that of rats. However, the mean concentrations in the alveoli of the two species were reversed; rats were predicted to have a 12-fold higher concentration in the alveoli compared to humans. Modeled NO₂ concentrations in the oxidant-sensitive terminal airways, considered to be the 16th or 17th airway generation in rats and humans, was not estimated. Sensitivity analysis indicated that tidal volume, respiratory rate and surface area differences between species had a significant impact on airway concentrations.

Investigations using a rat *in vitro* model determined that inhaled NO₂ does not penetrate the epithelial liquid lining to directly react with epithelial tissue, suggesting that chemical reaction products of NO₂ formed in the liquid lining are the cause of pulmonary injury (Postlethwait *et al.*, 1991; Postlethwait and Bidani, 1994). Subsequent studies in the rat found that glutathione (GSH) and ascorbate were the primary liquid lining absorption substrates for NO₂, and that these substrates could then generate reactive oxygen species which may contribute to NO₂ -induced cellular injury (Postlethwait *et al.*, 1995; Velsor and Postlethwait, 1997).

Given that levels of these anti-oxidants and other constituents in the surface liquid lining that react with NO₂ is species-specific, estimating human dosimetry and the resulting health effects based on animal exposure studies is not likely to be a straightforward extrapolation (Postlethwait and Bidani, 1994). In addition, cellular sensitivity is likely to show interspecies variability due to differences in defense and repair mechanisms and other physiological/metabolic parameters, resulting in further difficulties for quantitative animal-to-human extrapolation of effective NO₂ concentrations (WHO, 1997). Nevertheless, information on dosimetry alone has been beneficial in interpreting the toxic effects of NO₂ exposure.

Once NO₂ is absorbed in the lungs, chemical reaction products of NO₂ are dispersed in the blood and other body fluids (Goldstein *et al.*, 1977). Saul and Archer (1983) estimated that 9.6 μmol of nitrite (NO₂⁻) is formed in the respiratory tract of the rat per ppm NO₂ per 24-hour exposure. The nitrite formed was a result of the interaction of NO₂ with cellular components in the lung. Although not a major pathway of NO₂ absorption in the lung, reaction with water in the lung resulted in nitrate formation (NO₃⁻), which showed a linear increase in urine with increasing NO₂ concentration.

8.2.1 Summary

Dosimetry modeling studies are used to estimate the amount or rate of nitrogen dioxide (NO₂) absorbed by pulmonary target sites and to estimate NO₂ exposures necessary to produce oxidant injury in various

regions of the lung. An understanding of the dosimetry of NO₂ can assist in estimating animal-to-human extrapolation. These models support the animal and human exposure studies in that the primary site of lung damage due to inhalation of NO₂ is the bronchiolar-alveolar duct region, also called centriacinar region. Dosimetry modeling has also estimated tissue dose of inhaled NO₂ in various species at different airway levels and in alveoli. Although pharmacodynamic differences (e.g., cellular defense and repair mechanisms) in oxidant protection exist between species, dosimetry modeling indicates that humans may receive 2-4 times greater maximal tissue dose of NO₂ at the centriacinar region relative to experimental animals.

8.3 Respiratory Tract Effects

8.3.1 Morphological Effects

Animal studies reviewed in the previous NO₂ review (CARB, 1992) indicated that morphological changes in airway structure, primarily in the central acini, may occur with prolonged exposures to concentrations in the range of 0.34-1.0 ppm. Like ozone, high levels of NO₂ also result in hypertrophy of alveolar septa, with associated increases in both thickness and cellularity of the alveolar epithelium and interstitium. This injury is focal in nature and generally limited to alveoli and septal tips immediately adjacent to the terminal airways (i.e., the centriacinar region).

In key reports previously summarized, Kubota *et al.* (1987) observed increased thickness of the air-blood barrier and a relative increase in alveolar thickness in rats continuously exposed to 0.40 ppm NO₂ for up to 27 months. No statistically significant increase of morphometric indices were found in rats exposed to 0.04 ppm NO₂ under the same protocol, although an increasing trend was noted. Hayashi *et al.* (1987) observed type II cell hypertrophy, interstitial edema in alveolar walls, increased thickness of alveolar septa, pinocytotic vesicles of endothelial cells in capillaries, and fibrous pleural thickening in rats during 19-month exposure to 0.5 ppm NO₂.

In recent reports not previously summarized, rats were run intermittently on a treadmill while exposed to 0.6 ppm NO₂ for 3 hours to determine if exercise can induce acute toxic effects at high ambient NO₂ levels (Mautz *et al.*, 1988). This exercise level raised the metabolic gas exchange by a factor of about two over resting metabolism. Morphometric quantification for focal lung lesions and changes in numbers of nuclei in the walls of alveolar ducts and septae of centriacinar units indicated no difference compared to clean air controls.

In rats exposed continuously for 1 or 3 days to 0.8 ppm NO₂, morphological changes characterized by interstitial thickening with mononuclear cell infiltration in central acini was not evident, although higher NO₂ concentrations (5 and 10 ppm) resulted in these effects (Muller *et al.*, 1994). Assessment of airway epithelium proliferative activity using 5-bromo-2'-deoxyuridine (BrdU) labeling index and silver nucleolar organizer region (AgNOR) number methods revealed increased proliferation in bronchiolar epithelium with 1- and 3-day exposure to 0.8 ppm NO₂, suggesting oxidant injury occurred to this region of the epithelium (Barth *et al.*, 1994). Cellular proliferative activity is measured by incorporation of radiolabeled BrdU into dividing cells, and by silver staining (AgNOR technique) of cellular proteins important for transcription of rRNA genes. Proliferative activity of alveolar and bronchial epithelium was increased only at higher NO₂ concentrations (5 and 10 ppm).

Morphological changes resulting from 15-week intermittent nose-only exposure (2 hr in the morning, 2 hr in the afternoon, 5 days/wk) to 0.5 ppm NO₂ was studied in young ferrets during the period of most rapid lung development from age 6 weeks through 20 weeks (Rasmussen and McClure, 1992). In exposed animals, septal wall thickness and parenchyma cellularity (nuclear area density) was increased while alveolar diameter and cross-section area were decreased compared to controls. Ferrets were considered a better model than rodents for the developmental study due to their similarities in lung structure with humans and their relatively rapid growth. The validity of the ferret model relative to the rat model has been experimentally investigated in ozone exposure studies. Sterner-Kock *et al.* (2000) looked at ozone-induced lung damage and found that the lesions in ferrets more closely resembled the damage in primates than the rats.

Microthrombus formation by NO₂ was observed in lung capillary endothelium of mice exposed to 0.35 ppm NO₂ 7 hr/day, 5 days/wk for 6 weeks (Richters and Richters, 1989). The pulmonary lesion was thought to play a role in facilitation of blood-borne cancer cell metastasis to lungs following intravenous infusion of mouse melanoma cells.

In a morphometric study of lung changes in newborn mice exposed intermittently (6 hr/day, 5 days/wk) to 0.3 ppm NO₂ for 6 weeks, the number (hyperplasia) of type II cells was increased (Sherwin and Richters, 1985). It was postulated that the type II cell increase had occurred as a result of NO₂-induced type I cell damage. The type II cell field area/alveolar wall area ratio progressively decreased through 4- and 10-weeks post-exposure, and was significantly different from controls at 10 weeks post-exposure.

The authors suggested the impairment of type II growth coupled with a small persistent increase in alveolar wall area represented possible permanent changes in lung structure resulting from early life exposure to NO₂. A follow-up study exposed 3-week old mice to 0.25 ppm NO₂ (7 hr/day, 5 days/wk, for 6 wk) with a longer post-exposure period of 32 weeks (Sherwin and Richters, 1995b). A trend towards increased type II cell size (hypertrophy) and hyperplasia was seen immediately after exposure and at 10-weeks post-exposure, but was only significantly increased at 32-weeks post-exposure. The type II cell changes and the small, but not statistically significant increased alveolar wall measurements from both studies suggested long-term lung remodeling effects due to NO₂ exposure (Sherwin and Richters, 1985; Sherwin and Richters, 1995b).

Exposure to NO₂ has also been implicated in the development of emphysema-like changes in experimental animals, though the evidence has been mixed (CARB, 1992). In humans, emphysema is characterized by abnormally enlarged alveoli and destructive changes to alveolar walls before, or without, the presence of fibrosis. Mercer *et al.* (1995) suggested that early studies implicating NO₂ as causing emphysema-like damage might have been the result of NO₂ exposure atmospheres contaminated with NO. High NO exposures have resulted in enlargement of air spaces and destruction of alveolar septa in experimental animals.

To clarify this question, Mercer *et al.* (1995) used morphometric techniques to examine alveolar septa of rats following a nine-week simulated urban pattern of exposure to pure NO₂ (background of 0.5 ppm with two daily 1-hour peaks rising to 1.5 ppm, with 2-hour downtime for maintenance). Contamination of the atmosphere with NO was negligible. High ambient NO₂ exposure did not alter the absolute volume, surface area, or thickness of any of the alveolar septal components compared to controls. Although a non-significant increase in alveolar septal defects was noted (primarily fenestrations), no alterations were found in the connective tissue matrix or interstitial cell population. In addition, there were no significant differences in number of the major parenchymal cell types, including type I and type II cells. A parallel exposure study with NO using the same simulated pattern of exposure and concentrations resulted in focal degeneration of interstitial cells, interstitial matrix, and connective tissue fibers, producing the initial steps of emphysema-like destruction of alveolar septa (Mercer *et al.*, 1995). However, a follow-up study with higher concentrations of NO (2 and 6 ppm) indicated that inhaled NO produces a pattern of injury similar to that of other oxidants, such as ozone and NO₂, and does not produce an emphysema-like injury (Mercer, 1999).

A 78-week NO₂ exposure study in rats utilizing a similar exposure pattern to that of Mercer *et al.* (1995) (16-hour background of 0.5 ppm, with exposure rising to 1.5 ppm and declining to background over a 6-hour period five days/wk, and daily 2-hour downtime for maintenance) did not detect any significant histopathological changes in the epithelial cell make-up and characteristics in the proximal alveolar region (Tepper *et al.*, 1993). Likewise, continuous exposure of rats to 0.04 or 0.4 ppm NO₂ for 17 months did not produce evidence of alveolar or bronchiolar epithelial cell hyperplasia, although a higher NO₂ concentration (4 ppm) produced slight increases in these morphological parameters (Ichinose *et al.*, 1991).

8.3.2 Summary

Acute exposure studies of 1 ppm or less have not resulted in measurable morphometric changes to lung tissue of experimental animals, although cell labeling techniques have detected increased cell proliferation of bronchiolar epithelium with acute exposure to 0.8 ppm NO₂. With intermittent daily exposures, morphometric changes in alveolar tissue components (i.e. thickened alveolar walls, increased

cellularity, altered epithelial cell volumes) occurred during lung development in young mice exposed to 0.3 ppm for 6 weeks, and in young ferrets exposed to 0.5 ppm for 15 weeks, indicating that the developing lung may be the most sensitive target for NO₂ toxicity.

Microthrombus formation by NO₂ was observed in lung capillary endothelium of mice exposed intermittently to 0.35 ppm NO₂ for 6 weeks. Chronic exposure studies simulating high urban exposures of 0.5 ppm with 2 daily spikes to 1.5 ppm NO₂ have not observed morphological changes in centriacinar region tissue of adult rats. However, a six-week exposure study in rats under similar exposure conditions resulted in morphometric changes in alveolar tissue (e.g., increased volume). Moreover, chronic, continuous exposure to 0.4-0.5 ppm NO₂ in other rat studies have observed thickening of alveolar walls and other possible inflammatory changes.

Table 8.1. Morphological Effects of Nitrogen Dioxide

NO ₂ Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
Acute/short-term exposure studies						
0.6	3 hr, while exercising	M	7 weeks	Rat (Sprague-Dawley)	No quantified change in focal lung lesions or number of nuclei in walls of alveolar ducts and septae in centriacinar units of the lung.	Mautz et al. (1988)
0.8	Continuous, 1, 3 days	M	Young Adult *	Rat (Sprague-Dawley)	Increased proliferation of bronchiolar epithelium (BrdU-labeling index and AgNOR-number methods) at 1 and 3 days; no increase in proliferative activity in bronchial and alveolar epithelium.	Barth et al. (1994)
0.8	Continuous, 1, 3 days	M	Young Adult	Rat (Sprague-Dawley)	No change in interstitial thickness or mononuclear cell infiltration in central acini.	Muller et al. (1994)
Subchronic/chronic exposure studies						
0.04 0.4	Continuous, 9, 18 and 27 months	M	8 weeks	Rat (JCL/Wistar)	0.04 ppm: No statistically significant change in morphometric indices, although a trend for increased AMT and increased relative alveolar wall thickness was observed. 0.4 ppm: Increased arithmetic mean thickness (AMT) of the alveolar wall and increased relative alveolar thickness at 18 months. Qualitative observations of hypertrophy and hyperplasia of bronchial mucosa, thickening of walls in the centriacinar region, increased cell infiltration, and an increase of Clara cells.	Kubota et al., (1987)
0.04 0.4	Continuous, 17 months	M	6 weeks	Rat (Wistar)	No evidence of alveolar or bronchiolar epithelial cell hyperplasia.	Ichinose et al. (1991)
0.25	7 hr/day, 5 days/wk, 6 weeks	M	3 weeks	Mouse (Swiss-Webster)	Type II cell hyperplasia and hypertrophy significant at 32-weeks post exposure, but not significant immediately after exposure and at 10 weeks post-exposure.	Sherwin and Richters (1995b)
0.3	6 hr/day, 5 days/wk, 6 weeks	M	Begun last wk of gestation	Mouse (Swiss-Webster)	Type II cell hyperplasia immediately following exposure; no changes 4 weeks postexposure; decreased type II cell field area/alveolar wall area ratio at 10-weeks postexposure.	Sherwin and Richters (1985)
0.35	7 hr/day, 5 days/wk,	M	5 weeks	Mouse (C57BL/6J)	Microthrombus formation in lung capillary endothelium	Richters and Richters (1989)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
	6 weeks					
0.5	4 hr/day, 5 days/wk, 15 weeks	M F	6 weeks	Ferret (Mustela putorius furo)	Increased septal wall thickness and parenchyma cellularity; decreased alveolar diameter and cross-section area.	Rasmussen and McClure (1992)
0.5	Continuous, 7 or 15 days, and 1, 2, 4, 6, 12, or 19 months	NS **	NS	Rat (Wistar)	At 2-4 mo.: pinocytotic vesicles in the capillary endothelial cells followed by interstitial edema in the alveolar walls At 4 mo.: hypertrophy of type II alveolar cells. At 6 mo.: increased width of alveolar wall At 12 mo.: marked edema of alveolar interstitium At 19 mo.: slight fibrous thickening of the pleura	Hayashi et al. (1987)
0.5 base with two daily 1 hr peaks to 1.5	7 days/wk 9 weeks	M	7 weeks	Rat (Fisher 344)	No change in absolute volume, surface area, and thickness of alveolar septal components; no change in connective tissue matrix or interstitial cell population; no change in type I and type II cell number.	Mercer et al. (1995)
0.5 base with 2 hr peak to 1.5	5 day/wk, 1, 3, 13, 52, 78 weeks	M	9 weeks	Rat (Fisher 344)	No histopathological changes in the epithelial cell make-up and characteristics in the proximal alveolar region (quantitative morphometric techniques not shown).	Tepper et al. (1993)

* 'Young Adult' designation indicates only the weight of animals, not age, were reported, and that the weight of the animals suggested they were at least young adults (likely not immature or aged) when the experiment began.

** Not stated – age, weight and/or strain of animals not reported.

8.3.3 Inflammation and Lung Permeability Changes

Several studies used histochemical techniques or bronchoalveolar lavage (BAL) fluid analysis to detect oxidant-induced airway inflammation following exposure of animals to NO₂. Acute NO₂ concentrations above ambient levels (about 2 ppm or greater) have been shown to induce an inflammatory response in lung airways, characterized by recruitment of inflammatory cells and increased permeability to serum fluid and proteins (WHO, 1997; Menzel and Meacher, 1999). Animal NO₂ exposure studies have noted the focal effect of pulmonary injury and have employed histochemical techniques to localize the areas of pulmonary effects (Rasmussen, 1994). Thus, it should be kept in mind that BAL and whole lung homogenate analysis include mostly undamaged portions of the lung and thus may fail to detect the focal nature of NO₂-induced pulmonary inflammation at low NO₂ concentrations.

In findings of NO₂-induced lung effects summarized in the previous review (CARB, 1992), increased serum proteins were observed in BAL fluid of guinea pigs following continuous 7-day exposure to 0.4 ppm NO₂ (Sherwin and Carlson, 1973). Selgrade *et al.* (1981) observed increased protein in BAL fluid of vitamin C-depleted guinea pigs exposed to 1.0 ppm, but not 0.4 ppm, NO₂ continuously for 72 hrs. Additionally, mice exposed to 0.5 ppm NO₂ continuously for 10 days showed increased protein content in homogenized lung tissue (Sherwin and Layfield, 1976). The mouse study also found a progressive increase in mean lung protein values of controls with time, and that NO₂ exposure accentuated the increase in lung protein. The authors postulated NO₂ may act to accentuate injury of lungs already compromised by unidentified infectious or chemical insults.

In recent studies, acute exposure of mice to 0.7 ppm NO₂ for 2 hours did not elicit any changes in BAL cell number or type compared to the control group (Hubbard *et al.*, 2002). BAL fluid cell population analysis of rats acutely exposed to 0.5 ppm NO₂ for 4 or 8 hours, or intermittently exposed (8 hr/day, 5 days/wk) for 5 or 10 days indicated that no significant influx of inflammatory cells into lung airways and alveolar spaces had occurred (Robison *et al.*, 1993).

Likewise, continuous (24 hr/day) exposure of rats to 0.8 ppm NO₂ for 1 or 3 days did not result in histological evidence of edema, inflammatory cells, and sloughed epithelial cells in the centriacinar region (Barth *et al.*, 1994), or alter total number of lavageable cells, protein content, or number of alveolar macrophages (AM) and granulocytes in BAL fluid (Muller *et al.*, 1994).

However, under the same exposure regimen, higher NO₂ concentrations (5 or 10 ppm) did result in histological evidence of inflammation and increased inflammatory cell numbers in BAL fluid (Barth *et al.*, 1994; Muller *et al.*, 1994). With subchronic NO₂ exposure, increased numbers of inflammatory cells and necrotic cells were present in alveolar lumina and interstitium of young ferrets exposed intermittently (4 hr/day, 5 days/wk) to 0.5 ppm NO₂ for 15 weeks (Rasmussen and McClure, 1992). However, the authors suggested that some of the pathological changes might have been due to respiratory infections. Whether NO₂ enhanced susceptibility to infectious organisms was not discussed.

Rats run on a treadmill while exposed to 0.6 ppm NO₂ for 3 hours did not reveal histopathological evidence of pulmonary inflammation in the form of increased inflammatory cells, shed epithelial cells, or plasma proteins in centriacinar units (Mautz *et al.*, 1988). This exercise level had raised the metabolic gas exchange by a factor of about two over resting metabolism.

One-hour exposures of anesthetized, mechanically ventilated guinea pigs to combined cold dry air (-30°C) and 1 ppm NO₂ resulted in a greater proportion of macrophages and decreased proportion of neutrophils in BAL fluid when compared to cold dry air controls (Halinen *et al.*, 2000b). Subfreezing ambient temperatures are thought to enhance respiratory effects due to oxidant gases. However, total white cell counts were not determined and morphological evidence of airway inflammation was not observed.

In an *in vitro* study investigating NO₂-induced alterations of epithelial barrier and ion transport properties, guinea pig tracheobronchial epithelial monolayers exposed to 0.5 and 1.0 ppm NO₂ for 1 or 4 hours resulted in increased short-circuit current (SCC) at 1.0 ppm, but did not alter transepithelial resistance at either concentration (Robison and Kim, 1995). The increased SCC indicated that active ion transport across the airway epithelium had been stimulated by NO₂-induced

alterations of cell membrane function. This increased ion transport was suggestive of an increased cell membrane-bound Na⁺, K⁺-ATPase turnover rate (see Biochemical Effects Section). Transepithelial resistance, used to detect transepithelial fluid leakage as a result of oxidant damage, was affected only by higher NO₂ concentrations (>2 ppm, 1 hr).

8.3.3.1 Summary

No pulmonary inflammatory effects using histochemical techniques or BAL fluid analysis have been observed in animals following acute or short-term repeated NO₂ exposures of 1.0 ppm or less. However, cell labeling techniques have shown increased proliferative activity in bronchiolar epithelium following 24 hr exposure to 0.8 ppm NO₂, suggesting cellular injury and repair processes have occurred. With longer exposures, pulmonary inflammation has been observed in guinea pigs exposed to 0.5 ppm NO₂ continuously for 10 days, and in young ferrets exposed intermittently to the same concentration for 15 weeks.

Table 8.2. Inflammation and Lung Permeability Changes

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.4	Continuous, 7 days	M	NS*	Guinea pig (white, short-hair)	Increased protein content of lung lavage fluid	Sherwin and Carlson (1973)
0.47	Continuous, 10, 12, and 14 days	M	NS	Mouse (NS)	Increased mean protein value in homogenized lung tissue of all exposed animals	Sherwin and Layfield (1976)
0.4 1.0	Continuous, 72 hr	M	Young Adult	Guinea Pig (Hartley COBS)	No effect at 0.4 ppm; increase in lung lavage protein in vitamin C-depleted animals at 1.0 ppm. No change in protein or lipid content of lung lavage fluid in normal animals at either concentration.	Selgrade et al. (1981)
0.4	Continuous 7 days					
0.5	4 hr/day, 5 day/wk, 15 wk	M F	6 weeks	Ferret (Mustela putorius furo)	Increased number of inflammatory and necrotic cells in alveolar lumen and interstitium.	Rasmussen and McClure (1992)
0.5	4, 8 hr, 8 hr/day, 5 day/wk, 5, 10 days	M	Young Adult**	Rat (Sprague-Dawley)	No change in BAL fluid differential cell counts of inflammatory cells or in cell population totals for AMs and monocytes.	Robison et al. (1993)
0.6	3 hr, while exercising	M	7 weeks	Rat (Sprague-Dawley)	No histopathological change in inflammatory cell number, shed epithelial cell number, or plasma protein accumulation in centriacinar units.	Mautz et al. (1988)
0.7	2 hr	M F	Young Adult	Mouse (C57Bl/6)	No change in BAL fluid cell number or cell type.	Hubbard et al. (2002)
0.8	Continuous, 1, 3 days	M	Young Adult	Rat (Sprague-Dawley)	No histological evidence of edema, inflammatory cell increases, or sloughed epithelial cells in centriacinar region.	Barth et al. (1994)
0.8	Continuous, 1, 3 days	M	Young Adult	Rat (Sprague-Dawley)	No alteration of total number of lavageable cells, protein content, or number of AMs and granulocytes in BAL fluid.	Muller et al. (1994)
1	1 hr	M	NS	Guinea pig (Dunkin-	Combined with cold (-30°C) dry air, no morphological evidence of airway inflammation; AM/PMN ratio percent distribution increased in	Hälänen et al. (2000b)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
				Hartley)	BAL fluid. Total cell counts not determined.	
0.5 1	1, 4 hr (in vitro)	M	Young Adult	Guinea pig (Hartley)	At 1 ppm: Increased active ion transport across tracheobronchial epithelial monolayers, no change in transepithelial resistance (fluid leakage).	Robison and Kim (1995)

8.3.4 Biochemical Effects

Changes in pulmonary biochemistry as a result of NO₂ exposure are associated with potential mechanisms of toxic action or with detection of altered activities of protective and repair mechanisms. Some of these biochemical effects are a result of changes at the molecular level, but may not reflect measurable changes at the cellular level (i.e., cell injury or cell death). The various biochemical changes observed with exposure to NO₂ include release of lipid peroxides and aldehydes as a result of cell membrane damage, functional changes in cell membrane bound proteins and lung surfactant components, changes in the synthesis or content of structural proteins and protease inhibitors, alterations in anti-oxidant enzymes and arachidonate metabolites, and detection of enzymes associated with cell injury and death.

NO₂ is thought to initiate lipid peroxidation of unsaturated fatty acids in epithelial cell membranes, possibly leading to cell injury or death. In previously summarized studies by CARB (1992), increases in thiobarbituric acid reactant levels, an indirect measure of lipid peroxide generation, were not detected in lung homogenates of rats and guinea pigs that were exposed continuously to 0.4 ppm NO₂ for 2 weeks (Ichinose and Sagai, 1989). Anti-oxidant enzyme activities were also unchanged by exposure. Analyzing lung homogenates for what is usually a focal-type oxidant injury likely dilutes any potential findings. However, NO₂ exposure under the same exposure protocol did show increased lipid peroxidation and depletion of GSH-peroxidase activity with longer term NO₂ exposure (Sagai *et al.*, 1984). Other key studies by this research group showed that lipid peroxidation, as indicated by increased ethane exhalation, occurred with chronic exposure to 0.04, 0.12 or 0.4 ppm NO₂ continuously for 9 to 18 months.

In recent studies not previously reviewed, *in vitro* work by Robison and colleagues investigated the release of toxic aldehydic compounds, which are formed when NO₂ oxidizes cellular constituents such as polyunsaturated fatty acids. It is thought that aldehydes could amplify the damage originally caused by NO₂ reaction with cellular membranes (Robison *et al.*, 1995). Rat alveolar macrophages (AM) exposed *in vitro* to 0.5 or 1 ppm NO₂ for 4 hours generated one or more aldehydes including butanal, glycolaldehyde, 4-hydroxynonenal, pentanal, pental, and hexanal. The NO₂-induced release of aldehydes parallels the loss of essential AM function, such as superoxide generation and production of leukotrienes (see Lung Host Defense Section). Generation of aldehydes from guinea pig airway epithelial monolayers also occurred following exposure to 1 ppm NO₂ for 1 hour (Robison *et al.*, 1996). Glycolaldehyde was a major aldehydic product generated from exposure of both rat AMs and guinea pig airway epithelium to NO₂ (Robison *et al.*, 1995; Robison *et al.*, 1996).

Oxidant stress has also generally been associated with inhibition of the activity of the membrane bound pump, Na⁺, K⁺-adenosinetriphosphatase (Na⁺, K⁺-ATPase). Studies by Robison and colleagues investigated the action of NO₂ on Na⁺, K⁺-ATPase, which is found in most eukaryotic cells and plays a vital cellular role by maintaining intracellular Na⁺ and K⁺ concentrations. Alterations in pump activity by NO₂ may have effects on cellular viability and mucociliary clearance. One-hour exposure of guinea pig tracheobronchial epithelial monolayers to 1 ppm NO₂ increased ouabain-sensitive ⁸⁶Rb uptake, an index of Na⁺, K⁺-ATPase activity (Robison and Kim, 1996). Ouabain is an inhibitor of the Na⁺, K⁺ ATPase pump and Rb acts as a surrogate for K⁺. However, ⁸⁶Rb uptake in the presence of ouabain was decreased compared to the air-control value. The increase of ouabain-sensitive ⁸⁶Rb uptake found with NO₂ exposure is indicative of enhanced Na⁺, K⁺-ATPase activity, while the decrease of uptake in the presence of ouabain suggests that NO₂ may inhibit the activities of other K⁺-transport pathways. NO₂ exposure also increased the Na⁺, K⁺-ATPase binding constant (K_d) but did not affect specific [³H]ouabain-binding capacity (B_{max}) (Robison and Kim, 1996). The increase in K_d suggests a decreased pump affinity for ouabain resulting from NO₂-induced lipid peroxidation of the enzyme.

A number of studies have explored the interaction of NO₂ with lung surfactant components, including proteins and phospholipids, which overlie the alveolar epithelium. Pulmonary surfactant lines the alveoli to lower surface tension at the air-liquid interface, thus preventing alveolar collapse during end expiration and reducing the work of breathing. Disruption of surfactant components by oxidant gases could result in impaired lung function. However, the majority of these studies exposed experimental animals to NO₂ concentrations only as low as 3 ppm. In one study that did investigate

lung surfactant effects at concentrations relevant to this review, exposure of rats to 0.8 ppm NO₂ for 1 or 3 days did not result in an alteration of phospholipid composition or impair surface tension lowering capacity of lung lining fluid, although higher NO₂ concentrations (5 or 10 ppm) did affect these parameters (Muller *et al.*, 1994).

Inhalation of oxidant gases by experimental animals has resulted in increased collagen synthesis rate and collagen content in the lung. Excess accumulation of lung collagen in exposed animals is a hallmark of pulmonary fibrosis and can lead to impaired lung function. In ferrets exposed intermittently (nose only, 2 hr in the morning and 2 hr in the afternoon, 5 days/wk) to 0.5 ppm NO₂ from six-weeks of age to 20 weeks of age while the lung was developing, whole lung collagen content was unaffected, though higher concentrations (10 ppm) did increase collagen content (Rasmussen and McClure, 1992).

The ferret lung was chosen for toxicological study because the structure and development of the ferret lung is more similar to human lung structure and development relative to rodents. The validity of the ferret model relative to the rat model has been experimentally investigated in ozone exposure studies. Sterner-Kock *et al.* (2000) looked at ozone-induced lung damage and found that the lesions in ferrets more closely resembled the damage in primates than the rats. Further experiments investigating the effects of NO₂ on lung collagen content also used histochemical techniques to localize the areas of collagen deposition (Rasmussen, 1994). Using the same exposure regimen, juvenile ferrets exposed to 0.5 ppm NO₂ for 8 or 15 weeks did not exhibit increased collagen deposition in submucosa of respiratory bronchiolar epithelium, though again, the high NO₂ concentration (10 ppm) did increase collagen deposition. Whole lung collagen content was also measured in one lung lobe; lung collagen content was unaffected by exposure to 0.5 ppm NO₂ using this measurement technique.

Sherwin and Richters (1995a) measured elastin content in alveolar tissue following 6-week intermittent exposure (7 hr/day, 5 days/wk) of mice to 0.25 ppm NO₂. Exposure began when the lungs of the 3-week old mice were still developing. Immediately after exposure, elastin fiber number and field area were increased relative to controls. However, elastin fiber number and fiber area per lung field were decreased at 10 weeks post-exposure. By 32-weeks post-exposure these elastin measurements were of borderline significance, although ratios of elastin number/alveolar wall area and elastin area/alveolar wall area were significantly increased. The changes in elastin measurements following NO₂ exposure suggest impairment of lung development and growth, with initial fragmentation of elastin and/or new fiber synthesis, followed by elastin proliferation and decreased alveolar wall area at 32-weeks post-exposure.

Inhaled oxidants, including NO₂, may cause lung damage by inactivating the proteinase inhibitors that normally protect the lung from proteolysis. Failure of proteinase inhibitors to properly control the proteinases, which originate from phagocytic cells such as the polymorphonuclear leukocytes (PMN), can result in the destruction of structural protein elastin in the lung and lead to emphysema. To study the effects of NO₂ on alpha-1-proteinase inhibitor (α 1-PI), a principal proteinase inhibitor in the lung, rats were exposed to NO₂ (0.5 ppm background with 2 hr peaks of 1.5 ppm) for 12 and 18 months and examined for reductions in α 1-PI in lung lavage fluid (Johnson *et al.*, 1990). NO₂ did not result in measurable reductions in the functional activity of α 1-PI, indicating that NO₂ does not change α 1-PI levels.

In vitro studies in rats have shown that ascorbate and GSH are primary scavengers of NO₂ found in BAL fluid (Ben-Jebria *et al.*, 1998). Pulmonary increases in protective anti-oxidant enzymes and substances have been measured following animal exposure to oxidant gases. Increases in lung anti-oxidant levels are thought to contribute to the attenuation of lung injury with continued exposure to oxidant gases. Ichinose and Sagai (1989) measured levels of various anti-oxidant substances and enzymes from lung homogenates of rats and guinea pigs continuously exposed to 0.4 ppm NO₂ for 2 weeks. The levels of anti-oxidant substances ascorbate, vitamin E, and non-protein sulfhydryls, and anti-oxidant protective enzymes glutathione peroxidase, glutathione reductase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, glutathione S-transferase, and superoxide dismutase (SOD) in both species were unaffected by the NO₂ exposure. It should be kept in mind that the morphological lesions for NO₂ are focal, therefore caution should be used in interpreting

negative data from BAL or whole lung homogenates. However, previously reviewed reports by these researchers employing longer term exposures have observed measurable increases in some anti-oxidants (Sagai *et al.*, 1984).

Peroxidation products generated from the action of NO₂ on membrane polyunsaturated fatty acids of epithelial cells are thought to significantly affect the regulation of arachidonate metabolism in the airways. Activation of arachidonate metabolism is important in the host's inflammatory response and alteration of this pathway may impede defense against microbial infection. Acute NO₂ exposure (0.5 ppm) in rats resulted in reduced BAL fluid levels of leukotriene B₄ (LTB₄), thromboxane B₂ (TxB₂), prostaglandin E₂ (PGE₂), and prostaglandin F₂α (PGF₂a) at 4 hours of exposure, but all had returned to air-control levels with 8 hours of exposure (Robison *et al.*, 1993). Airway epithelium is known to synthesize PGE₂ and PGF₂a while alveolar epithelium primarily synthesizes PGE₂, PGF₂a and TxB₂. AMs synthesize LTB₄, as well as other arachidonate metabolites. With continued exposure (8 hr/day, 5 days/wk) to 0.5 ppm NO₂ for 5 or 10 days, metabolite levels of TxB₂ and PGF₂a were reduced in BAL fluid on day 5, but had recovered to air-control levels by day 10.

In vitro cellular injury in response to NO₂ exposure has been assessed through measurement of lactate dehydrogenase (LDH) activity. LDH is an intracellular enzyme; the presence of LDH extracellularly indicates cell injury or death. Residual cellular content of LDH activity in rat AMs was unaffected by NO₂ exposures as high as 1 ppm for up to 4 hours, indicating membrane integrity (i.e., cell viability) was unaffected by the oxidant gas (Robison *et al.*, 1990; Robison and Forman, 1993).

Much higher NO₂ concentrations (20 ppm, 1 hour) were needed to alter LDH activity and reduce cell viability (Robison *et al.*, 1990). In a preliminary *in vitro* technique that allowed direct cell exposure with a minimal barrier for contact between NO₂ and a rat bronchopulmonary cell culture, a dose-dependent increase in LDH release was found with 3-hour NO₂ exposures in the range of 80 ppb to 360 ppb (Knebel *et al.*, 1998). At the highest concentration (360 ppb), several other measures of cell injury including number of cells, mitochondrial activity, esterase activity, glutathione S-transferase activity, and DNA synthesis, were lower than the control group. However, the apparent lack of a sufficient number of test runs prevented statistical analysis.

8.3.4.1 Summary

Changes in pulmonary biochemistry due to NO₂ exposure may result in altered activities of protective and repair mechanisms in the lung. The most sensitive test observed decreased arachidonate metabolite levels in BAL fluid with acute and short-term exposure of rats to 0.5 ppm NO₂. Altered arachidonate metabolite levels may be related to NO₂-induced peroxidation and injury to lung epithelium, and could result in the reduced ability of the lung to fight off microbial infections. Longer term, repeated exposures to 0.25 ppm NO₂ in mice during lung development have resulted in alterations of structural protein (i.e., elastin) in lung tissue.

Chronic continuous exposure of rats to 0.4 ppm NO₂ resulted in increased lipid peroxidation, indirectly measured as increased thiobarbituric acid reactant levels, and depletion of GSH-peroxidase activity in lungs. Increased ethane exhalation, also an indicator of pulmonary lipid peroxidation, was observed with chronic continuous exposures as low as 0.04 ppm NO₂ for 9 months. *In vitro* studies allow researchers to explore oxidant effects that may be difficult to observe in the intact animal. *In vitro*, acute NO₂ exposure in the range of 0.5-1.0 ppm have resulted in alterations in pulmonary cell membrane ion pump activity vital to cellular stability and function, and generation of potentially toxic aldehydes related to peroxidation and damage of lung epithelial cells.

Table 8.3. Biochemical Effects of Nitrogen Dioxide

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.04 0.4	Continuous, 9, 18 or 27 mo.	M	8 weeks	Rat (JCL:Wistar)	Increased ethane exhalation at 0.04 and 0.4 ppm at all time points and increased lipid peroxidation by TBA method at 0.4 ppm after 18 mo.; decreased GSH-peroxidase activity at 0.4 ppm after 18 mo.	Sagai et al. (1984)
0.04 0.12 0.4	Continuous, 6, 9 and 18 mo.	M	NS	Rat (JCL:Wistar)	At 6 mo. – increased ethane exhalation at 0.4 ppm. At 9 and 18 mo. – increased ethane exhalation at all exposure concentrations.	
0.25	7 hr/day, 5 days/wk, 6 weeks	M	3 weeks	Mouse (Swiss-Webster)	Immediately after exposure: increased alveolar elastic fiber number and field area. 10 weeks post-exposure: decreased alveolar elastic fiber number and field area per lung field. 32 weeks post-exposure: increased ratios of elastin number/alveolar wall area and elastin area/alveolar wall area.	Sherwin and Richters (1995a)
0.4	Continuous, 2 weeks	M M	10 weeks 10 weeks	Rat (Wistar) Guinea pig (Hartley)	In both species: no increase in lipid peroxidation (TBA method); no alteration in whole lung homogenate levels of anti-oxidant substances (ascorbate, vitamin E, and non-protein sulfhydryls) and anti-oxidant protective enzymes (GSH peroxidase, GSH reductase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, GSH S-transferase, and SOD).	Ichinose and Sagai (1989)
0.5	4 hr/day, 5 days/wk, 15 weeks	M F	6 weeks	Ferret (<i>Mustela putorius furo</i>)	No alteration of whole lung collagen content	Rasmusse n and McClure (1992)
0.5	4 hr/day 5 days/wk, 8, 15 weeks	M F	6 weeks	Ferret (<i>Mustela putorius furo</i>)	No histochemical evidence of increased deposition of collagen in submucosa of respiratory bronchiolar epithelium or alteration of whole lung collagen content.	Rasmusse n, 1994

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.5	4, 8 hr 8 hr/day, 5 days/week, 5, 10 days	M	Young Adult	Rat (Sprague-Dawley)	Reduced BAL fluid levels of LTB4, TxB2, PGE2, and PGF2a at 4 hours of exposure, all returned to air-control levels at 8 hours of exposure. Reduced BAL fluid levels of TxB2 and PGF2a on day 5, but had recovered to air-control levels by day 10. LTB4 and PGE2 levels were unchanged.	Robison et al. (1993)
0.5 base with 2 hr peak to 1.5	7 days/wk 12, 18 months	M	NS	Rat (Fischer 344)	No change in alpha-1-proteinase inhibitor in lung lavage fluid	Johnson et al. (1990)
0.8	Continuous, 1, 3 days	M	Young Adult	Rat (Sprague-Dawley)	No alteration of phospholipid composition or impairment of surface tension lowering capacity of lung lining fluid	Müller et al. (1994)
In vitro studies						
0.089 0.189 0.359	3 hr (in vitro)	NS	Fetal	Rat (Han-Wistar)	Preliminary data suggests dose-dependent increase in LDH release in bronchopulmonary cell culture. At the highest concentration, decreased cell number, decreased mitochondrial, esterase and GSH S-transferase activity, and decreased DNA synthesis.	Knebel et al. (1998)
0.1 1	1 hr (in vitro)	NS	Young Adult	Rat (Sprague-Dawley)	No change in residual cellular content of LDH activity in AMs.	Robison et al. (1990)
0.5 1	4 hr (in vitro)	M	Young Adult	Rat (Sprague-Dawley)	At both concentrations: generation of one or more aldehydes from oxidation of cellular constituents in isolated AMs.	Robison et al. (1995)
1	1 hr (in vitro)	M	Young Adult	Guinea pig (Hartley)	Generation of aldehydes from airway epithelial monolayers, primarily glycoaldehyde.	Robison et al. (1996)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
1	1 hr (in vitro)	M	Young Adult	Guinea pig (Hartley)	In tracheobronchial monolayers: Increased ouabain-sensitive ^{86}Rb uptake, suggestive of enhanced Na^+ , K^+ -ATPase membrane pump activity; decreased ^{86}Rb uptake in the presence of ouabain, suggesting inhibition of activities of other K^+ -transport pathways; increased binding constant (K_d); did not alter specific $[^3\text{H}]$ ouabain-binding capacity (B_{max}).	Robison and Kim (1996)
1	2, 4 hr (in vitro)	M	Young Adult	Rat (Sprague-Dawley)	No change in residual cellular content of LDH activity in AMs.	Robison and Forman (1993)

8.3.5 Lung Host Defense

The host defense system in the respiratory tract of humans and animals protects against infectious and particulate deposition primarily by utilizing two well-coordinated systems, the mucociliary system and the immune system. The animal data provides a basis for comparison relevant to humans because the pulmonary defense systems function similarly in both animals and humans. Although the respiratory defense mechanisms act in concert to protect the lung, various aspects of the integrated system are discussed separately below. The clearance section discusses the effect of NO₂ on removal of inhaled particles. The section on alveolar macrophages discusses the effects of NO₂ exposure on the functions of these cells that help to clear the lungs of debris and particles. The section on interaction with infectious microorganisms discusses the effect of NO₂ exposure on defense against viral or bacterial exposure.

8.3.5.1 Clearance

Exposure of mice to a NO₂ concentration of 1 ppm (but not at 2 ppm or higher) for 4 hours stimulated the loss of ³⁵S-labeled *Mycoplasma pulmonis* from lung tissue, thereby implying that NO₂ had a mild accelerating effect on mucociliary transport (Davis *et al.*, 1991). The trend toward accelerated loss of radiolabeled *M. pulmonis* was also apparent in mice exposed to 0.5 ppm for four hours but was not statistically significant. This finding is consistent with a report presented in the previous NO₂ review by Vollmuth *et al.* (1986), in which an increased rate of particles were cleared from the alveolar region during a 14-day measurement period following a single 2-hr exposure to either 0.3 or 1.0 ppm NO₂, or to 2-week daily exposure to 1.0 ppm NO₂. These observations are compatible with the mild irritant effect of NO₂ at low concentrations (< 1 ppm) and may be an indicator of the oxidants biological activity in lung tissue (Davis *et al.*, 1991).

Tracer particle clearance was measured in airways of young ferrets exposed intermittently (nose only, 4 hr/day, 5 days/wk for 8 or 15 wk) to 0.5 or 10 ppm NO₂ during postnatal respiratory tract development (Rasmussen *et al.*, 1994). Thoracic clearance, which includes the lungs and lower trachea, was reduced at both NO₂ concentrations, but only significantly at the high concentration. Duration of exposure did not alter the clearance rate. Head airways clearance, which included the nose to the upper trachea, was unaffected at either concentration.

8.3.5.2 Alveolar Macrophages

AMs are the primary cellular defense system in the lower lung. Following exposure to inhaled or blood-borne antigens, AMs phagocytize foreign antigens and secrete mediators that recruit and activate inflammatory cells in the lung, thus amplifying their role in host defense. Impairment of AM's by NO₂ or other toxic agents can have a significant effect on host defense by affecting their phagocytic abilities, membrane integrity, mobility, and enzymatic capacity. Previously reviewed studies indicated that repeated 2-day exposure to 0.3 ppm NO₂ 2 hrs/day reduced AM phagocytosis and mobility in rabbits (Schlesinger, 1987). However, 2-day exposure to 1.0 ppm NO₂ 2 hrs/day resulted in enhanced AM phagocytosis.

To examine how AMs adapt themselves to oxidant exposure, rats were exposed to 0.4 ppm NO₂ continuously for 12 weeks, after which AMs were collected by pulmonary lavage and analyzed for metabolic and population changes (Mochitate *et al.*, 1992). Specific activities of glucose-6-phosphate dehydrogenase of the peroxidative metabolic pathway and pyruvate kinase of the glycolytic pathway were unaffected by NO₂ exposure. The population of AMs lavaged per rat lung was also unchanged by NO₂ exposure, although higher exposures (1.2 and 4.0 ppm) did increase AM numbers.

Peroxidation products generated from the action of NO₂ on membrane polyunsaturated fatty acids are thought to significantly affect the regulation of arachidonate metabolism in AMs. In addition, AMs are particularly vulnerable to oxidant gases, since they contain significant levels of polyunsaturated fatty acids in their cell membranes and lining fluid generally does not cover and help protect AMs.

Activation of arachidonate metabolism is important in the host's inflammatory response and alteration of this pathway may impede defense against microbial infection. For example, decreased AM leukotriene B₄ (LTB₄) synthesis may potentially impair the recruitment of neutrophils that are essential to resolving bacterial infections (Robison *et al.*, 1993). LTB₄ is considered the most potent chemotactic factor synthesized by AMs.

Acute exposure of rats to 0.5 ppm NO₂ for 4 or 8 hrs depressed *ex vivo* release of LTB₄ by AMs at 4 hrs but did not affect thromboxane B₂ (TxB₂) release (Robison *et al.*, 1993). With AM *ex vivo*-stimulated release by the calcium ionophore A23187, a potent stimulant of synthesis of cyclooxygenase and 5-lipoxygenase products, 5-hydroxyeicosatetraenoate (5-HETE) was depressed only at 4 hrs and LTB₄ and TxB₂ were depressed only at 8 hrs. *Ex vivo* stimulation of LTB₄ release by zymosan-activated rat serum (ZAS), a stimulant of LTB₄ production, was not acutely affected by NO₂ exposure.

With intermittent exposure to NO₂ (8 hr/day, 5 days/wk) for 5 or 10 days, TxB₂ production was unchanged, but LTB₄ production was depressed on day 5 (Robison *et al.*, 1993). AMs stimulated *ex vivo* by A23187 following repeated NO₂ exposure did not affect release of 5-HETE, LTB₄, or TxB₂ on day 5, but increased 5-HETE release on day 10. *Ex vivo* AM stimulation of LTB₄ release by ZAS following repeated NO₂ exposure showed marginally depressed production that was only statistically significant following 5 days of NO₂ exposure. Robison *et al.* (1993) also examined BAL fluid levels of LTB₄ and TxB₂ from NO₂-exposed rats (see Biochemical Effects Section), which showed a parallel to *ex vivo* depression of AM production of these metabolites and suggest altered activation of arachidonate metabolism and impeded defense against pulmonary infection.

In a manner similar to the depression of arachidonate metabolism, stimulated *ex vivo* AM superoxide production was depressed with 4 hour exposure of rats to 0.5 ppm NO₂ and remained lower than air control levels through 10 days of exposure (Robison *et al.*, 1993). The release of superoxide anion by AMs on a target cell, such as bacteria or tumor cells, is an important factor in the cytotoxic action of AMs. The researchers suggested that the depression of AM function may have significant impacts on the ability of these cells to defend against bacterial infection or secrete compounds that play important regulatory roles with inflammatory reactions in the lung.

In *in vitro* experiments, rat AMs exposed to 0.1 or 1.0 ppm NO₂ for one hour did not alter production of LTB₄ (Robison *et al.*, 1990). Similarly, exposure of rat AMs to 0.1 ppm NO₂ for one hour or 1.0 ppm NO₂ for up to 4 hours did not significantly affect arachidonate metabolite levels of TxB₂, 12-hydroxyheptadecatrienoic acid (12-HHT), or monohydroxyeicosatetraenoate isomers (monoHETEs) released in the medium (Robison and Forman, 1993).

When AMs were chemically stimulated by the ionophore A23187 to synthesize and release arachidonate metabolites following 1-hour *in vitro* exposure to 0.1 and 1.0 ppm NO₂, no changes were observed in formation of LTB₄, TxB₂, 12-HHT, or monoHETEs compared to non-exposed, A23187-treated controls (Robison *et al.*, 1990; Robison and Forman, 1993). However, 2- and 4-hour exposure to 1 ppm NO₂ enhanced A23187-stimulated production of LTB₄, and 12-HHT (2-hr exposure only), and TxB₂ (4-hour exposure only) (Robison and Forman, 1993).

The apparent dissimilarities in arachidonate metabolism between *ex vivo* (depressed AM release of arachidonate metabolites) and *in vitro* (no effect or stimulated release of arachidonate metabolites) exposures of AMs may be related to differences of exposure time and concentration as well as to effective level of NO₂ that actually interacts with these cells (Robison *et al.*, 1993). However, similar to the *ex vivo* findings of Robison *et al.* (1993), *in vitro* exposure of rat AMs to 1.0 ppm, but not 0.1 ppm NO₂ significantly depressed superoxide production in response to PMA stimulation (Robison *et al.*, 1990).

The capacity of AMs to release LTB₄ and other chemotactic agents that consequently recruit PMNs to a site of inflammation may be an important defense mechanism in the lung. Robison *et al.* (1990) observed that PMN migration *in vitro*, in response to products derived from rat AMs exposed *in vitro* to 0.1 or 1.0 ppm NO₂ for up to 4 hours, was not significantly increased with or without stimulation by

A23187. Increased PMN migration resulted only from higher NO₂ exposures (5 ppm, 1 hour), which also corresponded to the concentration where increased synthesis of LTB₄ by AMs was seen.

Glutathione (GSH) is an important cellular defense against oxidant stress. Robison *et al.* (1993) exposed rats to 0.5 ppm, 8 hr/day for 0.5, 1, 5 and 10 days and observed no change in GSH or oxidized GSH (GSSG) content of AMs from the exposed animals. AMs also release nitric oxide (NO), which exerts regulating and/or cytotoxic effects depending on the concentration acting on the target cell. Alteration of NO production by AMs may effect antimicrobial defense or modify the immune response. Bovine AMs exposed *in vitro* to 0.2 ppm for 2 hours resulted in increased NO production both in the absence and presence of lipopolysaccharide (LPS) stimulation (Hockele *et al.*, 1998). The authors suggested that NO₂ may affect AM inducible NO-synthase mRNA expression, the enzyme responsible for NO production, by activation of a transcription factor or by altered arachidonate metabolism.

8.3.5.3 Interaction with Infectious Microorganisms

Only a few new reports investigated the potential for compromised respiratory defense against bacterial or viral challenge as a result of exposure to NO₂ concentrations of 1.0 ppm or less. Previously reviewed studies (CARB, 1992) indicated that acute exposure to 1.0 ppm NO₂ or chronic exposure to 0.5 ppm NO₂ could enhance susceptibility to challenge by microorganisms.

In a key study, greater mortality from *streptococcus* infection occurs in mice exposed 5 days/week for one year to 0.2 ppm NO₂ with 0.8 ppm one-hr spikes twice per day (Miller *et al.*, 1987). Exposure to a background concentration of 0.2 ppm alone did not enhance mortality. The results indicate that the presence of spikes of NO₂ contributed significantly to the toxicity. This study is also notable because the respiratory defense system was significantly compromised in the absence of pulmonary morphological changes in these animals.

Ehrlich *et al.* (1979) observed that mice exposed to 0.5 ppm NO₂ (3 hr/day, 5 days/week) for 3 or 6 months followed by exposure to *streptococcus* aerosol resulted in increased mortality and decreased survival time. However, exposure to mice under the same protocol, but reexposed to 0.5 ppm NO₂ for 14 days following *streptococcus* challenge only resulted in a non-significant trend towards increased mortality and shorten survival time. In earlier experiments, mice exposed to 0.5 ppm NO₂ for 6, 18, or 24 hr/day followed by airborne challenge to *K. pneumoniae* resulted in increased mortality at 6 months, but not at 1 or 3 months, in all exposed groups, and at 12 months for the 24 hr/day exposed group (Ehrlich and Henry, 1968).

In recent reports not previously summarized, acute exposure of mice to 0.5, or 1 ppm NO₂ for four hours did not impair the capability of the mouse lung mycoplasmacidal defense system to kill *Mycoplasma pulmonis* (Davis *et al.*, 1991). However, higher concentrations (5 or 10 ppm) did impair bacterial killing of *M. pulmonis*. AM viability, as measured by trypan blue exclusion, was reduced only at NO₂ levels that affected intrapulmonary killing of the bacteria.

With prolonged NO₂ exposure, lungs and trachea of mice did not show increased mycoplasmal growth of *M. pulmonis* or increased formation of pneumonic lesions with continuous exposure to 1 ppm NO₂ for up to 28 days following infection (Nisizawa *et al.*, 1988). In addition, NO₂ exposure had no effect on serum antibody titers to *M. pulmonis*. These findings suggest that increased severity of *M. pulmonis* infection and related decreased function of AM's do not occur at low NO₂ concentrations (<1ppm).

In a study of the effect of NO₂ exposure on susceptibility to viral respiratory infection, exposure to 1 ppm NO₂ did not enhance susceptibility of mice to murine cytomegalovirus infection, although higher NO₂ concentrations (5 ppm) did enhance infection (Rose *et al.*, 1989). NO₂ exposures were 6 hr/day for 2 days prior to viral infection and 4 consecutive days the day after viral infection. Reinfection 1 month following the primary infection of NO₂ -exposed mice did not enhance pulmonary infection in mice that were exposed to less than 5 ppm NO₂. NO₂ exposure in viral-infected mice did not increase observable effects on lung structure at any concentration.

8.3.5.4 Summary

The host defense system in the respiratory tract protects against infectious and particulate deposition primarily by utilizing two well-coordinated systems, the mucociliary system and the immune system. The animal studies on lung host defense show that AMs, the primary cellular defense system against pulmonary infection, are probably the most sensitive indicator of NO₂ toxicity in the lung. *Ex vivo* release of arachidonate metabolites and superoxide was depressed by acute and short-term repeated exposure of rats to 0.5 ppm NO₂. Alteration of arachidonate metabolites, related to NO₂-induced damage to AM cell membranes, may impede the ability of the lung to protect itself from microbial infection. Reduced superoxide release by AMs, which kills infectious organisms, may also impede lung defense.

In interaction studies with infectious microorganisms, acute exposure to 1.0 ppm NO₂ or chronic exposure to 0.5 ppm NO₂ in mice have shown enhanced susceptibility to challenge by microorganisms. Greater mortality from *streptococcus* infection was demonstrated in mice exposed for one year to 0.2 ppm NO₂ with 0.8 ppm one-hr spikes twice per day. A single 2-hr exposure to 0.3 ppm NO₂ in rabbits has been shown to increase mucociliary transport in the lung, suggesting a mild irritant affect by NO₂. *In vitro*, acute NO₂ exposure (1 ppm) decreased superoxide release by AMs similar to that seen in *in vivo* results, but *in vitro* studies either had no effect or enhanced release of arachidonate metabolites from AMs, which conflicted with similar studies performed *in vivo*.

Table 8.4. Lung Host Defense Effects of Nitrogen Dioxide: Clearance, Alveolar Macrophages, and Interactions with Infectious Microorganisms

NO ₂ Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
Effects on Clearance						
0.3 1.0	2 hrs	M	NS	Rabbit (New Zealand White)	Increased alveolar clearance of particles ($p < 0.05$) on days 4 and 5 post-exposure following 0.3 ppm (2 hrs), and on days 4-6, 9-11, and 13 post exposure following 1.0 ppm (2 hrs).	Vollmuth et al. (1986)
1.0	2 hrs/day, 14 days				At 1.0 ppm (2 hr/day, 14 days): Increased alveolar clearance of particles ($p < 0.05$) on days 3-6, 8, 10, 12 and 14 post-exposure.	
0.5 1	4 hr	NS	8-10 weeks	Mouse (C57BL/6N)	No change in clearance at 0.5 ppm. 1 ppm accelerated mucociliary clearance of ³⁵ S-labeled Mycoplasma pulmonis from lung tissue.	Davis et al. (1991)
0.5	4 hr/day, 5 days/wk, 8, 15 weeks	M F	6 weeks	Ferret (Mustela putorius furo)	Thoracic clearance (lungs and lower trachea) and head airways clearance (nose and upper trachea) of tracer particles was unaffected at either duration of exposure.	Rasmussen et al. (1994)
Effects on Alveolar Macrophages						
0.3 1.0	2 hr/day, 2, 6, or 13 days	M	NS	Rabbit (New Zealand White)	Examined 24 hr after end of exposure: 0.3 ppm: decreased AM phagocytosis and mobility with 2-day exposure 1.0 ppm: increased AM phagocytosis with 2-day exposure. No change in cell number or viability at either concentration	Schlesinger (1987)
0.4	Continuous, 12 weeks	M	21 weeks	Rat (Jcl: Wistar)	No change in population of AMs lavaged per rat lung; no change in specific activities of glucose-6-phosphate dehydrogenase and pyruvate kinase in lavaged AMs.	Mochitate et al. (1992)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.5	4, 8 hr 8 hr/day, 5 days/week, 5, 10 days	M	Young Adult	Rat (Sprague-Dawley)	<p>Ex vivo release of arachidonate metabolites from AMs: depression of LTB4 at 4 hr and no change in TxB2; with A23187-stimulated AM release, 5-HETE depressed at 4 hr and LTB4 and TxB2 depressed at 8 hr; no change in LTB4 release with ZAS-stimulation of AMs; PMA-stimulated AM superoxide release depressed at both time points; AM GSH and GSSG content unchanged at both time points.</p> <p>Ex vivo release of arachidonate metabolites from AMs: depression of LTB4 release at day 5 and no change in TxB2 release; with A23187-stimulated AM release, no change in LTB4 and TxB2 and 5-HETE increased at day 10; LTB4 release depressed on day 5 with ZAS-stimulation of AMs; PMA-stimulated AM superoxide release depressed at both time points; AM GSH and GSSG content unchanged at both time points.</p>	Robison et al. (1993)
0.1 1.0	1 hr (in vitro)	NS	Young Adult	Rat (Sprague-Dawley)	No change in unstimulated and stimulated (A23187) AM release of LTB4 at either NO ₂ concentration; depressed AM superoxide production in response to PMA stimulation at 1.0 ppm.	Robison et al. (1990)
0.1 1.0	1-4 hr (in vitro)				No change in PMN migration in response to products derived from exposed AMs.	
0.1 1	1 hr (in vitro) 1, 2, 4 hr (in vitro)	NS	Young Adult	Rat (Sprague-Dawley)	<p>At 0.1 ppm: no change in production of arachidonate metabolites TxB2, 12-HHT, or monoHETEs by NO₂-exposed AMs with or without stimulation by A23187.</p> <p>At 1 ppm: no change in production of LTB4, TxB2, 12-HHT, or monoHETEs from unstimulated AMs at all exposure durations; in AMs stimulated with A23187, no change in LTB4, TxB2, 12-HHT, or monoHETEs with prior 1-hr exposure, LTB4 and 12-HHT production was increased with prior 2-hr exposure, LTB4 and TxB2 was increased with prior 4-hr exposure.</p>	Robison and Forman (1993)
0.2	2 hr (in vitro)	NS	1-4 years	Cattle	Increased NO production by AMs, both in the absence and presence of LPS stimulation.	Höckele et al. (1998)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
Interaction with Infectious Microorganisms						
0.2 0.2 plus 0.8 spike twice per day for 1 hr	5 days/week, 1 year	F	6-8 weeks	Mouse (CD-1)	Significantly greater mortality from streptococcus infection with spike exposures to 0.8 ppm; no difference in group exposed to 0.2 ppm background only.	Miller et al. (1987)
0.5 0.5 daily for 3 hr plus 0.1 base	Peak exposure (0.5 ppm) 3 hr/d, 5 d/wk for 1, 2, 3 or 6 mo.	F	8-10 weeks	Mouse (CD ₂ F ₁)	NO ₂ exposure followed by challenge with <i>Streptococcus</i> aerosol and kept for 14 d in clean air – decreased survival time and increased mortality at 3 and 6 mo. with peak only exposures, and at 6 mo. with base + peak exposures. NO ₂ exposure followed by challenge with <i>Streptococcus</i> aerosol, then reexposed for 14 d to NO ₂ – no statistically significant difference in mortality or survival time at either exposure.	Ehrlich et al. (1979)
0.5	24, 18, or 6 hr/day, 7 days/week for 1, 3, 6, 9 or 12 mo	F	Young Adult	Mouse (Swiss alibino)	NO ₂ exposure followed by challenge with <i>K. Pneumoniae</i> aerosol and kept for 14 d in clean air – Increased mortality at 6 mo for all exposed groups, and at 12 mo for the continuously exposed group.	Ehrlich and Henry (1968)
0.5 1	4 hr	NS	8-10 weeks	Mouse (C57BL/ 6N)	No change in intrapulmonary killing of <i>Mycoplasma pulmonis</i> .	Davis et al. 1991
1	Continuous, 7, 14, 21, 28 days	F	4 weeks	Mouse (SPF ddY)	No change in mycoplasmal growth of <i>M. pulmonis</i> or formation of pneumonic lesions in lungs and trachea at any time point; no change in serum antibody titers to <i>M. pulmonis</i> .	Nisizawa et al. 1988

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
1	6 hr/day, 2 days prior to viral infection and 4 consecutive days following viral infection	NS	4-6 weeks	Mouse (CD-1)	No enhancement of murine cytomegalovirus infection or lung structure effects with initial infection or reinfection 1 month following initial infection.	Rose et al. (1989)

8.3.6 Effects on Pulmonary Function

A few recent studies performed pulmonary function tests in experimental animals following NO₂ exposure. Similar to the findings in the previous CARB review (1992), serious adverse functional injury has not been associated with mean NO₂ exposures at or below the level of the standard (0.25 ppm), although reports show pulmonary function changes above the standard. In past key studies, decreased vital capacity was observed in mice exposed to a background concentration of 0.2 ppm NO₂ with 0.8 ppm 1-hr spikes twice per day (Miller *et al.*, 1987). Background exposure to 0.2 ppm alone did not result in any changes in pulmonary function. Stevens *et al.* (1988) exposed adult and 1-day-old rats to 0.5 ppm NO₂ with daily 1-hr spikes to 1.5 ppm for 6 weeks. Adult rats experienced a decrease in lung compliance after 6 weeks, but recovered by 3 weeks post-exposure. Neonatal rats showed an increase in lung volume and compliance after 3 weeks of exposure but not after six weeks of exposure.

In recent reports not previously summarized, Kobayashi and Miura (1995) exposed guinea pigs to 0.06, 0.5, or 1.0 ppm NO₂ continuously for 6 or 12 weeks to examine the subchronic effects of NO₂ on pulmonary specific airway resistance (S_{Raw}) and induced hyperresponsiveness. Although 6 and 12-week NO₂ exposure did not cause a significant increase in S_{Raw} compared to baseline values, 13% (2 of 15) of the animals following 12 week exposure to 1 ppm NO₂ did have an S_{Raw} over twice that found prior to exposure. S_{Raw} is a measure of the amount of work necessary for breathing; resistance increases when airways are constricted or narrowed, increasing the work necessary to breath. Pulmonary airway hyperresponsiveness, as assessed by histamine aerosol challenge, was increased only with 12 weeks of exposure to 1.0 ppm NO₂.

In a more comprehensive analysis of a report reviewed previously (CARB, 1992), a near-lifetime NO₂ exposure study in rats simulating an outdoor diurnal urban profile (16-hour background of 0.5 ppm, with exposure rising to 1.5 ppm and declining to background over a 6-hour period five days per week, and daily 2-hour downtime for maintenance) was conducted to determine if lung dysfunction suggestive of degenerative lung disease was produced (Tepper *et al.*, 1993). NO₂ exposure for 78 weeks did not result in lung function alterations in nitrogen washout, compliance, lung volume, or diffusion capacity of carbon monoxide. However, a decrease in the delta forced expiratory flow at 25% of forced vital capacity (Δ FEF25%) occurred at 78 weeks of exposure.

The Δ FEF25% measures the effort-independent portion of the maximum flow-volume curve; a decrease in this measure would suggest a breathing pattern change perhaps associated with lung tissue abnormalities. Other breathing pattern changes at or just below statistical significance also suggested increased resistance of breathing indicative of oxidant-induced premature aging. However, these lung function alterations were reversible during a 17-week clean-air postexposure period, strongly supporting the conclusion that this pattern of NO₂ exposure did not result in degenerative lung disease.

Exposure to cold air, a known cause of airway constriction of asthmatic airways, was combined with exposure to NO₂ in anesthetized, paralyzed, and mechanically hyperventilated guinea pigs to test for enhanced lung function responses under simulated mild to moderate exercise conditions (Halinen *et al.*, 2000a). First exposure to cold dry air (-30°C) causes bronchoconstriction (i.e., reduced peak expiratory flow) in guinea pigs, but with repeated 10-minute cold dry air exposures for up to 4 consecutive times, bronchoconstriction became attenuated. However, combined 10-minute exposures with 1 ppm NO₂ counteracted the attenuation of bronchoconstriction induced by repeated cold dry air exposure.

With 60-minute exposures to cold dry air + 1 ppm NO₂ or warm humid air + 1 ppm NO₂, no significant bronchoconstriction was observed, likely due to adaptive mechanisms against prolonged bronchoconstriction (Halinen *et al.*, 2000b). However, the decline in tidal volume that occurs with normal ventilation of warm humid air and with hyperventilation of cold dry air was nearly abolished with exposure of NO₂ with either cold air or warm air. It was postulated that the NO₂ -induced increase in tension in the small bronchioles prevented them from collapsing and closing the airway.

Exposure of young ferrets nose only to 0.5 ppm NO₂ for 4 hr total per day (2 hr in the morning, 2 hr in the afternoon), 5 days/wk for 15 weeks during lung development (age 6-20 weeks), induced a

small but statistically significant increase in the ratio of total lung volume to body weight (Rasmussen and McClure, 1992). Higher NO₂ concentrations (10 ppm) resulted in greater lung volume increases, suggesting a dose-related effect on lung function. Young mice exposed intermittently (7 hr/day, 5 days/wk) to NO₂ for 6 weeks during lung development did not show changes in lung volume, as measured by water displacement, immediately after exposure or at 10 and 32 weeks post-exposure (Sherwin and Richters, 1995a).

8.3.6.1 Summary

The only acute exposure studies on pulmonary function explored the effect of NO₂ in combination with cold, dry air or warm, humid air. NO₂ (1 ppm) reduced the lungs ability to attenuate the airway constrictive effects of repeated, 10 min exposures to cold dry air. This may have relevance for asthmatics in cold environments. However, NO₂ did not enhance this airway constrictive effect with longer exposures (1-hour) to cold, dry air and warm, humid air.

With longer exposures, decreased vital capacity was observed in mice exposed to a background concentration of 0.2 ppm NO₂ with 0.8 ppm 1-hr spikes twice per day. Background exposure to 0.2 ppm alone did not result in any changes in pulmonary function. In adult and 1-day-old rats exposed to 0.5 ppm NO₂ with daily 1-hr spikes to 1.5 ppm for 6 weeks, the adult rats experienced a decrease in lung compliance after 6 weeks, but recovered by 3 weeks post-exposure. Neonatal rats showed an increase in lung volume and compliance after 3 weeks of exposure but not after six weeks of exposure. A chronic exposure study in rats employed a number of pulmonary function tests during NO₂ exposure (background of 0.5 ppm with daily peak rising to 1.5 ppm), but the deficits on the work of breathing were marginal and disappeared soon after exposure ended suggesting this level of NO₂ does not result in permanent, degenerative lung injuries.

Table 8.5. Pulmonary Function Effects of Nitrogen Dioxide

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.06 0.5 1.0	Continuous, 6, 12 weeks	M	10 weeks	Guinea pig (Hartley)	At 6 weeks: no change in SRaw compared to baseline SRaw; no effect on airway hyperresponsiveness with histamine challenge. At 12 weeks: No change in SRaw compared to baseline SRaw values, though 2 of 15 animals exposed to 1 ppm for 12 weeks had increased SRaw over twice that prior to exposure; induced airway hyperresponsiveness with histamine challenge at 1.0 ppm only.	Kobayashi and Miura, (1995)
0.2 0.2 plus 0.8 spike twice per day for 1 hr	5 days/week, 1 year	F	6-8 weeks	Mouse (CD-1)	0.2 ppm + 0.8 ppm spike group: trend toward decreased vital capacity that was also significantly different from 0.2 ppm background group.	Miller et al. (1987)
0.25	7 hr/day, 5 days/wk, 6 weeks	M	3 weeks	Mouse (Swiss-Webster)	No change in lung volume at 0, 10, or 32 weeks post-exposure.	Sherwin and Richters (1995a)
0.5	4 hr/day, 5 days/wk, 15 weeks	M F	6 weeks	Ferret (Mustela putorius furo)	Increase in the ratio of total lung volume to body weight.	Rasmussen and McClure (1992)
0.5 base with 2 hr peak to 1.5	5 day/wk, 1, 3, 13, 52, 78 weeks	M	9 weeks	Rat (Fisher 344)	Decreased Δ FEF25% at 78 weeks that was reversible with 17-week postexposure period; no change in nitrogen washout, compliance, lung volume, or diffusion capacity of CO.	Tepper et al. (1993)
1	10 min, with cold dry air and simulated exercise, repeated up to 4 times	M	NS	Guinea pigs (Dunkin-Hartley)	Normal attenuation of bronchoconstriction with consecutive 10 min cold dry air (-30°C) exposures is counteracted when NO ₂ exposure was included.	Hälinen et al. (2000a)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
1	1 hr, with cold dry air or warm humid air	M	NS	Guinea pigs (Dunkin-Hartley)	No significant bronchoconstriction observed; normal decline in tidal volume during 60-min exposures to cold dry air (-30°C) or warm humid air was nearly abolished when NO ₂ was included.	Hälinen et al. (2000b)

8.3.7 Effects on the Pulmonary Immune Response and Interaction with Allergens

NO₂ and other common air pollutants have been implicated as having the potential to increase the risk of sensitization to airborne allergens and enhance the airway responsiveness in subjects predisposed to asthma. Allergic asthma is characterized by airway hyperresponsiveness to a variety of specific and nonspecific stimuli, chronic pulmonary eosinophilia, elevated serum IgE levels, and excessive airway mucus production. IgE antibody is produced during allergic sensitization, which is regulated by cytokines and T lymphocytes. Antigen binds to IgE on mast cells and triggers the release of histamine and other molecules resulting in the allergic response. NO₂-induced alterations in cytokine and T lymphocyte levels may disrupt IgE regulation and lead to allergic diseases.

Pulmonary allergic response effects at NO₂ concentrations below or near 1 ppm were not reported previous to the first NO₂ review (CARB, 1992). However, suppressed T cell responsiveness in both pulmonary and extra-pulmonary sources has been observed following acute or short-term exposure to NO₂ concentrations starting at 5 ppm (Holt *et al.*, 1979; Joel *et al.*, 1982), or following subchronic exposure to NO₂ concentrations starting at 0.25 ppm (Richters and Damji, 1988).

Morphological changes suggestive of mast cell degranulation was observed in rats acutely exposed to 0.5 ppm NO₂ for 4 hrs or 1.0 ppm for 1 hr (Thomas *et al.*, 1967). These changes were not apparent 24-27 hrs post-exposure. The granules of mast cells in the lung contain a broad variety of pharmacologically active chemicals which are capable of initiating acute inflammation, as well as the bronchoconstriction typically associated with asthma. In one of the most sensitive acute changes reported in the lung following NO₂ exposure, the number of mast cells in the bronchi of rats was observed to increase with exposure to 0.2 ppm NO₂ for 3 hours (Hayashi and Kohno, 1985). However, mast cell number had returned to normal levels by day 7 of continuous exposure. Statistical analysis was not provided for changes in mast cell number.

Similar changes in mast cell number were also observed with continuous exposure to 0.4 and 0.5 ppm NO₂ although the number of mast cells appeared to continue rising through 6 days of exposure to 0.5 ppm NO₂. A transient, but statistically significant, decrease in histamine content of the rat trachea was observed after 45-60 min of exposure to 0.5 ppm NO₂ during continuous exposure for 7 days (Hayashi and Kohno, 1985; Hayashi *et al.*, 1987). This change coincided with the appearance of alcian blue positive cells, suggesting a decrease of histamine as a result of an increase in the alcian blue-staining immature forms of mast cells

Since 1992, a number of pulmonary immune studies have been conducted in several animal species including guinea pigs, rats, mice and rabbits. Because pulmonary immune effects in humans following NO₂ exposure is the primary basis of the NO₂ standard recommendations, animal studies using NO₂ concentrations above 1 ppm are also reviewed.

Historically, the guinea pig has been the model of choice and both specific immunologic responses and pulmonary sensitivity (reactivity) to inhaled or intratracheally administered allergens have been measured (Luster *et al.*, 2000). Although the guinea pig has some significant immunological differences compared to humans (e.g., IgG1 vs. IgE reagenic antibodies), it has been proven to be a predictable model for humans given the limited comparative data available. Airway smooth muscle hyperresponsiveness of main bronchi to stimuli (acetylcholine, neurokinin A, electrical stimulation), airway microvascular leakage, and airway influx of eosinophils and neutrophils have been demonstrated in guinea pigs following acute exposure to a high concentration of NO₂ (18 ppm for 4 hr) (Papi *et al.*, 1999).

As discussed in the Pulmonary Function Section, continuous 12-week exposure, but not 6-week exposure, to 1 ppm NO₂ increased airway hyperresponsiveness to histamine challenge in guinea pigs (Kobayashi and Miura, 1995). Another study in guinea pigs used exposures of 5 ppm NO₂ for 6 weeks (4 hr/day, five days/week), during which the animals were sensitized with two IP injections of *Candida albicans* and later exposed to a provocative challenge with aerosolized *C. albicans* (Kitabatake *et al.*, 1995). The NO₂-exposed animals exhibited delayed-type dyspneic symptoms compared to the control group, suggesting enhancement of allergenic respiratory disease by NO₂.

Ohashi *et al.* (1998) exposed guinea pigs to 9 ppm NO₂ (6 hr/day, 12 times over 13 days) during which some of the animals were passively sensitized and then challenged with intratracheally administered antigen at the end of exposure. NO₂ exposure resulted in ciliary depression and accumulation of significant numbers of eosinophils to the epithelium of the trachea. Neither passive sensitization nor antigen challenge with NO₂ exposure developed more prominent pathological changes than NO₂ exposure alone. However, antigen-antibody interaction with NO₂ resulted in considerably increased injury of tracheal epithelium that was thought to be related to the presence of activated eosinophils and free eosinophil-specific granules in the tracheal mucosa of this group.

The mouse is currently the model of choice by most immunologists when examining the allergic response (Gershwin, 2003). Exposure of animals to ovalbumin (OVA) has frequently been used to model allergic airway disease in humans and is known to elicit similar physiologic and immunologic outcomes. Exposure to irritant gases such as NO₂ is thought to modulate the development and/or outcome of allergic airway disease. In mice sensitized by intraperitoneal OVA injections followed by daily 1-hour OVA aerosol inhalation challenge, airway inflammation was produced characterized by increased BAL fluid cellularity and eosinophil levels (Hubbard *et al.*, 2002). Eosinophils are a prominent feature of allergic airway disease and participate through the release of inflammatory cytokines, granular proteins, and oxidants. However, when daily OVA aerosol challenge for 3 or 10 days was followed immediately each day by exposure to 0.7 or 5 ppm NO₂ (3 day exposure only) for 2 hours, BAL cellularity and eosinophil levels were significantly reduced. Histopathological analysis confirmed the reduction in pulmonary inflammation. These results were contrary to expectations and the differences with comparison to the clinical data was attributed to risk factors or triggers (e.g., exposure to other pollutants, respiratory-tract infections) encountered in humans but not in the animal models, and/or to the temporal relationship between NO₂ exposure and OVA exposure. The developmental stage during exposure (adult mice versus children) may also explain the incongruity between these results and observations in humans.

A similar study exposed OVA-immunized mice to 5 or 20 ppm NO₂ for 3 hr just after intranasal challenge with OVA or saline (Proust *et al.*, 2002). Acute NO₂ exposure produced contrasting effects on the development of asthma-related responses in OVA-immunized mice, depending on the concentration of NO₂. Increased epithelial permeability and bronchopulmonary hyperreactivity to methacholine occurred at 20 ppm NO₂ compared with air or 5 ppm NO₂. These effects were likely related to epithelial injury resulting at the high NO₂ concentration. Eosinophilia was present in air and 20 ppm groups, but a marked reduction in eosinophilia was observed at 5 ppm. NO₂ exposure had no effect on production of IL-4 in BAL fluid or IgE titers in serum compared to air controls.

However, BAL fluid levels of IL-5 were enhanced by exposure to 20 ppm NO₂ and reduced by exposure to 5 ppm NO₂ compared to air controls. Serum IL-4 and IL-5 are indicators of allergic inflammation. Serum IgG1 titers were increased in the 5 ppm group compared to the 20 ppm and air groups. Finally, mucosal metaplasia was observed by histological analysis in the OVA-air group, but did not develop in groups exposed to NO₂, likely due to oxidative denaturation of the mucus. The researchers speculated that NO₂ at lower concentrations (5 ppm) may reduce the capacity of AMs to respond to immunological stimuli, similar to what was shown by Robison *et al.* (1993), thus explaining the decreased allergic responses observed at this level.

Poynter *et al.* (2006) exposed mice to 5 or 25 ppm NO₂ for longer periods of time (6 hr/day for 1, 3 or 5 days) following sensitization and challenge with OVA to generate airway inflammation. In mice immunized and challenged with OVA, inhalation of 25 ppm NO₂ caused a marked augmentation of eosinophilic inflammation and terminal bronchiolar lesions, and was still present in 5-day-exposed mice up to 20 days postcessation. Significantly, exposure to 25 ppm alone was sufficient to cause airway hyperresponsiveness following methacholine challenge, a cardinal feature of asthma. Exposure to 5 ppm NO₂ elicited no pathological findings over that produced by sensitization and challenge by OVA alone.

In order to investigate the pulmonary immune effects of NO₂ (0.5 and 1.0 ppm) on IgG1, and IgG2a antibody production and interleukin levels in BAL fluid, mice were intermittently treated with ovalbumin (OVA) aerosol at 3-wk intervals during continuous exposure to NO₂ for 12 weeks (Fujimaki *et al.*, 1998). Half of the mice had been pre-immunized with OVA prior to NO₂ exposure. In

mice without pre-immunization, BAL fluid production of IgG2a was suppressed at both NO₂ concentrations and production of IgG1 was suppressed at 1.0 ppm. In mice that were pre-immunized with OVA, production of both antibodies appeared to be stimulated, but only IgG1 levels at 1.0 ppm were statistically significant.

Alterations in levels of interleukins-4, -10, and -12 (IL-4, IL-10, IL-12, respectively) were also investigated in BAL fluid of mice with or without pre-immunization. These cytokines are generated by various lymphocytes and are known to promote the secretion of antibodies. However, the only alteration observed in cytokine levels was a reduction of IL-4 in animals exposed to 1.0 ppm NO₂ without pre-immunization. These results indicated that the timing of NO₂ exposure in relation to immunization appears to be an important factor for how the antigen-specific immune system responds.

In another murine model, Hussain *et al.* (2004) sensitized the animals to OVA by IP injections on days 1 and 7 and were challenged with aerosolized OVA on days 13 and 14. Some mice were exposed to NO₂ at 2 ppm for 24 hours before undergoing OVA challenge. Despite increased epithelial damage in OVA-exposed mice, NO₂ exposure did not alter the expression of allergen-induced airway response as measured by bronchial reactivity to inhaled methacholine or changes in airway eosinophilia, although increased tone of respiratory smooth muscle was observed. Contrary to expectations, NO₂ exposure reduced epithelial mucin in OVA-exposed mice. The authors suggested such short-term NO₂ exposures do not significantly alter airway hyperreactivity, and mirrors similar findings in *in vitro* studies.

A study in mice to investigate the effect of oxidative stress induced by vitamin E-deficiency and/or NO₂ inhalation on allergen-sensitized type 1 (respiratory sensitization) allergy responses has been conducted (Mi *et al.*, 2002). NO₂ inhalation (5-6 ppm for 2 weeks) had little or no effect on enhancement of serum IgE levels in unsensitized, vitamin E-deficient mice, and 2,4-dinitrochlorobenzene-sensitized mice that were vitamin E-deficient. However, NO₂ exposure markedly enhanced serum IgE levels in trimellitic anhydride (TMA)-sensitized mice that were either vitamin E-deficient or fed the control diet.

Three-hour exposure of Brown Norway rats to 5 ppm NO₂ after both a priming injection and pulmonary challenge with allergen enhanced specific immune responses to House dust mite allergen (HDM) (i.e., increased IgG, IgE, IgA in BAL fluid) and increased the number of inflammatory cells in the lung (Gilmour *et al.*, 1996). The Brown Norway rat has increasing use in allergy research because of their high IgE responder status compared to other strains of rat. Lymphocyte responsiveness to antigen in the spleen and mediastinal lymph nodes was also significantly higher in these NO₂-exposed rats.

Single NO₂ exposures after either phase resulted in variable responses but no trend toward suppression or enhancement of any immune parameters was discernable. The authors suggested that the NO₂-enhanced immunity and subsequent inflammation may involve the release of mediators from the lung tissue as well as increasing antigen translocation to lymphoid tissue underlying the epithelial surfaces of the lung.

Histamine release stimulated with IgE or the calcium ionophore A23187 was investigated *ex vivo* in lung mast cells of Wistar rats and guinea pigs that were exposed continuously to 1.0 ppm NO₂ for 12 weeks (Fujimaki and Nohara, 1994). NO₂ had no effect on the number of mast cells or on IgE- and A23187-mediated histamine release in either species, although higher NO₂ exposures (4 ppm) in guinea pigs did produce an increase in IgE-mediated histamine release.

The role of airborne pollutants in the programming of the immune system during infancy is one likely factor that can determine whether and to what extent allergic sensitization can occur. Rabbits immunized within 24 hr of birth with an antigen together with the adjuvant Al(OH)₃ induces preferential production of antigen specific IgE antibodies that exhibit several features in common with asthmatic individuals. In a study on the interaction of allergens with NO₂ in neonatal animals, rabbits were immunized within 24 hours of birth by IP injection of HDM antigen in Al(OH)₃ gel, and exposed to 4 ppm NO₂ for 2 hr/day, 5 days/week for 3 months (Douglas *et al.*, 1995).

NO₂ had no effect on total cell and differential cell counts recovered in BAL fluid, and airways responsiveness via histamine or methacholine provocation compared to HDM-immunized rabbits. NO₂ exposure also had no effect on cutaneous responsiveness to intradermal antigen, or serum IgE levels assessed by the passive cutaneous anaphylaxis reaction. The researchers theorized that the lack of influence of NO₂ may be related to the choice of exposure route for immunization, IP injection as opposed to aerosol-delivery of antigen.

In vitro studies have failed to demonstrate any effect of acute exposure to NO₂ on bronchial smooth muscle from allergic guinea pigs (Chitano *et al.*, 1994). NO₂ also had no effect on the *in vitro* smooth muscle contractile response of guinea pig bronchi in the presence of tachykinins, indicating that NO₂ does not directly cause an increase in bronchial smooth muscle responsiveness (Chitano *et al.*, 1995).

8.3.8 Summary

Because of the high sensitivity of human asthmatics exposed to NO₂, a number of studies have investigated the effect of NO₂ on allergic asthma in animal models. Many factors in addition to NO₂ exposure could have a large impact on how NO₂ affects the lung's immune response to antigens. Some factors likely involved include exposure to other pollutants besides NO₂, presence of current or past respiratory-tract infections, the temporal relationship between NO₂ exposure and antigen exposure, and developmental stage of animal during exposure.

At low NO₂ concentrations (≤ 1.0 ppm), indicators of allergic asthma in antigen-sensitized animal models were either negative or produced effects contrary to the original hypothesis. However, exposure to higher concentrations of NO₂ (about 5 ppm and greater) have more consistently produced one or more indicators of allergic asthma including, enhancement of delayed-type dyspneic symptoms, increased serum IgE levels, increased pulmonary eosinophilia and epithelial injury, and increased bronchial hyperresponsiveness.

Table 8.6. Pulmonary Immune Response Effects and Interaction of Nitrogen Dioxide with Allergens

NO ₂ Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.2 0.4 0.5	Continuous, 30 min to 7 days 20 min to 7 days 5 min to 6 days	M	Young adult	Rat (Wistar)	Qualitative increase of mast cell number in bronchi by 3 hrs of exposure to 0.2 ppm. Reduction in mast cell number to normal levels by day 6 at 0.2 and 0.4 ppm. Continued increase in mast cell number through day 6 of exposure to 0.5 ppm. At 0.5 ppm: decreased histamine content (p<0.05) in rat trachea at 45 and 60 min time points.	Hayashi and Kohno (1985); Hayashi et al. (1987)
0.5 1	4 hr 1 hr	M	Young Adult	Rat (Sprague-Dawley)	At both concentrations: Morphological changes in lung mast cells suggestive of degranulation immediately after exposure. Mast cells of exposed rats appeared normal 24-27 hr post-exposure.	Thomas et al. (1967)
0.5 1.0	Continuous, 12 weeks	F	4 weeks	Mouse (BALB/c)	In pre-OVA immunized and non-pre-OVA immunized mice, OVA aerosol treatment was performed at 3-week intervals during exposure. Without pre-OVA immunization: BAL fluid reductions of IgG2a at 0.5 and 1.0 ppm, and IgG1 at 1.0 ppm; BAL fluid reduction of IL-4 at 1.0 ppm; no change IL-10 or IL-12 levels. With pre-OVA immunization: BAL fluid increase in IgG1 at 1.0 ppm; no change in IgG2a, IL-4, IL-10, IL-12 levels.	Fujimaki et al. (1998)
0.7 5	2 hr/day, 3, 10 days	M F	Young Adult	Mouse (C57Bl/6)	In OVA-sensitized mice, exposure for 3 days (0.7 and 5 ppm) or 10 days (0.7 ppm) reduced OVA-induced (OVA challenge immediately before air or NO ₂ exposure) BAL cellularity and eosinophil levels, and reduced histopathological evidence of OVA-induced pulmonary inflammation.	Hubbard et al. (2002)
1.0 2.0 4.0	Continuous, 12 weeks	M NS	10 weeks 10 weeks	Rat (Wistar) Guinea pig (Hartley)	In rats: no change in number of mast cells; reduction in IgE-mediated histamine release at 2 ppm; no change in A23187-mediated histamine release. In guinea pigs: no change in number of mast cells; increased IgE-mediated histamine release at 4 ppm; no change in A23187-mediated histamine release.	Fujimaki and Nohara (1994)

NO ₂ Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
2	24 hr	F	6-8 weeks	Mouse (BALB/c)	OVA-sensitized on days 1 and 7, challenged with aerosolized OVA on days 13 and 14, animals exposed to NO ₂ prior to OVA challenge. Compared to immunized/challenged mice, NO ₂ increased airway smooth muscle tone, but had no effect on percent airway eosinophils, hyperreactivity via methacholine challenge, or airway goblet cell hyperplasia.	Hussain et al. (2004)
4	2 hr/day, 5 days/week, 3 months	M F	< 1 day	Rabbit (New Zealand White)	Immunized IP with house dust mite antigen and Al(OH) ₃ adjuvant gel, then exposed to NO ₂ . Compared to immunized rabbits, NO ₂ had no effect on airway inflammation, airway responsiveness via histamine or methacholine provocation, or on serum IgE levels as assessed by the passive cutaneous anaphylaxis reaction.	Douglas et al. (1995)
4.76	4 hr/day, 5 days/week, 6 weeks	M	Young Adult	Guinea pig (Hartley)	Animals sensitized to <i>Candida albicans</i> IP on day 1 and week 4 of NO ₂ exposure, and then challenged with <i>C. albicans</i> inhalation after end of exposure. NO ₂ enhanced delayed-type dyspneic symptoms (tachypnea) in sensitized guinea pigs.	Kitabatake et al. (1995)
5 20	3 hr	M	6-7 weeks	Mouse (BALB/c)	OVA-immunized mice intranasally challenged with OVA just before NO ₂ exposure. At 5 ppm compared to OVA-air controls: No effect on bronchopulmonary hyperresponsiveness, epithelial permeability, neutrophilia, serum IL-4, and serum IgE. Reduced eosinophilia, serum IL-5 and mucosal metaplasia. Increased serum IgG1. At 20 ppm compared to OVA-air controls: increased bronchopulmonary hyperresponsiveness, epithelial permeability, neutrophilia, and serum IL-5. No effect on eosinophilia, serum IL-4, or serum IgE and IgG1 levels. Reduced mucosal metaplasia.	Proust et al. (2002)

NO ₂ Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
5 25	6 hr/day for 1, 3, or 5 days	NS	NS	Mouse (C57BL/6)	Exposure to 25 ppm alone for 3 days increased airway hyperresponsiveness (AHR) following methacholine challenge. In mice immunized and challenged with OVA, 25 ppm caused marked augmentation of eosinophilic inflammation and terminal bronchiolar lesions. At 20 days postcessation of 5-day 25 ppm mice, eosinophilic and neutrophilic inflammation, pulmonary lesions, and AHR still present. 5 ppm NO ₂ elicited no pathological findings over that produced by sensitization and challenge by OVA alone.	Poynter et al. (2006)
5	3 hr	F	7 weeks	Rat (Brown Norway)	Immunized IP with HDM antigen and Bordetella pertussis adjuvant, then challenged 2 weeks later with intratracheal injection of antigen NO ₂ exposure after both immunization and challenge: increased serum IgE, local IgA, IgG and IgE compared to air controls; increased inflammatory cells in lungs and lymphocyte responsiveness to antigen in spleen and mediastinal lymph nodes. Single NO ₂ exposures after either phase: variable responses but no trend toward suppression or enhancement of all immune parameters.	Gilmour et al. (1996)
5-6	Continuous, 2 weeks	M	3 weeks	Mouse (BALB/c)	At beginning of exposure, mice were sensitized to either DNCB or TMA on skin, then challenged with same solutions on ears 7 days later. Serum IgE levels collected at end of exposure were enhanced by NO ₂ exposure in TMA-sensitized mice that were fed either control diet or a vitamin E-deficient diet.	Mi et al. (2002)
9	6 hr/day, 12 exposures over 13 days	F	Young Adult	Guinea Pig (Hartley)	Passively sensitized by anti-benzylpenicilloil bovine gamma globulin guinea pig serum i.v. on day 7 of exposure, and then challenged by intratracheally with antigen 1 day after exposure. NO ₂ decreased ciliary activity, increased tracheal eosinophilia. NO ₂ plus antigen-antibody treatment increased tracheal epithelial damage by activated eosinophils.	Ohashi et al. (1998)
18	4 hr	M	Young Adult	Guinea Pig (Hartley)	Exposure enhanced bronchial hyperresponsiveness ex vivo to acetylcholine, electrical field stimulation, neurokinin A, but not to histamine. Airway influx of eosinophils and neutrophils	Papi et al. (1999)

8.4 Systemic Effects

Exposure to NO₂ may result in health effects on organs and tissues outside of the lung. It is unlikely inhalation of NO₂ will result directly in a systemic effect, but rather, is the result of reaction products of NO₂ or endogenously produced hormone-like substances generated through pulmonary oxidant injury. The reports summarized here, some of which were reviewed previously by CARB (1992), examined the hematology, cardiovascular and systemic immune systems for NO₂-induced effects.

Miller *et al.* (1980) reported interference with the detoxification process in the liver of mice, measured as an increase in pentobarbital-induced sleeping time, at exposures as low as 0.25 ppm NO₂ for three hours.

Splenic and peripheral blood lymphocyte responses following NO₂ exposure have been studied. Splenic lymphocytes have important roles in immune surveillance, antigen recognition, and overall control of immune responses. Shifts in different lymphocyte populations could lead to abnormal cellular interactions and responses to infectious microorganisms and neoplastic cells. Richters and Damji (1988) examined the effects of NO₂ on murine splenic T-lymphocyte subpopulations and natural killer cells. Natural killer activity targets neoplastic and virus-infected cells and is considered an immediate defense mechanism or an innate immune response.

Exposures included 0.25 ppm NO₂ for 7 weeks and 0.35 ppm NO₂ for 12 weeks on two separate groups of mice. Although only the Thy-1.2-positive T-lymphocytes of total spleen cells were statistically significantly reduced ($p < 0.05$) following exposure to 0.25 ppm NO₂, percentages of all T-lymphocyte subpopulations tested and natural killer cells were lower in spleens of mice exposed to NO₂. This report provided the first evidence linking alterations in T-lymphocyte subpopulations and natural killer cells to NO₂ exposure at ambient levels.

Kuraitis and Richters (1989) observed an increase in mean percent spleen weight ($p < 0.05$) at week 6 in 2 of 6 groups of mice exposed to 0.35 ppm NO₂ 8 hr/day, 5 days/week for up to 16 weeks. However, the mean percent spleen weights were reduced at weeks 9 and 16. No effect on spleen weight was seen in groups of mice exposed for 8 and 12 weeks. The total number of spleen cells and the total number of splenocytes were reduced at 9 and 12 weeks of exposure, but not with 6 weeks of exposure.

The total number of spleen RBCs were reduced only with 12 weeks of exposure. The relative mean percent of IgM-positive lymphocytes in the spleen was reduced with 12 week exposure to 0.35 ppm NO₂, suggesting this lymphocyte population may be responsible for the observed changes in splenocyte counts. A shift in peripheral blood leukocyte counts were also noted in mice exposed to NO₂ for 8 weeks, exhibiting an increase in neutrophils and a decrease in lymphocytes.

In further studies, Richters and Damji (1990) observed a persistent reduction in splenic T-lymphocyte subpopulations that was statistically significant ($p < 0.05$) for the T-helper/inducer (CD4+) lymphocytes following exposure of AKR mice to 0.25 ppm (7 hr/day, 5 days/week) for 181 days. AKR mice are susceptible to spontaneous lymphoma, but the NO₂-exposed mice showed a slowed progression of spontaneous lymphoma and increased survival. It was hypothesized that NO₂ suppresses the T-lymphocytes that give rise to AKR lymphoma, thus reducing the progression of the disease.

Fujimaki *et al.* (1982) observed suppression of the splenic primary antibody response to sheep RBCs in mice exposed to 0.4 ppm NO₂ continuously for 4 weeks. The suppression was thought to be either an inactivation of T and B cells and/or changes of the ratio of the number of T and B cells in the spleens of exposed mice. Mice exposed to 0.5 ppm NO₂ continuously for 3 to 12 months show duration dependent splenic T and B cell suppression (Maigetter *et al.*, 1978).

In a chronic study, rats were exposed to an urban pattern of NO₂ (16-hour background of 0.5 ppm, with exposure rising to 1.5 ppm and declining to background over a 6-hour period five days per week, and daily 2-hour downtime for maintenance) for 1, 3, 13, 52, and 78 weeks, following which several immune response tests were conducted (Selgrade *et al.*, 1991). NO₂ did not affect splenic T-cell responses to the mitogens concanavalin A (ConA) and phytohemagglutinin (PHA), or the splenic B-cell response to the mitogen *Salmonella typhimurium* glycoprotein. Peripheral blood lymphocyte responses to T-cell mitogens PHA and ConA were also unaffected by NO₂ exposure. No

histopathologic effects in spleen, lymph nodes, thymus, or bone marrow were observed. NO₂ did suppress splenic natural killer cell activity, but only at the 3-week exposure time point.

Changes in D-2,3-diphosphoglycerate (2,3-DPG) content of RBCs in guinea pigs was investigated following continuous exposure to 0.36 ppm NO₂ for 1 week (Mersch *et al.*, 1973). The preliminary results showed a significant increase in RBC 2,3-DPG content in exposed animals, suggesting 2,3-DPG dissociation from hemoglobin and decreased oxygen binding.

Heart and blood pressure effects resulting from NO₂ exposure have also been investigated. Under the same chronic exposure conditions as that used by Selgrade *et al.* (1991), no significant exposure-related consequences were observed in repeated measures analysis of the electrocardiogram (ECG) at either 52- or 78-week exposure evaluation (Tepper *et al.*, 1993). In a separate study, 60-minute exposures to 1 ppm NO₂ and cold dry air (-30°C) in anesthetized, paralyzed, and mechanically hyperventilated guinea pigs did not alter blood pressure and heart rate compared to cold dry air controls, although exposure to cold dry air itself induced increases in both blood pressure and heart rate (Halinen *et al.*, 2000b). Inhalation of NO₂ is thought to enhance the bronchoconstrictive effects of cold dry air in hyperventilating asthmatic subjects.

Human studies have suggested a link between NO₂ exposure and cardiovascular effects in patients with diabetes mellitus and those with cardiovascular diseases who have high risks of atherogenesis. Takano *et al.* (2004) exposed a male rat model susceptible to cardiovascular-type diseases, the Otsuka Long-Evans Tokushima Fatty rat, to 0, 0.16 or 0.8 ppm NO₂ continuously from 8 to 32 weeks of age. Another group of rats, Long-Evans Tokushima rats, were exposed to the same NO₂ regimen and served as normal controls. Exposure to 0.16 ppm NO₂ increased triglycerides and decreased HDL and the HDL/total cholesterol ratio in the obese rat model, whereas NO₂ exposure only decreased HDL levels in the normal controls.

These changes suggest increased atherogenic cardiovascular effects. At 0.8 ppm, blood levels of HDL were increased in both rat strains compared to their identical strain controls. However, no dose-response effect was seen for any measure of atherogenic disease. The authors speculated that the dose-response curve for atherogenic indicators in the rats is U-shaped (i.e., signs of atherogenesis occurring predominantly at only low or high exposures) due to integrated toxicologic responses dependent on multiple underlying processes.

8.4.1 Summary

Because NO₂ has its primary action in lung tissues, fewer studies have investigated systemic effects due to NO₂ exposure. Acute NO₂ exposures to 1 ppm and chronic exposures to 0.5 ppm (with daily 2 hr peak to 1.5 ppm) has not resulted in blood pressure and heart rate changes. However, interference with the detoxification process in the liver of mice, measured as increased pentobarbital-induced sleeping time, has been observed with acute exposure to 0.25 ppm NO₂. Systemic immune system cells and organs showed no differences with long-term exposure of rats to 0.5 ppm NO₂ with daily peaks to 1.5 ppm, with the exception of a transient reduction in splenic natural killer activity early in exposure. Reduction in natural killer activity may result in increased susceptibility to viral infections or neoplastic disease.

In mice, subchronic exposures to 0.25-0.35 ppm NO₂ suppressed splenic T-lymphocyte subpopulations and altered spleen weight. Splenic lymphocytes have important roles in immune surveillance, antigen recognition, and overall control of immune responses. An obese rat strain exposed to 0.16 ppm NO₂ for 24 weeks exhibited changes in blood levels of triglycerides, HDL, and HDL/total cholesterol ratio suggestive of atherogenic cardiovascular effects. This study indicates that animals with compromised health may be sensitive models for NO₂-induced toxicity.

Table 8.7. Systemic Effects of Nitrogen Dioxide

NO ₂ Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.16 0.8	Continuous, 24 weeks	M	8 weeks	Rat (Long Evans "fatty" and 'normal')	Normal rats: Decreased HDL blood levels at 0.16 and 0.8 ppm (no dose-response effect). Fatty rats: Decreased HDL, HDL./total cholesterol ratio, and increased triglycerides in blood at 0.16 ppm. Decreased HDL blood levels at 0.8 ppm. No dose-reponse effect seen.	Takano et al. (2004)
0.25	7 hr/day, 5 days/wk, 181 days	F	5 weeks	Mouse (AKR/cum)	Decreased splenic T-helper/inducer lymphocytes; trend towards decreased T-lymphocytes. Decrease in development and progression of spontaneous T-cell lymphoma.	Richters and Damji (1990)
0.25- 0.35	8 hr/day, 5 days/wk for up to 16 weeks	M	4 weeks	Mouse (C57BL/6J)	Increased mean percent spleen weight at 6 wks, decreased at 9 and 16 wks; decreased total number of spleen cells and splenocytes at 9 and 12 wks, decreased spleen RBCs and mean relative percent of IgM-positive lymphocytes at 12 wks, but no change in theta-positive lymphocytes; decreased spleen mean lymphoid nodule area at 16 wks; lower percent lymphocytes and higher percent neutrophils in differential peripheral blood cell counts at 8 wks.	Kuraitis and Richters (1989)
0.25	7 hr/day, 5 days/wk, 7 weeks	F	6 weeks	Mouse (AKR/cum)	Decreased T-lymphocyte subpopulation of total spleen cells ($p < 0.05$). Trend towards decreased natural killer cells and other splenic lymphocyte subpopulations.	Richters and Damji (1988)
0.35	7 hr/day, 5 days/wk, 12 weeks	M	6 weeks	Mouse (C57BL/6j)	Trend towards suppression in all 3 T-cell subpopulations tested	
0.36	Continuous, 1 week	NS	NS	Guinea Pig (NS)	Increased RBC D-2,3-diphosphoglycerate content	Mersch et al. (1973)
0.4	Continuous, 4 weeks	M	7 weeks	Mouse (BALB/c)	Suppression of the splenic primary antibody response to sheep RBCs	Fujimaki et al. (1982)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.5 0.1 with 3-hr spikes, 5 days/week of 0.25, 0.50 or 1.0	Continuous 1,3,6,9,12 months Continuous, 1,3,6,9,12 months	M	10-11 weeks	Mouse (CD-1)	Mitogenic response of splenic lymphocytes, when stimulated by phytohemagglutinin (PHA) or bacterial lipopolysaccharide, was generally depressed after 3-12 month exposure at all exposure levels. The decrease was not related to NO ₂ concentration or duration of exposure, except for continuous exposure to 0.5 ppm NO ₂ , where the decrease in PHA stimulation index was related to the duration of exposure. Further statistical analysis not provided.	Maigaetter et al. (1978)
0.5 base with 2 hr peak to 1.5	5 days/wk with peak, 7 days/wk with base, 1, 3, 13, 52, 78 weeks	M	10 weeks	Rat (Fisher 344)	No change in splenic T-cell or peripheral blood lymphocyte response to ConA, and PHA; no change in splenic B-cell response to S. typhimurium glycoprotein; no effects in spleen, lymph nodes, thymus, or bone marrow; reduced splenic natural killer cell activity at 3 wks.	Selgrade et al. (1991)
0.5 base with 2 hr peak to 1.5	5 days/wk with peak, 7 days/wk with base, 52, 78 weeks	M	9 weeks	Rat (Fisher 344)	No change in ECG.	Tepper et al. (1993)
1	1 hr, with cold dry air (-30°C)	M	NS	Guinea pigs (Dunkin-Hartley)	No change in blood pressure and heart rate compared to cold dry air controls.	Hälönen et al. (2000b)

8.5 Effects on Development

This section covers the scientific literature that investigated both the pulmonary and extra-pulmonary effects of NO₂ during pre and postnatal development. There has been an increasing awareness in recent years that children may be more susceptible than adults to the harmful effects of air pollutants. A major rationale children may be more susceptible is that their developing organs, in particular, the pulmonary system, is still immature.

Lung development is both a pre and postnatal process. The lung is largely formed during gestation; however, following birth there is a significant period of cellular differentiation and proliferation as the lung continues to grow until adolescence (Smiley-Jewell and Van Winkle, 2004). It is becoming apparent from epidemiologic and animal studies that the developing lung is especially susceptible to toxic pollutants present in the environment and that, in many cases, it has limited repair potential compared to the adult lung. Lung repair in the growing individual is complicated by the fact that reparative and developmental phases overlap following injury; this may result in alterations in the normal course of lung maturation.

Cyclic exposure studies in infant monkeys to another oxidant gas, ozone, have been shown to alter postnatal maturation of the lung. Changes include loss in the number of strictly conducting airways, reduction of distal airway size, altered smooth muscle bundle orientation, and hyperinnervation and irregular epithelial nerve distribution in intrapulmonary airways (Fanucchi *et al.*, 2006; Kajekar *et al.*, 2006). One likely result of these ozone-induced pulmonary changes in the monkeys is a marked increase in baseline airway resistance (Schelegle *et al.*, 2003).

Two reports examined the effects of prenatal NO₂ exposure on the offspring. Pregnant rats were exposed to NO₂ in concentrations of 0.05, 0.10, 1, and 10 mg/m³ (0.03, 0.05, 0.5, and 5 ppm, respectively) for 6 hrs/day throughout gestation (Tabacova *et al.*, 1985). Maternal toxicity data was not presented. There was a transient decrease in pup body weight on postnatal day 21 (PN21) at 5 ppm, but no effect on viability. For physical maturation parameters, a dose-dependent delay in eye opening and incisor eruption was observed that was statistically significant in the 0.5 and 5 ppm NO₂ groups. For neuromotor development, pups in the 5 ppm group showed decreased rate and increased latency of righting, reduced air righting reflex, decreased hindlimb support success rate and increased latency for hindlimb support, and reduced reactivity to auditory startle response.

A reduction in negative geotaxis was observed in the 0.5 and 5 ppm groups. The most sensitive indicator of neuromotor effects was postural and gait differences in open field behavior (i.e., number of squares entered, number of head and body raisings, posture and gait characteristics, duration of passivity periods, and latency) observed in pups prenatally exposed to 0.05 ppm NO₂. These effects also appeared to be dose-related. No significant differences in motor activity were noted by PN21. However, reduced horizontal and vertical motor activity and a tendency toward reduced exploratory behavior were noted in the 5 ppm group at 1 and 2 months of age, and reduced number of rearings in the 0.5 ppm at one month of age.

Biochemical parameters of the livers of offspring were also investigated by Tabacova *et al.* (1985). Statistically significant decreases in cytochrome P-450, aminopyrine-N-demethylase activity, and oxygen consumption were observed in livers of pups exposed prenatally to 5 ppm NO₂. In addition, increased lipid peroxides were also found in pup livers in the 0.5 and 5 ppm groups. Hexobarbital sleeping time, an indicator of liver drug-metabolizing ability, was increased in pups of the 0.5 and 5 ppm groups.

In another prenatal exposure study, pregnant mice were exposed to 22 or 45 ppm NO₂ from gestational day 7 to 18 (Singh, 1988). Visible signs of maternal toxicity were not observed, but other endpoints of maternal toxicity (e.g., reduced weight gain) were apparently not explored. Decreased birth weight was observed in offspring of both exposed groups. Neonatal behavioral development was also altered in both groups, with exposure resulting in reduced righting reflex and aerial righting score. Although reduced, negative geotaxis and activity scores of prenatally exposed pups were not statistically significantly different from control groups when measured on PN10 and 28.

The majority of studies investigating the NO₂ effects in young animals dealt with postnatal exposures. In young rats and guinea pigs, exposure to NO₂ during lung development generally shows greater resistance to injury than mature animals. In particular, weaning appears to be the critical time point for changes in responsiveness to oxidant injury. For example, exposure of one-, five-, or ten-day-old rats to 14 ppm NO₂ continuously for 24 to 72 hours caused only a slight loss in cilia in the terminal bronchioles, while the destruction of epithelial cells occurred in similarly exposed 20 to 40-day-old rats (Stephens *et al.*, 1978). In a separate study, Stephens *et al.* (1982) estimated that the nursing rat pup was 5 to 6 times more resistant than young adult rats to cellular and tissue injury. However, in terms of lethality, rat pups appear to be only about twice as resistant as young adults during the first 96 hrs of continuous exposure.

In a similar study, Azoulay-Dupuis *et al.* (1983) exposed 5, 10, 21, 45, 55, and 60-day-old rats and guinea pigs to 10 ppm NO₂ for three days to investigate age-related histopathological alterations. NO₂ exposure in rats caused slight fibrous deposits in alveoli and some loss of cilia in the airway on the older three groups, but not in rats 21 days old or less. Guinea pigs of all ages were affected more severely than rats, but both histopathology and mortality were worse among older groups. The guinea pigs showed an increase in type II cell deterioration at all ages, and a deterioration of both type I cells and capillary endothelium leading to an alveolar and interstitial edema in adults only.

Lunan *et al.* (1977) exposed 10- to 50-day-old rats continuously to 14 ppm NO₂ for three days and measured the glucose-6-phosphate dehydrogenase (G6PDH) content of the rat lungs. One function of G6PDH is to supply reduced nicotinamide adenine dinucleotide phosphate (NADPH), which is needed to reduce lipid peroxides formed in cell membranes as a consequence of oxidant gas exposure. Only rats older than 10 days of age at end of exposure responded with an elevation of G6PDH. Thus, it was thought that the response of G6PDH levels in the lung does not explain the increased resistance of early postnatal rats to NO₂ exposure, and that the disposal of the toxic lipid peroxides generated by NO₂ does not involve G6PDH and the glutathione shuttle.

Similar to other oxidant gases such as ozone and hyperbaric oxygen, there is evidence that prolonged exposure to NO₂ delays lung maturation. Exposure of rats from birth to 15 ppm NO₂ continuously for up to 75 days reduced the number of parenchymal airspaces between 10 and 45 days of age, but the deficit was made up by 75 days of age (Freeman *et al.*, 1974). This finding suggests a delayed or slower rate of alveolar maturation caused by NO₂ inhaled during lung development, but can be transient and does not necessarily persist into adulthood.

Three early life studies began NO₂ exposures in rats at one month of age. The lungs of one-month-old rats are in a rapid, "equilibrated" growth phase which would not resemble lung development in either the newborn or adult rats at the start of exposure (Mauderly *et al.*, 1987). Freeman *et al.* (1969) exposed groups of one-month-old rats continuously to 15 ppm NO₂ anywhere from 1 to 20 weeks. The animals were then allowed to recover in clean air for 0, 8, 20, or 52 weeks. The NO₂ exposures resulted in increased lung weight and hypertrophy of bronchiolar and alveolar epithelium, which tended towards normal recovery soon after the exposures.

In the aged rats 52 weeks following NO₂ exposures of 4 weeks or longer, lung weights were greater than the control group. Increased staining for collagen and elastin was observed in alveolar tissue, which was most noticeable in the ductal areas. Septal walls were sometimes attenuated and fractured in the longer-exposed rats. These results indicate some alterations in lung structure occur as a result of early life NO₂ exposure that is not apparent until later in life.

In the study by Juhos *et al.* (1980), one month old rats were exposed continuously to 15 ppm NO₂ for up to 17 months. In exposed rats, increased lung volume and lesions of small airways and adjacent alveolar tissue was observed. The diameters of the respiratory bronchioles were also decreased. Concurrent exposures of similarly-exposed adult rats with mature lung development was not conducted in these studies to determine if these changes primarily occur due to exposure in immature lungs.

The third study performed morphometric evaluations on the lungs of 1, 3, 12, and 21 months-old rats exposed to 0.1, 0.5, 3 and 10 ppm NO₂ continuously for one month (Kyono and Kawai, 1982). Both dose and age dependent effects were observed on the arithmetic mean thickness of the air-blood

barrier (AMT). Increased AMT was highest in the 1-month-old groups and decreased in order in the 3-month-old and 12-month-old groups, then increased again in the 21-month-old groups. The increased AMT resulted from the dose-dependent increase of total volume density of alveolar wall tissue and the dose-dependent decrease of the surface area of the alveolar wall.

Statistically significant increased volume density of alveolar wall tissue in 1-month-old rats occurred at 0.5 ppm NO₂ and was primarily due to an increase of the interstitial matrix. There was also a tendency for increased volume density of type I and II cells in the alveolar walls of 1-month-old and 21-month-old rats with increasing dose. For the 3- and 12-month-old rats, no change or decreased volume density of type I and II cells were observed at NO₂ concentrations below 10 ppm. Slight hyperplasia and decreased number of endothelial cells were observed in 1-month-old rats at NO₂ exposures over 0.5 ppm and was considered to imply a mild lesion of the endothelium.

Chang *et al.* (1986) exposed one-day-old (juvenile) and six-week-old (adult) rats to 0.5 ppm NO₂ 23 hrs/day, 7 days/week for 6 weeks. Two daily one-hour spikes to 1.5 ppm NO₂ were applied 5 days/week to simulate urban daily exposures. Adult rats were as sensitive, or more sensitive to NO₂ injury than were juvenile rats. Body weights were not affected in either exposure group. The epithelial type II cells in both exposed groups showed hypertrophy and spreading to cover more alveolar surface. This change resulted in significant decreases in the fraction of alveolar surface covered by type I cells.

The relative number of type II cells in the proximal alveolar region was unchanged. However, the pattern of response in juvenile and adult rats was different with respect to changes in type II cell size and thickness. The mean size of type II cells increased only 16% in the juvenile rats, but increased 79% in adult rats. The mean cell thickness of the type II cells decreased in exposed juvenile rats, but increased nonsignificantly in exposed adult animals. Additionally, adult rats showed a 24% increased total volume of alveolar tissue due to increased volume of type II epithelium (108%), fibroblasts (23%), interstitial matrix (20%) and alveolar macrophages (130%).

The exposed juvenile and adult rats were also examined for changes in lung volumes, compliance and efficiency of ventilation distribution between the juvenile and adult animals (Stevens *et al.*, 1988). Additional exposure groups included juvenile and adult rats exposed continuously to 1.0 and 2.0 ppm NO₂ with twice daily one-hour spikes equal to three times the baseline concentration. The juveniles initially appeared more susceptible to changes in lung function, which showed increased lung compliance after 3-week exposure to 1.0 and 2.0 ppm NO₂. However, lung compliance was unchanged after 6-week exposure. The exposed adults, on the other hand, exhibited normal pulmonary function until 6 weeks of exposure when the lungs became slightly stiffer (i.e., decreased compliance). This change was in direct correlation to NO₂ concentration, and was statistically significant for rats exposed to 1.0 and 2.0 ppm NO₂.

In a longer exposure study, Mauderly *et al.* (1987) exposed rats to 9.5 ppm NO₂ 7 hrs/day, 5 days/week for 6 months either throughout the period of development beginning at conception or as adults 6 months of age. Unlike the shorter exposure study by Chang *et al.* (1986), the longer NO₂ exposures at a higher steady concentration used in the present study caused few adverse effects discernable in either age group by the evaluations applied. Normal lung development, as reflected in the size and functional efficiency at adulthood, was not affected by NO₂ in the study. There was no age or exposure-related differences in the rate of clearance of radiolabeled particles from the lungs of either age group. There were no exposure-related differences in the mean linear intercepts of terminal air spaces or in the calculated lung internal surface areas of either age group that would indicate histological evidence for thickening of airways, interstitial tissue or alveolar septae.

NO₂ slightly reduced body weight (10%) and altered airway fluid enzymes of both age groups, with a greater number of statistically significant differences detectable in the enzyme levels of animals exposed as adults. The cytoplasmic enzymes, lactate dehydrogenase and glutathione peroxidase, the lysosomal enzyme, acid phosphatase, and the Type II cell-associated enzyme, alkaline phosphatase, were significantly increased in exposed adults. The airway fluid results suggested that phagocytosis, or the death of phagocytic cells, and cell injury in general were greater in the adults.

The response was not accompanied by an influx of leukocytes; the total cell numbers were not altered by exposure.

A number of developmental NO₂ exposure studies have been conducted in young mice and hamsters. In general, exposure of these animals to NO₂ during lung development result in equal or greater pulmonary injury compared to exposures of mature animals. In addition, young mice appear to be a more sensitive species to NO₂ exposure relative to young rats.

Three-day-old hamsters exposed continuously to 30 ppm NO₂ for ten days resulted in reduced lung elastic recoil and reduced alveolar surface area at 52 weeks of age (Lam *et al.*, 1983). Similarly exposed 21-day-old hamsters did not exhibit these effects at 52 weeks of age. The peak proliferative activity of the hamster lung occurs between 2 and 10 days of life, suggesting that the mild bronchiolitis produced by NO₂ exposure during a critical period in postnatal lung growth resulted in long-term differences in lung structure and function.

In a morphometric study of lung changes in newborn mice exposed intermittently (6 hr/day, 5 days/wk) to 0.3 ppm NO₂ for 6 weeks, the number (hyperplasia) of type II cells was increased (Sherwin and Richters, 1985). Mean type II cell area increased (hypertrophy) but fell short of statistical significance. It was postulated that the increased size and number of type II cells had occurred as a result of NO₂-induced type I cell damage.

The type II cell field area/alveolar wall area ratio progressively decreased through 4- and 10-weeks post-exposure, and was significantly different from controls at 10 weeks post-exposure. The authors interpreted this finding to be the result of NO₂-induced type II cell swelling followed by impairment of normal type II cell growth, and represented a possible permanent change in lung structure resulting from exposure during lung development.

Follow-up studies exposed 3-week old weanling mice to 0.25 ppm NO₂ (7 hr/day, 5 days/week, for 6 weeks) with a longer post-exposure period of 32 weeks (Sherwin and Richters, 1995a; Sherwin and Richters, 1995b). A trend towards increased type II cell size and number was seen immediately after exposure and at 10-weeks post-exposure, but was only significantly increased at 32-weeks post-exposure. Increases in alveolar wall intercepts ($p=0.08$) and alveolar wall perimeter ($p=0.09$) of borderline significance at 0 week postexposure suggested impaired function as a result of splitting and fragmentation of alveolar walls. Recovery of the alveolar wall parameters occurred at the 32-week postexposure period but was still increased above control levels. Alveolar elastic tissue and measurements were also performed in the mice.

Immediately after exposure, elastin fiber number and field area were increased relative to controls. However, elastin fiber number and fiber area per lung field were decreased at 10 weeks post-exposure. By 32-weeks post-exposure these elastin measurements were of borderline significance relative to the controls, although ratios of elastin number/alveolar wall area and elastin area/alveolar wall area were significantly increased. The changes in elastin measurements following NO₂ exposure indicate initial fragmentation of elastin and/or new fiber synthesis, followed by elastin proliferation and decreased alveolar wall area at 32-weeks post-exposure. No changes in lung volume measured by water displacement were found in the exposed mice at 0, 10, or 32 weeks postexposure.

The results of the elastic tissue and alveolar wall measurements in mice imply impairment of lung development and growth and altered repair processes as a result of NO₂ exposure. The persistence of the cell and wall alterations 32 weeks after NO₂ exposure ended further implies some degree of permanent structural and/or functional lung changes. Although concurrent exposures in adult mice were not conducted in either of the above mouse studies, an earlier study by Sherwin and Richters (1982) exposed adult mice (actual age not reported) to 0.34 ppm NO₂, 6 hr/day, 5 days/week for 6 weeks and examined similar lung morphology endpoints. Similar to the results in newborn and weanling mice, NO₂ exposure in adult mice resulted in an increased number of type II cells, an increased area (or volume) of type II cells, and a greater alveolar wall area immediately after exposure. However, changes at later postexposure time points were not examined in adult mice, so

it is unclear if the changes are as persistent as those found in NO₂-exposed newborn and weanling mice.

The extrapulmonary effects of NO₂ have also been investigated in young mice. Intermittent exposure (8 hr/day, 5 days/week for 3-12 weeks) to 0.25-0.30 ppm NO₂ in newborn and adult mice resulted in significantly lower body weight gains in the young animals, indicating greater sensitivity of the newborn mice for this endpoint relative to adults (Richters *et al.*, 1987). However, spleen weight in newborn and adult mice showed similar increases following exposure to 0.35 ppm NO₂, 8 hr/day, 5 days/week for 6 weeks (Kuraitis *et al.*, 1981).

The spleens of exposed newborn mice were also examined for other changes. NO₂ exposure resulted in increased size of spleen lymphoid nodules, a smaller increase in spleen cell number per given weight increment of spleen, and a visibly greater predominance of red cells in the red pulp. One explanation for the spleen changes was that NO₂ reaction products reached the bloodstream and caused increased phagocytic activity or macrophage number in the spleen and contributed to spleen weight changes.

Rasmussen and colleagues investigated effects of NO₂ exposure in young ferrets, another relatively sensitive animal species. Ferrets were chosen due to similarities in lung structure and development compared to the human lung. However, these studies did not compare similarly-exposed adult ferrets with mature lungs to determine if any lung changes observed in the juvenile ferrets are primarily due to NO₂ exposure during lung development. The animals were exposed intermittently (nose only, 2 hr in the morning and 2 hr in the afternoon, 5 days/week) to 0.5 or 10 ppm NO₂ from 6-weeks of age to 20 weeks of age during the period of most rapid lung development (Rasmussen and McClure, 1992).

In the 0.5 ppm group, increased numbers of inflammatory cells and necrotic cells were present in alveolar lumina and interstitium. Morphological changes at 0.5 ppm included increased septal wall thickness and parenchyma cellularity and decreased alveolar diameter and cross-section area. There was also a small, but statistically significant increase in the ratio of total lung volume to body weight. Whole lung collagen content was increased only at 10 ppm. The researchers noted that the ferret species used (*Mustela putorius furo*) generally exhibits low grade lung lesions likely related to respiratory infections. Whether NO₂ exposure resulted in the enhancement of respiratory inflammation in affected ferrets was not investigated.

Because small changes in collagen deposition in the alveolar region may be undetectable by gross, whole lung homogenate methods, further experiments in NO₂ induced lung collagen content used histochemical techniques to localize the areas of collagen deposition (Rasmussen, 1994). Using the same exposure regimen, 6-week-old ferrets exposed to 0.5 or 10 ppm NO₂ for 8 or 15 weeks resulted in increased collagen deposition in submucosa of respiratory bronchiolar epithelium, but was only statistically significant in the 10 ppm group. Removal from exposure after 8 weeks and allowing a 7-week recovery did not result in a decrease in collagen-stained areas.

Whole lung collagen content measured in one lung lobe was not sensitive enough to detect collagen differences among the treatment groups. Tracer particle clearance was also measured in airways of 6-week-old ferrets exposed to 0.5 or 10 ppm NO₂ under the same regimen for 8 or 15 weeks (Rasmussen *et al.*, 1994). Thoracic clearance, which includes the lungs and lower trachea, was reduced at both NO₂ concentrations, but only significantly in the 10 ppm group. Duration of exposure did not alter the clearance rate. Head airways clearance, which included the nose to the upper trachea, was unaffected at either concentration.

8.5.1 Summary

The few prenatal exposure studies conducted in animals indicate that exposures as low as 0.05 ppm NO₂ may result in behavioral changes of rat offspring. Prenatal exposure to 0.5 ppm provided stronger evidence of delayed physical and behavioral maturation, although it was unclear what factor maternal toxicity had on these endpoints. The cumulative findings from the postnatal NO₂ exposure

studies suggest that mice are one of the most sensitive species. NO₂ exposure at concentrations as low as 0.25 ppm resulted in a persistent, if not permanent, alteration of both the epithelial cell population of the mouse alveolus and the supporting tissues of the lung parenchyma.

Separate exposure studies in adult mice under similar exposure conditions indicate that they are equal to or less sensitive than the young, although it is unknown if the pulmonary changes would persist significantly beyond the end of exposure in adult mice. Most postnatal exposure studies used the rat as the animal model. However, rats in which exposure began prior to weaning were less sensitive than adults by the pulmonary endpoints used. On the other hand, one study exposed rats for one month just after weaning and compared them to similarly exposed 3- and 12-month-old adult rats. An overall increase in the volume density of alveolar wall tissue was observed at 0.5 ppm in 1-month old rats, but not in the adult rats, suggesting greater sensitivity of young rats exposed during their rapid pulmonary growth phase.

Besides sensitivity differences due to species or age at beginning of exposure, the developmental studies also showed that NO₂ exposure pattern and post-exposure follow-up examination are important factors in detecting injury. For example, essentially no morphological pulmonary changes were found in juvenile and adult rats exposed intermittently to 9.5 ppm NO₂ for 6 months. However, exposure to 0.5 ppm NO₂ with twice daily peaks to 1.5 ppm for 6 weeks resulted in alveolar changes favoring the oxidant resistant type II cells. Many of the developmental studies examined animals soon after NO₂ exposure ended. However, results by Freeman *et al.* (1969) in rats and Sherwin and Richters (1995b) in mice indicate that some alterations in lung structure that occur as a result of early life NO₂ exposure do not become apparent until later in life.

Cyclic exposure to ozone in infant monkeys has resulted in serious alterations in lung postnatal maturation that are likely permanent. The pulmonary endpoints examined that resulted in changes (i.e., loss in the number of strictly conducting airways, altered smooth muscle bundle orientation, reduction of distal airway size, and hyperinnervation and irregular epithelial nerve distribution in intrapulmonary airways) and the exposure pattern were largely different than those investigated in the NO₂ exposure studies, so some NO₂-induced pulmonary changes may have been missed - most of the NO₂ exposure studies examined alveolar tissue for changes, not distal conducting airway tissue where most changes appear to occur in ozone-exposed monkeys.

However, there are some similarities between the ozone results and findings from the NO₂-exposure studies in young animals. Similar to the ozone-exposed monkeys, Juhos *et al.* (1980) observed reduced respiratory bronchiole diameters in one-month-old rats exposed chronically to 15 ppm NO₂. Also, an altered pattern of interstitial elements resulting from exposure during lung maturation occurred in both ozone-exposed monkeys and NO₂-exposed ferrets and mice (i.e., altered smooth muscle bundle orientation in monkeys; altered orientation or abundance of elastin in respiratory bronchioles of ferrets and in alveolar tissue of mice). Finally, both the ozone and NO₂ studies indicate there is a persistent, if not permanent, change in the structure of developing pulmonary epithelium of young animals exposed to an oxidant gas.

Table 8.8. Developmental Effects of Nitrogen Dioxide

NO ₂ Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
Pre-natal exposure studies						
0.03 0.05 0.5 5	6 hrs/day throughout gestation (21 days)			Rat (Wistar)	At 0.05 ppm and above: postural and gait changes in open field behavior At 0.5 ppm and above: delayed eye opening and incisor eruption; reduced number of rearings; increased liver lipid peroxides and hexobarbitol sleeping time A 5 ppm: reduced pup body weight; decreased rate and increased latency of righting reflex; reduced air righting reflex; decreased success rate and increased latency of hindlimb support; reduced auditory startle and horizontal and vertical motor activity; decreased liver P-450 activity. Maternal toxicity data not presented	Tabacova et al. (1985)
22 45	Continuous, day 7-18 of gestation			Mouse (CD-1)	In all groups: decreased birth weight; reduced righting reflex and aerial righting score No visible signs of maternal toxicity	Singh (1988)
Post-natal exposure studies						
0.1 0.5 3 10	Continuous, 1 month	F	1,3,12, 21 months	Rat (JCL-SD)	Arithmetic mean thickness of air-blood barrier highest in 1-mo old groups and decreased in order from 21-, 3-, to 12-mo old groups; volume density of alveolar wall in 1 mo and 21-mo age groups increased significantly at 3 ppm and above; number of type II cells at 10 ppm increased in 1-, 3-, and 21-mo-old groups and decreased in 12-mo-old groups; increased number of interstitial cells in 1- and 21-mo-old groups.	Kyono and Kawai (1982)
0.25-0.30	8 hr/day, 5 days/week, 1 to 12 weeks	M	Newborn and adult	Mouse (Swiss-Webster)	Six independent experiments, 3 in newborns and 3 in adults. Body weights of newborns were more sensitive than adults and showed significantly lower body weight gains	Richters <i>et al.</i> , 1987

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.25	7 hr/day, 5 days/wk, 6 weeks	M	3 weeks	Mouse (Swiss-Webster)	Type II cell hyperplasia and hypertrophy significant at 32-weeks post exposure, but not immediately after exposure or at 10 weeks post-exposure. Increased alveolar elastic fiber number and field area immediately after exposure, 10 weeks post-exposure: decreased alveolar elastic fiber number and field area per lung field, 32 weeks post-exposure: increased ratios of elastin number/alveolar wall area and elastin area/alveolar wall area.	Sherwin and Richters (1995a,b)
0.34	6 hr/day, 5 days/week, 6 weeks	M	Adult	Mouse (Swiss-Webster)	Increased number and area (or volume) of type II cells; greater alveolar wall area immediately after exposure.	Sherwin and Richters (1982)
0.3	6 hr/day, 5 days/wk, 6 weeks	M	Begun last wk of gestation	Mouse (Swiss-Webster)	Type II cell hyperplasia immediately following exposure; no changes 4 weeks postexposure; decreased type II cell field area/alveolar wall area ratio at 10-weeks postexposure.	Sherwin and Richters (1985)
0.35	8 hr/day, 5 days/week, 6 weeks	M	Newborn and adult	Mouse (Swiss-Webster)	Similar increased in % spleen weight for both newborns and adults. In newborns only, increased spleen lymphoid nodules, increased spleen cell number per given weight, and greater predominance of red cells in the red pulp.	Kuraitis et al. (1981)
0.5 with 2 daily 1 hr spikes of 1.5, 5 days/week	7 days/week, 6 weeks	M	1 day, 6 weeks	Rat (F344)	Few morphometric changes in 1-day-old animals; mean type II size increased 16%. In 6-week-old animals, mean type II size increased 79%; total volume of alveolar tissue increased 24% due to increases in volume of type II epithelium (108%), fibroblasts (23%), interstitial matrix (20%), and alveolar macrophages (130%).	Chang <i>et al.</i> (1986)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.5 1.0 2.0 with 2 daily 1 hr spikes 3 times background, 5 days/week	7 days/week, 3 and 6 weeks	M	1 day, 6 weeks	Rat (F344)	In 1-day-old group, lung compliance increased at 1.0 and 2.0 ppm at 3 weeks, but was unchanged at 6 week exposure interval. In 6-week-old group, compliance decreased at 2.0 ppm at 6 weeks and was correlated to an overall thickening of alveolar interstitium and septal tissue. No difference from controls for this pulmonary function change after 3-week recovery period.	Stevens <i>et al.</i> , 1988
0.5 10	4 hr/day 5 days/wk, 8 or 15 weeks	M F	6 weeks	Ferret (Mustela putorius furo)	At both levels: Increased number of inflammatory and necrotic cells in alveolar lumen and interstitium; increased alveolar wall thickness and decreased alveolar chord and cross-section area; increased nuclear density in lung parenchyma; increased ratio of total lung volume to body weight. At 10 ppm only: increased lung collagen content per mg protein; increased deposition of collagen in submucosa of respiratory bronchiolar epithelium, and no recovery 7-weeks post-exposure; reduced thoracic clearance (lungs and lower trachea) No change in whole lung collagen content or head airways clearance (nose and upper trachea) at either level.	Rasmussen and McClure (1992); Rasmussen (1994); Rasmussen <i>et al.</i> (1994)
2 10	Continuous, 3 days	M F	5,10,21,45,55,60 days, and adults	Rat (Wistar) Guinea Pig (Dunkin Hartley)	At 2 ppm: no histological alterations in rats; alveolar inflammation and edema in guinea pigs beginning at 45 days of age. At 10 ppm: in rats, fibrinous deposits in alveoli and occasional loss of cilia in tracheal and bronchiolar epithelium beginning at 45 days of age; in guinea pigs, severe pulmonary injury at all ages, and increasing mortality with increasing age and exposure.	Azoulay-Dupuis <i>et al.</i> (1983)
9.5	7 hrs/day, 5 days/week, 6 months	M	1 day, 6 mo.	Rat (F344)	Reduced body weight in both age groups; slightly altered airway fluid enzymes (lactate dehydrogenase, glutathione peroxidase, acid phosphatase, alkaline phosphatase) in both age groups with greater differences in the adult group No change functional lung efficiency, lung clearance rate, or mean linear intercepts of terminal air spaces in either age group.	Mauderlay <i>et al.</i> (1987)

NO2 Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
14	Continuous, 24, 48, 72 hrs	M F	1,5,10, 15,20,25,30,35,40 days	Rat (Sprague-Dawley)	Increasing evidence of mild injury of epithelial cells in the terminal bronchioles between ages 8 and 20 days. Loss of cilia is severe and cellular hypertrophy significant in 20-day-olds. Injury response reaches plateau by 40 days of age. Injury to alveolar epithelium begins in 20-day-olds and increases with age.	Stephens et al. (1978)
14	Continuous, 3 days	M F	10,15,20,25,30,35 days	Rat (Sprague-Dawley)	No elevation in G6PDH enzyme from homogenized lungs until day 15 of age; enzyme level doubled over controls at age 30 to 40 days.	Lunan <i>et al.</i> (1977)
20	Continuous 2 weeks		30 days		G6PDH peaked at 3 days and declined slightly by end of two-week exposure.	
15	Continuous, up to 75 days	M F	Day 1 up to 75 days of age	Rat (Hilltop)	Reduced number of parenchymal air spaces between 10 and 45 days of age; no difference by day 75 of age.	Freeman <i>et al.</i> , (1974)
15	Continuous, 1,4,10,16,20 weeks	(NS)	1 month	Rat (NS)	Exposures of 4 weeks or longer resulted in greater lung weight by 52 weeks following end of exposure. Increased staining for collagen and elastin in alveolar tissue.	Freeman <i>et al.</i> (1969)
15	Continuous, 1,2,3,4 weeks, 2,3,4,5,7,13,15, 17 mo.	M	1 month	Rat (NS)	Increased lung volume and lesions of small airways; hypersecretion and aggregation of cellular debris in terminal bronchioles; decreased diameter of terminal bronchioles after first week of exposure.	Juhos <i>et al.</i> (1980)
15 25 50 60	Intermittent 4 hr exposure with 1.5 hr break for up to 72 hrs.	M F	10 and 60 days	Rat (Sprague-Dawley)	Nursing pups are 5 to 6 times more resistant to cellular injury compared to 60-day-old animals. In terms of lethality, nursing pups are only about twice as resistant as 60-day-old animals.	Stephens et al. (1982)
30	Continuous, gradual increase to 30 ppm over 3 days, then exposed 7 days	M	3-day-old, 21-day-old	Hamster (NS)	After 1 yr of age, animals exposed as 3-day-olds showed increased lung volume at 25 cm H ₂ O pressure (V ₂₅), decreased transpulmonary pressure at 60% of V ₂₅ , and increased alveolar mean linear intercept. No change in exposed 21-day-old animals at 1 yr of age.	Lam <i>et al.</i> , 1983

8.6 Genotoxic, Mutagenic and Carcinogenic Effects

While the carcinogenic effects of NO₂ are not the focus of the standard, animal studies in this area of research are summarized here to provide a comprehensive review of the literature. As discussed in the introduction, most relevant experimental animal data on the mutagenic and carcinogenic effects of NO₂ is reviewed regardless of exposure level. This is because the development of cancer by a carcinogenic substance is considered a non-threshold event, unless scientifically established otherwise. In order to quantify the carcinogenic effect, animal carcinogenicity studies use several doses usually greater than the doses that people would be typically exposed to. The results are then extrapolated to the doses that people are exposed to. Thus, any exposure to a cancer-causing substance, no matter how small, will result in some incremental increase in cancer risk.

8.6.1 Genotoxicity and Mutagenicity Studies

NO₂ alone (Victorin and Stahlberg, 1989) or in combination with NO (Arroyo *et al.*, 1992) has been shown to cause mutations in bacterial assays in the range of 6-15 ppm. Higher concentrations tended to be bacteriotoxic. *In vitro* studies in V79 hamster cells have induced chromatid-type aberrations, sister-chromatid exchanges or DNA single strand breaks with NO₂ concentrations as low as 5 ppm (Tsuda *et al.*, 1981; Gorsdorf *et al.*, 1990), though one study observed increased sister-chromatid exchanges with NO₂ concentrations >1 ppm, but not 0.5 ppm (Shiraishi and Bandow, 1985). Single strand breaks have also been observed in rat AMs exposed to NO₂ (20 ppm, 2 hr) (Walles *et al.*, 1995). A recent report by Kelman *et al.* (1997) indicated that NO₂ may not be directly mutagenic. No mutagenic potential was observed when bacterial DNA was exposed to NO₂. However, when the bacterial DNA was exposed to a reaction product of NO and NO₂, dinitrogen trioxide (N₂O₃), base pair mutations occurred.

In genotoxicity studies with NO₂ *in vivo*, exposure of rats continuously for 3 days to 1.2 ppm NO₂ did not cause a significant increase in DNA single-strand breaks or stimulate poly (ADP-ribose)synthetase activity in lavaged AMs (Bermudez *et al.*, 1999; Bermudez, 2001). Poly(ADP-ribose) synthetase is an enzyme crucial for DNA repair; increased activity of this enzyme would indicate DNA damage has occurred. However, lung cells from rats exposed to 8-27 ppm NO₂ for 3 hours showed increased mutation frequency to ouabain resistance beginning at 15 ppm, and increased chromosome aberrations beginning at 8 ppm (Isomura *et al.*, 1984).

A mouse micronucleus assay was negative for genotoxic effects in bone marrow after inhalation of 20 ppm NO₂ for 23 hours (Victorin *et al.*, 1990). A previously reviewed report (CARB, 1992) also observed no genotoxic effects in non-pulmonary cells (spermatocytes and lymphocytes) following 6-hour exposure to 0.1-10 ppm NO₂ (Gooch *et al.*, 1977).

8.6.2 Carcinogenicity and Co-carcinogenicity Studies

No published reports employing classical carcinogenesis whole-animal bioassays were found. Since the previous NO₂ review (CARB, 1992), the most comprehensive long-term NO₂ carcinogenicity study had been conducted by Ichinose *et al.* (1991). The primary purpose of this study was to investigate the possible tumor enhancement by NO₂ with co-exposure to a lung carcinogen (see Interactions of NO₂ with Other Co-occurring Pollutants section). However, groups of rats were also continuously exposed to 0.04, 0.4 or 4 ppm NO₂-only for 17 months and then examined for nasal tumors (20 animals per group) and lung tumors (10 animals per group). No respiratory tract tumors and no increased incidence in non-pulmonary organ tumors were observed.

A less-than-lifetime exposure study investigated the effect of NO₂ and other substances in an experimental animal, which has a high spontaneous incidence of pulmonary tumors and is susceptible to lung tumor formation following exposure to some carcinogens. Intermittent NO₂ exposure (6 hr/day, 5 days/wk) of A/J mice for 6 months to 10 ppm, but not 1 or 5 ppm, induced a small but statistically significant increase in the frequency (tumors per mouse) and incidence (tumors per tumor-bearing mouse lung) of lung adenomas (Adkins *et al.*, 1986). However, a dose-response

effect was not apparent and the large variability in lung adenoma development among control groups was such that the data may be too ambiguous to conclude from this one study that NO₂ enhances tumor formation.

Alternatively, Richters and Damji (1990) have shown that the toxic action of NO₂ can also inhibit the formation of spontaneous tumors. Intermittent exposure to 0.25 ppm NO₂ for up to 181 days slowed the progression of spontaneous lymphoma and increased survival in AKR mice. A persistent reduction in splenic T-lymphocyte subpopulations (Thy-1.2+, CD4+) was also observed in NO₂-exposed mice. Since T-lymphocytes are the cells that give rise to AKR lymphoma, it was hypothesized that NO₂ adversely affects cells of the immune system, thus reducing the progression of spontaneous lymphoma in AKR mice.

In a study of NO₂-induced modulation of metastatic cell burden in lung, exposure of mice to 0.35 ppm NO₂ (7 hr/day, 5 days/wk) for six weeks followed by intravenous infusion of mouse melanoma cells facilitated blood-borne cancer cell metastasis to lungs (Richters and Richters, 1989). Microthrombus formation by NO₂, observed in lung capillary endothelium of the mice, was suggested to have a role in retention and establishment of the cancer cells in the lung. This work supports previously reported studies by the researchers of NO₂-induced increases of lung tumors in mice after injection of melanoma cells (CARB, 1992).

In a co-carcinogen study, hamsters received subcutaneous 20 mg/kg injections of the respiratory tract carcinogen N-nitrosodiethylamine (DEN) twice a week while being exposed continuously to 15 ppm NO₂ for up to 6 months (Witschi *et al.*, 1993). All surviving animals were sacrificed 8 months after beginning of exposure. No respiratory system tumors or increased incidence of non-pulmonary organ tumors were found in animals exposed only to 15 ppm NO₂. Exposure to DEN alone induced pulmonary tumors but no evidence for co-carcinogenesis with NO₂ was observed. In fact, NO₂ exposure actually delayed or inhibited the formation of lung, nasal, and tracheal tumors and extended survival compared to animals exposed to DEN alone.

In another co-carcinogen study, rats were given a single IP injection of the respiratory tract carcinogen N-bis(2-hydroxypropyl)nitrosamine (BHPN) followed by continuous exposure to clean air, 0.04, 0.4 or 4 ppm NO₂ for 17 months (Ichinose *et al.*, 1991). Nasal tumors were observed in nearly all animals receiving BHPN, regardless of exposure regimen. Exposure to 4 ppm NO₂ + BHPN resulted in a small but nonsignificant increase in lung tumors. All other groups, including groups exposed to NO₂ alone, did not display increased tumors in lungs or other organs. Combined exposures to pollutants including NO₂ have also been investigated for tumor promoting effects and are reviewed in Co-occurring Pollutants section below.

8.6.3 *In vivo* formation of carcinogens from NO₂

Previously reviewed studies (CARB, 1992) observed increased endogenous synthesis of carcinogenic nitrosamines in animals following treatment with nitrosamine precursors and inhalation of ambient levels of NO₂. Presumably, *in vivo* nitrosation of the precursor by NO₂ reaction products (i.e., nitrous or nitric acid) occurs to form the carcinogenic agent (Kano *et al.*, 1990; CARB, 1992). Rubenchik *et al.* (1995) found the carcinogen N-nitrosodimethylamine (NDMA) in whole body mouse powder following inhalation of NO₂ (4-4.5 ppm, 1 hr) by mice that were also administered the NDMA precursor amidopyrine.

In addition, inhalation of NO₂ by mice (4-4.5 ppm, 1 hr) and rats (0.4 ppm, 8 hr/day, 5 days/wk for 2 months) with co-exposure to orally administered amidopyrine and sodium nitrite enhanced formation of NDMA in blood. Intravenous administration of amidopyrine in rabbits acutely exposed to NO₂ (50 ppm) also led to the formation of NDMA in the blood (Kosaka *et al.*, 1987). Intermittent inhalation of 20 ppm radiolabelled-NO₂ (¹⁵NO₂) by mice for 4 days with daily gavage of morpholine resulted in the *in vivo* formation of the potent carcinogen N-nitrosomorpholine (Van Stee *et al.*, 1995). About 98% of the resulting N-nitroso nitrogen originated from the inhaled ¹⁵NO₂.

Administration of various non- or weakly mutagenic polycyclic aromatic hydrocarbons (PAH's) to experimental animals during exposure to NO₂ has also resulted in endogenously-formed mutagenic

nitro-PAH's, as detected with the Ames Salmonella test. In mice, IP injection of pyrene during 4-day exposure to 5 or 10 ppm NO₂ resulted in highly mutagenic nitropyrene metabolites found in the urine (Kano *et al.*, 1990). The urinary mutagenicity was dependent on both the dose of NO₂ and the pyrene concentration.

At higher NO₂ concentrations, mutagenic metabolites were also found in the urine of mice, rats, hamsters and guinea pigs following IP injection of various PAH's during exposure to 20 ppm NO₂ for 2 days (Miyaniishi *et al.*, 1996). Urine from mice treated with PAH's + clean air showed almost no mutagenic activity. Oral administration of ascorbic acid and α -tocopherol decreased the urinary mutagenicity of mice treated with both pyrene and NO₂ (Miyaniishi *et al.*, 1996).

8.7 Atmospheric Formation of Mutagenic Reaction Products

While not a principal topic of this review, laboratory investigations have produced highly mutagenic nitro-PAHs following the reaction of NO₂ at concentrations of 10 ppm or less with various PAH's in the presence of photoirradiation (Hisamatsu *et al.*, 1986; Hisamatsu *et al.*, 1989; Sasaki *et al.*, 1995; Ishii *et al.*, 2000). A number of species of nitrated PAH's are known to exist in polluted ambient air and could likely have arisen with the reaction of PAH's and NO₂ with sunlight. Various alkenes (i.e., propene, butadiene, ethane), when combined with NO₂ (0.2-0.25 ppm) and UV-irradiation, also produced or enhanced mutagenic activity in bacterial assays (Victorin and Stahlberg, 1989).

8.7.1 Summary

While the carcinogenic effects of NO₂ are not the focus of the standard, animal studies in this area of research are summarized here to provide a comprehensive review of the literature. NO₂ has been shown to be genotoxic and mutagenic in some, but not all, bacterial and animal test systems. The limited number of carcinogenicity and co-carcinogenicity studies that have been conducted were negative for lung cancer. In a study that investigated NO₂'s ability to modify lung tumor development, high NO₂ exposures (10 ppm) increased tumor frequency and incidence in a tumor-susceptible mouse strain, but there was a lack of a clear dose-response effect and high variability in lung adenoma development existed among several control groups.

In contrast to these data, the cytotoxic action of NO₂ had a beneficial effect on spontaneous T-lymphocyte-derived tumor development, presumably by suppressing the immune system in tumor-susceptible mice. NO₂ has been shown to facilitate blood-borne cancer cell metastasis resulting from infusion of mouse melanoma cells. Several reports show that NO₂ may have an indirect carcinogenic action, through the *in vivo* formation of nitrosamines and through formation of airborne carcinogenic compounds via atmospheric reactions with UV irradiation and volatile and semi-volatile organic substances. However, no reports exist that attempt to address indirect risk contribution scenarios for ambient levels of NO₂.

Table 8.9. Carcinogenic or Co-carcinogenic Effects of Nitrogen Dioxide

NO ₂ Concentration (ppm)	Exposure	Gender	Age	Species (Strain)	Effects	Reference
0.04 0.4 4	Continuous, 17 months	M	6 weeks	Rat (Wistar)	NO ₂ alone: No respiratory tract tumors or increased incidence of non-pulmonary organ tumors. BHPN treatment prior to exposure: No increase in pulmonary and non-pulmonary tumors except for a non-significant increase in BHPN-induced lung tumors at 4 ppm.	Ichinose et al. (1991)
0.25	7 hr/day, 5 days/wk, 181 days	F	5 weeks	Mouse (AKR/cum)	Reduces progression of spontaneous lymphoma.	Richters and Damji (1990)
0.35	7 hr/day, 5 days/wk, 6 weeks	M	5 weeks	Mouse (C57BL/6J)	Facilitates blood-borne cancer cell metastasis resulting from infusion of mouse melanoma cells following exposure.	Richters and Richters (1989)
1 5 10	6 hr/day, 5 days/wk, 6 months	Probably all F	6-10 weeks	Mouse (A/J)	No effect at 1 and 5 ppm; increased frequency and incidence of spontaneous lung adenomas at 10 ppm only.	Adkins et al. (1986)
15	23 hr/day, 7 days/wk, 4, 6 months	M	6-8 weeks	Hamster (Syrian golden)	NO ₂ alone: no respiratory system tumors and no increased incidence of non-pulmonary organ tumors. DEN treatment twice a week during exposure: NO ₂ delayed or inhibited DEN-induced formation of lung, trachea, and nasal cavity tumors.	Witschi et al. (1993)

8.8 Interactions of Nitrogen Dioxide with Other Co-occurring Pollutants

This section summarizes the interactive effects of NO₂ exposure in combination with other air pollutants at near-ambient concentrations, relative to NO₂ exposure alone. Since most people are exposed to several air pollutants simultaneously or sequentially, experimental studies that reproduce these complex interactions can represent more realistic environmental conditions than studies with NO₂ alone. Pollutants can interact toxicologically in three basic modes: additive, more than additive (synergistic), or less than additive (antagonistic).

Potentiation is a sub-classification of synergism and refers to a situation in which a pollutant that does not elicit a response when acting alone nevertheless increases the effect of another co-occurring pollutant. While antagonism implies lesser risk, some antagonistic interactions may increase the risk of disease through diminished protective or reparative abilities. The major air pollutants that have been combined with NO₂ in exposure studies include ozone, sulfur oxides (i.e., sulfuric acid, sulfur dioxide, sulfates), and particulate matter, including complex mixtures containing numerous pollutants.

8.8.1 Non-cancer Toxicological Studies with Co-occurring Pollutants

Continuous exposure to 0.4 ppm NO₂ and 0.4 ppm ozone for 2 weeks resulted in a synergistic increase in lipid peroxides based on thiobarbituric acid reactant levels in lung homogenates of guinea pigs, but not rats (Ichinose and Sagai, 1989). Exposure to NO₂ and ozone alone did not induce altered lipid peroxide levels in either species. Analysis of anti-oxidant substance levels and enzyme activities from lung homogenates revealed synergistic or additive increases in non-protein sulfhydryls, ascorbate, glucose-6-phosphate dehydrogenase, and glutathione peroxidase with combined oxidant exposure primarily in rats only. Exposure to NO₂ or ozone alone had little or no effect on anti-oxidant levels in either species. These findings suggest that guinea pigs are more sensitive than rats to oxidants due to their poor induction ability of antioxidants, resulting in greater lipid peroxide generation in lungs.

In a lifetime exposure study by the same authors, rats were exposed to ozone, ozone + 0.04 ppm NO₂, and ozone + 0.4 ppm NO₂ for up to 22 months and examined for pulmonary biochemical effects (Sagai and Ichinose, 1991). Ozone exposure duration was 10 hr/day, with a mean of 0.05 ppm and a daily peak level of 0.1 ppm. NO₂ exposures were continuous. Thiobarbituric acid values, an index of lipid peroxidation, had synergistically increased in the ozone/NO₂ mixtures at 9 months, but were similar to control values after 18 and 22 months of exposure.

Ozone alone and NO₂ alone from a previous study (Sagai *et al.*, 1984) did not alter thiobarbituric acid values. In general, both ozone/NO₂ groups and the ozone-only group showed increased lung vitamin E and nonprotein sulfhydryl contents at 9 months, which decreased to control or below control levels at 18 and 22 months. Whole lung antioxidant protective enzyme activities (mainly GSH enzymes and SOD) did not show any changes from control values in any groups during exposure. However, NADPH cytochrome P-450 peroxidase activity was reduced at 5-9 months in the high exposure group relative to control values. This enzyme is very sensitive to membrane lipid peroxides. A few pulmonary adenomas were observed in the combined exposure groups but were not statistically different from control values.

The effect of NO₂ was evaluated in a guinea pig model of asthmatic dyspnea in which inhalation of ammonium sulfate increased the sensitization to antigen challenge (Kitabatake *et al.*, 1992). Animals were exposed to ammonium sulfate (0.3 mg/m³) with or without NO₂ (0.2 ppm), 2 hrs/day, 5 days/wk for approximately 9 weeks. During the last two weeks of exposure, the animals were exposed to an albumin spray solution (3 times per week) following NO₂/ammonium sulfate exposure. While exposure to ammonium sulfate alone in antigen-sensitized animals increased asthmatic dyspnea (immediate type) sensitization and nonspecific responsiveness of the airways to acetylcholine challenge, the combined exposure with NO₂ had no additional effect on asthmatic sensitization in the guinea pigs.

Mautz *et al.* (2001) examined cumulative and adaptive responses of 3 concentrations of a simulated Southern California air pollutant mixture in rats intermittently exposed (4 hr/day, 3 days/wk) for 4 weeks. Direct comparisons with NO₂ exposure alone were not performed. Exposure to the high dose (0.6 ppm ozone, 0.4 ppm NO₂, 0.2 mg/m³ ammonium bisulfite (ABS), 0.12 mg/m³ carbon particles (C), 0.1 mg/m³ nitric acid (HNO₃)) exacerbated irritant-induced rapid shallow breathing responses while exposure to the medium concentration (0.3 ppm ozone, 0.2 ppm NO₂, 0.1 mg/m³ ABS, 0.06 mg/m³ C, 0.05 mg/m³ HNO₃) showed diminished responses over the 4-week exposure period.

Lavaged AMs showed dose-dependent depressions of Fc-receptor binding and phagocytosis that was significantly decreased at the medium (Fc-binding) or high (phagocytosis) concentrations. The pollutant atmospheres did not alter respiratory tract clearance of tracer particles but bronchoalveolar permeability, measured as total protein in BAL fluid, and histological evidence of parenchymal inflammation was increased at the high concentration.

Epithelial cell proliferation labeling, a marker of cell injury, showed a dose-dependent increase at all respiratory tract levels but was markedly elevated in the nose and terminal bronchioles of the high concentration group. It was indicated that exposure to the lower levels of pollutants induced a response that then attenuates on repeated exposure, but higher doses delivered in repetition result in an exacerbated response.

Combined exposure to filtered diesel exhaust, including the ultra fine particle fraction (<0.2 μm) and 0.79 ppm gaseous phase NO₂ emissions, during the fetal period has been investigated. Prior to filtering the total diesel exhaust, the test atmosphere contained 1.73 mg/m³ particulate matter. Rats exposed to both total and filtered diesel exhaust with NO₂ during the fetal period (7th day of gestation until 3 days after birth) or the suckling period (4th to 22nd day after birth) showed elevated titers of IgE against pollen (Watanabe and Ohsawa, 2002). Exposure after weaning had little or no effect, suggesting insufficient development of the immune system occurs during fetal and neonatal periods.

In a similar exposure study, male rats exposed to total or filtered diesel exhaust containing 0.80 ppm (high dose) or 0.10 ppm (low dose) NO₂ during gestation (gestational day 7 to birth) showed decreased daily sperm production at maturity, due to an insufficient number of Sertoli cells (Watanabe, 2005). The total diesel exhaust prior to filtering contained 1.71 mg/m³ (high dose) or 0.17 mg/m³ (low dose) particulate matter.

These studies indicate that the ultra fine diesel particulate fraction may be primarily responsible for the toxic insults in fetal and neonatal rats. While the contribution of NO₂ to allergic reactivity is unknown since NO₂-only and particulate-only groups were not included, the studies suggest enhancement of allergic reactivity may be, in part, due to synergism of NO₂ and other air pollutants interacting with environmental allergens.

8.8.2 Genotoxicity/Carcinogenicity Studies with Co-occurring Pollutants

In genotoxicity studies with combined exposures to NO₂ and ozone *in vivo*, rats continuously exposed for 3 days to 0.3 ppm ozone and 1.2 ppm NO₂ showed a significant increase in DNA single-strand breaks and stimulated poly (ADP-ribose)synthetase activity in the lavaged cells (primarily AMs and PMNs) (Bermudez *et al.*, 1999; Bermudez, 2001).

Poly(ADP-ribose) synthetase is an enzyme crucial for DNA repair; increased activity of this enzyme would indicate DNA damage had occurred. Exposure to 1.2 ppm NO₂ alone did not alter these parameters compared to control values. The combined oxidant exposure showed a synergistic interaction for stimulated poly (ADP-ribose)synthetase activity (Bermudez, 2001). However, the number of DNA single-strand breaks was similar for combined oxidant exposure and ozone alone (Bermudez *et al.*, 1999).

Combined exposure to oxidant pollutants including NO₂ have been investigated for tumor promoting effects. Rats were given a single IP injection of the respiratory tract carcinogen BHPN followed by exposure to 0.05 ppm ozone, 0.05 ppm ozone + 0.4 ppm NO₂, or 0.4 ppm NO₂ + 1 mg/m³ sulfuric acid for 13 months followed by post-exposure period of 11 months (Ichinose and Sagai, 1992).

Ozone exposure duration was 10 hr/day, with a mean of 0.05 ppm and a daily peak level of 0.1 ppm. All other pollutant exposures were continuous. No lung or nasal tumors were found in groups exposed to the oxidant gases without BHPN.

Exposure to BHPN followed by exposure to the ozone/NO₂ mixture resulted in a slight, but significant increase in lung tumors over groups exposed to BHPN alone. The other pollutant groups also enhanced lung tumor incidence resulting from BHPN exposure but were not statistically significant. Nasal tumors were observed in all animals treated with BHPN, which was the primary cause of death.

The previous review (CARB, 1992) noted that Richter and co-workers demonstrated that inhalation of 0.3-0.8 ppm NO₂ will facilitate melanoma cell metastasis to the lungs of exposed animals. In addition to this research, Richters (1988) has shown that exposure of mice to a combination of NO₂ and ozone at 0.35 ppm and 0.15 ppm, respectively, 7 hr/day, 5 days/wk for 12 weeks results in facilitation (70% increase) of blood-borne melanoma cell metastasis. Exposure to 0.15-0.3 ppm ozone alone does not facilitate melanoma cell metastasis. However, it was unclear whether synergism or an additive effect was displayed with combined oxidant exposure because the increase in the number of nodules with combined exposure was within the range observed with NO₂-only exposure (Richters, 1986).

Hamsters were exposed 19 hr/day, 5 days/wk to 5 ppm NO₂ and 10 ppm SO₂ combined for up to 18 months to determine if the irritant gases enhance tumor induction caused by a single injection (3 or 6 mg/kg) of the respiratory carcinogen diethylnitrosamine (DEN) (Heinrich *et al.*, 1989). Exposure to NO₂ alone was not performed. While exposure to DEN alone caused upper respiratory tract tumors, no enhancement in tumors were observed with exposure to the combined gases and DEN. In addition, exposure to the gas combination alone did not increase the tumor rate.

In studies investigating the tumor promoting effects of combined NO₂/particle exposures, rats were intratracheally administered 0.2 ml suspensions of diesel exhaust particle extracts (DEP) (once per week for four weeks) at the beginning of exposure to 6 ppm NO₂ 16 hr/day for 10 months (Ohyama *et al.*, 1999). Another group of rats were treated in a similar fashion but were exposed to 6 ppm NO₂ and 4 ppm SO₂ combined. Exposure to NO₂ alone was not performed.

The lungs of all rats were analyzed for tumors and DNA adducts following an 8-month clean air post-exposure period. While DEP alone did not result in DNA adducts or any lung tumors, exposure to DEP + NO₂ and DEP + the combined gases produced both DNA adducts and a significant increase in rats with alveolar adenomas. However, there were significantly fewer rats with adenomas in the combined gas group compared to the NO₂ group.

Using a similar exposure regimen, Ito *et al.* (1997) examined the lungs of rats for pulmonary endocrine cell hyperplasia and papillomas following intratracheal injections (once per week for four weeks, beginning at week 0) of an extract of suspended particulate matter (SPM) from urban air and co-exposure to 6 ppm NO₂ or 4 ppm SO₂, or both gases combined. A significant fraction of human lung cancers are known to originate from pulmonary endocrine cells. Gas exposures were 16 hr/day for 11 months, with analysis occurring in the 18th experimental month. Treatment with SPM-only increased the incidence of endocrine cell hyperplasia and induced a non-significant increase in endocrine cell papillomas. Co-exposure to the NO₂/SO₂ mixture or NO₂ alone had no effect on the incidence of endocrine cell hyperplasia and papillomas caused by SPM treatment.

8.8.3 Summary

Experimental studies that reproduce multi-pollutant interactions can represent more realistic environmental conditions than studies with NO₂ alone. The non-carcinogenic toxicological studies of multi-pollutant mixtures including NO₂ demonstrate that these multi-pollutant interactions are quite complex, and that the degree of non-cancer toxicological interaction is dependent on a variety of factors, including timing and intensity of exposure as well as the animal species. Two-week exposures of rats to NO₂ (0.4 ppm) and ozone (0.4 ppm) resulted in synergistic or additive increases

in anti-oxidant substances and enzymes in lung and no increase in lipid peroxides. However, guinea pigs under the same exposure regimen produced the opposite effect: no change in pulmonary anti-oxidant levels but increased lipid peroxide formation.

Long-term exposures in rats to NO₂ (0.4 or 0.04 ppm) and ozone (0.05 average with daily 0.1 ppm peak) did demonstrate a synergistic increase in lipid peroxides, but in general, no change in anti-oxidant substances and enzymes. A repeated 4-week exposure to a complex mixture of urban-type pollutants, including 0.2-0.4 ppm NO₂, observed inflammatory, morphological, and pulmonary function effects. Combined diesel exhaust and NO₂ exposure during fetal and neonatal development have observed reproductive and immunological effects. However, in some cases, the co-pollutant studies did not include groups exposed to NO₂ alone or without NO₂ to determine the contribution of NO₂ to the effect.

The carcinogenic interaction of NO₂ with other pollutants has also been studied. NO₂ (6 ppm) combined with a diesel exhaust particle (DEP) extract produced a synergistic increase in DNA adducts and alveolar adenomas over DEP treatment alone. A similar long-term multi-pollutant study including exposure to suspended particulate matter (SPM) did not observe an increased incidence of endocrine cell hyperplasia and papillomas with SPM/NO₂ or SPM/NO₂/SO₂ mixtures over that caused by SPM treatment alone. Co-carcinogenicity studies with multi-pollutant mixtures yielded ambiguous results. NO₂ (0.4 ppm) and ozone (0.05 ppm average) mixtures in rats produced a marginally significant co-carcinogenic action with the pulmonary carcinogen BHPN, while mixtures of NO₂ and H₂SO₄ with BHPN in rats and NO₂ and SO₂ with the pulmonary carcinogen DEN in mice did not.

Table 8.10. Interactions of Nitrogen Dioxide with Other Co-occurring Pollutants

Pollutant Concentration (ppm)	Exposure	Gender/Age	Species (Strain)	Effects/Interaction	Reference
Toxicological, Non-cancer Effects					
NO ₂ : 0.4 O ₃ : 0.4	Continuous, 2 weeks	M 10 wks	Rat (Wistar)	Rat: No change in lipid peroxides in lung homogenates; synergistic or additive increase in non-protein sulhydryls, ascorbate, glucose-6-phosphate dehydrogenase, and glutathione peroxidase.	Ichinose and Sagai (1989)
		NS 10 wks	Guinea pig (Hartley)	Guinea pig: synergistic increase in lipid peroxides in lung homogenates; no effect on above listed anti-oxidant substance levels or enzyme activities.	
NO ₂ : 0.04 O ₃ : 0.05 NO ₂ : 0.4 O ₃ : 0.05	NO ₂ continuous, O ₃ was 0.05 average with daily 0.1 ppm peak, 5, 9, 13, 18, 22 months	M 7 wks	Rat (Wistar)	Dose-dependent, synergistic increase in lipid peroxides in lung homogenates at 5-9 months, though the low exposure group did not reach statistical significance at 5 months; no change in lipid peroxides with ozone or NO ₂ alone; NADPH cytochrome P-450 peroxidase activity reduced at 5-9 months in high exposure group; no change in nonprotein sulhydryl and vitamin E contents compared to ozone alone; no change in other antioxidant enzyme activities.	Sagai and Ichinose (1991)
NO ₂ : 0.2 Ammonium sulfate: 0.3 mg/m ³	2 hr/day, 5 days/wk, 9 weeks	M Young Adult	Guinea pigs (NS)	Animals sensitized to antigen (albumin) 3x/week during last 2 weeks of exposure: combined NO ₂ /ammonium sulfate exposure had no additional effect on asthmatic dyspnea (immediate type) sensitization and nonspecific responsiveness of the airways to acetylcholine challenge compared to exposure to ammonium sulfate alone.	Kitabatake et al. (1992)
Three mixtures of: NO ₂ , O ₃ , ammonium bisulfite (ABS), carbon particles (C), HNO ₃	4 hr/day, 3 days/wk, 4 weeks	M NS	Rat (Fisher 344/N)	High exposure group (NO ₂ , 0.4 ppm; O ₃ , 0.6 ppm; ABS 0.2 mg/m ³ ; C, 0.12 mg/m ³ ; HNO ₃ , 0.1 mg/m ³): continued exposure exacerbated irritant induced rapid shallow breathing; reduced AM phagocytosis; increased total protein in BAL fluid; showed histological evidence of pulmonary inflammation; increased epithelial cell proliferation. Lower exposure groups (NO ₂ , 0.2 or 0.1 ppm; O ₃ , 0.3 or 0.15 ppm; ABS, 0.1 or 0.05 mg/m ³ ; C, 0.06 or 0.03 mg/m ³ ; HNO ₃ , 0.05 or 0.03 mg/m ³) show attenuation in responses with continued exposure.	Mautz et al. 2001

Pollutant Concentration (ppm)	Exposure	Gender/Age	Species (Strain)	Effects/Interaction	Reference
0.79 ppm NO2 with either total (1.73 mg/m3) or filtered diesel exhaust	6 hr/day, GD7 to day 3 after birth (fetal), Day 4-22 (suckling), Day 23-41 (weaning)	M	Rat (Fisher F344 /DuCrj)	Fetal period exposure: increased IgE titers against pollen in immunized rats and decreased thymus and spleen weights in both total and filtered diesel exhaust groups. Suckling period exposure: increased IgE titers against pollen in immunized rats and increased testosterone levels in both total and filtered diesel exhaust groups. Decreased thymus weight in filtered diesel group only. Weaning period exposure: no effect seen.	Watanabe and Ohsawa (2002)
0.10 or 0.80 ppm NO2 with 1.71 or 0.17 mg/m3 total (or filtered) diesel exhaust, respectively	6 hr/day, GD7 to birth	M	Rat (Fisher F344 /DuCrj)	At day 96 after birth in exposure groups: decreased daily production of sperm, spermatids, and Sertoli cells; increased spermatids/Sertoli cells ratio and follicle-stimulating hormone levels.	Watanabe (2005)
Carcinogenic and Co-carcinogenic Effects					
Group 1 O3: 0.05 Group 2 O3: 0.05 NO2: 0.4 Group 3 NO2: 0.4 H2SO4: 1 mg/m3	13 months NO2, H2SO4: Continuous, O3: 10 hr/day, mean 0.05 ppm with daily 0.1 ppm peak,	M 6 wks	Rat (Wistar)	Combined exposures without BHPN: No respiratory tract tumors over length of experiment (24 months). BHPN treatment prior to exposure: small increase in lung tumors in all pollutant exposure groups but was only statistically significant with the O3/NO2 mixture.	Ichinose and Sagai (1992)

Pollutant Concentration (ppm)	Exposure	Gender/Age	Species (Strain)	Effects/Interaction	Reference
Group 1 NO2: 0.35 O3: 0.15 Group 2 O3: 0.15	7 hr/day, 5 days/wk, 12 weeks	M 5 wks	Mouse (C57Bl/6J)	Combined exposure increased blood borne melanoma cell metastasis to lungs by 70% over controls receiving only melanoma cell infusion. Exposure to ozone alone had no effect on melanoma cell metastasis.	Richters (1988)
NO2: 5 SO2: 10	19 hr/day, 5 days/wk, 18 months	M F 10 wks	Hamster (Syrian golden)	Combined exposure without DEN: no increase in tumor rate. DEN treatment 2 weeks after initiation of exposure: no enhancement in respiratory tract tumors compared to DEN alone.	Heinrich et al. (1989)
Group 1 NO2: 6 Group 2 NO2: 6 SO2: 4	16 hr/day, 10 months with 8 month clean air post- exposure period	M 6 wks	Rat (F344/Jcl)	Weekly DEP extract instillation during first month of exposure: No pulmonary DNA adducts or lung tumors with DEP alone; DEP + NO2 and DEP + combined gases increased pulmonary DNA adducts and alveolar adenomas; Exposure to NO2 alone without DEP was not performed.	Ohyama et al. (1999)
Group 1 NO2: 6 Group 2 NO2: 6 SO2: 4	16 hr/day, 11 months with 7 month clean air post- exposure period	M 5 wks	Rat (Fisher 344)	Weekly instillation of extract of SPM from urban air during first month of exposure: Co-exposure to NO2 or the NO2/SO2 mixture did not enhance pulmonary endocrine cell hyperplasia and papillomas caused by SPM treatment alone. Exposure to NO2 alone without SPM was not performed.	Ito et al. (1997)

8.9 Toxicological Effects in Human In Vitro Test Systems

In vitro studies with human tissues and cells can examine mechanistic questions not amenable to *in vivo* studies and further the support for controlled human and epidemiological findings. Because *in vitro* data generate another level of uncertainty for interpretation of NO₂ exposures occurring *in vivo*, these types of studies are generally not used as the basis for dose-response assessment and standard setting. This is due to kinetic limitations and the lack of naturally occurring defense mechanisms such as the presence of a protective epithelial lining fluid and endogenous reducing agents or oxidant scavengers that are normally present *in vivo*.

Respiratory tract cell lines, such as human bronchial epithelial cells (HBECs), are often used for *in vitro* experiments, and are obtained as bronchial biopsy specimens from volunteers. Lung airway segments are also used and are obtained from patients that had undergone thoracotomy for lung cancer. Additionally, alveolar macrophages collected and isolated by lung lavage techniques from volunteers are also used for *in vitro* studies.

The inflammatory effects of NO₂ have been investigated using cell cultures. NO₂-exposed HBEC monolayers (0.1 to 0.4 ppm) exhibited increased permeability to ¹⁴C-labelled bovine serum albumin (BSA), decreased electrical resistance (another indicator of lost epithelial membrane integrity), increased release of ⁵¹Cr from prelabeled cells (indicative of cell damage), and increased release of the inflammatory mediator leukotriene C₄ (LTC₄) (Devalia *et al.*, 1993b; Bayram *et al.*, 1999).

HBEC cultures exposed to 0.4 ppm NO₂ for 6 hr have also been shown to increase the release of the proinflammatory cytokines including interleukin (IL)-8, granulocyte/macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor- α (TNF- α), RANTES, and soluble intercellular adhesion molecule-1 (sICAM-1) (Devalia *et al.*, 1993a; Bayram *et al.*, 1999). Loratadine, an antihistamine, reduced the release of cytokines from NO₂-exposed HBEC's when added to the culture (Bayram *et al.*, 1999). Exposure of HBECs to 0.8 ppm NO₂ for 6 hr does not release cytokines to the same extent as observed with 0.4 ppm NO₂, probably as a result of the cytotoxic action of NO₂ under these conditions (Devalia *et al.*, 1993a).

Ritter *et al.* (2001) exposed human lung fibroblasts and HBECs to various concentrations of NO₂ to investigate effects on metabolic activity and viability. Even though viability was reduced in exposed HBEC cultures, tetrazolium salt cleavage, ATP and ATP/ADP ratio were increased with 1 hr NO₂ exposures at 0.075 ppm, then showed a dose-dependent decrease with increasing NO₂ concentration. Lung fibroblast cells showed an increase in the ATP/ADP ratio with 2 hr exposure to 0.15 and 0.3 ppm NO₂. However, GSH and GSSG/GSH-ratio was unaffected by NO₂ exposure in both cell lines. Increased ATP content and ATP/ADP ratio of cells are likely due to enhanced metabolic activity, while increased tetrazolium salt cleavage is an indicator of increased mitochondrial activity.

The cytotoxicity of NO₂ was investigated in A549 human pulmonary type-II-like epithelial cell lines and human skin fibroblasts using a MTS (tetrazolium salt cleavage method), NRU (neutral red uptake) and ATP assays (Bakand *et al.*, 2006). Concentration-dependent effects were observed in both human cells exposed to NO₂ concentrations from 2.5 to 10 ppm for 1 hr. At 2.5 ppm, exposure resulted in a significant decrease in cell viability of both human cells in nearly all *in vitro* assays. The cell viability of A549 lung derived cells was significantly reduced in a time-dependent way with increasing exposure times of 30, 60 and 120 min at 5 ppm.

Airway diseases such as asthma may be modulated by NO₂ by increasing the release of proinflammatory mediators from epithelial cells, which form the first line of defense against inhaled irritants. Exposure of HBEC cultures from atopic subjects with mild asthma or nonatopic nonasthmatic subjects to 0.2 and/or 0.4 ppm NO₂ for 6 hr increased the release of the inflammatory mediators IL-8 and sICAM-1 (Bayram *et al.*, 2001). While, there was no significant difference between the release of inflammatory mediators from HBECs cultured from asthmatic and nonasthmatic subjects, there was a significant enhancement of GM-CSF released from asthmatic HBEC cultures compared to the non-NO₂-exposed control group.

In addition, the potent proinflammatory chemokine RANTES which affects the activity of eosinophils, was only released by HBECs of asthmatic subjects and increased with exposure to 0.2 ppm NO₂. In further research by this group, NO₂-induced epithelial permeability was shown to be greater in HBEC cultures of asthmatics compared to non-asthmatics (Bayram et al., 2002). Cell cultures from asthmatics exposed to 0.4 ppm NO₂ for 6 hr exhibited decreased electrical resistance, but had no effect on electrical resistance of HBEC cultures from non-asthmatics. Similarly, increased movement of ¹⁴C-BSA occurred across asthmatic cell cultures, but not non-asthmatic cell cultures, after 6 hr exposure to 0.2 or 0.4 ppm NO₂.

A few *in vitro* studies investigated airway hyperresponsiveness of bronchial smooth muscle following NO₂ exposure. Under isometric conditions (i.e., force development), Ben-Jebria *et al.* (1992) observed increased airway smooth muscle contractions of human bronchial segments in response to carbachol, histamine, and substance P following exposure to 2.0 ppm NO₂ for 30 min. Following 1 ppm NO₂ for 30 min, no increase in smooth muscle contractions in response to carbachol was found.

The contractile response of human bronchial ring segments to acetylcholine, neurokinin A (NKA), and substance P were studied by Chitano *et al.* (1996) under isotonic conditions (i.e., shortening capacity) after exposure to 2.5 ppm NO₂ for 4 hr. The response to NKA was also studied in ring segments, with or without epithelium, following exposure to 7 ppm NO₂. None of the experimental exposures caused alterations of the human bronchial smooth muscle shortening capacity, suggesting that NO₂ does not directly cause an increase of bronchial smooth muscle responsiveness by this process.

The acute effects of NO₂ on ciliary activity of human respiratory cells *in vitro* has been investigated. Decreased ciliary activity may reduce muciliary transport in lung airways. Devalia *et al.* (1993b) observed a gradual reduction in ciliary beat frequency of HBECs with increasing NO₂ concentration (0.1, 0.4, 0.8, or 2.0 ppm for 20 min), with a significant reduction occurring only at 2.0 ppm. However, NO₂ concentrations from 3 to 15 ppm for 30 min was found to cause a concentration-dependent increase of ciliary beat frequency in nasal ciliated cells (Riechelmann *et al.*, 1994; Kienast *et al.*, 1996b). With 2 hr exposure to 15 ppm, a non-significant reduction of ciliary beat frequency was observed (Riechelmann *et al.*, 1994). It was speculated that the initial increase in ciliary beat frequency of nasal ciliated cells represents a "stress reaction" induced by the oxidative pathomechanisms of NO₂ (Kienast *et al.*, 1996b).

In another nasal tissue *in vitro* study, histamine, a marker of mast cell degranulation, was significantly elevated in culture medium of nasal mucosa tissue samples exposed to 200 or 800 µg/m³ (0.17 or 0.67 ppm, respectively) NO₂ for 24 hr (Schierhorn *et al.*, 1999). This result shows that NO₂ can trigger an inflammatory reaction of human nasal mucosa in organ culture.

In vitro exposure studies have also been carried out on human alveolar macrophages (AM) lavaged from bronchoalveolar airways. Exposure of AMs to oxidant gases can result in cell activation associated with the secretion of various bioactive products and mediators which can cause interstitial lung injury. AMs exposed to 0.1, 0.3, or 0.5 ppm NO₂ for 30, 60, or 120 min yielded a dose-dependent elevation of spontaneous reactive oxygen intermediates (Kienast *et al.*, 1994). However, extending exposure time from 30 up to 120 min yielded nearly constant values of spontaneous reactive oxygen intermediates.

The NO₂-induced release of cytokines (IL-1β, IL-6, IL-8, TNF-α, transforming growth factor-β (TGF-β)) involved in AM inflammatory reactions was also investigated (Kienast *et al.*, 1996a). NO₂ exposure (0.1 to 0.5 ppm) of non-stimulated AMs for 30 min did not result in changes in IL-1β, IL-6, TNF-α and TGF-β release, but decreased release of IL-8. However, NO₂ exposure caused a concentration-dependent decrease in lipopolysaccharide (LPS)-stimulated IL-1β, IL-6, IL-8, and TNF-α release. Cytotoxicity measured by Trypan blue exclusion was not detected in AMs exposed to NO₂.

In a co-pollutant exposure study, AM exposure *in vitro* to particles and fibers alone results in increased release and mRNA expression of IL-1β, IL-6, IL-8 and TNF-α (Drumm *et al.*, 1999). When particle and fiber exposure is combined with 1 ppm NO₂ for 30 min, cytokine mRNA expression and

release from AMs was reduced. It was postulated in these studies that NO₂-induced cell membrane lipid peroxidation and consecutive reactive oxygen intermediate release may be responsible for the observed decrease in cytokine specific mRNA expression and cellular release of cytokines.

8.9.1 Summary

In vitro test systems using human bronchial epithelial cells (HBEC) and human lung fibroblasts have shown an increase in excretion of proinflammatory cytokines, and cell membrane damage in response to NO₂ exposure. Decreased viability was observed in lung fibroblasts and HBECs exposed to NO₂. A significant enhancement of release of molecules associated with allergy from HBECs of asthmatic (but not non-asthmatic) individuals has also been observed with NO₂ exposure. Nasal mucosal tissue in culture exposed to NO₂ exhibited increased histamine release, which is associated with response to allergens. Alveolar macrophages exposed to NO₂ released reactive oxygen species, and a number of inflammatory mediators. These *in vitro* studies provide mechanistic support for the observed enhancement of response to allergen in asthmatics.

Table 8.11. Toxicological Effects of Nitrogen Dioxide in Human *In Vitro* Test Systems

NO ₂ Concentration (ppm)	Exposure	Tissue	Subjects (n)	Effects	Reference
0.075 0.15 0.3 0.6 1.2	1 or 2 hr	Human lung fibroblasts and human bronchial epithelial cells	Not stated	HBECs: dose-dependent decrease in viability; increased tetrazolium salt cleavage, ATP, and ATP/ADP ratio at lowest exposure, then decreasing with increasing concentration. GSH and GSSG/GSH ratio unaffected by exposure. Human lung fibroblasts (0.15 and 0.3 ppm only): increased ATP/ADP ratio at both concentrations. Other measures (cell viability, tetrazolium salt cleavage, ATP, GSH, and ATP/ADP and GSSG/GSH ratios) between 40 and 80% of controls at both concentrations.	Ritter et al. (2001)
0.1 0.2 0.4	6 hr	Cultured human bronchial epithelial cells	28 (13 non-atopic, non asthmatic; 15 with atopic mild asthma)	Nonasthmatics: increased release of IL-8 and sICAM-1 at 0.2 and 0.4 ppm compared to non-NO ₂ -exposed HBECs. Asthmatics: increased release of IL-8, sICAM-1 and RANTES at 0.2 ppm, and increased release of GM-CSF at 0.2 and 0.4 ppm, compared to non-NO ₂ -exposed HBECs. No significant difference between the release of inflammatory mediators IL-8, sICAM-1 and GM-CSF from HBECs cultured from asthmatic and nonasthmatic subjects.	Bayram et al. (2001)
0.1 0.2 0.4	2, 6 hr	Cultured human bronchial epithelial cells	28 13 non-atopic, non asthmatic; 15 with atopic mild asthma	Nonasthmatics: no effect on electrical resistance (ER) or movement of ¹⁴ C-BSA across cell cultures. Asthmatics: decreased ER at 6 hr exposure to 0.4 ppm; decreased ER at 0.1 ppm for 6 hr and at 0.4 ppm 18 hr after exposure compared to non-atopic non-asthmatics. Increased ¹⁴ C-BSA across cell cultures at 0.2 and 0.4 ppm for 6 hr; increased ¹⁴ C-BSA at 0.2 and 0.4 ppm 18 hr after exposure compared to non-atopic non-asthmatics.	Bayram et al. (2002)
0.1 0.3 0.5	30, 60, 120 min	Lavaged, isolated AMs	8 smokers without acute bronchitis	Dose-dependent elevation of reactive oxygen intermediate (ROI) production from AMs and peripheral blood mononuclear cells, both spontaneous and phorbolmyristate acetate-induced, with 30 and 120 min exposure. Production of ROIs was not affected by increasing exposure time above 30 min.	Kienast et al., (1994)

NO ₂ Concentration (ppm)	Exposure	Tissue	Subjects (n)	Effects	Reference
0.1 0.3 0.5	30 min	Lavaged, isolated AMs	9 lung cancer patients without acute bronchitis or pneumonia	No effect on unstimulated AM release of IL-1 β , IL-6, TNF- α , and TGF- β . NO ₂ induced a concentration-dependent decrease in IL-8 release from unstimulated AMs. NO ₂ induced a concentration-dependent decrease in IL-1 β , IL-6, IL-8, and TNF- α from LPS-stimulated AMs. Release of TGF- β was unaffected in both unstimulated and LPS-stimulated AMs.	Kienast et al., (1996a)
0.1 0.4 0.8 2.0	20 min	Cultured human bronchial epithelial cells	12	Increased permeability to ¹⁴ C-BSA at 0.1 ppm and above; increased release of ⁵¹ Cr from prelabelled cells at 0.4 ppm and above. Increased arachidonic acid metabolite LTC ₄ at 0.4 and 0.8 ppm (0.1 ppm level not tested). Increasing attenuation of ciliary beat frequency with increasing NO ₂ concentration; significantly attenuated at 2.0 ppm.	Devalia et al., (1993a)
0.17 0.67	24 hr	Cultured human nasal mucosa	21	Increased release of histamine at both exposure concentrations.	Schierhorn et al. (1999)
0.4	6 hr	Cultured human bronchial epithelial cells	6	Decreased electrical resistance; incubation with loratadine had no effect. Increased release of IL-8, RANTES and sICAM-1; incubation with loratadine (0.25 to 25 μ M) attenuated release of the cytokines.	Bayram et al. (1999)
0.4 0.8	6 hr	Cultured human bronchial epithelial cells	7	0.4 ppm: increased release of proinflammatory cytokines GM-CSF, IL-8, and TNF- α 0.8 ppm: increased release of GM-CSF, but not to the same extent as with 0.4 ppm NO ₂ .	Devalia et al. (1993b)
1	30 min	Lavaged, isolated AMs	6 lung cancer patients without acute bronchitis or pneumonia	Exposure to particles or fibers resulted in increased IL-1 β -, IL-6-, IL-8-, and TNF- α -specific expression and increased release of these cytokines from AMs. With co-exposure to NO ₂ , both mRNA expression and release of these cytokines were reduced in AMs.	Drumm et al., (1999)
1 2	30 min	Bronchial segments	5	Following 1.0 ppm, no isometric contractions of smooth muscle in response to carbachol. Following 2.0 ppm, increased isometric contraction of smooth muscle in response to carbachol, histamine, or substance P.	Ben-Jebria et al. (1992)
2.5 7	4 hr	Bronchial segments	12	No change in contractile response of bronchial rings to acetylcholine, neurokinin A or substance P under isotonic conditions (2.5 ppm only), or to bronchial segments with or without epithelium to neurokinin A (7 ppm).	Chitano et al. (1996)

NO ₂ Concentration (ppm)	Exposure	Tissue	Subjects (n)	Effects	Reference
2.5 5 10	1 hr	A549 pulmonary type II-like epithelial cells and skin fibroblasts	Epithelial lung carcinoma cell line (A549) and skin biopsies from healthy subjects	Concentration-dependent reduction in cell viability in both human cells that was statistically significant at 2.5 ppm by most cytotoxicity assays. Cell viability of A549 lung derived cells significantly reduced in a time-dependent way after increasing the exposure time from 30 min to 120 min at 5 ppm.	Bakand et al. (2006)
3 6 9 12 15	30, 120 min	Human nasal respiratory (ciliated) cells	25	Significant, concentration-dependent increase of ciliary beat frequency with 30 min exposure. Exposure to 15 ppm for 120 min resulted in a non-significant decrease of ciliary beat frequency compared to control values.	Riechelmann et al. (1994)
3 6 9 12 15	30 min	Human nasal respiratory (ciliated) cells	12	Significant, concentration-dependent increase of ciliary beat frequency, with a statistically significant 32% increase at 15 ppm compared to control values.	Kienast et al., (1996b)

8.10 Summary of Relevant Effects

The health effects data of NO₂ exposures in animals that is pertinent to the re-evaluation of the one-hour ambient air quality standard and the establishment of an annual average standard has been thoroughly reviewed. The focus of the animal health effects review was on those studies conducted at levels of NO₂ considered relevant for decision-making processes (i.e., exposures \leq 1.0 ppm NO₂). In terms of the most relevant studies, those that employed intermittent or episodic-type exposures, similar to urban exposures to NO₂, represent more realistic exposure conditions compared to studies employing continuous 24 hr/day exposures. Adaptive responses of the lung will be different depending on whether intermittent or continuous exposure was used. Those studies identified below as using intermittent exposure conditions, particularly the subchronic/chronic studies, should be considered most relevant for risk assessment purposes, because typical human exposure is intermittent.

8.11 Effects reported at or below 0.25 ppm

No reports since the last review (CARB, 1992) have observed health effects resulting from acute or short-term repeated exposures at or below the level of the current 1-hr standard (0.25 ppm). However, this may be partly due to the small number of studies with exposures at this level, or the necessity to employ higher concentrations to elicit statistically significant effects by the methods used. The most sensitive measure of acute changes in lung occurred with 24 hr exposure to 0.8 ppm NO₂, resulting in increased proliferation of bronchiolar, but not alveolar, tissue determined by cell labeling methods in rats (Barth *et al.*, 1994). Previously reviewed studies have reported exposure to 0.2 ppm NO₂ for as little as three hours resulted in increased mast cell number in the bronchi of rats (Hayashi and Kohno, 1985); Miller *et al.* (1980) reported interference with the detoxification process in the liver at exposures of 0.25 ppm NO₂ for three hours; and Iqbal *et al.* (1981) reported the biosynthesis of a carcinogenic compound associated with NO₂ exposures of 0.20 ppm for four hours.

Recent longer-term studies have observed morphological and biochemical changes in developing mice with NO₂ concentrations at 0.25 ppm. Six-week intermittent exposure to 0.25 ppm beginning at 3 weeks of age, during which lung development is still occurring, resulted in increased number and size of alveolar Type II cells (Sherwin and Richters, 1995b). While this effect was noted immediately following exposure, it was not statistically significant until 32 weeks post-exposure. Type II cell alterations long after NO₂ exposure has ended suggest permanent structural changes have occurred to alveolar tissue. In addition to these effects, there were alterations in measures of elastic fiber abundance in alveolar tissue up to 10 weeks post-exposure, with increased ratios of elastin number/alveolar wall area and elastin area/alveolar wall area at 32 weeks post-exposure (Sherwin and Richters, 1995a). The increased amount and density of elastin in alveolar tissue would suggest an interstitial fibrotic consequence resulting from exposure during lung development.

Other developmental studies finding effects at low concentrations of NO₂ include reduced body weight gain in newborn mice exposed intermittently to 0.25 ppm NO₂ for 3 or 12 weeks (Richters *et al.*, 1987). Tabacova *et al.* (1985) noted behavioral changes in rat offspring exposed to as low as 0.05 ppm NO₂ during gestation, including postural and gait differences in open field behavior at 1 or 2 months of age.

In comparison, chronic exposure studies using adult animals concentrations did not observe morphometric changes in alveolar tissue components even with exposure levels above 0.25 ppm (Tepper *et al.*, 1993; Mercer *et al.*, 1995). Beginning NO₂ exposure at a more mature stage of animal development (7 weeks or young adult) may have missed the most sensitive period for oxidant injury in the lung. In addition, these studies used rats, rather than mice, which may be more resistant to oxidant injury.

A few studies have observed changes in adult animals with NO₂ exposures of 0.25 ppm or less. Continuous exposure of rats to concentrations as low as 0.04 ppm for 9 months or more resulted in increased ethane exhalation, a measure of increased lipid peroxidation (Sagai *et al.*, 1984). However, no evidence of morphometric changes to alveolar or bronchiolar epithelium was found at this level or in similarly exposed animals (Kubota *et al.*, 1987; Ichinose *et al.*, 1991). Intermittent exposure of young adult mice to 0.25 ppm NO₂ for 7-26 weeks resulted in changes of the spleen, including altered weight and decreased T-lymphocyte subpopulations (Richters and Damji, 1988; Richters and Damji, 1990).

Animal models sensitive to specific diseases also appear more susceptible to low NO₂ concentrations. Obese rat strains prone to cardiovascular-type diseases exhibited increased blood levels of triglycerides and decreased HDL and HDL/total cholesterol ratio when exposed continuously to 0.16 ppm NO₂ for 24 weeks (Takano *et al.*, 2004). A related normal rat strain similarly exposed only showed decreased HDL levels.

In an animal *in vitro* study, exposure to NO₂ (0.2 ppm, 2 hrs) altered NO production in AMs. NO plays a role in antimicrobial defense or modification of the immune response (Hockele *et al.*, 1998). However, comparable *in vivo* studies of NO₂ effects on NO production in AMs are lacking. In human *in vitro* test systems, Schierhorn *et al.*, (1999) observed elevated histamine in culture medium, a marker of mast cell degranulation, following 1 day exposure of nasal mucosa to 0.17 ppm NO₂. Notably, mast cell degranulation and increased mast cell number have been observed in lung tissue of rats acutely exposed to 0.5 and 0.2 ppm NO₂, respectively (Thomas *et al.*, 1967; Hayashi and Kohno, 1985).

In other human *in vitro* studies, acute exposure of human bronchial epithelial cells (HBEC) to NO₂ concentrations as low as 0.2 ppm from asthmatic and non-asthmatic individuals showed increased release of certain inflammatory mediators from HBECs of asthmatic individuals only (Bayram *et al.*, 2001). Also, NO₂-induced increases in cell permeability were greater in HBECs from asthmatic individuals relative to non-asthmatic individuals (Bayram *et al.*, 2002). *In vitro* data provide valuable insight into potentially sensitive indicators of acute NO₂-induced cellular injury. However, *in vitro* data generate another level of uncertainty for interpretation of NO₂ exposures resulting in toxic effects in intact animals and may produce inconsistent results when compared to *in vivo* studies. Thus, *in vitro* studies provide mechanistic information but are not useful for dose-response assessment.

8.12 Effects reported at concentrations above 0.25 ppm

The majority of the reports covered in this review exposed animals to NO₂ concentrations between 0.25 and 1.0 ppm. However, numerous studies have observed pulmonary and extra-pulmonary effects at NO₂ concentrations near 0.25 ppm (*e.g.*, between 0.25 and 0.5 ppm) and are highlighted below.

One of few acute effects observed at low NO₂ concentrations was a transient increase in alveolar clearance rate 4 to 5 days after exposure to 0.3 ppm NO₂ for 2 hrs (Vollmuth *et al.*, 1986). This finding suggests a mild irritant action on lung tissue. Epithelial cell labeling techniques have noted increased cell proliferation in bronchiolar tissue (but not alveolar tissue) with one-day continuous exposure to 0.8 ppm (Barth *et al.*, 1994). However, no measurable pulmonary inflammatory effects have been measured with acute exposures of 1 ppm or lower.

Significantly, exposure of exercising rats to 0.6 ppm for 3 hours had no effect on measures of pulmonary inflammation (Mautz *et al.*, 1988). Dosimetric calculations show that exercise can appreciably increase the dose of NO₂ reaching the sensitive centriacinar pulmonary region (Miller *et al.*, 1982). These data would indicate that conventional methods of measuring NO₂-induced pulmonary inflammation (*e.g.*, cell and protein analysis of BAL fluid) may be relatively insensitive for measuring pulmonary injury resulting from ambient level NO₂ exposure. In multi-day exposure studies, pulmonary inflammation was detected in the form of increased protein content of BAL fluid with continuous exposure of mice and guinea pigs to NO₂ levels as low as 0.40 ppm for 7-10 days (Sherwin and Carlson, 1973; Sherwin and Layfield, 1976).

Significantly, alteration of AM function appears to be a more sensitive indicator of NO₂ exposure than indicators of pulmonary inflammation. Exposure of rabbits to 0.3 ppm 2 hr/day for 2 days resulted in decreased AM phagocytosis and mobility (Schlesinger, 1987). AMs are the primary cellular defense system in the lower lung. Impairment of AM's by NO₂ may have a significant effect on their ability to protect the host from pulmonary infections. Additionally, transient reductions in levels of particular arachidonate metabolites in BAL fluid following acute and short-term exposure to 0.5 ppm NO₂ also suggests the potential for impeding the host's defense against microbial infection (Robison *et al.*, 1993).

These findings are supported by similar results from *ex vivo* studies that noted reduced release of arachidonate metabolites and superoxide from AMs exposed acutely to 0.5 ppm. These effects on AM function by NO₂ have not translated into an enhancement of pulmonary infection by microorganisms with acute exposures of less than 1 ppm, although prolonged exposures to 0.5 ppm or 0.2 ppm with 2 daily spikes to 0.8 ppm have increased mortality to bacterial infection (Ehrlich and Henry, 1968; Ehrlich *et al.*, 1979; Miller *et al.*, 1987).

In support of low NO₂ exposures (at or below 0.25 ppm) resulting in altered lung development, morphometric changes in alveolar tissue components (i.e. thickened alveolar walls, increased cellularity, altered epithelial cell volumes) occurred during lung development in young mice intermittently exposed to 0.3 ppm for 6 weeks, and in young ferrets intermittently exposed to 0.5 ppm for 15 weeks, indicating that the developing lung may be the most sensitive target for NO₂ toxicity (Sherwin and Richters, 1985; Rasmussen and McClure, 1992).

Overall, the evidence indicates young rats appear to be less sensitive to NO₂ than young mice, with pre-weaned rats being more resistant to oxidant pulmonary injury when compared to similarly exposed adult rats (Chang *et al.*, 1986). However, stage of lung development may play a key role in NO₂ sensitivity. Kyono and Kawai (1982) observed greater morphometric alterations in alveolar tissue of 1-month-old rats exposed to 3 and 10 ppm NO₂ compared to 3- and 12-month-old rats exposed under the same conditions.

In one of the few pre-natal exposure studies, Tabacova *et al.* (1985) observed delays in developmental milestones (e.g., eye opening, incisor eruption), behavioral changes (reduced number of rearings), increased liver lipid peroxides and increased hexobarbital sleeping time in rat offspring following gestational exposure to 0.5 ppm NO₂. Considering pre-natal effects were seen at 0.5 ppm, and even at 0.05 ppm, it's surprising that this was the only pre-natal exposure study located in the literature that exposed animals to ambient level NO₂.

In adult mice, long-term intermittent exposures in the range of 0.25-0.35 ppm have resulted in pulmonary and extra-pulmonary changes, including alveolar epithelial alterations, reduced weight gain, and suppression of splenic T-lymphocytes (Sherwin and Richters, 1982; Richters *et al.*, 1987; Kuraitis and Richters, 1989). There is also evidence that exposure of mice to 0.35 ppm NO₂ for 6 weeks may cause damage to the pulmonary microvasculature, thus permitting an injected murine melanoma cell line to take hold and increase metastatic lung burden (Richters and Richters, 1989). This would suggest that adult mice, similar to young mice, may be relatively sensitive to low level NO₂ exposure compared to other rodent species.

Chronic exposure studies simulating high urban exposures of 0.5 ppm with 2 daily spikes to 1.5 ppm NO₂ have not observed morphological changes in centriacinar region tissue of adult rats (Tepper *et al.*, 1993; Mercer *et al.*, 1995). However, six-week exposure under similar exposure conditions resulted in morphometric changes in alveolar tissue suggestive of an irritant-type response (Chang *et al.*, 1986). Moreover, chronic, continuous exposure to 0.4-0.5 ppm NO₂ in other rat studies have observed thickening of alveolar walls and other possible inflammatory changes, suggesting prolonged exposures in this range may be a threshold for measurable morphometric changes in the lung of adult rats (Hayashi *et al.*, 1987; Kubota *et al.*, 1987). However, it should be kept in mind that these exposure studies employed continuous 24 hr/day-type exposure conditions that does not represent the episodic nature of typical human urban NO₂ exposure.

Because of the high sensitivity of human asthmatics exposed to NO₂, a number of studies have investigated the effect of NO₂ on allergic asthma in animal models. Acute NO₂ exposure studies on pulmonary function in cold air or warm humid air environments show that NO₂ (1 ppm) reduces the normal attenuation in bronchoconstriction in guinea pigs that occurs with exposure to these environments, suggesting an increased potential for asthmatic episodes in susceptible animals (Halinen *et al.*, 2000b; Halinen *et al.*, 2000a). Many factors in addition to NO₂ exposure could have a large impact on how NO₂ affects the lung's immune response to antigens, which may be the reason low NO₂ concentrations (<1.0 ppm), produced negative or contrary effects in antigen-sensitized animal models (Fujimaki *et al.*, 1998; Hubbard *et al.*, 2002).

However, exposure to higher concentrations of NO₂ (about 5 ppm and greater) have more consistently produced one or more indicators of allergic asthma including, enhancement of delayed-type dyspneic symptoms, increased serum IgE levels, increased pulmonary eosinophilia and epithelial injury, and increased bronchial hyperresponsiveness (Kitabatake *et al.*, 1995; Gilmour *et al.*, 1996; Ohashi *et al.*, 1998; Papi *et al.*, 1999; Mi *et al.*, 2002).

Pulmonary function changes with prolonged exposure include increased airway hyperresponsiveness with histamine challenge after exposure of guinea pigs to 1.0 ppm NO₂ for 6 weeks (Kobayashi and Miura, 1995), and a transient reduction in Δ FEF_{25%} with intermittent NO₂ exposure (0.5 ppm base with daily 2 hr peaks of 1.5 ppm) for 78 weeks (Tepper *et al.*, 1993). The most sensitive indicator of pulmonary function change was observed in a study by Miller *et al.* (1987), in which background exposure to 0.2 ppm + 0.8 ppm spikes twice per day for one hour resulted in decreased vital capacity after one year of exposure. However, continuous exposure to 0.2 ppm NO₂ alone did not result in any pulmonary function changes.

NO₂ has been shown to be genotoxic and mutagenic in some bacterial and animal test systems, but no standard carcinogen animal bioassays with a sufficient number of animals/group could be located in the literature. However, the available carcinogenicity and co-carcinogenicity studies that have been conducted were negative or ambiguous for lung cancer (Adkins *et al.*, 1986; Ichinose *et al.*, 1991; Witschi *et al.*, 1993). The cytotoxic action of NO₂ had a beneficial effect on spontaneous tumor development presumably by suppressing the immune system in AKR mice and reducing T-lymphocyte-derived lymphoma (Richters and Damji, 1990).

NO₂ may have an indirect carcinogenic action, through the *in vivo* formation of nitrosamines and through formation of airborne carcinogenic compounds via atmospheric reactions with UV irradiation and volatile organic substances. However, assessing carcinogenic risk from these secondary source contributions would be a complex exercise and has not been attempted. Co-exposure of NO₂ with carcinogenic substances has also been studied. NO₂ (6 ppm) combined with a diesel exhaust particle (DEP) extract produced a synergistic increase in DNA adducts and alveolar adenomas over DEP treatment alone (Ohyama *et al.*, 1999).

Co-carcinogenicity studies with multi-pollutant mixtures yielded mixed results. NO₂ (0.4 ppm) and ozone (0.05 ppm average) mixtures in rats produced a small co-carcinogenic action with BHPN while mixtures of NO₂ and H₂SO₄ with BHPN in rats and NO₂ and SO₂ with DEN in mice did not (Heinrich *et al.*, 1989; Ichinose and Sagai, 1992). Thus, the overall potential for animal carcinogenicity, and by extrapolation, human carcinogenicity, at ambient air levels of NO₂ is uncertain.

The few relevant non-carcinogenic toxicological studies of multi-pollutant mixtures reviewed herein demonstrate that NO₂ may show additive or synergistic effects with other pollutants, but may also show no effect over and above that attributable to the other pollutant(s). The degree of interaction has been shown to be dependent on a variety of factors. For example, exposing rats to NO₂ (0.4 ppm) and ozone (0.4 ppm) for 2 weeks resulted in synergistic or additive increases in anti-oxidant substances and enzymes in lung and no increase in lipid peroxides (Ichinose and Sagai, 1989). Guinea pigs under the same exposure regimen produced the opposite effect; no change in pulmonary anti-oxidant levels but increased lipid peroxide formation.

However, longer exposures in rats to NO₂ (0.4 or 0.04 ppm) and ozone (0.05 average with daily 0.1 ppm peak) did demonstrate a synergistic increase in lipid peroxides, but in general, no change in

anti-oxidant substances and enzymes. Thus, pollutant interactions may be dependent on the timing and intensity of exposure as well as the species. In a study of prolonged intermittent exposure to a complex mixture, the high exposure group (0.4 ppm NO₂, 0.6 ppm ozone, and aerosols of carbon particles, nitric acid, and ammonium bisulfite) showed continued inflammatory, morphological, and pulmonary function effects while a lower exposure group (0.2 ppm NO₂, 0.3 ppm ozone, and lower levels of the individual aerosols) showed attenuation of these responses with continued exposure (Mautz *et al.*, 2001). The authors suggest that certain health effects exhibit synergism when oxidant or acid-forming gases are combined with particles.

The animal *in vitro* data comprising biochemical effects of NO₂ on airway epithelium and AMs demonstrate toxic effects at acute NO₂ concentrations of 0.5-1.0 ppm. The *in vitro* data provide valuable insight into potentially sensitive indicators of acute NO₂-induced cellular injury. However, *in vitro* data generate another level of uncertainty for interpretation of NO₂ exposures resulting in toxic effects in intact animals and may produce inconsistent results when compared to *in vivo* studies. As shown by Robison and colleagues (Robison *et al.*, 1990; Robison and Forman, 1993; Robison *et al.*, 1993), conflicting findings on release of arachidonate metabolites by AMs occurred in response to NO₂ exposure, depending on whether the exposure was *in vivo* or *in vitro*. Therefore, while *in vitro* assays provide mechanistic information, no conclusions will be drawn from these types of studies at this time with regard to dose-response assessment of NO₂ in intact animals.

8.13 Conclusions

The findings from the animal exposure studies in this review suggest cause for concern, and support the epidemiological and controlled human exposure studies presented elsewhere in this document. NO₂ exposure has been shown to enhance allergic asthma in sensitive human populations at or below the level of the existing 1-hr standard (0.25 ppm). Although *in vitro* studies were not used for dose-response assessment, acute exposure of human bronchial epithelial cells (HBEC) from asthmatic subjects to 0.2 ppm NO₂ showed significant enhancement of certain inflammatory mediators and increased cell permeability compared to HBECs from non-asthmatic individuals.

While adult animal *in vivo* models for allergic asthma have not demonstrated consistent enhancement of allergic responses with NO₂ concentrations below about 5 ppm, many of the animal models for allergic airway disease elicit similar physiologic and immunologic outcomes as that found in human cases of the disease at concentrations at or above 5 ppm.

Some early studies have observed effects at very low NO₂ exposure concentrations, including behavioral changes in offspring following gestational exposure to 0.05 ppm, increased ethane exhalation (indicating increased pulmonary lipid peroxidation) with chronic exposure to 0.04 ppm, and increased bronchial mast cell number with acute exposure to 0.2 ppm. Surprisingly little follow-up has been conducted by other researchers to verify and interpret these findings considering histamine release has been observed in cultured human nasal mucosa acutely exposed to NO₂ concentrations as low as 0.17 ppm. Further investigation of these potential NO₂-induced toxicological endpoints should be pursued.

Significantly, alteration of AM function appears to be a more sensitive indicator of pulmonary oxidant damage by NO₂ than indicators of pulmonary oxidant inflammation. Potentially deleterious alterations in AM function have been observed with acute and short-term NO₂ exposures in the range of 0.3-0.5 ppm. Because AMs are a critical component of the host lung defense against infectious microorganisms, additional research in this field is also warranted.

Although prolonged exposures were necessary to produce the effect, key non-cancer health concerns in sensitive animal models have been observed at or below the level of the one-hour NO₂ standard (0.25 ppm) in two areas: (1) an animal model sensitive to cardiovascular-type diseases, and (2) the developing lung of young animals. NO₂ and other air pollutants have been linked with an increased risk of premature cardiovascular disease deaths in humans. An obese rat strain exposed to 0.16 ppm NO₂ for 24 weeks exhibited changes in blood levels of tryglycerides, HDL, and HDL/total cholesterol ratio suggestive of atherogenic cardiovascular effects (Takano *et al.*, 2004).

As in humans, this animal study indicates compromised health may be one of the most sensitive factors for NO₂-induced toxicity. During postnatal lung development, potentially permanent structural changes to the lung may occur with exposure to 0.25 ppm NO₂ (Sherwin and Richters, 1995a; Sherwin and Richters, 1995b). This evidence is supported by other reports of lung alterations following intermittent exposure to low concentrations of NO₂ in mice (0.3 ppm) and ferrets (0.5 ppm) during lung development (Sherwin and Richters, 1985; Rasmussen and McClure, 1992). Additional NO₂ exposure studies would be valuable in supporting the findings of atherogenic cardiovascular effects in sensitive animal models and altered lung morphology during lung development.

The comprehensive review into developmental effects of NO₂ has also provided some promising areas for future work. The rodent studies indicate that mice are more sensitive than rats with exposure to NO₂ during lung development, suggesting the mouse may be a superior model for NO₂ lung development exposure studies. The stage of lung development when NO₂ exposure occurs is also important. Greater morphometric alterations in alveolar tissue was observed in 1-month-old rats exposed to 3 and 10 ppm NO₂ compared to 3- and 12-month-old rats exposed under the same conditions. The lungs of one-month-old rats are in a rapid, "equilibrated" growth phase which does not resemble lung development in either newborn or adult rats. The developmental studies also showed that NO₂ exposure pattern and post-exposure follow-up examination are important factors in detecting injury.

An exposure pattern employing twice daily NO₂ peaks simulating urban patterns of exposure appeared to result in more pulmonary alterations than exposures to intermittent, but steady, exposures of similar or higher NO₂ concentrations. Regarding the importance post-exposure examination, some researchers have noted that early life exposure to NO₂ resulted in lung alterations that did not become apparent until later in life, suggesting the need for later follow-up examination in developmental studies. Finally, cyclic exposure to ozone in infant monkeys has resulted in serious alterations in lung postnatal maturation that are likely permanent. Similar studies incorporating exposure to NO₂ may also be justified.

While the interaction of NO₂ and antigen exposure during infancy has not been thoroughly investigated, co-occurring pollutant studies indicate that NO₂ and diesel exhaust combined with an allergen during lung development is a sensitive target for immune and reproductive system toxicity (Watanabe and Ohsawa, 2002; Watanabe, 2005). The NO₂-diesel co-pollutant studies did not include groups exposed to NO₂ or diesel exhaust alone, so it is unclear if NO₂ could have had an additive or synergistic action, or potentiated the toxic effect of diesel exhaust. NO₂ animal exposure studies both alone and with co-occurring pollutants/antigens may help in understanding the contribution of NO₂ to human toxicity in polluted atmospheres, particularly to the developing lung.

The interaction of HDM antigen and episodic ozone exposure during lung development in monkeys has been shown to synergistically enhance allergic-reactive airway disease (Evans *et al.*, 2003; Schelegle *et al.*, 2003; Larson *et al.*, 2004; Kajekar *et al.*, 2006).

Episodic ozone exposure represents an exposure scenario closer to typical intermittent free ranging human exposure than continuous 24 hr/day exposure studies. Intermittent exposure also results in different pulmonary adaptive responses compared to continuous exposure. A number of NO₂ animal studies described in this review, particularly earlier studies, used continuous exposure conditions in their experiments. Less weight should be given to these types of studies and future studies should employ episodic-type NO₂ exposures that will more closely mimic typical human exposure patterns.

8.14 References

- Adkins, B., Jr., Van Stee, E. W., Simmons, J. E., and Eustis, S. L. 1986. Oncogenic response of strain A/J mice to inhaled chemicals. *J Toxicol Environ Health* 17: 311-322.
- Arroyo, P. L., Hatch-Pigott, V., Mower, H. F., and Cooney, R. V. 1992. Mutagenicity of nitric oxide and its inhibition by antioxidants. *Mutat Res* 281: 193-202.
- ATS. 1996. American Thoracic Society. Committee of the Environmental and Occupational Health Assembly of the American Thoracic Society. Health Effects of Outdoor Air Pollution. Part 2, *American Journal of Respiratory Critical Care Medicine*. 153: 477-498.
- Azoulay-Dupuis, E., Torres, M., Soler, P., and Moreau, J. 1983. Pulmonary NO₂ toxicity in neonate and adult guinea pigs and rats. *Environ Res* 30: 322-339.
- Bakand, S., Winder, C., Khalil, C., and Hayes, A. 2006. An experimental in vitro model for dynamic direct exposure of human cells to airborne contaminants. *Toxicol Lett* 165: 1-10.
- Barth, P. J., Muller, B., Wagner, U., and Bittinger, A. 1994. Assessment of proliferative activity in type II pneumocytes after inhalation of NO₂ by AgNOR-analysis. *Exp Toxicol Pathol* 46: 335-342.
- Bauer, M. A., Utell, M. J., Morrow, P. E., Speers, D. M., and Gibb, F. R. 1986. Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. *Am Rev Respir Dis* 134: 1203-1208.
- Bayram, H., Devalia, J. L., Khair, O. A., Abdelaziz, M. M., Sapsford, R. J., Czarlewski, W., Campbell, A. M., Bousquet, J., and Davies, R. J. 1999. Effect of loratadine on nitrogen dioxide-induced changes in electrical resistance and release of inflammatory mediators from cultured human bronchial epithelial cells. *J Allergy Clin Immunol* 104: 93-99.
- Bayram, H., Rusznak, C., Khair, O. A., Sapsford, R. J., and Abdelaziz, M. M. 2002. Effect of ozone and nitrogen dioxide on the permeability of bronchial epithelial cell cultures of non-asthmatic and asthmatic subjects. *Clin Exp Allergy* 32: 1285-1292.
- Bayram, H., Sapsford, R. J., Abdelaziz, M. M., and Khair, O. A. 2001. Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients in vitro. *J Allergy Clin Immunol* 107: 287-294.
- Ben-Jebria, A., Marthan, R., and Savineau, J. P. 1992. Effect of in vitro nitrogen dioxide exposure on human bronchial smooth muscle response. *Am Rev Respir Dis* 146: 378-382.
- Ben-Jebria, A., Satchithanandam, L., Gusic, R. J., Gervais, T. R., and Ultman, J. S. 1998. Kinetics of protein depletion in rat bronchoalveolar lavage fluid following in vitro exposure to nitrogen dioxide. *Environmental Toxicology and Pharmacology* 6: 177-185.
- Bermudez, E. 2001. Detection of poly(ADP-ribose) synthetase activity in alveolar macrophages of rats exposed to nitrogen dioxide and ozone. *Inhal Toxicol* 13: 69-84.
- Bermudez, E., Ferng, S. F., Castro, C. E., and Mustafa, M. G. 1999. DNA strand breaks caused by exposure to ozone and nitrogen dioxide. *Environ Res* 81: 72-80.
- CARB. 1992. Review of the One-Hour Ambient Air Quality Standard for Nitrogen Dioxide. Technical Support Document. California Air Resources Board, California Environmental Protection Agency, Sacramento CA.
- Chang, L. Y., Graham, J. A., Miller, F. J., Ospital, J. J., and Crapo, J. D. 1986. Effects of subchronic inhalation of low concentrations of nitrogen dioxide. I. The proximal alveolar region of juvenile and adult rats. *Toxicol Appl Pharmacol* 83: 45-61.
- Chitano, P., Coser, E., Lucchini, R. E., Papi, A., Saetta, M., Maestrelli, P., Faggian, D., Plebani, M., Ciaccia, A., Fabbri, L. M., and et al. 1994. In vitro exposure to nitrogen dioxide (NO₂) does not alter

bronchial smooth muscle responsiveness in ovalbumin-sensitized guinea-pigs. *Pulm Pharmacol* 7: 251-257.

Chitano, P., Lucchini, R. E., Calabro, F., Saetta, M., Maestrelli, P., Fabbri, L. M., and Mapp, C. E. 1996. Isotonic smooth muscle response in human bronchi exposed in vitro to nitrogen dioxide. *Eur Respir J* 9: 2294-2297.

Chitano, P., Lucchini, R. E., Coser, E., Papi, A., Saetta, M., Maestrelli, P., Ciaccia, A., Fabbri, L. M., and Mapp, C. E. 1995. In-vitro exposure of guinea pig main bronchi to 2.5 ppm of nitrogen dioxide does not alter airway smooth muscle response. *Respir Med* 89: 323-328.

Davis, J. K., Davidson, M., and Schoeb, T. R. 1991. Murine respiratory mycoplasmosis: a model to study effects of oxidants. Research Report Number 47. *Res Rep Health Eff Inst*: 1-29; discussion 31-43.

Devalia, J. L., Campbell, A. M., Sapsford, R. J., Rusznak, C., Quint, D., Godard, P., Bousquet, J., and Davies, R. J. 1993a. Effect of nitrogen dioxide on synthesis of inflammatory cytokines expressed by human bronchial epithelial cells in vitro. *Am J Respir Cell Mol Biol* 9: 271-278.

Devalia, J. L., Sapsford, R. J., Cundell, D. R., Rusznak, C., Campbell, A. M., and Davies, R. J. 1993b. Human bronchial epithelial cell dysfunction following in vitro exposure to nitrogen dioxide. *Eur Respir J* 6: 1308-1316.

Douglas, G. J., Price, J. F., and Page, C. P. 1995. The effect of prolonged exposure to NO₂ from birth on airways responsiveness in rabbits sensitized at birth. *Eur Respir J* 8: 246-252.

Ehrlich, R., Findlay, J. C., and Gardner, D. E. 1979. Effects of repeated exposures to peak concentrations of nitrogen dioxide and ozone on resistance to streptococcal pneumonia. *J Toxicol Environ Health* 5: 631-642.

Ehrlich, R., and Henry, M. C. 1968. Chronic toxicity of nitrogen dioxide. I. Effect on resistance to bacterial pneumonia. *Arch Environ Health* 17: 860-865.

Evans, M. J., Fanucchi, M. V., Baker, G. L., Van Winkle, L. S., Pantle, L. M., Nishio, S. J., Schelegle, E. S., Gershwin, L. J., Miller, L. A., Hyde, D. M., Sannes, P. L., and Plopper, C. G. 2003. Atypical development of the tracheal basement membrane zone of infant rhesus monkeys exposed to ozone and allergen. *Am J Physiol Lung Cell Mol Physiol* 285: L931-939.

Fanucchi, M. V., Plopper, C. G., Evans, M. J., Hyde, D. M., Van Winkle, L. S., Gershwin, L. J., and Schelegle, E. S. 2006. Cyclic exposure to ozone alters distal airway development in infant rhesus monkeys. *Am J Physiol Lung Cell Mol Physiol* 291: L644-650.

Freeman, G., Crane, S. C., and Furiosi, N. J. 1969. Healing in rat lung after subacute exposure to nitrogen dioxide. *Am Rev Respir Dis* 100: 662-676.

Freeman, G., Juhos, L. T., Furiosi, N. J., Mussenden, R., and Weiss, T. A. 1974. Delayed maturation of rat lung in an environment containing nitrogen dioxide. *Am Rev Respir Dis* 110: 754-759.

Fujimaki, H., and Nohara, O. 1994. Changes in the Response of Lung Mast Cells Isolated from Rats and Guinea Pigs Exposed to Nitrogen Dioxide. *Inhal Toxicol* 6: 515-520.

Fujimaki, H., Ohmori, T., Ushio, H., and Saneyoshi, K. 1998. Timing of low-level NO₂ exposure alters antigen-specific IgE, IgG1, and IgG2a antibody production in mice. *Inhal Toxicol* 10: 1079-1093.

Fujimaki, H., Shimizu, F., and Kubota, K. 1982. Effect of subacute exposure to NO₂ on lymphocytes required for antibody responses. *Environ Res* 29: 280-286.

Gershwin, L. J. 2003. Effects of air pollutants on development of allergic immune responses in the respiratory tract. *Clin Dev Immunol* 10: 119-126.

Gilmour, M. I., Park, P., and Selgrade, M. J. 1996. Increased immune and inflammatory responses to dust mite antigen in rats exposed to 5 ppm NO₂. *Fundam Appl Toxicol* 31: 65-70.

- Goldstein, E., Peek, N. F., Parks, N. J., Hines, H. H., Steffey, E. P., and Tarkington, B. 1977. Fate and distribution of inhaled nitrogen dioxide in rhesus monkeys. *Am Rev Respir Dis* 115: 403-412.
- Gooch, P. C., Luippold, H. E., Creasia, D. A., and Brewen, J. G. 1977. Observations on mouse chromosomes following nitrogen dioxide inhalation. *Mutat Res* 48: 117-119.
- Gorsdorf, S., Appel, K. E., Engeholm, C., and Obe, G. 1990. Nitrogen dioxide induces DNA single-strand breaks in cultured Chinese hamster cells. *Carcinogenesis* 11: 37-41.
- Halinen, A. I., Salonen, R. O., Pennanen, A. S., and Kosma, V. M. 2000a. Combined respiratory effects of cold air with SO₂ or NO₂ in repeated 10-minute exposures of hyperventilating guinea pigs. *Inhal Toxicol* 12: 671-691.
- Halinen, A. I., Salonen, R. O., Pennanen, A. S., and Kosma, V. M. 2000b. Combined respiratory effects of cold air with SO₂ or NO₂ in single 1-hour exposures of hyperventilating guinea pigs. *Inhal Toxicol* 12: 693-713.
- Hayashi, Y., and Kohno, T. 1985. A pathological study on effects of nitrogen dioxide on the respiratory system in rats. In: *Experimental studies on health effects of nitrogen dioxides*. Special Research Project of Environmental Science, Ministry of Education Science and Culture, Japan Researches on Human Health Effects. Kagawa Medical School, Kagawa, Japan. (Environ. Science Res. Report, B233-R20-1). 31-44.
- Hayashi, Y., Kohno, T., and Ohwada, H. 1987. Morphological effects of nitrogen dioxide on the rat lung. *Environ Health Perspect* 73: 135-145.
- Hazucha, M. J. 1999. Controlled exposure to ozone, nitrogen oxides and acids, S. T. Holgate, J.M. Samet, H. S. Koren, R. L. Maynard eds. *Air Pollution and Health*, The center for Environmental Medicine and Lung Biology, The University of North Carolina at Chapel Hill, CHapel Hill, NC. p. 511-29.
- Heinrich, U., Mohr, U., Fuhst, R., and Brockmeyer, C. 1989. Investigation of a potential cotumorogenic effect of the dioxides of nitrogen and sulfur, and of diesel-engine exhaust, on the respiratory tract of Syrian golden hamsters. Research Report No. 26. *Res Rep Health Eff Inst*: 1-27.
- Hisamatsu, Y., Nishimura, T., Tanabe, K., and Matsushita, H. 1986. Mutagenicity of the photochemical reaction products of pyrene with nitrogen dioxide. *Mutat Res* 172: 19-27.
- Hisamatsu, Y., Shida, Y., and Matsushita, H. 1989. Mutagenicity of the reaction products of carbazole in the presence of nitrogen dioxide and nitrocarbazole. *Mutat Res* 226: 55-59.
- Hockele, V., Kruger, E., Krug, H. F., and Seidel, A. 1998. In vitro effects of nitrogen dioxide on the release of nitric oxide by bovine alveolar macrophages. *Toxicol Lett* 96-97: 53-57.
- Holt, P. G., Finlay-Jones, L. M., Keast, D., and Papadimitrou, J. M. 1979. Immunological function in mice chronically exposed to nitrogen oxides (NO_x). *Environ Res* 19: 154-162.
- Hubbard, A. K., Symanowicz, P. T., Thibodeau, M., Thrall, R. S., Schramm, C. M., Cloutier, M. M., and Morris, J. B. 2002. Effect of nitrogen dioxide on ovalbumin-induced allergic airway disease in a murine model. *J Toxicol Environ Health A* 65: 1999-2005.
- Hussain, I., Jain, V. V., O'Shaughnessy, P., Businga, T. R., and Kline, J. 2004. Effect of nitrogen dioxide exposure on allergic asthma in a murine model. *Chest* 126: 198-204.
- Ichinose, T., Fujii, K., and Sagai, M. 1991. Experimental studies on tumor promotion by nitrogen dioxide. *Toxicology* 67: 211-225.
- Ichinose, T., and Sagai, M. 1989. Biochemical effects of combined gases of nitrogen dioxide and ozone. III. Synergistic effects on lipid peroxidation and antioxidative protective systems in the lungs of rats and guinea pigs. *Toxicology* 59: 259-270.
- Ichinose, T., and Sagai, M. 1992. Combined exposure to NO₂, O₃ and H₂SO₄-aerosol and lung tumor formation in rats. *Toxicology* 74: 173-184.

- Iqbal, Z. M., Dahl, K., and Epstein, S. S. 1981. Biosynthesis of dimethylnitrosamine in dimethylamine-treated mice after exposure to nitrogen dioxide. *J Natl Cancer Inst* 67: 137-141.
- Ishii, S., Hisamatsu, Y., Inazu, K., Kobayashi, T., and Aika, K. 2000. Mutagenic nitrated benzo[a]pyrene derivatives in the reaction product of benzo[a]pyrene in NO₂-air in the presence of O₃ or under photoirradiation. *Chemosphere* 41: 1809-1819.
- Isomura, K., Chikahira, M., Teranishi, K., and Hamada, K. 1984. Induction of mutations and chromosome aberrations in lung cells following in vivo exposure of rats to nitrogen oxides. *Mutat Res* 136: 119-125.
- Ito, T., Ohyama, K., Kusano, T., Usuda, Y., Nozawa, A., Hayashi, H., Ohji, H., Kitamura, H., and Kanisawa, M. 1997. Pulmonary endocrine cell hyperplasia and papilloma in rats induced by intratracheal injections of extract from particulate air pollutants. *Exp Toxicol Pathol* 49: 65-70.
- Joel, D. D., Chandra, P., and Chanana, A. D. 1982. Effects Of NO₂ on immune responses in pulmonary lymph of sheep. *J Toxicol Environ Health* 10: 341-348.
- Johnson, D. A., Winters, R. S., Lee, K. R., and Smith, C. E. 1990. Oxidant effects on rat and human lung proteinase inhibitors. Research Report Number 37. *Res Rep Health Eff Inst*: 1-39.
- Juhos, L. T., Green, D. P., Furiosi, N. J., and Freeman, G. 1980. A quantitative study of stenosis in the respiratory bronchiole of the rat in NO₂-induced emphysema. *Am Rev Respir Dis* 121: 541-549.
- Kajekar, R., Pieczarka, E. M., Smiley-Jewell, S. M., Schelegle, E. S., Fanucchi, M. V., and Plopper, C. G. 2006. Early postnatal exposure to allergen and ozone leads to hyperinnervation of the pulmonary epithelium. *Respir Physiol Neurobiol*.
- Kanoh, T., Fukuda, M., Hayami, E., Kinouchi, T., Nishifuji, K., and Ohnishi, Y. 1990. Nitro reaction in mice injected with pyrene during exposure to nitrogen dioxide. *Mutat Res* 245: 1-4.
- Kelman, D. J., Christodoulou, D., Wink, D. A., Keefer, L. K., Srinivasan, A., and Dipple, A. 1997. Relative mutagenicities of gaseous nitrogen oxides in the supF gene of pSP189. *Carcinogenesis* 18: 1045-1048.
- Kienast, K., Knorst, M., Lubjuhn, S., Muller-Quernheim, J., and Ferlinz, R. 1994. Nitrogen dioxide-induced reactive oxygen intermediates production by human alveolar macrophages and peripheral blood mononuclear cells. *Arch Environ Health* 49: 246-250.
- Kienast, K., Knorst, M., Muller-Quernheim, J., and Ferlinz, R. 1996a. Modulation of IL-1 beta, IL-6, IL-8, TNF-alpha, and TGF-beta secretions by alveolar macrophages under NO₂ exposure. *Lung* 174: 57-67.
- Kienast, K., Riechelmann, H., Knorst, M., Haffner, B., Muller-Quernheim, J., Schellenberg, J., and Ferlinz, R. 1996b. Combined exposures of human ciliated cells to different concentrations of sulfur dioxide and nitrogen dioxide. *Eur J Med Res* 1: 533-536.
- Kitabatake, M., Murase, S., Masuji, A., Tomita, Y., Satoh, M., and Yoshida, K. 1992. Effects of mixed exposure to ammonium sulfate aerosol and nitrogen dioxide on guinea pigs with experimental asthma. Report of the Environmental Science, Mie Univ. 16: 13-20.
- Kitabatake, M., Yamamoto, H., Yuan, P. F., Manjurul, H., Murase, S., and Yamauchi, T. 1995. Effects of exposure to NO₂ or SO₂ on bronchopulmonary reaction induced by *Candida albicans* in guinea pigs. *J Toxicol Environ Health* 45: 75-82.
- Kleinman, M. T., and Mautz, W. J. 1991. The effects of exercise on dose and dose distribution of inhaled automotive pollutants. Research Report Number 45. *Res Rep Health Eff Inst*: 1-40; discussion 41-50.
- Knebel, J. W., Ritter, D., and Aufderheide, M. 1998. Development of an in vitro system for studying effects of native and photochemically transformed gaseous compounds using an air/liquid culture technique. *Toxicol Lett* 96-97: 1-11.

- Kobayashi, T., and Miura, T. 1995. Concentration- and time-dependent increase in specific airway resistance after induction of airway hyperresponsiveness by subchronic exposure of guinea pigs to nitrogen dioxide. *Fundam Appl Toxicol* 25: 154-158.
- Kosaka, H., Uozumi, M., and Nakajima, T. 1987. Induction of SOS functions in *Escherichia coli* and biosynthesis of nitrosamine in rabbits by nitrogen dioxide. *Environ Health Perspect* 73: 153-156.
- Kubota, K., Murakami, M., Takenaka, S., Kawai, K., and Kyono, H. 1987. Effects of long-term nitrogen dioxide exposure on rat lung: morphological observations. *Environ Health Perspect* 73: 157-169.
- Kuraitis, K. V., and Richters, A. 1989. Spleen cellularity shifts from the inhalation of 0.25-0.35 PPM nitrogen dioxide. *J Environ Pathol Toxicol Oncol* 9: 1-11.
- Kuraitis, K. V., Richters, A., and Sherwin, R. P. 1981. Spleen changes in animals inhaling ambient levels of nitrogen dioxide. *J Toxicol Environ Health* 7: 851-859.
- Kyono, H., and Kawai, K. 1982. Morphometric study on age-dependent pulmonary lesions in rats exposed to nitrogen dioxide. *Ind Health* 20: 73-99.
- Lam, C., Kattan, M., Collins, A., and Kleinerman, J. 1983. Long-term sequelae of bronchiolitis induced by nitrogen dioxide in hamsters. *Am Rev Respir Dis* 128: 1020-1023.
- Larson, S. D., Schelegle, E. S., Walby, W. F., Gershwin, L. J., Fanucchi, M. V., Evans, M. J., Joad, J. P., Tarkington, B. K., Hyde, D. M., and Plopper, C. G. 2004. Postnatal remodeling of the neural components of the epithelial-mesenchymal trophic unit in the proximal airways of infant rhesus monkeys exposed to ozone and allergen. *Toxicol Appl Pharmacol* 194: 211-220.
- Lunan, K. D., Short, P., Negi, D., and Stephens, R. J. 1977. Glucose-6-phosphate dehydrogenase response of postnatal lungs to NO₂ and O₂. In: *Pulmonary Macrophage and Epithelial Cells. Proceedings of the 16th Annual Hartford Symposium, Series 43.*: pp. 236-247.
- Luster, M. I., Simeonova, P., Gallucci, R., Matheson, J., Yucesoy, B., and Sugawara, T. 2000. Overview of immunotoxicology and current applications to respiratory diseases. *Immunopharmacology* 48: 311-313.
- Maigetter, R. Z., Fenters, J. D., Findlay, J. C., Ehrlich, R., and Gardner, D. E. 1978. Effect of exposure to nitrogen dioxide on T and B cells in mouse spleens. *Toxicology Letters* 2: 157-161.
- Mauderly, J. L., Bice, D. E., Carpenter, R. L., Gillett, N. A., Henderson, R. F., Pickrell, J. A., and Wolff, R. K. 1987. Effects of inhaled nitrogen dioxide and diesel exhaust on developing lung. Research Report No. 8. *Res Rep Health Eff Inst*: 3-37.
- Mautz, W. J., Kleinman, M. T., Bhalla, D. K., and Phalen, R. F. 2001. Respiratory tract responses to repeated inhalation of an oxidant and acid gas-particle air pollutant mixture. *Toxicol Sci* 61: 331-341.
- Mautz, W. J., Kleinman, M. T., Phalen, R. F., and Crocker, T. T. 1988. Effects of exercise exposure on toxic interactions between inhaled oxidant and aldehyde air pollutants. *J Toxicol Environ Health* 25: 165-177.
- Menzel, D. B., and Meacher, D. M. 1999. Ozone and nitrogen dioxide. In: *Reactive Oxygen Species in Biological Systems: An Interdisciplinary Approach*. Gilbert, D. L. and C. A. Colton Eds. Kluwer Academic Publishers, Norwell, MA :pp. 335-66.
- Mercer, R. R. 1999. Morphometric analysis of alveolar responses of F344 rats to subchronic inhalation of nitric oxide. *Res Rep Health Eff Inst*: 1-15; discussion 17-19.
- Mercer, R. R., Costa, D. L., and Crapo, J. D. 1995. Effects of prolonged exposure to low doses of nitric oxide or nitrogen dioxide on the alveolar septa of the adult rat lung. *Lab Invest* 73: 20-28.
- Mersch, J., Dyce, B. J., Haverback, B. J., and Sherwin, R. P. 1973. Diphosphoglycerate content of red blood cells. *Arch Environ Health* 27: 94-95.

- Mi, H., Hiramoto, K., Kujirai, K., Ando, K., Ikarashi, Y., and Kikugawa, K. 2002. Effects of vitamin E-deficiency and/or nitrogen dioxide inhalation on allergen-sensitized type IV and type I allergy responses of mice. *Journal of Health Science* 48: 22-29.
- Miller, F., Overton, J., Kimbell, J., and Russell, M. 1992. Regional Respiratory Tract Absorption of Inhaled Reactive Gases. Health Effects Research Lab., Research Triangle Park, NC. Environmental Toxicology Div.; Chemical Industry Inst. of Toxicology, Research Triangle Park, NC. Duke Univ. Medical Center, Durham, NC. Center for Extrapolation Modelling. 29 Jun 1992. 88p. Report: EPA/600/A-92/179.
- Miller, F. J. 1994. Dosimetry of Inhaled Gases. Jenkins, P. G., Et Al. (Ed.). International Programme on Chemical Safety Ipcs Joint Series, 18. Respiratory Toxicology and Risk Assessment; International Symposium, Hanover, Germany, October 6-9, 1992. Xiv+396p. Wissenschaftliche Verlagsgesellschaft Mbh: Stuttgart, Germany. Isbn 3-8047-1327-0.; 0 (0). 1994. 111-144. .
- Miller, F. J., Graham, J. A., Illing, J. W., and Gardner, D. E. 1980. Extrapulmonary effects of NO₂ as reflected by pentobarbital-induced sleeping time in mice. *Toxicol Lett* 6: 267-274.
- Miller, F. J., Graham, J. A., Raub, J. A., Illing, J. W., Menache, M. G., House, D. E., and Gardner, D. E. 1987. Evaluating the toxicity of urban patterns of oxidant gases. II. Effects in mice from chronic exposure to nitrogen dioxide. *J Toxicol Environ Health* 21: 99-112.
- Miller, F. J., Overton, J. H., Jr., Myers, E. T., and Graham, J. A. 1982. Pulmonary dosimetry of nitrogen dioxide in animals and man. In: *Air Pollution by Nitrogen Oxides. Proceedings of the US-Dutch International Symposium, Maastricht, The Netherlands, May 24-28, 1982.* T. Schneider and L. Grant Eds. Elsevier Scientific Publishing Co. New York, NY :pp. 377-85.
- Miyanishi, K., Kinouchi, T., Kataoka, K., Kanoh, T., and Ohnishi, Y. 1996. In vivo formation of mutagens by intraperitoneal administration of polycyclic aromatic hydrocarbons in animals during exposure to nitrogen dioxide. *Carcinogenesis* 17: 1483-1490.
- Mochitate, K., Ishida, K., Ohsumi, T., and Miura, T. 1992. Long-term effects of ozone and nitrogen dioxide on the metabolism and population of alveolar macrophages. *J Toxicol Environ Health* 35: 247-260.
- Muller, B., Schafer, H., Barth, P., and von Wichert, P. 1994. Lung surfactant components in bronchoalveolar lavage after inhalation of NO₂ as markers of altered surfactant metabolism. *Lung* 172: 61-72.
- Nisizawa, T., Saito, M., Nakayama, K., Nishihara, T., Imai, S., and Nakagawa, M. 1988. Effects of nitrogen dioxide on Mycoplasma pulmonis infection and humoral immune responses in mice. *Jpn J Med Sci Biol* 41: 175-187.
- Ohashi, Y., Nakai, Y., Okamoto, H., Sugiura, Y., Ohno, Y., Hashimoto, M., and Uozumi, M. 1998. Nitrogen dioxide modifies allergic inflammation in tracheal mucosa. *Acta Otolaryngol Suppl* 538: 227-232.
- Ohyama, K., Ito, T., and Kanisawa, M. 1999. The roles of diesel exhaust particle extracts and the promotive effects of NO₂ and/or SO₂ exposure on rat lung tumorigenesis. *Cancer Lett* 139: 189-197.
- Overton, J. H., and Graham, R. C. 1995. Simulation of the uptake of a reactive gas in a rat respiratory tract model with an asymmetric tracheobronchial region patterned on complete conducting airway cast data. *Comput Biomed Res* 28: 171-190.
- Overton, J. H., Jr. 1984. Physicochemical processes and the formulation of dosimetry models. *J Toxicol Environ Health* 13: 273-294.
- Overton, J. H., Jr., and Graham, J. A. 1990. Modeling the uptake of nitrogen dioxide in the lower respiratory tracts of humans and laboratory animals: present status and future needs. *Proceedings, Annual Meeting - Air & Waste Management Association 83rd(Vol. 8):90-147.2*, 14 pp.

- Papi, A., Amadesi, S., Chitano, P., Boschetto, P., Ciaccia, A., Geppetti, P., Fabbri, L. M., and Mapp, C. E. 1999. Bronchopulmonary inflammation and airway smooth muscle hyperresponsiveness induced by nitrogen dioxide in guinea pigs. *Eur J Pharmacol* 374: 241-247.
- Postlethwait, E. M., and Bidani, A. 1994. Mechanisms of pulmonary NO₂ absorption. *Toxicology* 89: 217-237.
- Postlethwait, E. M., Langford, S. D., and Bidani, A. 1991. Transfer of NO₂ through pulmonary epithelial lining fluid. *Toxicol Appl Pharmacol* 109: 464-471.
- Postlethwait, E. M., Langford, S. D., Jacobson, L. M., and Bidani, A. 1995. NO₂ reactive absorption substrates in rat pulmonary surface lining fluids. *Free Radic Biol Med* 19: 553-563.
- Postlethwait, E. M., and Mustafa, M. G. 1989. Effect of altered dose rate on NO₂ uptake and transformation in isolated lungs. *J Toxicol Environ Health* 26: 497-507.
- Poynter, M. E., Persinger, R. L., Irvin, C. G., Butnor, K. J., van Hirtum, H., Blay, W., Heintz, N. H., Robbins, J., Hemenway, D., Taatjes, D. J., and Janssen-Heininger, Y. 2006. Nitrogen dioxide enhances allergic airway inflammation and hyperresponsiveness in the mouse. *Am J Physiol Lung Cell Mol Physiol* 290: L144-152.
- Proust, B., Lacroix, G., Robidel, F., Marliere, M., Lecomte, A., and Vargaftig, B. B. 2002. Interference of a short-term exposure to nitrogen dioxide with allergic airways responses to allergenic challenges in BALB/c mice. *Mediators Inflamm* 11: 251-260.
- Rasmussen, R. E. 1994. Localization of increased collagen in ferret lung tissue after chronic exposure to nitrogen dioxide. *Toxicol Lett* 73: 241-248.
- Rasmussen, R. E., Mannix, R. C., Oldham, M. J., and Phalen, R. F. 1994. Effects of nitrogen dioxide on respiratory tract clearance in the ferret. *J Toxicol Environ Health* 41: 109-120.
- Rasmussen, R. E., and McClure, T. R. 1992. Effect of chronic exposure to NO₂ in the developing ferret lung. *Toxicol Lett* 63: 253-260.
- Richters, A. 1986. The role of NO₂ and O₃ in cancer metastasis and in systemic adverse effects. Final report. Prepared for California Air Resources Board, ARB Contract A4-064-33. Submitted May 1, 1986 :34 pages.
- Richters, A. 1988. Effects of nitrogen dioxide and ozone on blood-borne cancer cell colonization of the lungs. *J Toxicol Environ Health* 25: 383-390.
- Richters, A., and Damji, K. S. 1988. Changes in T-lymphocyte subpopulations and natural killer cells following exposure to ambient levels of nitrogen dioxide. *J Toxicol Environ Health* 25: 247-256.
- Richters, A., and Damji, K. S. 1990. The relationship between inhalation of nitrogen dioxide, the immune system, and progression of a spontaneously occurring lymphoma in AKR mice. *J Environ Pathol Toxicol Oncol* 10: 225-230.
- Richters, A., and Richters, V. 1989. Nitrogen dioxide (NO₂) inhalation, formation of microthrombi in lungs and cancer metastasis. *J Environ Pathol Toxicol Oncol* 9: 45-51.
- Richters, A., Richters, V., and Sherwin, R. P. 1987. Influence of ambient level NO₂ exposure on newborn and adult mice body weights. *J Environ Pathol Toxicol Oncol* 7: 65-72.
- Riechelmann, H., Kienast, K., Schellenberg, J., and Mann, W. J. 1994. An in vitro model to study effects of airborne pollutants on human ciliary activity. *Rhinology* 32: 105-108.
- Ritter, D., Knebel, J. W., and Aufderheide, M. 2001. In vitro exposure of isolated cells to native gaseous compounds--development and validation of an optimized system for human lung cells. *Exp Toxicol Pathol* 53: 373-386.
- Robison, T. W., Duncan, D. P., and Forman, H. J. 1990. Chemoattractant and leukotriene B₄ production from rat alveolar macrophages exposed to nitrogen dioxide. *Am J Respir Cell Mol Biol* 3: 21-26.

- Robison, T. W., and Forman, H. J. 1993. Dual effect of nitrogen dioxide on rat alveolar macrophage arachidonate metabolism. *Exp Lung Res* 19: 21-36.
- Robison, T. W., Forman, H. J., and Thomas, M. J. 1995. Release of aldehydes from rat alveolar macrophages exposed in vitro to low concentrations of nitrogen dioxide. *Biochim Biophys Acta* 1256: 334-340.
- Robison, T. W., and Kim, K. J. 1995. Dual effect of nitrogen dioxide on barrier properties of guinea pig tracheobronchial epithelial monolayers cultured in an air interface. *J Toxicol Environ Health* 44: 57-71.
- Robison, T. W., and Kim, K. J. 1996. Enhancement of airway epithelial Na⁺,K⁺-ATPase activity by NO₂ and protective role of nordihydroguaiaretic acid. *Am J Physiol* 270: L266-272.
- Robison, T. W., Murphy, J. K., Beyer, L. L., Richters, A., and Forman, H. J. 1993. Depression of stimulated arachidonate metabolism and superoxide production in rat alveolar macrophages following in vivo exposure to 0.5 ppm NO₂. *J Toxicol Environ Health* 38: 273-292.
- Robison, T. W., Zhou, H., and Kim, K. J. 1996. Generation of glycolaldehyde from guinea pig airway epithelial monolayers exposed to nitrogen dioxide and its effects on sodium pump activity. *Environ Health Perspect* 104: 852-856.
- Rose, R. M., Pinkston, P., and Skornik, W. A. 1989. Altered susceptibility to viral respiratory infection during short-term exposure to nitrogen dioxide. Research Report No. 24. *Res Rep Health Eff Inst*: 1-24.
- Rubenchik, B. L., Glavin, A. A., Galenko, P. M., Kilkichko, A. A., Oleinick, I. O., and Artemov, K. V. 1995. Gaseous nitrogen dioxide increases the endogenous synthesis of carcinogenic N-nitrosodimethylamine in animals. *J Environ Pathol Toxicol Oncol* 14: 111-115.
- Sagai, M., and Ichinose, T. 1991. Biochemical effects of combined gases of nitrogen dioxide and ozone. IV. Changes of lipid peroxidation and antioxidative protective systems in rat lungs upon life span exposure. *Toxicology* 66: 121-132.
- Sagai, M., Ichinose, T., and Kubota, K. 1984. Studies on the biochemical effects of nitrogen dioxide. IV. Relation between the change of lipid peroxidation and the antioxidative protective system in rat lungs upon life span exposure to low levels of NO₂. *Toxicol Appl Pharmacol* 73: 444-456.
- Sasaki, J., Arey, J., and Harger, W. P. 1995. Formation of mutagens from the photooxidations of 2-4-ring PAH. *Environmental Science & Technology* 29: 1324-1335.
- Saul, R. L., and Archer, M. C. 1983. Nitrate formation in rats exposed to nitrogen dioxide. *Toxicol Appl Pharmacol* 67: 284-291.
- Schelegle, E. S., Miller, L. A., Gershwin, L. J., Fanucchi, M. V., Van Winkle, L. S., Gerriets, J. E., Walby, W. F., Mitchell, V., Tarkington, B. K., Wong, V. J., Baker, G. L., Pantle, L. M., Joad, J. P., Pinkerton, K. E., Wu, R., Evans, M. J., Hyde, D. M., and Plopper, C. G. 2003. Repeated episodes of ozone inhalation amplifies the effects of allergen sensitization and inhalation on airway immune and structural development in Rhesus monkeys. *Toxicol Appl Pharmacol* 191: 74-85.
- Schierhorn, K., Zhang, M., Matthias, C., and Kunkel, G. 1999. Influence of ozone and nitrogen dioxide on histamine and interleukin formation in a human nasal mucosa culture system. *Am J Respir Cell Mol Biol* 20: 1013-1019.
- Schlesinger, R. B. 1987. Intermittent inhalation of nitrogen dioxide: effects on rabbit alveolar macrophages. *J Toxicol Environ Health* 21: 127-139.
- Selgrade, M. K., Daniels, M. J., and Grose, E. C. 1991. Evaluation of Immunotoxicity of an Urban Profile of Nitrogen Dioxide: Acute, Subchronic, and Chronic Studies. *Inhal Toxicol* 3: 389-403.
- Selgrade, M. K., Mole, M. L., Miller, F. J., Hatch, G. E., Gardner, D. E., and Hu, P. C. 1981. Effect of NO₂ inhalation and vitamin C deficiency on protein and lipid accumulation in the lung. *Environ Res* 26: 422-437.

- Sherwin, R. P., and Carlson, D. A. 1973. Protein content of lung lavage fluid of guinea pigs exposed to 0.4 ppm nitrogen dioxide. *Arch Environ Health* 27: 90-93.
- Sherwin, R. P., and Layfield, L. J. 1976. Protein leakage in the lungs of mice exposed to 0.5 ppm nitrogen dioxide. *Arch Environ Health* 31: 116-118.
- Sherwin, R. P., and Richters, V. 1982. Hyperplasia of Type 2 pneumocytes following 0.34 ppm nitrogen dioxide exposure: quantitation by image analysis. *Arch Environ Health* 37: 306-315.
- Sherwin, R. P., and Richters, V. 1985. Effect of 0.3 ppm ozone exposure on type II cells and alveolar walls of newborn mice: an image-analysis quantitation. *J Toxicol Environ Health* 16: 535-546.
- Sherwin, R. P., and Richters, V. 1995a. Effects of 0.25 ppm nitrogen dioxide on the developing mouse lung. Part 2: Quantitation of elastic tissue and alveolar walls. *Inhal Toxicol* 7: 1183-1194.
- Sherwin, R. P., and Richters, V. 1995b. Effects of 0.25 PPM nitrogen dioxide on the developing mouse lung. Part 1: Quantitation of type 2 cells and measurements of alveolar walls. *Inhal Toxicol* 7: 1173-1182.
- Shiraishi, F., and Bandow, H. 1985. The genetic effects of the photochemical reaction products of propylene plus NO₂ on cultured Chinese hamster cells exposed in vitro. *J Toxicol Environ Health* 15: 531-538.
- Singh, J. 1988. Nitrogen dioxide exposure alters neonatal development. *Neurotoxicology* 9: 545-549.
- Smiley-Jewell, S., and Van Winkle, L. S. 2004. Repair of environmental lung injury during development. In: *The Lung: Development, Aging and the Environment*. R. Harding, K. Pinkerton, C. Plopper eds.: pp. 353-362.
- Stephens, R. J., Sloan, M. F., Groth, D. G., Negi, D. S., and Lunan, K. D. 1978. Cytologic responses of postnatal rat lungs to O₃ or NO₂ exposure. *Am J Pathol* 93: 183-200.
- Stephens, R. J., Tallent, C., Hart, C., and Negi, D. S. 1982. Postnatal tolerance to NO₂ toxicity. *Exp Mol Pathol* 37: 1-14.
- Sterner-Kock, A., Kock, M., Braun, R., and Hyde, D. M. 2000. Ozone-induced epithelial injury in the ferret is similar to nonhuman primates. *Am J Respir Crit Care Med*. 162(3 Pt 1):1152-1156.
- Stevens, M. A., Menache, M. G., Crapo, J. D., FJ, M. I., and Graham, J. A. 1988. Pulmonary function in juvenile and young adult rats exposed to low-level NO₂ with diurnal spikes. *J Toxicol Environ Health* 23: 229-240.
- Tabacova, S., Nikiforov, B., and Balabaeva, L. 1985. Postnatal effects of maternal exposure to nitrogen dioxide. *Neurobehav Toxicol Teratol* 7: 785-789.
- Takano, H., Yanagisawa, R., Inoue, K., Shimada, A., Ichinose, T., Sadakane, K., Yoshino, S., Yamaki, K., Morita, M., and Yoshikawa, T. 2004. Nitrogen dioxide air pollution near ambient levels is an atherogenic risk primarily in obese subjects: a brief communication. *Exp Biol Med (Maywood)* 229: 361-364.
- Tepper, J. S., Costa, D. L., Winsett, D. W., Stevens, M. A., Doerfler, D. L., and Watkinson, W. P. 1993. Near-lifetime exposure of the rat to a simulated urban profile of nitrogen dioxide: pulmonary function evaluation. *Fundam Appl Toxicol* 20: 88-96.
- Thomas, H. V., Mueller, P. K., and Wright, R. 1967. Response of rat lung mast cells to nitrogen dioxide inhalation. *J Air Pollut Control Assoc* 17: 33-35.
- Tsuda, H., Kushi, A., Yoshida, D., and Goto, F. 1981. Chromosomal aberrations and sister-chromatid exchanges induced by gaseous nitrogen dioxide in cultured Chinese hamster cells. *Mutat Res* 89: 303-309.
- Tsujino, I., Kawakami, Y., and Kaneko, A. 2005. Comparative simulation of gas transport in airway models of rat, dog, and human. *Inhal Toxicol* 17: 475-485.

- Van Stee, E. W., Sloane, R. A., Simmons, J. E., Moorman, M. P., and Brunnemann, K. D. 1995. Endogenous formation of N-nitrosomorpholine in mice from 15NO₂ by inhalation and morpholine by gavage. *Carcinogenesis* 16: 89-92.
- Velsor, L. W., and Postlethwait, E. M. 1997. NO₂-induced generation of extracellular reactive oxygen is mediated by epithelial lining layer antioxidants. *Am J Physiol* 273: L1265-1275.
- Victorin, K., Busk, L., Cederberg, H., and Magnusson, J. 1990. Genotoxic activity of 1,3-butadiene and nitrogen dioxide and their photochemical reaction products in *Drosophila* and in the mouse bone marrow micronucleus assay. *Mutat Res* 228: 203-209.
- Victorin, K., and Stahlberg, M. 1989. Mutagenic activity of ultraviolet-irradiated mixtures of nitrogen dioxide and propene or butadiene. *Environ Res* 49: 271-282.
- Vollmuth, T. A., Driscoll, K. E., and Schlesinger, R. B. 1986. Changes in early alveolar particle clearance due to single and repeated nitrogen dioxide exposures in the rabbit. *J Toxicol Environ Health* 19: 255-266.
- Wallis, S. A., Victorin, K., and Lundborg, M. 1995. DNA damage in lung cells in vivo and in vitro by 1,3-butadiene and nitrogen dioxide and their photochemical reaction products. *Mutat Res* 328: 11-19.
- Watanabe, N. 2005. Decreased number of sperms and Sertoli cells in mature rats exposed to diesel exhaust as fetuses. *Toxicol Lett* 155: 51-58.
- Watanabe, N., and Ohsawa, M. 2002. Elevated serum immunoglobulin E to *Cryptomeria japonica* pollen in rats exposed to diesel exhaust during fetal and neonatal periods. *BMC Pregnancy Childbirth* 2: 2.
- WHO. 1997. World Health Organization. Nitrogen Oxides (Second Edition). Environmental Health Criteria 188, Geneva.
- Witschi, H., Breider, M. A., and Schuller, H. M. 1993. Failure of ozone and nitrogen dioxide to enhance lung tumor development in hamsters. Synopsis of Research Report Number 60. *Res Rep Health Eff Inst*: 1-25; discussion 27-38.

9 Effects of Nitrogen Dioxide on Vegetation

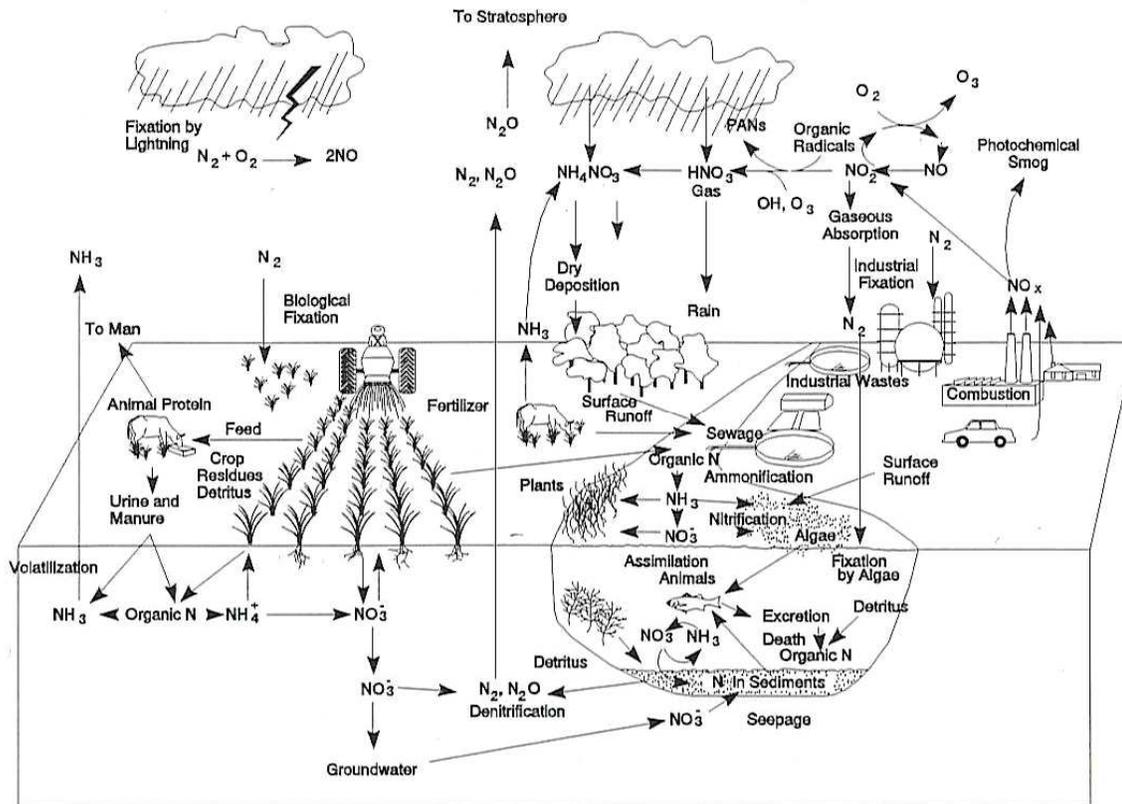
9.1 Introduction

Nitrogen (N) is one of the most important elements for plant and animal life, comprising 2 to 4 per cent of body mass, and is a major element in proteins, enzymes, nucleic acids, and other biochemical components of cells and tissues. Nitrogen is also one of the most abundant elements in the atmosphere and lithosphere, yet paradoxically, N is perhaps the essential element most limiting to plant growth and reproduction. The reason for this apparent scarcity is that N is not available for assimilation by plants until it has been “fixed”; that is, transformed chemically or biologically into oxidized or reduced ionic forms that can be transported across cellular membranes. The chemical and biological transformations of N from atmospheric N₂ and soil organic N into ionic forms and back to the atmosphere constitute a suite of complex interrelated processes known as the nitrogen cycle (Figure 9.1). Prior to industrialization, most of the fixed N compounds available for plant growth were produced by biological processes, particularly by microorganisms in the soil or those in symbiotic relationships with the roots of higher plants. A small amount of oxidized N was produced in the atmosphere by high-energy processes, such as lightning and photochemical oxidation. Because of this relative scarcity, plants evolved highly efficient mechanisms to assimilate fixed N compounds from their environment. One extreme example of this is the evolution in acidic N-poor environments of a number of carnivorous plants that extract fixed N from animals trapped in highly modified leaves (Juniper et al. 1989). Within plants, N is conserved by translocation from senescing leaves to actively growing plant parts. Within ecosystems N is conserved by tight coupling of biogeochemical cycles. Indeed, a key indicator of ecosystem health is the low level of leakage of fixed N from the system (Fenn et al. 1998).

Industrialization has greatly increased the amount of fixed N compounds in the atmosphere, in soils, and in water (Figure 9.1), leading to concerns about adverse impacts on plant and ecosystem health in response to higher concentrations of some of these potentially toxic compounds. Adverse effects on plant growth and productivity have long been observed from exposure to elevated concentrations of NO₂. The U.S. Environmental Protection Agency (USEPA) in 1971 established a national ambient air quality standard of 0.053 parts per million (ppm) as an annual average in order to protect health and welfare, including plants, from adverse effects of exposure to NO₂. California also established an ambient air quality standard for NO₂ at 0.25 ppm averaged for one hour, to protect human health and welfare. The criteria for the national standard were last reviewed by the USEPA in 1993 (USEPA 1993) and by California in 1992 (California Air Resources Board (CARB) 1992). Based on extensive reviews of the best available information from the literature, these reviews concluded that the current air quality standards would protect vegetation from adverse effects of the direct impact of ambient NO₂. However, indirect effects of wet and dry N deposition to ecosystems and landscapes, particularly in remote areas and national parks (National Academy of Sciences 2004), remain a concern. In California, as well as in most of North America, the most important photochemical air pollutant is ozone (O₃). The adverse effects of O₃ on crops (Heck et al. 1988) and forests (Miller and McBride 1998) have been well documented, but many avenues of research on O₃ effects remain to be explored. In contrast, no ongoing studies of the short-term or long-term direct effects of NO or NO₂ have been conducted in the U.S. in many years. The current experimental literature is based primarily on studies conducted in Europe, where research on the effects of NO and NO₂ has remained a high priority (Davison and Cape 2003).

The objective of this review of the literature on the effects of NO₂ on plants is to provide a critical evaluation of recent peer-reviewed studies of the direct effects of NO₂ on injury and growth of plants and indirect effects of reactive N deposition on ecosystems and landscapes in California, and to provide the background information necessary for the evaluation of those studies.

Figure 9.1 Schematic diagram of the nitrogen cycle, emphasizing human activities that affect fluxes of nitrogen. (US EPA 1993)



9.2 Sources and Atmospheric Chemistry of Nitrogen Compounds

9.2.1 Emissions, Transformations, and Transport of Nitrogen Oxides

Emissions of nitrogen oxides vary widely among the different air basins in California, from less than 10 tons per day (T/d) in the lightly populated northeast to over 1,000 T/d in the South Coast Air Basin (Table 9.1). This three-order-of-magnitude variation reflects the large differences in automobile density and usage from urban to rural areas, because approximately 80 per cent of NO_x emissions are derived from mobile sources (CARB 2004). Statewide, emissions of NO_x from all sources totaled 3,595 T/d in 2000. This represented a 30 per cent decline in emissions of NO_x from a peak of 5,130 T/d in 1980. Because of the implementation of effective control strategies, the decline in NO_x emissions is expected to continue for the foreseeable future.

Table 9.1 Estimated NO_x emissions from California Air Basins, 2002, in tons per day. (Data from CARB 2004).

Air Basin	Mobile Sources	Total
Great Basin Valleys	4.7	6.1
Lake County	6.9	8.3
Lake Tahoe	4.6	5.3
Mojave Desert	101.5	219.5
Mountain Counties	45.4	56.4
North Central Coast	62.1	92.7
North Coast	45.2	58.5
Northeast Plateau	20.4	24.2
Sacramento Valley	218.3	273.5
Salton Sea	45.7	55.2
San Diego	199.1	220.5
San Francisco Bay	508.6	621.8
San Joaquin Valley	360.8	525.0
South Central Coast	100.9	128.3
South Coast	938.8	1088.2

Chapter 2 summarizes the atmospheric chemistry of nitrogen oxide and related compounds.

In the presence of volatile organic compounds (VOC) such as hydrocarbons and aldehydes from automotive emissions and other sources, NO₂ participates in additional O₃ formation through a series of highly complex photochemical reactions (Finlayson-Pitts and Pitts 1986). The amount of O₃ generated by these photochemical reactions is highly variable, and is a function of the mixing ratios of NO₂ and VOCs (Atkinson 2000). In polluted urban air plumes with high NO_x concentrations and high NO_x/VOC ratios, one molecule of NO_x will produce on the order of 10 or fewer molecules of O₃. In rural or wilderness areas, where NO_x emissions are low and a higher proportion of VOCs are derived from biogenic emissions, photochemical formation of O₃ is enhanced, and one molecule of NO_x can produce upwards of 100 molecules of O₃ (Pierce et al. 1998). The enhanced efficiency of O₃ formation from NO_x at high VOC ratios may in part explain the relatively high concentrations of O₃ observed in remote mountain air sheds in California (Tonnesen et al. 2003). Recent remote-sensing satellite measurements of world-wide tropospheric O₃ formation confirm that surface O₃ is more sensitive to emissions of NO_x than to VOC emissions in most regions of the northern hemisphere in summer (Martin et al. 2004). The significant exceptions to these observations are the Los Angeles Basin and certain industrial areas in Germany. Differences in urban/rural NO_x/VOC mixing ratios and the subsequent variation in O₃ formation have consequences for control strategies to reduce ozone formation through reductions in chemical precursors; such a discussion is beyond the scope of this report.

These photochemical reactions also lead to the formation of additional secondary air pollutants, such as peroxyacetyl nitrate (PAN), (see Chapter 2) that also have adverse impacts on human health and plant injury (Temple et al. 1998). PAN is thermally unstable, but in the cold upper troposphere PAN is stable for periods of 5 to 20 hours and can be transported long distances downwind of the urban pollutant plume to

remote areas. Nitric acid vapor (HNO₃) is an additional secondary air pollutant with potentially adverse effects on vegetation (Bytnerowicz et al. 1998a). It is formed in polluted atmospheres through the reaction of NO₂ with hydroxyl radicals (see Chapter 2).

Ammonia (NH₃) is an additional source of N deposition to landscapes. Sources of NH₃ are primarily agricultural operations including livestock feed lots, application of liquid ammonia fertilizers, and fertilizer production plants. Effects of NH₃ on plants will not be discussed in this review, except in connection with the deposition of atmospheric N.

Transport of nitrogen oxides from sources in urban coastal and Central Valley cities in California to inland valleys, deserts, and mountains is principally governed by sea-land wind patterns and mountain-valley wind flows (Fujioka et al. 1998). During the summer, when pollutant levels are highest, the prevailing winds are from the west-northwest and sweep air masses through the gap in the Coast Range at San Francisco Bay and through the Los Angeles area to the valleys and mountain ranges to the east. Daytime heating of west-facing slopes generates local anabatic winds that drive polluted air masses to mountain crests. Less is known of the transport of polluted air masses through drainages and passes to eastern deserts and plateaus, although recent modeling studies suggest that transport of pollutants across the Transverse Ranges and the Sierra Nevada may occur (Tonnesen et al. 2003). Estimates of the loss of NO_x in transported air masses in the South Coast Air Basin suggest a lifetime on the order of 10 hours for NO_x (Finlayson-Pitts and Pitts 1986). During this period the polluted air mass can be transported from tens to hundreds of kilometers downwind of urban sources.

9.2.2 Spatial and Temporal Patters of NO₂ Concentrations

Ambient air concentrations of NO₂ throughout the state generally reflect NO_x emissions, although because of long-range transport the gradients of NO₂ concentrations from urban to rural areas are not as steep as those of the emissions. Maximum annual average NO₂ concentrations in 2001 ranged from 0.007 ppm in the North Central Coast Air Basin to 0.041 ppm in the South Coast Air Basin (SoCAB) (Table 9.2).

Table 9.2 Ambient air concentrations of NO₂ in California Air Basins in 2001, in parts per million (ppm). (Data from CARB 2004).

Air Basin	Max Annual Average	Max 1 hr	Peak 1 hr*
Lake Tahoe	0.011	0.09	0.068
Mojave Desert	0.024	0.102	0.099
North Central Coast	0.007	0.071	0.046
Sacramento Valley	0.019	0.085	0.097
Salton Sea	0.017	0.086	0.071
San Diego	0.022	0.148	0.126
San Francisco Bay	0.025	0.114	0.105
San Joaquin Valley	0.024	0.099	0.106
South Central Coast	0.020	0.124	0.086
South Coast	0.041	0.251	0.216

The peak 1-hour indicator represents the maximum concentration expected to occur once per year, on average, based on statistical calculations using three years of ambient data from the site.

Maximum one-hour concentrations ranged from 0.052 ppm in the North Coast to 0.251 ppm in South Coast. As with emissions data, long-term trends in ambient NO₂ concentrations show significant declines from maxima in the 1980's (CARB 2004). Peak hourly concentrations have declined by about 50 per cent from 1975 to 2001 (Figure 9.2). The highest ambient NO₂ concentrations in the state are present in the highly urbanized SoCAB, but even here, peak concentrations have declined from 0.414 ppm in 1982 to 0.216 ppm in 2001 (Figure 6.3). In forested regions of the state, hourly mean concentrations of NO₂ did not exceed 0.050 ppm (Bytnerowicz and Fenn 1996). In summary, all areas of the state are currently designated as in attainment for the California NO₂ ambient air quality standard, and most areas are also in attainment of the national NO₂ standard.

Because of the complexities of the photochemical reactions outlined above, NO₂ is typically only one component of a polluted air mass. In addition, the presence of other oxides of nitrogen, O₃, PAN, NO₃⁻, SO₂, or other air pollutants should be considered in evaluating the response of plants to a single pollutant. The co-occurrence of air pollutant mixtures in the atmosphere has been reviewed extensively (Lefohn et al. 1987; USEPA 1993). Lefohn (1992) summarized the literature and concluded that the number of co-occurrences of hourly average concentrations equal to or greater than 0.03 ppm for NO₂, O₃, and SO₂ was infrequent at most air quality reporting sites in the U.S. Using a minimum co-occurrence of 0.05 ppm for mixtures of NO₂ and SO₂, Lefohn and Tingey (1984) reported that the number of such events was rare. Even in areas where O₃ concentrations were elevated, for example in California, the number of co-occurrences of O₃ and NO₂ above 0.05 ppm was also rare, and the three-pollutant mixture of NO₂/O₃/SO₂ above 0.05 ppm was almost non-existent (Lefohn and Tingey 1984). In England, where co-occurrences of elevated concentrations of NO₂ and SO₂ have been reported during the winter months (Lane and Bell 1984), these co-occurrences accounted for <1% of the monitoring period. Most studies of the effects of pollutant mixtures on plants have used concentrations and exposure intervals higher or greater than those found to occur under ambient pollutant conditions. Results from some of these studies are reported in Section 9.4, but it should be emphasized that conclusions concerning the effects of pollutant mixtures on plants remain, with few exceptions, observations from laboratory experiments.

9.3 Pollutant Uptake

9.3.1 Uptake of NO_x from the Atmosphere

Current air quality standards, both national and state, are expressed as mixing ratios or partial pressures in the atmosphere and are given in units of ppm or µg/m³. However, plants do not respond to atmospheric concentrations of pollutants but to the rate and amount of pollutant absorbed into the plant (Runeckles 1992). The obvious exception to this statement, damage to epicuticular waxes, trichomes, and cuticles by HNO₃ or by NO₂, will be discussed in a later section. Foliar uptake of gaseous pollutants has long been modeled as a series of resistances, as molecules diffuse across the leaf boundary layer, through the stomata, across the sub-stomatal cavity, to the mesophyll tissue layer of the leaf (Figure 9.2). A few studies have shown that NO₂ can move through detached plant cuticles (Lendzian and Kerstiens 1988). However most experiments using gas-exchange measurements (Darrall 1989; Saxe 1986a; Thoene et al. 1991) or stable ¹⁵N isotopes (Okano et al. 1988) have shown that stomatal resistance is the primary determining factor in controlling the rate of foliar absorption of NO₂.

Numerous external environmental factors, such as light intensity, temperature, vapor pressure deficit (VPD), wind speed, and plant water and nutrient status affect stomatal resistance. Exposure to NO₂ by itself can interfere with stomatal responses, but only at unrealistically high (ca. 1 ppm) concentrations (Carlson 1983). At concentrations near ambient and up to 0.43 ppm, NO₂ had little or insignificant effects on stomata (McAinsh et al. 2002; Sinn et al. 1984). Endogenous or genetic factors, such as stomatal size, density, behavior, and distribution on the leaf also control the amount and rate of entry of a gaseous pollutant into the leaf (Okano et al. 1988). The interactions of these external and endogenous controls on stomatal function and its consequences for uptake of pollutants have been the subjects of numerous studies and the literature has been extensively reviewed, particularly with regard to the uptake of O₃ (Darrall 1989; Runeckles 1992). In general, factors conducive to greater rates of stomatal conductance (1/resistance) result in increased pollutant uptake. Conversely, stress factors such as low light conditions and drought can significantly reduce the effects of exposure to NO₂ (Srivastava et al. 1975; Murray 1984; Stulen et al. 1998). The importance of plant water status in the regulation of gaseous pollutant entry into leaves has been amply demonstrated in both laboratory and field studies with respect to the impact of O₃

on plant injury, growth, and productivity of crops (Heagle et al. 1988) and trees (Temple et al. 1993). Comparable field studies have not been conducted with NO_2 , but by analogy with O_3 , the responses of plants to NO_2 are likely to be regulated by the rate of entry of the pollutant into plant leaves, which is under stomatal control.

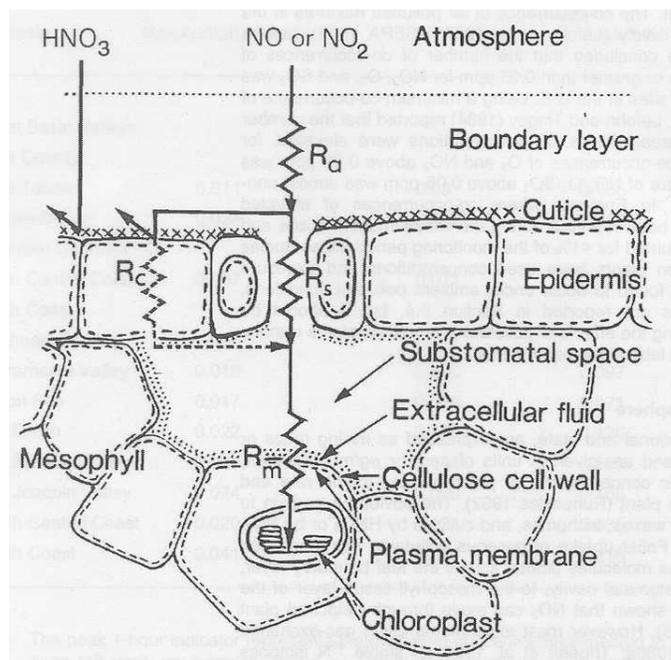


Figure 9.2 Pathways for the entrance of gaseous nitrogen oxides from the atmosphere into leaf tissues and cells. (From US EPA, 1993). The layer of still air or boundary layer imposes a resistance (R_a) that depends on a number of factors including wind speed. Access is then limited by the degree of stomatal opening (R_s) or to a much lesser extent by penetration through the cuticle of epidermal layers (R_c). The mesophyll resistance (R_m) consists of a number of different components before at the major sites of reaction are encountered.

9.3.2 Biochemical Reactions

The complex chemical and enzymatic reactions of NO and NO_2 in plants have been recently reviewed (USEPA 1993; Lea et al. 1994; Stulen et al. 1998). In brief, once past the stomata, gaseous NO_2 in the substomatal cavity is rapidly absorbed into the apoplast, forming ionic nitrate and nitrite. Nitrite can also be formed by the dissolution of NO in the apoplast. Nitrate can be stored in the vacuole or reduced to nitrite in the cytoplasm by the activity of the enzyme nitrate reductase (NR). Induction of NR in numerous plant tissues, including needles of conifers exposed to NO_2 , has been amply demonstrated (Norby et al. 1989; Thoene et al. 1991). Nitrite is transported into the chloroplast, where it is reduced to ammonium (NH_4^+) by the enzyme nitrite reductase (NiR). NiR is regenerated in the chloroplast by the light reactions of photosynthesis. The NH_4^+ is assimilated into amino acids by the glutamine synthase/glutamine oxoglutarate aminotransferase cycle (GS/GOGAT) in the chloroplast. Assimilation of NO or NO_2 produces protons (H^+), which must be neutralized to maintain acid/base equilibria in the cell. The stoichiometry of these reactions was discussed by Raven (1988), who calculated that one mole of foliar-supplied NO_x would yield 0.22 mole of excess H^+ . Once the proton gradient across membranes has been altered, ion transport is inhibited. High concentrations of NO_2 have also been shown to inhibit lipid biosynthesis, which could also affect membrane integrity (Lea et al. 1994).

Several lines of evidence indicate that the rates of de-acidification and NR activity influence plant responses to NO_2 , and that plants with higher rates of NR activity may be less susceptible to the toxic effects of nitrite and nitrate generated by the rapid absorption of NO_2 . In bean plants (*Phaseolus vulgaris* L.) exposed to $^{15}\text{NO}_2$, after three hours, the labeled N was primarily in the protein and nucleic acid fraction of the leaf (63% of total labeled N), with 33% in the amino acid fraction, and < 5% as nitrite (Rogers et al. 1979). However, when plants were exposed to exceptionally high concentrations of labeled NO_2 (> 3 ppm), a much higher fraction of the label accumulated in nitrate, suggesting that detoxification mechanisms had been saturated (Yu et al. 1988). In barley plants (*Hordeum vulgare* L.) selected for

resistance to NO₂, the degree of resistance to growth reduction was directly proportional to rates of NR activity in the different lines (Srivastava et al. 1994). Pioneer or “weedy” plant species showed higher potential for foliar nitrate assimilation and higher levels of NR activity than slow growing species (Soares et al. 1995). Plants with a higher capacity to assimilate foliar nitrate apparently generated higher amounts of OH⁻, which increased the buffer capacity of the leaf. The observation that plants exposed to NO₂ in the dark accumulated greater amounts of nitrite and showed greater amounts of foliar injury than those exposed in the light (Amundson and MacLean 1982) is consistent with a light-generated, photosynthesis-dependent pathway for the detoxification of absorbed NO₂.

9.3.3 Assimilated NO₂ as a Source of Available Plant Nitrogen

Land plants have evolved mechanisms for the acquisition of fixed N from both below ground and above ground sources (Raven 1998; Wellburn 1990). The assimilation of N from NO₂ through leaves has been amply demonstrated by use of ¹⁵N-labelled NO₂ (Rogers et al. 1979; Okano et al. 1988). This observation led to speculation regarding the possible role of ambient NO₂ as a direct source of N fertilization for crop plants and native vegetation. This question has been addressed by numerous studies of N uptake from both root and aerial sources that show significant interactions between the two sources of N. Assimilation of N and transport within the plant are controlled by active transport carriers, so these processes are limited by the availability of active transport sites and ATP-energized enzyme systems. Thus, uptake of N from atmospheric deposition is a function of the total availability of N from all sources, and soil N in particular. Crop plants furnished with adequate supplies of N show little response to additional N from NO₂ (Srivastava and Ormrod 1984). In an extensive series of experiments, Stulen et al. (1998) showed that rapidly-growing spinach plants (*Spinacea oleracea* L.), cultivated in conditions of sub-optimal root N, derived less than 10 per cent of total plant N from “realistic” concentrations of NO₂ (< 0.3 ppm). Plants grown with adequate supplies of N to the roots assimilated very little additional N from atmospheric NO₂.

Plants exposed to atmospheric N in the form of rain acidified with HNO₃ also derived relatively little of total plant N from this source. Experiments with bean plants (*Phaseolus vulgaris* L.) (Evans et al. 1986) and red spruce (*Picea rubens* Sarg.) seedlings (Bowden et al. 1989) exposed to rain acidified with ¹⁵NO₃⁻ to pH as low as 2.7 showed that these plants derived less than 1.5 per cent of total N required for new growth from water droplets deposited on foliage. Dry deposition of N onto plant and soil surfaces may be a significant source of N for native plants in N-poor soils after washout by rain events. This subject will be discussed in a later section.

9.4 Effects of NO₂ on Plants

9.4.1 Foliar Injury

9.4.1.1 Foliar Injury Symptoms

Foliar injury symptoms specifically attributed to ambient NO₂ have not been observed in the field, except in the vicinity of strong point sources or accidental spillages (Taylor and McLean 1970; Wellburn 1990; Bytnerowicz et al. 1998a,b). Foliar injury symptoms on plants observed in response to experimental exposures to high concentrations of NO₂ resemble those induced by exposures to SO₂. Early symptoms on broad-leaved plants include cellular damage leading to a dull water-soaked appearance on inter-veinal or marginal areas of the upper leaf surface, followed by tissue collapse and bleaching to a pale to dark brown color (Taylor and McLean 1970; Bytnerowicz et al. 1998b). The initial onset of a water-soaked appearance in leaves in response to NO₂ is consistent with disruption of cellular membranes and electrolyte leakage as the primary point of toxicity of NO₂. On narrow-leaved plants, particularly grasses, injury consists of narrow bands or stripes of yellow to tan necrotic tissue between the parallel leaf veins or on leaf tips and margins. On conifers, acute NO₂ injury first appears at the needle tip as a gray-green water-soaked area, then the injury progresses toward the leaf base, darkening with age to dark brown or reddish-brown (USEPA 1993).

Foliar injury on plants exposed in the field to high doses of NO₂ from point sources are more variable than those just described. Leaves on elm trees (*Ulmus carpinifolia*) growing close to a fertilizer factory showed symptoms of desiccation between leaf veins. Foliar injury on oak trees (*Quercus robur* L.) exposed to high concentrations of NO₂ from a fertilizer factory in Poland consisted of pale tan to brown marginal and tip necrosis on leaf lobes. Initial symptoms on Scots pine (*Pinus sylvestris* L.) needles were excessive

elongation and shoot deformities (Oleksyn et al. 1994). About 1, 200 hectare of pine forest in the vicinity of this forest died from excessive toxic emissions from this factory.

Relatively little research has been conducted on foliar injury from other fixed N compounds. Seedlings of ponderosa pine (*Pinus ponderosa* Laws.) and California black oak (*Quercus kelloggii* Newb.) subjected to short-term exposures from 50 to 250 ppb of HNO₃ vapor for 12 hours showed significant changes in epicuticular structure and composition on pine needles at 50 ppb. Oak leaves appeared to be more resistant to HNO₃ vapor (Bytnerowicz et al. 1998b). These concentrations of HNO₃ are higher than those normally observed in mixed conifer forests of California, although daytime 12 hour concentrations as high as 18.4 ppb (46.7 µg/m³) have been recorded at Tanbark Flat, on the western slopes of the San Gabriel Mountains, upwind from Los Angeles (Bytnerowicz et al. 1998a). Foliar injury from exposure to NH₃ has been recorded principally in the vicinity of accidental spills or releases (Bytnerowicz et al. 1998a; Temple et al. 1979). Injury symptoms from ambient concentrations of NH₃ have not been reported.

9.4.1.2 Relative Susceptibility to NO₂

Lists of the relative susceptibilities of plant species to NO₂ have been reported previously (Taylor and McLean 1970; Bytnerowicz et al. 1998a). The most susceptible woody plant species exposed to NO₂ in the vicinity of a fertilizer plant in Poland were common juniper (*Juniperus communis* L.), Scots pine, and Norway spruce (*Picea abies* (L.) Karst.); resistant trees included box elder (*Acer negundo* L.), elm (*Ulmus carpinifolia* L), Norway maple (*Acer platanoides* L.), and willow (*Salix* spp.). Crop species susceptible to NO₂ include maize (*Zea mays* L.), pinto bean, and sunflower (*Helianthus annuus* L.); asparagus (*Asparagus officinalis* L.) and bush bean were resistant (Taylor and McLean 1970).

9.4.1.3 Dose-Response Relationships

The functional relationship between ambient concentrations of an air pollutant and a specific plant response, such as foliar injury, is complex. Factors such as inherent rates of stomatal conductance and detoxification mechanisms and external factors, including plant water status, light, temperature, humidity, and the particular pollutant exposure regime, all affect the amount of a pollutant needed to cause symptoms of foliar injury. Plant age and growing conditions, and experimental exposure techniques also vary widely among reports of experimental exposures of plants to NO₂. Nevertheless, a meta-analysis conducted by USEPA of over 50 peer-reviewed reports on the effects of NO₂ on foliar injury indicated that plants are relatively resistant to NO₂, especially in comparison to foliar injury caused by exposure to O₃ (USEPA 1993). With few exceptions, no visible injury was reported at concentrations below 0.20 ppm, and these occurred when the cumulative duration of exposures extended to 100 hours or longer (Figure 9.3). At 0.25 ppm, increased leaf drop was reported on navel orange trees (*Citrus sinensis* L. cv. 'Washington'), but only after exposures in excess of 1000 hours (Thompson et al. 1970). Green bean plants used as bio-indicators of NO₂ injury in Israel developed foliar injury symptoms when ambient concentrations exceeded 0.50 ppm (Donagi and Goren 1979). Only when concentrations exceeded 1 ppm did injury occur on most plants in less than one day (USEPA 1993). The EPA concluded from these results that foliar injury symptoms were unlikely to occur on even the most susceptible plant species at concentrations of NO₂ prevalent even in the most polluted areas of the U.S. No reports of plant exposures to NO₂ published since this meta-analysis have altered this conclusion.

9.4.2 Physiological, Growth, and Yield Responses

9.4.2.1 Effects of NO₂ on Photosynthesis

As discussed in Section 9.3, the primary mode of toxicity of NO₂ is the rapid increase in nitrite concentration within the chloroplast, thus altering pH equilibria and ion transport across membranes. Disruption of chloroplast membranes could lead to reductions in rates of photosynthesis and assimilation of carbon dioxide (CO₂). Reductions in rates of photosynthesis have been recorded in experimental exposures of plants to both NO and NO₂, but only at concentrations significantly higher than would normally be encountered in ambient air. For example, Sabaratnam et al. (1988) reported that soybean (*Glycine max* Merr. cv. 'Williams') exposed 7 hours/day for 5 days showed an increase in photosynthetic rates at a concentration of 0.20 ppm, but a reduction in net photosynthesis at a concentration of 0.50 ppm. Short-term exposures of soybean to 0.6 ppm NO₂ for 2 to 3 hours also had no effect on net photosynthesis (Carlson 1983). Most plants appear to be more susceptible to NO than to NO₂, as shown by Saxe (1986b), who exposed a variety of horticultural plants raised in greenhouses (species of *Hedera*,

Ficus, Hibiscus, Nephrolepis, and Dieffenbachia) to both NO and NO₂. He reported that reductions in net photosynthesis occurred at doses of NO that were 22 times less than that for NO₂. However, these reductions in net photosynthesis required concentrations as high as 1 ppm NO for 12 hours to elicit a response in these plants. Plants exposed to combinations of NO and NO₂ showed responses at concentrations of NO₂ lower than those previously reported for NO₂ acting alone. Lettuce (*Lactuca sativa* L. cv. 'Ambassador') grown in high concentrations of CO₂ (950 ppm) and NO (2 ppm), showed reductions in net photosynthesis of 15 to 20 per cent when exposed to 0.5 ppm NO₂ for 30 minutes (Caporn 1989). Such unusual atmospheric conditions might occur in greenhouses with malfunctioning space heaters, but are not expected under ambient conditions. In reviewing the extensive literature on the effects of NO₂ in combination with other gaseous air pollutants, particularly SO₂ and O₃, the USEPA (1993) concluded that combinations of pollutants can cause reductions in photosynthesis or foliar injury at concentrations lower than those associated with NO₂ acting alone, but the plant responses occur at concentrations much higher than are found in ambient air. In addition, the presence of NO₂ in combination studies did not produce symptoms different from those caused by the dominant pollutant, either SO₂ or O₃, so that a plant response produced by combinations of NO₂ with other air pollutants in the field would be difficult, if not impossible, to distinguish from those of the other single pollutants (USEPA 1993).

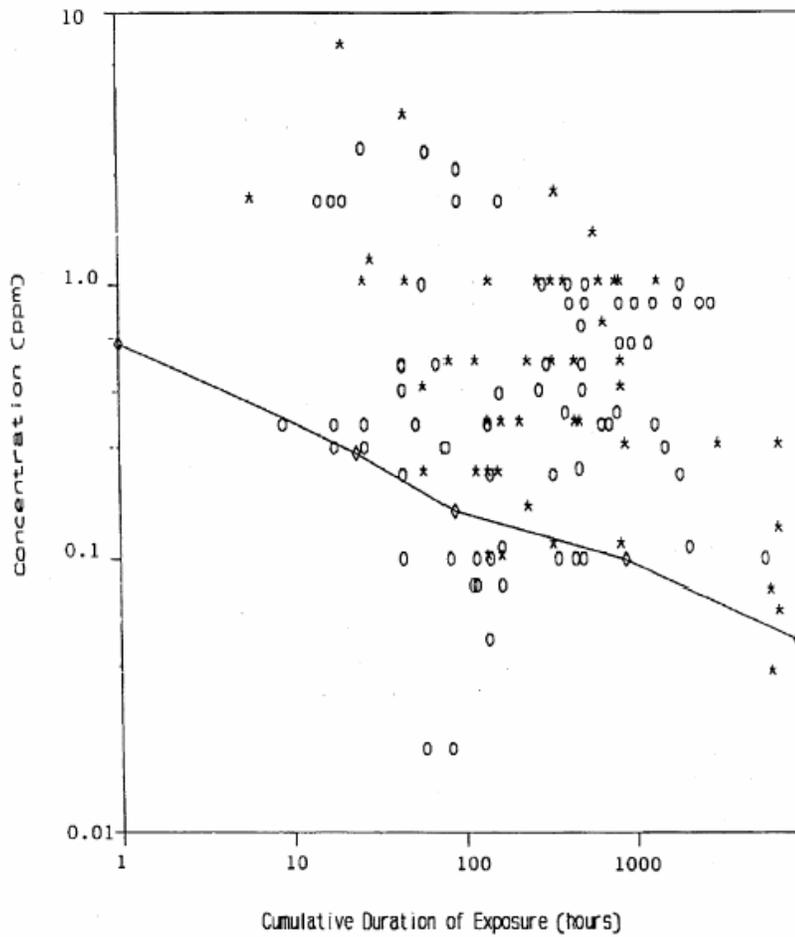


Figure 9.3. Occurrence (*) or absence (0) of foliar injury from exposure to nitrogen oxides in long-term experimental exposures. Solid line represents the upper limit of ambient NO₂ concentrations. Both X- and Y- axes are log scale. (From USEPA 1993)

9.4.2.2 Effects on Growth and Yield

Hundreds of studies have been conducted on the effects of NO₂ on growth and yield of plants. These studies varied widely in plant species, growing conditions, exposure equipment, concentrations, durations, exposure regimes, and environmental conditions during exposures. No clear dose-response surface relating exposure to NO₂ and reductions in growth and/or yield of plants has emerged from these experiments. However, based on the analysis conducted by the EPA (1993) of over 100 studies, a few generalizations can be offered.

9.4.2.2.1 Concentrations of NO₂ < 0.20 ppm

Several species of pasture grasses appear to be susceptible to reductions in growth by relatively low concentrations of NO₂, particularly when exposed during low-light conditions in the winter in England. For example, nearly continuous exposure to 0.1 ppm NO₂ for eight weeks significantly reduced growth of Kentucky blue grass (*Poa pratensis* L.) seedlings (Ashenden and Williams 1980; Whitmore and Mansfield 1983). Longer exposures up to 28 weeks at the same concentration (0.1 ppm) reduced growth of timothy (*Phleum pratense* L.) and perennial rye grass (*Lolium multiflorum* Lam.). Eight species of tree seedlings were exposed to 0.1 ppm NO₂ for six hours/day for 28 days, resulting in reduced shoot or root growth in two species, white ash (*Fraxinus americana* L.) and sweet gum (*Liquidambar styraciflua* L.), reduced height growth in two clones of loblolly pine (*Pinus taeda* L.), and no effects on the other species (Kress and Skelly 1982). No effects of NO₂ at 0.1 ppm or lower were observed on numerous other species, including potato (*Solanum tuberosum* L.), black poplar (*Populus nigra* L.), radish (*Raphanus sativus* L.), soybean, or peas (*Pisum sativum* L.) (US EPA 1993). No or only slight effects of 0.11 ppm NO₂ were reported on a variety of native California desert plants exposed for five hours/day for 12 to 32 weeks (Thompson et al. 1980). Inconsistent results were obtained using green bean seedlings exposed to 0.1 ppm NO₂. In one study (Srivastava and Ormrod 1986), plants exposed six hours/day for 14 days experienced significantly reduced shoot and root mass, relative to controls. However other studies reported no effect on bean seedlings from exposure to 0.1 ppm NO₂ or increased growth of bean seedlings exposed to 0.1 ppm NO₂ for seven hours/day, five days/week for three weeks (Sandhu and Gupta 1989).

9.4.2.2.2 Concentrations > 0.2 ppm

Negative effects of NO₂ on growth and yield appear on more plant species when concentrations of 0.2 ppm and above were used. For example, potato plants exposed to 0.2 ppm NO₂ for five hours/day, two days/week, for the length of the growing season (12 to 16 weeks) experienced significantly greater foliar senescence and leaf abscission, and 43 to 51 per cent lower yield of tubers, compared with controls (Sinn and Pell 1984). This study demonstrated that hourly concentrations of NO₂ < 0.25 ppm can have significantly adverse effects on growth and yield of potato. However, this experiment was conducted in controlled environment chambers under ideal growing conditions, so the relevance of this study to field conditions cannot be determined. Exposure to 0.25 ppm NO₂ three hours /day for six days in four weeks significantly decreased shoot length and leaf mass in two of eight cultivars of 1-year-old azaleas (*Rhododendron* spp.) (Sanders and Reinert 1982). However, other workers reported no effects or positive growth responses from long-term exposures to between 0.2 and 0.3 ppm NO₂ on soybean, green beans, tobacco (*Nicotiana tabacum* L.), tomato (*Lycopersicon esculentum* Mill.), and radish (USEPA 1993). No effects of NO₂ were observed on soybeans grown in field plots subjected to a series of 10 episodic exposures averaging 0.4 ppm for 2.5 or 3 hours (Irving et al. 1982). Numerous studies have reported negative effects on growth of a variety of plants exposed to 0.5 ppm NO₂ and above (US EPA 1993), but these concentrations are unrealistically high relative to current ambient levels of NO₂.

9.4.2.2.3 Pollutant Mixtures

Studies of the effects of NO₂ in combination with other harmful air pollutants, such as SO₂ and O₃, have shown that the addition of NO₂ at realistic concentrations to the air pollutant mix generally did not alter the growth or yield responses of plants to the other pollutants (USEPA 1993). Notable exceptions to this are the responses of pasture grasses to low-level, long-term exposures to combinations of NO₂ and SO₂ in England. Clones of Kentucky blue grass, timothy, perennial rye, and orchard grass exposed to 0.11 ppm SO₂ and/or 0.11 ppm NO₂ five days/week for 20 weeks showed significant reductions in shoot growth or tiller production in response to the combination of pollutants, and the interactive effects were greater than

the sum of the pollutants acting alone (Ashenden and Williams 1980). The adverse effects of the interacting pollutants were attributed at least in part to the low light and cool temperatures during the winter growing season, which reduced photosynthetic rates and may also have reduced rates of nitrite detoxification in the grasses. Continuous exposure of tomato (cv. 'Fireball') plants in controlled environment chambers to 0.11 ppm NO₂ in combination with 0.11 ppm SO₂ reduced leaf area and fresh weight of stems after 14 days and root fresh weight was reduced 65 per cent after 28 days of exposure, relative to controls (Marie and Ormrod 1984). In a similar experiment, growth of potato plants (cvs. 'Kennebec' and 'Russet Burbank') exposed to 0.11 ppm NO₂ and SO₂ for 7 to 14 days was reduced 60 per cent relative to control plants (Pettite and Ormrod 1988). Root fresh weight was reduced to a greater extent than stem fresh weight by the mixture of pollutants. In a zonal exposure system, field-grown soybeans experienced 10 episodes of combinations of NO₂ from 0.06 to 0.40 ppm and SO₂ from 0.13 to 0.42 ppm during the growing season in Illinois (Irving et al. 1982). NO₂ by itself had no effect on seed yield but the combination of pollutants reduced yields from 19 to 25 per cent. Studies that used combinations of NO₂ and O₃, or the three-way combination of NO₂, SO₂, and O₃ had results that generally did not differ significantly from those of O₃ acting alone (US EPA 1993). The results of these pollutant interaction studies suggest that under certain circumstances, such as low light intensity and low temperature, in which plants experience slow growth rates, long-term exposure to low levels of NO₂ in the range of 0.1 ppm, either alone or in combination with SO₂, can reduce plant growth and yields of some agricultural crops.

9.4.2.2.4 Effects of NO₂ on Woody Plants

Relatively little is known of the effects of NO₂ on tree growth; furthermore, most studies that have been conducted used very young tree seedlings. Rooted cuttings of white birch (*Betula alba* L) exposed to 0.04 ppm NO₂ for nine weeks showed increased growth relative to controls, but cuttings exposed to 0.05 ppm for four weeks showed no significant responses (Freer-Smith 1985). One-year-old cuttings of black poplar, downy birch (*Betula pubescens* Ehrh.), apple (*Malus pumila* Mill.), little-leaved linden (*Tilia cordata* Mill.), white alder (*Alnus incana* (L.) Moench.), and white birch exposed to concentrations of 0.1 ppm NO₂, 104 hours/week for 60 weeks showed either no growth response, or increased shoot height and mass, relative to control plants (Freer-Smith 1984). Norway spruce (*Picea abies* L.) seedlings exposed to 0.05, 0.10, or 0.20 ppm NO₂ in laboratory exposure chambers for 19 days showed small structural changes in epicuticular waxes but no effects on the feeding of spruce shoot aphids (*Cinara pilicornis* Hartig) (Viskari et al. 2000a,b). Effects of combinations of NO₂, SO₂, and O₃ on tree seedling growth have been reported in only a few studies. The addition of NO₂ to mixtures of SO₂ and O₃ reduced growth of sycamore (*Platanus occidentalis* L.) but slightly increased growth of loblolly pine seedlings (Kress and Skelly 1982). Yellow poplar (*Liriodendron tulipifera* L.) seedlings exposed to combinations of O₃ at 0.07 ppm, SO₂ at 0.06 ppm, and NO₂ at 0.01 ppm, six hours/day for 35 days had variable responses to the combinations (Mahoney et al. 1984). Addition of NO₂ to the pollutant mix did not reduce growth more than SO₂ + O₃, but combinations of NO₂ and SO₂ had greater adverse effects on growth than SO₂ acting alone. No reports are available on the effects of NO₂ on native California forest trees.

9.4.2.2.5 Effects of NO₂ on California Plants

Relatively few studies have been conducted on the effects of NO₂ on crops or native plants specific to California. Exposure to 0.11 ppm NO₂ five hours/day, five days/week, for up to 32 weeks had no effect on growth of a number of trees and shrubs native to the Mojave Desert of California, and NO₂ did not significantly alter plant responses to SO₂ (Thompson et al. 1980). A study of the effects of NO₂ on navel orange (cv. 'Washington') growing in large greenhouse-type field chambers found no effects on fruit yield after eight months of exposure at concentrations of NO₂ one or two times ambient in the Los Angeles Basin (Thompson et al. 1971). An earlier study had shown significant reductions in yield of navel oranges at NO₂ concentrations of 0.5 and 1 ppm for 290 days, and lower production at concentrations of 0.25 to 0.06 ppm NO₂ for 290 days (Thompson et al. 1970). Although these citrus studies had the advantage of using mature, fruit-bearing, field-grown trees, the type of enclosed field chamber used for the exposures may have increased the susceptibility of the orange trees to air pollutants. In addition, the concentrations of NO₂ used in these studies were generally higher than present ambient levels of NO₂.

Studies of crop plant responses to NO₂ using species commonly grown in California, such as potato and tomato, have been conducted on potted plants grown in controlled environment chambers (Pettite and

Ormrod 1988; Sinn and Pell 1984). The relevance of these results to field-grown plants in California is difficult to assess. No field exposures of crop plants to NO₂ have to date been conducted under growing conditions prevalent in California.

9.5 Ecological Effects of Nitrogen Deposition

9.5.1 The Nitrogen Cycle

The complex suite of biological and chemical transformations of atmospheric nitrogen to living and non-living components of global ecosystems is known as the nitrogen cycle (Figure 9.1). Prior to modern industrial and agricultural processes, free-living and symbiotic microorganisms added between 90 to 140 teragrams (Tg)¹ annually to pools of fixed N compounds (Vitousek et al. 1997). Lightning fixed an additional 5 to 10 Tg annually, falling to the ground as nitrate dissolved in rain, so the upper limit of total N fixed by natural processes is estimated at approximately 140 Tg per year. Human activities have effectively doubled the amount of fixed N added to global ecosystems. Application of chemical fertilizers currently adds about 80 Tg per year to soils, and this amount is increasing rapidly as developing countries increase use of chemical fertilizers for crop production. The increased use of nitrogen-fixing crops, such as soybean, dry bean (*Phaseolus* spp.), alfalfa (*Medicago sativa* L), and other legumes and the increased intensive cultivation of paddy rice have added an additional 40 Tg of fixed N to the pool. The burning of fossil fuels and other high-temperature combustion processes add an additional 20 Tg of fixed N to the atmosphere, mostly in the form of gaseous oxides of nitrogen. Finally, large-scale transformations of natural landscapes for human use; e.g., burning of forests and grasslands, draining of wetlands, clearing of land for crops and animal husbandry, and climate change, which could accelerate decomposition of frozen peatlands, can add an additional 10 to 20 Tg of fixed N to the nitrogen cycle. All together, human activities add to the nitrogen cycle each year an amount of fixed N equal to that of natural processes.

A significant fraction of this anthropogenic fixed N is returned to the atmosphere in the form of emissions of NO_x and NH₃. Approximately 80 per cent of all NO_x emissions and 70 per cent of NH₃ emissions are derived from human sources (Vitousek et al. 1997). Emissions from the burning of fossil fuels are estimated at 20 Tg per year; burning of forests and grasslands, 10 Tg per year; and volatilization of NO from soils, 5 to 20 Tg per year. Major sources of NH₃ emissions include volatilization from fertilizer applications (10 Tg per year); animal wastes (32 Tg per year); and forest burning (5 Tg per year). These emissions are re-deposited to terrestrial and aquatic ecosystems in the form of wet and dry gaseous and particulate nitrogen deposition.

¹ 1 Tg = 1 x 10⁶ metric tons

9.5.2 Sources of N Deposition to California Ecosystems

In California, the relative proportion of oxidized to reduced N contributing to the deposition of fixed N compounds is determined primarily by the proximity of urban/industrial versus agricultural sources (Bytnerowicz and Fenn 1996). At Tanbark Flat, on the western slope of the San Gabriel Mountains adjacent to Los Angeles, most of the atmospheric N was in the form of NO₂ and other oxidized N species derived from auto exhaust and other urban sources (Figure 9.4). At this site, the relative proportions of various N compounds varied from day to night, reflecting the influence of photochemical processes in the production of oxidized N pollutants (Grosjean and Bytnerowicz (1993). However, at Whitaker Forest, a site on the western slopes of the Sierra Nevada east of Fresno, NH₃ was the major component of total atmospheric N, and the proportions of the various N compounds showed relatively little difference from day to night (Bytnerowicz and Riechers 1995) (Figure 6). Emissions of NH₃ from agricultural and feed lot operations dominated the total N pollutant budget at this site in the Sierra Nevada. It is likely that this site in the Sierra Nevada is representative of the apportionment of N deposition at most forest and rural locations in the state (Bytnerowicz and Fenn 1996).

9.5.3 Rates of Nitrogen Deposition to California Ecosystems

Recent estimates of total N deposition to forested ecosystems and landscapes in California (Fenn et al. 2003a) show an enormous range of values, from 1.4 kg/ha/yr in Lassen National Forest to almost 100 kg/ha/yr at Camp Paivika, on the western slope of the San Bernardino Mountains (Figure 9.5). Northern

and central Sierra Nevada sites generally averaged less than 10 kg N/ha/yr, while locations in the southern Sierra Nevada and most sites in the San Gabriel and San Bernardino Mountains average less than 20 kg N/ha/yr. Lower elevation chaparral and mixed conifer forest sites on exposed western slopes of the Transverse Range receive from 20 to 45 kg N/ha/yr, the highest rate of total N deposition of any location in North America (Fenn et al. 2003a). Alpine and sub-alpine landscapes in the Sierra Nevada receive an average of 3 kg N/ha/yr (Sickman et al. 2001). Deposition of N in grasslands and coastal sage scrub in southern California ranges from about 3 kg/ha/yr at Lake Skinner to over 30 kg N/ha/yr in the Box Spring Mountains near Riverside (Padgett et al. 1999). No information is currently available for rates of N deposition in desert ecosystems or other locations north or east of the Sierra Nevada. Trends of N emissions and deposition vary among regions in California. In the South Coast Air Basin, N emissions have declined significantly in recent decades, through increased controls on automobile and other mobile sources, and because of changing land usages, as agricultural and dairy operations move out of the area (CARB 2004). Although historical data are absent, this decline in N emissions suggests that N deposition may have also declined in the area, as emissions and deposition are closely related (Fenn et al. 2003a). However, N deposition may be increasing in the Sierra Nevada as urbanization, intensive agriculture, dairy, and feed lot operations expand in the Central Valley. Wet deposition of nitrate and ammonium measured in Sequoia National Park, increased from 1981 to 2001 (Fenn et al. 2003a). This trend of increased N deposition in the Sierra Nevada is likely to continue as population increases and land use patterns extend development farther into rural and wildland areas in the state.

Figure 9.4. Relative contributions of oxidized and reduced nitrogen compounds to daytime and nighttime N deposition in Tanbark Flat, San Gabriel Mountains and Whitaker Forest, Sierra Nevada, California. (From Bytnerowicz and Fenn 1996)

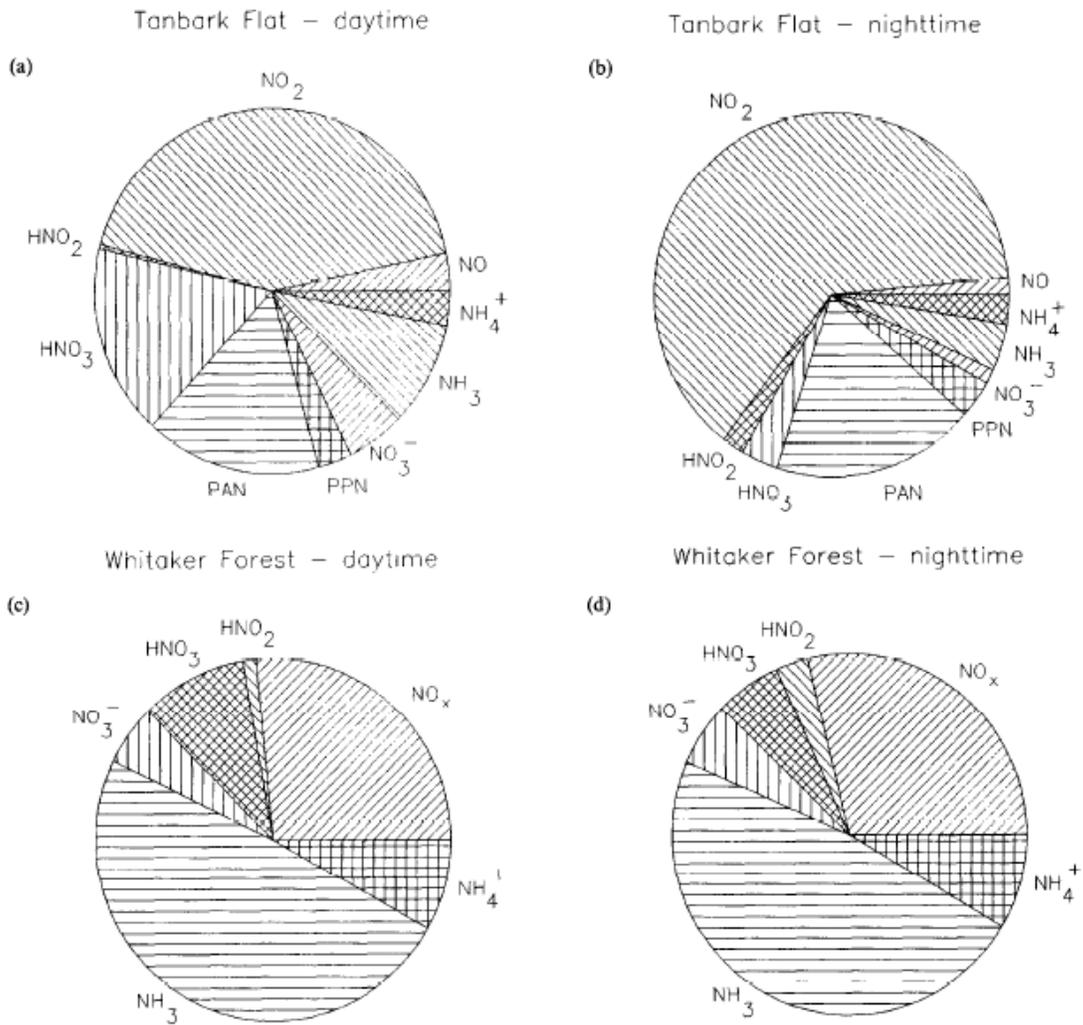
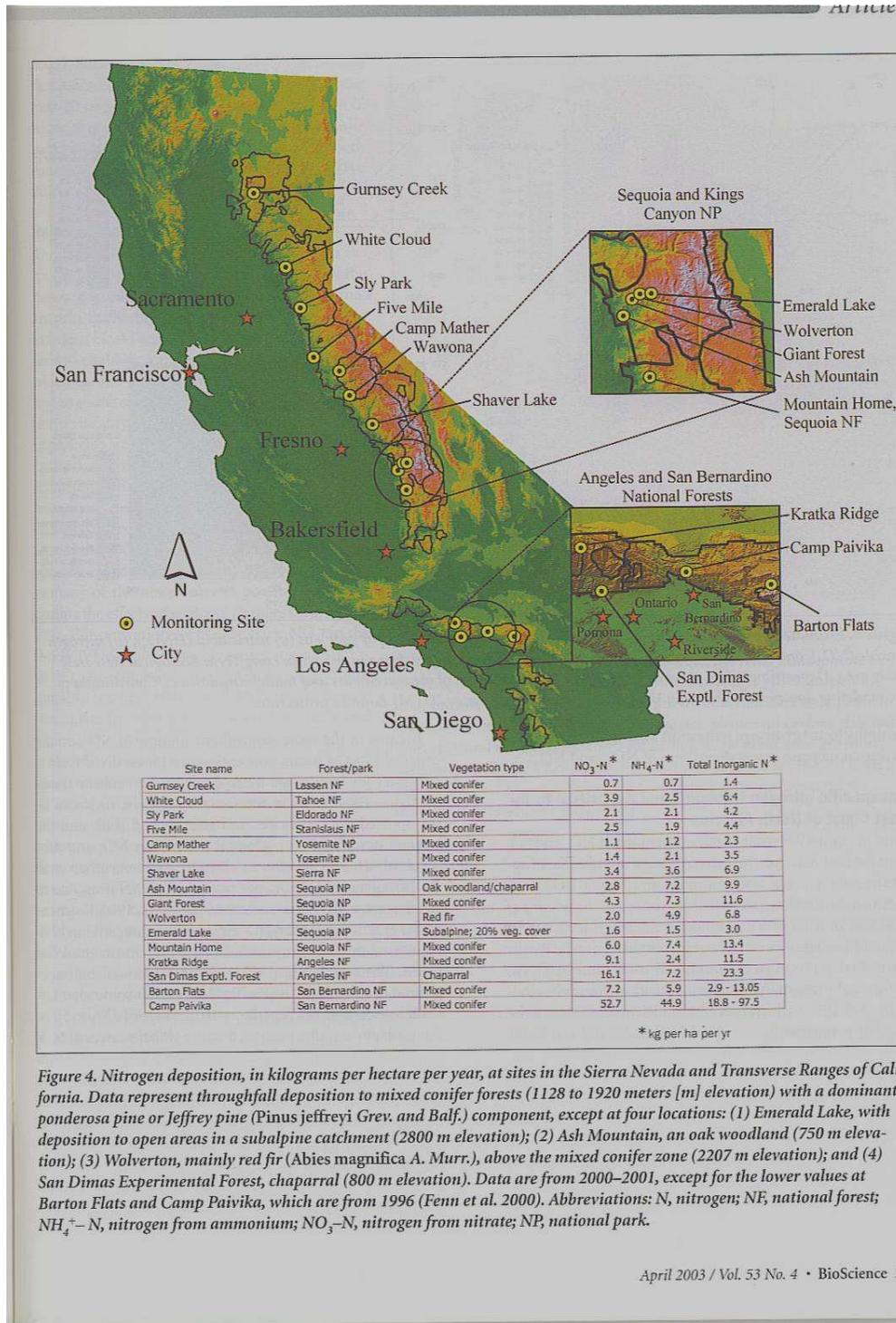


Figure 9.5. Nitrogen deposition, in kg/ha/y, at forested sites in the Sierra Nevada and Transverse Ranges in California. (From Fenn et al. 2003b)



9.5.4 Ecological Effects of Nitrogen Deposition in California.

The importance of atmospheric deposition of fixed nitrogen compounds in altering the structure and functioning of plant and aquatic communities on scales from local to global has been the subject of numerous recent reviews (Bytnerowicz and Fenn 1996; Vitousek et al. 1997; Fenn et al. 1998; Lee and Caporn 1998; Takemoto et al. 2001; Fenn et al. 2003a; Fenn et al. 2003b). A complete review of this literature is beyond the scope of this report, but a brief summary of the ecological effects of excess N deposition on some California ecosystems is given in Table 9.3.

Table 9.3 Ecological effects of nitrogen deposition on ecosystems in California (adapted from Fenn et al. 2003b).

I. Terrestrial ecosystems	References
* Decreased mycorrhizal community diversity in soils	Egerton-Warburton et al. 2001
* Decreased diversity of lichen communities	Nash and Sigal 1999
* Increased invasiveness of alien plant species	Weiss 1999
* Altered fire cycles; shrub to grass conversion	Vitousek et al. 1997
* Altered tree responses to ozone	Gulke and Balduman 1999
* Reduced root growth, increased needle turnover rates	Gulke et al 1998
* Reduced visibility in national parks	Fenn et al. 2003a
II. Aquatic ecosystems	
* Elevated nitrate in stream runoff	Fenn et al. 2003b
* Altered diatom community structure in alpine lakes	Fenn et al. 2003b
* Reduced water clarity and decreased water quality in oligotrophic lakes	Jassby et al. 1994

The highest rates of N deposition in California occur in the foothills and west-facing slopes of the mountains surrounding the Los Angeles Basin, and the clearest examples of the adverse effects of excess N deposition have been documented from these areas. Instances of nitrogen saturation, broadly defined as pools of available N in excess of demand from plant and microbial growth, leading to sustained losses of N from the ecosystem (Aber et al. 1989), have been observed in chaparral and mixed conifer forests in the San Gabriel and San Bernardino Mountains (Fenn et al. 1998). Evidence for N saturation includes extremely high concentrations of nitrate in streams and runoff from west-facing slopes following winter and spring rain events, extremely high concentrations of nitrate in soil solutions from forested sites, and high fluxes of NO from forest soils, equivalent to flux rates from fertilized croplands (Fenn et al. 1998; Fenn and Poth 1999). Higher concentrations of foliar N from nitrate have also been recorded from pine needles collected at Camp Paivika, at the western edge of the San Bernardino Mountains. These concentrations were higher than in pines at Camp Osceola farther east along the N deposition gradient, indicating that trees growing under conditions of heightened N deposition can assimilate the excess N in their tissues (Bytnerowicz and Fenn 1996).

As a consequence of exposure to both elevated N and high ambient O₃, ponderosa pines growing at the western edge of the San Bernardino Mountains exhibit accelerated senescence and premature abscission of older needles, but at the same time greater growth of new needles than trees growing farther east, at the lower end of the N and O₃ deposition gradient (Grulke and Balduman 1999). The deleterious effects of O₃ were compensated to some degree by N fertilization, allowing the trees to maintain a relatively rapid rate of growth, despite reductions in photosynthetic tissues caused by premature needle abscission and foliar O₃ injury (Takemoto et al. 2001). However, pines growing under conditions of high N and O₃ exhibited greatly reduced fine root biomass and belowground carbohydrate storage (Grulke et al. 1998), suggesting that these trees may be more vulnerable to drought stress than other with more balanced patterns of carbon allocation. The rapid turnover of pine foliage at these sites in the San Bernardino Mountains has led to greatly increased rates of litter accumulation on the forest floor, with consequence for tree seed germination, litter decomposition (Fenn and Dunn 1989), and reduced plant species diversity in the understory (Temple 2004). Preliminary data from sites in the Sierra Nevada suggest that Mountain Home may be the most N polluted location along the mountain range, with some evidence of decreased C:N ratios and increased rates of N mineralization in forest soils from this area (Fenn et al. 2003). The alterations in needle turnover and patterns of carbon allocation noted in pines from N saturated areas of the San Bernardino Mountains have not yet been observed in the Sierra Nevada.

Conversion of shrublands to grasslands is now occurring at a rapid pace in southern California (Minnich and Dezzani 1998). Deposition of N has been implicated in this change, although a wide variety of causes contribute to the conversion process (D'Antonio and Vitousek 1992). Changes in diversity and numbers of arbuscular mycorrhizal fungi have also been recorded along N deposition gradients in southern California Coastal sage scrub vegetation (Egerton-Warburton and Allen 2000). Large-spored species decreased in numbers as N deposition increased, while small-spored species, particularly *Glomus*, increased in number. Similar results were obtained from a chrono-sequence of archived soils that were collected from 1937 to the present at a site in the San Dimas Experimental Forest (Egerton-Warburton et al. 2001). Large-spored mycorrhizal species decreased in number as soil N concentrations increased from past to present soil collections. The small-spored *Glomus* mycorrhizae did not promote the growth of native shrub species, but did successfully inoculate the roots of the exotic annual grass *Bromus madritensis* (Sigenza 2000). Additional evidence for the role of N deposition in conversion of native shrub ecosystems to grasslands has come from studies along a gradient of N deposition (Padgett et al. 1999). Plots in the Box Springs Mountains, where the former dominant coastal sage scrub has been replaced by annual grasses and weedy mustards, receive an average of 30 kg N/ha/yr. Vegetation at Lake Skinner, where N deposition is only about 3 kg/ha/y, is still dominated by healthy stands of coastal sage scrub. Fertilization studies of native shrubs with high amounts of N indicate that some natives grow rapidly, then die in response to high N, but weedy annual grasses increase in productivity, especially during wet years (Allen et al. 1998). The increased growth of fire-prone annual grasses also contributes to a more rapid fire cycle, which leads to further conversion of shrubland to grasslands, with consequent losses in native plant and animal species diversity (D'Antonio and Vitousek 1992).

Other significant effects of N deposition in southern California include reductions in lichen diversity and numbers in the San Bernardino Mountains (Nash and Sigal 1999). The relative abundance of crustose nitrophilous lichen species has increased while large foliose lichens, particularly the gray-green species, have become locally extinct. This pattern of reduced lichen diversity has been recorded throughout southern and central California, and had been attributed to increased rates of N deposition, alone or in combination with O₃ and other air pollutants (Nash and Sigal 1999). More research is needed to establish the causal link between N deposition and changes in lichen distribution.

9.5.5 Critical Loads for Nitrogen Deposition

The concept of a critical load or critical level of a pollutant is based on the assumption that there exists a concentration or rate of deposition of a pollutant or interacting pollutants below which significant adverse effects do not occur in sensitive ecosystems (Rosenbaum et al. 1994). The concept also incorporates the idea that standards may be set at higher levels because of overriding social or economic factors, in which case the higher level of the pollutant is called the target level, and can be used as the basis for regulating emissions, if controlling to the critical load cannot be implemented. It is difficult to apply this concept to N deposition in California, because except for the intensively studied sites in southern California, relatively

little is known of the effects of N deposition on terrestrial and aquatic ecosystems in the state. In addition, California possesses an enormous diversity of vegetation and soil types and large-scale gradients in the types and amounts of N deposition. Based on results from southern California, it is suggested that at total N deposition rates of ca. 20 kg/ha/yr or greater, some evidence in changes in soil chemistry and spring runoff may be detected. Above 30 kg N/ha/yr, biological effects on soil microorganisms and sensitive native plants may be observed, and prolonged exposure to > 40 kg N/ha/yr may significantly affect plant community structure and function.

These suggested critical loads for California are generally higher those proposed to protect ecosystems from adverse effects of N deposition in Europe, because the humid climate and acidic soils of northern Europe may render some ecosystems more susceptible to N deposition. For example, loads of only 5 kg/ha/yr have been proposed to protect Northern European acidic peatlands, arctic and alpine heathlands, and moss/lichen communities from eutrophication (Sanders et al. 1995). More research is needed in California, particularly in sensitive high elevation ecosystems in the Sierra Nevada and in desert communities to determine if current levels of N deposition are having adverse effects on biological diversity or biogeochemical processes in these areas.

9.5.6 Oxides of Nitrogen and Global Climate Change

Evidence that human activities have increased the concentrations of certain greenhouse (heat-trapping) gases in the atmosphere has been accumulating for many years. The general consensus among climatologists and other researchers in the field is that these activities will raise the overall temperature of the earth by several degrees in the near to mid future (IPCC 2001). Naturally-occurring greenhouse gases include water vapor, carbon dioxide (CO₂), methane (CH₄), nitrous oxide (N₂O), and ozone (O₃). Water vapor is the most abundant greenhouse gas and it exerts the most influence on the atmosphere. However, the overall concentration of H₂O vapor in the atmosphere has not changed in recent years, and it is generally not affected by human activities. In contrast, the concentrations of the other natural greenhouse gases CO₂, CH₄, N₂O, and O₃ have increased significantly, particularly since the rise of industrial societies in the 18th C. Since about 1750, the tropospheric concentration of CO₂ has risen 31%, CH₄ 150%, and N₂O 16% (IPCC 2001). The global warming potential (GWP) of these gases, relative to the best-known greenhouse gas CO₂, is shown in Table 9.4). Nitrous oxide has over 300 times the GWP of CO₂, and CH₄ over 20 times, but CO₂ still accounts for over 80 per cent of all GWP (USEPA 2004). Since 1990, CO₂ emissions in the U.S. have increased 15.6%, N₂O emissions have increased 5.7%, while CH₄ emissions have declined 6.9% (Table 9.4). The largest single source of N₂O in the U.S. is agriculture, stemming primarily from microbial transformations of N-containing chemical fertilizers and manures used in the production of crops. Because of its high agricultural productivity, California contributes substantially to the total annual emissions of N₂O in the U.S.

Table 9.4 Global warming potential (GWP) relative to CO₂ and U.S. emissions of the major naturally-occurring greenhouse gases (data from USEPA 2004).

Gas	GWP	Emissions (2002)(Tg)*	Percent increase since 1990
CO ₂	1	5782.4	15.6
CH ₄	21#	598.1	-6.9
N ₂ O	310	415.8	5.7

* One Tg = 10⁶ metric tons.

Includes direct effect of CH₄ and indirect effects due to production of water vapor and tropospheric O₃

Numerous other anthropogenic emissions, particularly chlorofluorocarbons (CFCs), hydrochlorofluorocarbons (HCFCs), and halomethanes, are potent greenhouse gases and also have contributed directly to depletion of stratospheric O₃. However, a discussion of these materials is beyond the scope of this report. Several other gases emitted to the atmosphere via human activities, such as NO_x, do not have a direct effect on global warming, but they contribute indirectly through their photochemical transformations in the atmosphere.

The potential effects of increased CO₂, N₂O, and other greenhouse gases in the atmosphere have been subjects of intense research and a complete description of all possible scenarios is beyond the scope of this report. However, two recent publications have discussed possible changes to California's temperature and precipitation in response to elevated CO₂ and the impacts of such changes on the state's native vegetation and agriculture. A report prepared for the California Energy Commission (Wilson et al. 2003) used two climate change models to project changes to California's temperature and precipitation over the next 90 years. One model (Hadley Center Climate Model) predicted an average temperature increase of 3.3 degrees C for the state and a 58% increase in precipitation, mostly in the form of rain. A second model (Parallel Climate Model, PCM) predicted an average temperature increase of 2.4 degrees C and a reduction in precipitation of 21%. Based on these climate change scenarios, models of vegetation responses to the environment predicted that forests will migrate to higher elevations and the extent of alpine and sub-alpine vegetation will drastically decline. Shrublands and grasslands will increase in area, except for coastal sage scrub in southern California, which will be severely impacted as a consequence of increased urbanization and climate change. The frequency and intensity of fires in the State will increase, even if precipitation increases, because of increased fuel loads. Harvestable timber may increase in the near-term, but will significantly decline towards the end of the century. The effects of climate change on crops were difficult to assess, because of the wide variety of crop plants grown in the state. The models predicted a decrease in runoff water available for irrigation, due to a decrease in snowmelt. However, the possible increase in water use efficiency of plants in response to increased atmospheric concentrations of CO₂ might compensate for reductions in irrigation.

Using the same models (Hadley and PCM) running different emission scenarios, Hayhoe et al. (2004) predicted average temperature increases of 2.2 to 4.0 degrees C across the state by the end of the century, with greater warming in the northern and northeastern parts of the state. Precipitation was predicted to decrease, especially in the winter, by 15 to 30%, particularly along the north Pacific coast. The impact of these climate changes on agriculture was calculated for two of the most economically important sectors of the agricultural economy - dairy products and wine grapes. For dairy production, models predicted a reduction of up to 22% in milk production due to excessive temperatures in the Central Valley. Higher temperatures impacted wine grapes, causing their ripening one to two months earlier than present and adversely impacting the quality and perhaps quantity of fruit. Changes in the distribution of native vegetation were similar to those predicted by Wilson et al. (2003), except that the worst-case scenario predicted by Hayhoe et al. (2004) showed a reduction in alpine/sub-alpine forests of 75 to 90% and declines in the Sierra Nevada snowpack of 73 to 90%, both of which were significantly greater than those previously reported. These model predictions for California suggest that by the end of this century climate change will have profound impacts on natural ecosystems, agriculture, and the distribution and use of water throughout the state.

9.6 References

- Aber JD, Nadelhoffer KJ, Steudler P, Melillo JM. 1989. Nitrogen saturation in northern forest ecosystems. *BioScience* 39: 378-386.
- Allen EB, Padgett PE, Bytnerowicz A, Minnich R. 1998. Nitrogen deposition effects on coastal sage vegetation of southern California. In: Proc. International Symposium on Air Pollution and Climate Change Effects on Forest Ecosystems, Bytnerowicz A, Arbaugh MJ, Schilling SL, eds. Feb. 5-9, 1996, Riverside, CA USDA Forest Service PSW-GTR-166. pp. 131-139.
- Amundson RG, MacLean DC. 1982. Influence of oxides of nitrogen on crop growth and yield: an overview. In: *Air Pollution by Nitrogen Oxides*, Schneider T, Grant L, eds. Amsterdam: Elsevier Publ. Co., pp 501-510.
- Ashenden TW, Williams IAD. 1980. Growth reductions in *Lolium multiflorum* Lam. and *Phleum pratense* L. as a result of SO₂ and NO₂ pollution. *Environmental Pollution Ser. A*. 21: 131-139.
- Atkinson R. 2000. Atmospheric chemistry of VOC's and NO_x. *Atmospheric Environment* 35: 2063-2101.
- Bowden RD, Geballe GT, Bowden WB. 1989. Foliar uptake of ¹⁵N from simulated cloud water by red spruce (*Picea rubens*) seedlings. *Canadian J. Forest Research* 19: 382-386.
- Bytnerowicz A, Fenn ME. 1996. Nitrogen deposition in California forests: a review. *Environmental Pollution* 92: 127-146.
- Bytnerowicz A, Riechers G. 1995. Nitrogenous air pollutants in a mixed conifer stand of the western Sierra Nevada, California. *Atmospheric Environment* 29: 1369-1377.
- Bytnerowicz A, Dueck T, Godzik S. 1998a. Nitrogen oxides, nitric acid vapor, and ammonia. In: Flagler RB, ed. *Recognition of Air Pollution Injury to Vegetation, A pictorial atlas*. Air & Waste Management Assoc., Pittsburgh, PA pp. 5-1 to 5-6.
- Bytnerowicz A, Padgett P, Percy K, Krywult M, Riechers G, Hom J. 1998b. Direct effects of nitric acid on forest trees. In: Miller PR, McBride JE eds. *Oxidant Air Pollution Impacts in the Montane Forests of Southern California*. Springer, New York. pp.270-287.
- California Air Resources Board. 1992. Review of the One-Hour Ambient Air Quality Standard for Nitrogen Dioxide. Research Division, ARB, Sacramento, CA
- California Air Resources Board. 2004. Web page. The California Almanac of Emissions and Air Quality. 2004 edition.
- Caporn SJM. 1989. The effects of oxides of nitrogen and carbon dioxide enrichment on photosynthesis and growth of lettuce (*Lactuca sativa* L.). *New Phytologist* 111: 473-481.
- Carlson RW. 1983. Interaction between SO₂ and NO₂ and their effects on photosynthetic properties of soybean *Glycine max*. *Environmental Pollution, Ser. A* 32:11-38.
- D'Antonio CM, Vitousek PM. 1992. Biological invasions by exotic grasses, the grass/fire cycle, and global change. *Annual Review Ecology and Systematics* 23: 63-87.
- Darrall NM. 1989. The effect of air pollutants on physiological processes in plants. *Plant Cell Environment* 12:1-30.
- Davison AW, Cape JN. 2003. Atmospheric nitrogen compounds – issues related to agricultural systems. *Environment International* 29: 181-187.
- Donagi AE, Goren AI. 1979. Use of indicator plants to evaluate atmospheric levels of nitrogen dioxide in the vicinity of a chemical plant. *Environmental Science & Technology* 13: 986-989.
- Egerton-Warburton LM, Allen EB. 2000. Shifts in arbuscular mycorrhizal communities along an anthropogenic nitrogen deposition gradient. *Ecological Applications* 10: 484-496.

- Egerton-Warburton LM, Graham RC, Allen EB, Allen MF. 2001. Reconstruction of the historical changes in mycorrhizal fungal communities under anthropogenic nitrogen deposition. *Proc. Royal Soc. Biological Sciences* 268: 2479-2484.
- Evans LS, Canada DC, Santucci KA. 1986. Foliar uptake of ^{15}N from rain. *Environmental and Experimental Botany* 26: 143-146.
- Fenn ME, Dunn PH. 1989. Litter decomposition across an air pollution gradient in the San Bernardino Mountains. *Soil Science Soc. America J.* 53: 1560-1567.
- Fenn ME, Poth MA. 1999. Nitrogen deposition and cycling in Mediterranean forests: the new paradigm of nitrogen excess. In: Miller PR, McBride JE, eds. *Oxidant Air Pollution Impacts in the Montane Forests of Southern California*. Springer, New York. pp. 288-314.
- Fenn ME, Baron JS, Allen EB, et al. 2003a. Ecological effects of nitrogen deposition in the western United States. *BioScience* 53: 404-420.
- Fenn ME, Haeuber R, Tonnesen GS, et al. 2003b. Nitrogen emissions, deposition, and monitoring in the western United States. *BioScience* 53: 391-403.
- Fenn ME, Poth MA, Aber JD, et al. 1998. Nitrogen excess in North American ecosystems: Predisposing factors, ecosystem responses, and management strategies. *Ecological Applications* 8: 706-733.
- Fenn ME, Poth MA, Bytnerowicz A, Sickman JO, Takemoto BK. 2003. Effects of ozone, nitrogen deposition, and other stressors on montane ecosystems in the Sierra Nevada. In: Bytnerowicz A, Arbaugh MJ, Alonso R, eds. *Ozone Air Pollution in the Sierra Nevada: Distribution and Effects on Forests*. Elsevier, Amsterdam. pp. 111-155.
- Finlayson-Pitts BJ, Pitts JN Jr. 1986. *Atmospheric Chemistry: Fundamentals and Experimental Techniques*. J. Wiley Sons, New York.
- Freer-Smith PH. 1984. The responses of six broadleaved trees during long-term exposure to SO_2 and NO_2 . *New Phytologist* 97: 49-61.
- Freer-Smith PH. 1985. The influence of SO_2 and NO_2 on the growth, development and gas exchange of *Betula pendula* Roth. *New Phytologist* 99: 417-430.
- Fujioka FM, Roads JO, Chen S-C. 1998. Climatology. In: Miller PR, McBride JE eds. *Oxidant Air Pollution Impacts in the Montane Forests of Southern California*. Springer, New York. pp.28-43.
- Grosjean G, Bytnerowicz A. 1993. Nitrogenous air pollutants at a southern California mountain forest smog receptor site. *Atmospheric Environment* 27: 483-492.
- Gulke NE, Balduman L. 1999. Deciduous conifers: High N deposition and O_3 exposure effects on growth and biomass allocation in ponderosa pine. *Water, Air, Soil Pollution* 116: 235-248.
- Gulke NE, Anderson CP, Fenn ME, Miller PR. 1998. Ozone and nitrogen deposition reduces root biomass of ponderosa pine in the San Bernardino Mountains, California. *Environmental Pollution* 103: 63-73.
- Hayhoe K, Cayan D, Field CB, Frumhoff PC, Maurer EP, Miller NL, Moser SC, Schneider SH, Cahill KN, Cleland EE, Dale L, Drapek, R, Hanemann RM, Kalkstein LS, Lenihan J, Lunch CK, Neilson, RP, Sheridan SC, Verville JH. 2004. Emissions pathways, climate change, and impacts on California. *Proc. National Academy Sciences* 101: 12422-12427.
- Heagle AS, Kress LW, Temple PJ, Kohut RJ, Miller JE, Heggstad HE. 1988. Factors influencing ozone dose-yield response relationships in open-top field chamber studies. In:
- Heck WW, Taylor OC, Tingey, eds. 1988. *Assessment of Crop Loss from Air Pollutants*. Elsevier Publ. Co, London. pp. 141-179.
- IPCC 2001. *Climate Change 2001: The Science of Climate Change*. Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge U.K.

Irving PM, Miller JE, Xerikos PB. 1982. The effect of NO₂ and SO₂ alone and in combination on the productivity of field-grown soybeans. In: Air Pollution by Nitrogen Oxides. Schneider T, Grant L, eds. Elsevier Scientific Publ. Co, New York pp. 521-531.

Jassby AD, Reuter JE, Azler RP, Goldman CR, Hackley SH. 1994. Atmospheric deposition of nitrogen and phosphorus in the annual nutrient load of Lake Tahoe (California-Nevada) Water Resources Research 30: 2207-2216.

Juniper BE, Robins RJ, Joel DM. 1989. The Carnivorous Plants. London: Academic Press.

Kress LW, Skelly JM. 1982. Response of several eastern forest tree species to chronic doses of ozone and nitrogen dioxide. Plant Disease 66: 1149-1152.

Lane PI, Bell JNB. 1984. The effects of simulated urban air pollution on grass yield: Part 2-Performance of *Lolium perenne*, *Phleum pratense* and *Dactylis glomerata* fumigated with SO₂, NO₂ and /or NO. Environmental Pollution (Series A) 35: 97-124.

Lea PJ, Wolfenden J, Wellburn AR. 1994. Influence of air pollutants upon nitrogen metabolism. In: Alscher R, Wellburn AR, eds. Plant Responses to the Gaseous Environment, Chapman & Hall, London. Pp 279-299.

Lee JA, Caporn SJM. 1998. Ecological effects of atmospheric reactive nitrogen deposition on semi-natural terrestrial ecosystems. New Phytologist 139: 127-134.

Lefohn AS. 1992. The characterization of ambient ozone exposures. In: Lefohn AS, ed. Surface Level Ozone Exposures and their Effects on Vegetation. Lewis Publ. Co., Chelsea, MI. pp 31-92.

Lefohn AS, Tingey DT. 1984. The co-occurrence of potentially phytotoxic concentrations of various gaseous air pollutants. Atmospheric Environment 18: 2521-2526.

Lenzian KJ, Kerstiens G. 1988. Interactions between plant cuticles and gaseous air pollutants. Aspects of Applied Biology 17: 97-104.

Lefohn AS, Davis CE, Jones CK, Tingey DT, Hogsett WE. 1987. Co-occurrence patterns of gaseous air pollutant pairs at different minimum concentrations in the United States. Atmospheric Environment 21: 2435-2444.

Mahoney MJ, Skelly JM, Chevone BI, Moore LD. 1984. Response of yellow poplar (*Liriodendron tulipifera* L.) seedling shoot growth to low concentrations of O₃, SO₂, and NO₂. Canadian J. Forest Research 14: 150-153.

Marie BA, Ormrod DP. 1984. Tomato plant growth with continuous exposure to sulphur dioxide and nitrogen dioxide. Environmental Pollution Ser. A 33: 257-265.

Martin RV, Fiore AM, Van Donkelaar A. 2004. Space-based diagnosis of surface ozone sensitivity to anthropogenic emissions. Geophysical Research Letters 31: L06120 (www.agu.org/pubs/crossref/2004GL019416.shtml).

McAinsh MR, Evan NH, Montgomery LT, North KA. 2002. Calcium signaling in stomatal responses to pollutants New Phytologist 153: 441-447.

Miller PR, McBride JE, eds. 1998 Oxidant Air Pollution Impacts in the Montane Forests of Southern California. Springer, New York.

Minnich RA, Dezzani RJ. 1998. Historic decline of coastal sage scrub in the Riverside-Perris Plail, California. Western Birds 29: 366-391.

Murray AJS. 1984. Light affects the deposition of NO₂ to the Flacca mutant of tomato without affecting the rate of transpiration. New Phytologist 98: 447-450.

Nash TH, Sigal LL. 1999. Epiphytic lichens in the San Bernardino mountains in relation to oxidant gradients. In: Miller PR, McBride JE, eds. 1998 Oxidant Air Pollution Impacts in the Montane Forests of Southern California. Springer, New York. pp. 223-234.

- National Academy of Sciences 2004. Air Quality Management in the United States (2004). Board on Environmental Studies and Toxicology National Academies Press, Washington D.C.
- Norby RJ, Weerasuriya Y, Hanson PJ. 1989. Induction of nitrate reductase activity in red spruce needles by NO₂ and HNO₃ vapor. Canadian J. Forest Research 19: 889-896.
- Okano K, Machida T, Totsuka T. 1988. Absorption of atmospheric NO₂ by several herbaceous species: estimation by the ¹⁵N dilution method. New Phytologist 109: 203-210.
- Oleksyn J, Prus-Glowacki W, Giertych M, Reich PB. 1994. Relation between genetic diversity and pollution impact in a 1912 experiment with east European *Pinus sylvestris* provenances. Can. J. Forest Research 24: 2390-2394.
- Padgett PE, Allen EB, Bytnerowicz A, Minnich RA. 1999. Changes in soil inorganic nitrogen as related to atmospheric nitrogenous pollutants in southern California. Atmospheric Environment 33: 769-781.
- Pettite JM, Ormrod DP 1988. Effects of sulphur dioxide and nitrogen dioxide on shoot and root growth of Kennebec and Russet Burbank potato plants. American Potato J. 65: 517-527.
- Pierce T, Geron C, Bender L, Dennis RL, Tonnesen GS, Guenther A. 1998. Influence of isoprene emissions on regional ozone modeling. J. Geophysical Research 103: 25611-25629.
- Raven JA. 1988. Acquisition of nitrogen by the shoots of land plants: its occurrence and implications for acid-base regulation. New Phytol. 109: 1-20.
- Raven JA. 1998 The past, present and future of nitrogenous compounds in the atmosphere, and their interactions with plants. New Phytologist 139: 205-219.
- Rogers HH, Campbell JC, Volk RJ. 1979. Nitrogen-15 dioxide uptake and incorporation by *Phaseolus vulgaris* L. Science 206: 333-335.
- Rosenbaum BJ, Strickland TC, McDowell MK. 1994. Mapping critical levels of ozone, sulfur dioxide and nitrogen dioxide for crops, forests and natural vegetation in the United States. Water, Air, Soil Pollution 74: 307-319
- Runeckles VC. 1992. Uptake of ozone by vegetation. In: Lefohn AS, ed. Surface Level Ozone Exposures and their Effects on Vegetation. Lewis Publ. Co., Chelsea, MI. pp 157-188.
- Sabaratham S, Gupta G, Mulchi C. 1988. Nitrogen dioxide effects on photosynthesis in soybean. J. Environmental Quality 17: 143-146.
- Sanders GE, Skarby L, Ashmore MR. 1995. Establishing critical levels for the effects of air pollution on vegetation. Water Air Soil Pollution 85: 189-200.
- Sanders JS, Reinert RA. 1982 Screening azalea cultivars for sensitivity to nitrogen dioxide, sulfur dioxide, and ozone alone and in mixtures. J. American Society Horticultural Science 107: 87-90.
- Sandhu R, Gupta G. 1989. Effects of nitrogen dioxide on growth and yield of black turtle bean (*Phaseolus vulgaris* L. cv. 'Domino'). Environmental Pollution 59: 337-344.
- Saxe H. 1986a. Stomatal-dependent and stomatal –independent uptake of NO₂. New Phytologist 103: 199-205.
- Saxe H. 1986b. Effects of NO, NO₂, and CO₂ on net photosynthesis, dark respiration, and transpiration of pot plants. New Phytologist 103: 185-197.
- Sickman JO, Leydecker A, Melack JM. 2001. Nitrogen mass balances and abiotic controls of N retention and yield in high-elevation catchments of the Sierra Nevada, California, USA. Water Resources Research 37: 1445-1461.
- Siguenza C. 2000. Nitrogen deposition and soil microorganisms of *Artemisia californica* and exotic grasses in southern California. PhD dissertation, Botany and Plant Sciences Dep't., University of California, Riverside.

- Sinn JP, Pell EJ. 1984. Impact of repeated nitrogen dioxide exposures on composition and yield of potato foliage and tubers. *J. American Society Horticultural Science* 109: 481-484.
- Sinn JP, Pell EJ, Kabel RL. 1984. Uptake rate of nitrogen dioxide by potato plants. *J. Air Pollution Control Association* 34: 668-669.
- Soares A, Ming JY, Pearson J. 1995. Physiological indicators and susceptibility of plants to acidifying atmospheric pollutants: a multivariate approach. *Environmental Pollution* 87: 159-166.
- Srivastava HS, Ormrod DP. 1984. Effects of nitrogen dioxide and nitrate nutrition on growth and nitrate assimilation in bean leaves. *Plant Physiology* 76: 418-423.
- Srivastava HS, Ormrod DP. 1986. Effects of nitrogen dioxide and nitrate nutrition on nodulation, nitrogenase activity, growth, and nitrogen content of bean plants. *Plant Physiology* 81: 737-741.
- Srivastava HS, Joliffe PA, Runeckles VC. 1975. The effects of environmental conditions on the inhibition of leaf gas exchange by NO₂. *Canadian J. Botany* 53: 475-482.
- Srivastava HS, Wolfenden J, Lea PJ, Wellburn AR. 1994. Differential responses of growth and nitrate reductase activity in wild-type and NO₂-tolerant barley mutants to atmospheric NO₂ and nutrient nitrate. *J. Plant Physiology* 143:738-743.
- Stulen I, Perez-Soba M, DeKok LJ, Van der Eerden L. 1998. Impact of gaseous nitrogen on plant functioning. *New Phytologist* 139:61-70. Review. Pathways of gaseous N assimilation.
- Takemoto BK, Bytnerowicz A, Fenn ME. 2001. Current and future effects of ozone and atmospheric nitrogen deposition on California's mixed conifer forests. *Forest Ecology Management* 144: 159-173.
- Taylor OC, McLean DC. 1970. Nitrogen oxides and the peroxyacetyl nitrates. In: Jacobson JS, Hill AC, eds. *Recognition of Air Pollution Injury to Vegetation: a pictorial atlas*. Air Pollution Control Assoc., Pittsburgh, PA. pp. E1-E14.
- Temple, PJ, Harper DS, Pearson RG, Linzon SN. 1979. Toxic effects of ammonia on vegetation in Ontario. *Environmental Pollution* 13; 297-302.
- Temple PJ, Reichers GH, Miller PR, Lennox RW. 1993. Growth responses of ponderosa pine to long-term exposure to ozone, wet and dry acidic deposition, and drought. *Canadian J. Forest research* 23: 59-66.
- Temple PJ, Sun JE-J, Krause GHM. 1998. Peroxyacyl nitrates (PANs) and other minor pollutants. In: Flagler RB, ed. *Recognition of Air Pollution Injury to Vegetation: a pictorial atlas*. Air & Waste Management, Pittsburgh, PA pp. 6-1 to 6-21.
- Thoene B, Schroder P, Papin H, Egger A, Rennenberg H. 1991. Absorption of atmospheric NO₂ by spruce (*Picea abies* (L.) Karst.) trees. I. NO₂ influx and its correlation with nitrate reductase. *New Phytologist* 117: 575-585.
- Thompson CR, Hensel EG, Kats G, Taylor OC. 1970. Effects of continuous exposure of navel oranges to nitrogen dioxide. *Atmospheric Environment* 4: 349-355.
- Thompson CR, Kats G, Hensel EG. 1971. Effect of ambient levels of NO₂ on navel oranges. *Environmental Science Technology* 5: 1017-1019.
- Thompson CR, Kats G, Lennox RW. 1980. Effects of SO₂ and/or NO₂ on native plants of the Mojave Desert and eastern Mojave-Colorado desert. *J. Air Pollution Control Association* 30: 1304-1309.
- Tonnesen G, Wang Z, Omary M, Chien C-J. 2003. Formulation and application of regional air quality modeling for integrated assessments of urban and wildland pollution. In: Bytnerowicz A, Arbaugh MJ, Alonso R, eds. *Ozone Air Pollution in the Sierra Nevada: Distribution and Effects on Forests*. Elsevier, Amsterdam. pp. 299-324.
- USEPA 1993. Air Quality Criteria for Oxides of Nitrogen. EPA/600/8-91/049bF, Research Triangle Park, NC.

- Viskari EL, Holopainen T, Karenlampi L. 2000a Responses of spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions: II Ultrastructural changes and stomatal behaviour. *Environmental Pollution*: 107: 99-107.
- Viskari EL, Surakka J, Pasanen P, Mirme A, Kossi S, Ruuskanen J, Holopainen JK. 2000b Responses of spruce seedlings (*Picea abies*) to exhaust gas under laboratory conditions I Plant insect interactions. *Environmental Pollution* 107: 89-98.
- Vitousek PM, Aber JD, Howarth RW, Likens GE, et al. 1997. Human alteration of the global nitrogen cycle: sources and consequences. *Ecological Applications* 7: 737-750.
- Weiss SB. 1999. Cars, cows, and checkerspot butterflies: Nitrogen deposition and management of nutrient-poor grasslands for a threatened species. *Conservation Biology* 13: 1476-1486.
- Wellburn AR. 1990. Why are atmospheric oxides of nitrogen usually phytotoxic and not alternative fertilizers? *New Phytologist* 115: 395-429.
- Whitmore ME, Mansfield TA. 1983. Effects of long-term exposures to SO₂ and NO₂ on *Poa pratensis* and other grasses. *Environmental Pollution Ser. A* 31: 217-235.
- Wilson T, Williams L, Smith J, Medelson R. 2003. Global Climate Change and California: Potential Implications for Ecosystems, Health, and the Economy. Publ. 500-03-058CF. California Energy Commission, Sacramento, CA.
- Yu S, Li L, Shimazaki K. 1988. Response of spinach and kidney bean plants to nitrogen dioxide. *Environ. Pollut.* 55: 1-13.

10 Effects on Visibility

10.1 Introduction

Nitrogen dioxide contributes to reduction of visibility both directly, by selectively absorbing the shorter blue wavelengths of visible light, and indirectly by contributing to the formation of nitrate aerosols. Gaseous NO_2 turns air a reddish brown color, appearing as either a defined plume from a strong NO_x source or as a component of diffuse haze. Nitrate aerosols predominantly scatter light, creating a white haze. These two pollutants are often found together, and are contributors to the hazy-brown sky conditions observed in the South Coast Air Basin, the San Joaquin Valley, and elsewhere. The following discussion focuses on NO_2 , but the reader should bear in mind that, in actual outdoor atmospheres, these effects do not occur in isolation from those of other pollutants.

10.2 Visibility Reduction Due to NO_2

10.2.1 Human Visual Perception

This section summarizes the conceptual framework for relating the physical properties of a polluted atmosphere to perceived visual effects; more complete discussion of the physical and psycho-physiological phenomena of vision can be found in Tse et al. (2005), U.S.EPA (1979), and elsewhere. This discussion is adapted from these two works except where otherwise noted.

All the components of the atmosphere scatter, absorb, and reradiate photons, each in their own characteristic set of wavelengths; these effects impair visibility when they occur within the limited spectral range of human visual sensitivity, or the “photopic” response as shown in Figure 10.1. Figures 10.1 and 10.2 illustrate the relative spectral intensities of various day-light conditions and the relative response of a “normal” eye to equal intensities of radiation over the visible spectrum. Perceptibility of an object or scene is not strictly proportional to the total illumination. Rather it is a complex function of the nature of the scene (inherent color and contrast), the type and intensity of illumination

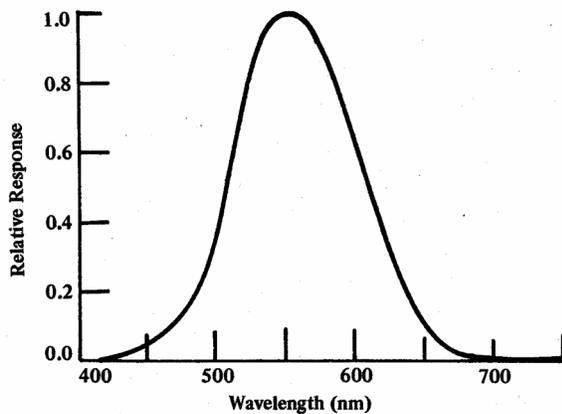


Figure 10.1 Relative spectral response of the American Standard Observer, normalized to 550 nm. (Todd and Zakia, 1969)..., the sensitivity of the eye, and the subjective interpretation of the scene by the brain, which corrects for much of the variation.

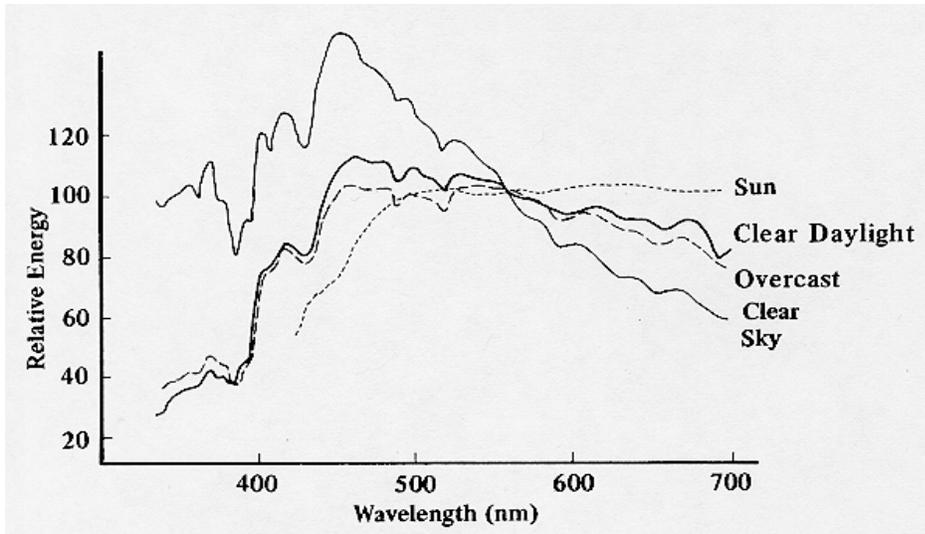


Figure 10.2 Relative Spectral Energy Distributions of Various Light Sources, normalized to green light (550 nm.) (Todd and Zakia, 1969)

10.2.2 Extinction of Light in the Atmosphere

With pollutants present, light traveling from the scene to the eye is reduced in intensity by scattering and absorption (see Figure 10.3), and extraneous light is scattered toward the eye (these processes also can have significant variation among different wavelengths) with the net results being reduced contrast, shifts in color of the scene, and the sensation of “haze” between the scene and the eye. If both the illumination and the composition of the air are homogeneous along the sight path, light intensity will decay exponentially with the product of concentration and distance from the source. The distance – concentration product of a pollutant along a sight path is termed the “optical thickness” for that pollutant.

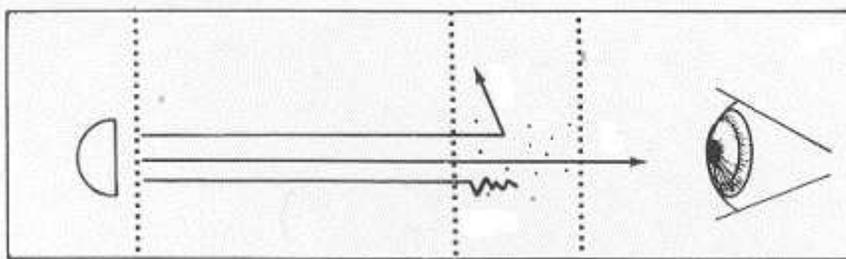


Figure 10.3 Simple case of extinction of beam of light. Some rays reach the observer, others are scattered or absorbed

The absorption and scattering of a beam of light in an air mass are termed “extinction.” For a given wavelength of light, the extinction coefficient, B_{ext} , is given by the sum of the respective partial extinction coefficients for absorption, B_{ab} and scattering, B_{scat} . All of these coefficients are proportional to the

concentration of the particular pollutant. Extinction measurements are in inverse distance units (e.g. km^{-1}) and usually given for a wavelength of 550 nanometers (nm) to coincide with the peak of the photopic response. The intensity of a beam, with source intensity I_0 , is reduced along a path length r to I_r , according to the formula $I_r/I_0 = e^{-r(B_{\text{ext}})}$ (see Figure 10.4).

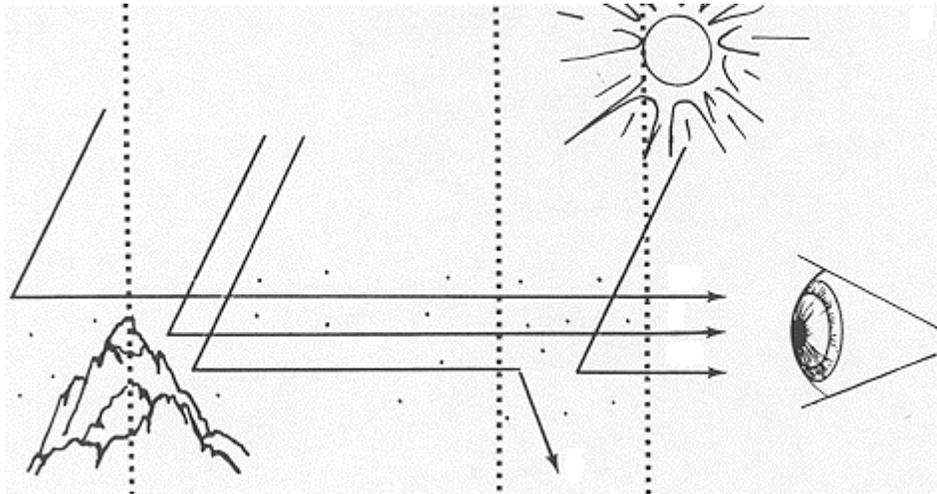


Figure 10.4 View of a distant scene in daylight. The target and the air are both illuminated by the sun. Light reflected off the target is reduced by scattering and absorption along the path to the observer. “Air Light,” perceived as haze, is sunlight scattered toward the observer; the horizon appears bright due to scattering of sunlight by a greater optical depth of air than the sight path to the target. (U.S.EPA 1979).

The extinction coefficient is an inherent physical property of an air mass, but it can be related to human perception by converting it to the equivalent reduction in contrast of a scene per unit distance, then calculating the distance at which the contrast would just equal the threshold of visual perception (generally accepted as 2%). The distance-contrast relationship is shown graphically in Figure 11-5.

The relationship between extinction and visual range, V_r , can be described as follows. V_r refers to the distance [km] at which a large black object disappears from view against the horizon sky. This relationship is called the Koschmeider formula:

$$V_r(\text{km}) = \frac{3.92}{B_{\text{ex}}}$$

This formula permits the use of a contrast measurement on a relatively close target (a few kilometers) to calculate the limit of visual range. This theoretical relationship is only an approximation of actual atmospheric conditions, since the assumptions of a black target, homogeneous air mass, and consistent illumination for all observations are virtually never met in actual measurements. This approximation is the basis for the use of teleradiometers to measure both V_r and B_{ext} , and to convert nephelometer measurements of B_{scat} to an upper-bound estimate of V_r .

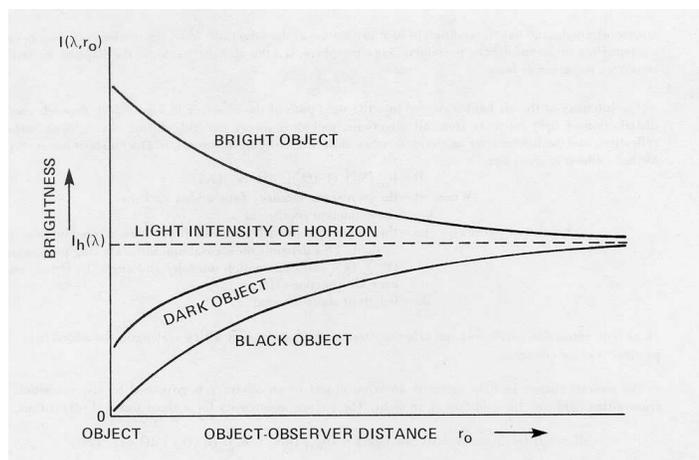


Figure 10.5 The effect of distance on the apparent brightness of an object. The optical thickness of the sight path approaches that of the horizon (virtually an infinite depth) as distance increases, resulting in decreasing the observed proportion of the target brightness contributed by the target, and increasing the proportion contributed by the air along the sight path (U.S.EPA 1979).

10.2.3 Optical properties of NO₂

NO₂ extinction occurs in two particular ways that fall within the general discussion above: by strong absorption of light at wavelengths less than about 700 nm termed B_{ag} ("ag" = absorption by gases) and, to a much smaller extent, by Rayleigh scattering, termed B_{sg} (magneto-electric interaction of gas molecules with passing photon, resulting in their redirection of "scattering"). The spectral distribution of these phenomena is shown in Fig. 11-6.

The absorption curve in Figure 10.6 would apply to a moderately polluted urban site; 0.1 ppm was equaled or exceeded at least once in 1990 at 53 stations in 20 California counties. Higher concentrations are geographically restricted; in 1990 the state 1-hr standard (.25 ppm) was only exceeded in the South Coast Air Basin (ARB, 1992).

Rayleigh scatter decreases with the fourth power of the wavelength, therefore the visual effect is to predominantly scatter short wavelength blue light (which causes the sky to appear blue). The blue-dominated absorption function of NO₂ has the opposite effect, and at sufficiently high concentrations (about 0.03 to 0.05 ppm, depending on the wavelength of the measurement) it equals the component of extinction due to molecular scattering. The radiance of the horizon for a given set of conditions is proportional to the ratio of B_{scat} to B_{ext}; thus adding absorption will darken the sky proportionally. Since B_{scat} is small in clean air, the perceived optical effect of NO₂ is greatest on clean air.

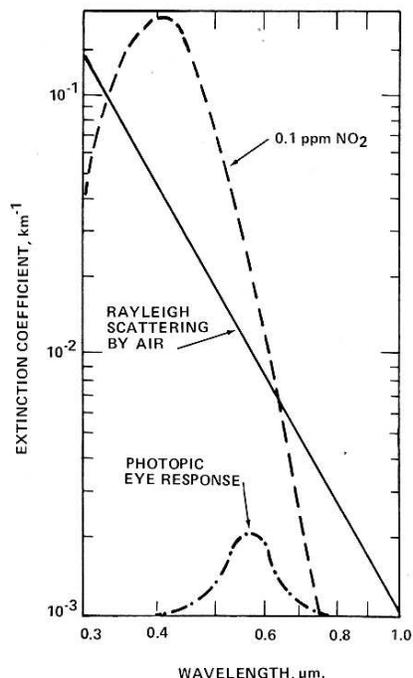


Figure 10.6 Comparison of the spectral distributions of Rayleigh scattering and NO₂ absorption. (EPA, 1979)

10.2.4 Coloration of the Atmosphere by NO₂

Latimer and colleagues developed the ratio of blue to red light reaching the observer as an index of NO₂-caused discoloration (U.S. EPA 1979). In a later publication (Latimer and Ireson, 1980), the blue/red ratio calculations have been refined so that they can be applied to both homogeneous and heterogeneous atmospheres. Looking through a homogeneous parcel of air, a blue/red ratio of one indicates no discoloration; as the NO₂ absorption increases, more blue light is removed and the B/R ratio decreases; at a ratio of about 0.9, discoloration becomes noticeable to an observer.

In an inhomogeneous atmosphere, for example viewing a NO_x plume or looking at layered haze, the discoloration can manifest itself as a dark band against a blue sky, or may appear reddish against clouds or a white haze. The effects of the NO₂ may also be masked by the re-introduction of scattered light into the line of sight between the NO₂ body and the observer. For the case of plumes, Latimer and Ireson (1980) developed extensive tables relating optical density of a plume, viewing distance, and "background" visual range to the plume's discoloration and contrast with the sky at the observer's location. The PLUVUE model, developed by Latimer for EPA, can estimate the visual impact of a plume from a stationary source under a wide range of meteorological and pollutant conditions.

10.2.5 Interactions of NO₂ with its Environment

The atmospheric chemistry of nitrogen compounds is quite complex, as discussed in Chapter 2 of this Technical Support Document.

In clean, dry air, oxidation of colorless NO to NO₂ makes a particle-free plume of NO-rich stack gas visible. The oxidation occurs initially by partial thermal oxidation, then slowly thereafter by reduction of ambient ozone. By contrast, near-ground-level NO_x emissions entering a polluted urban atmosphere,

containing an abundance of ozone and other reactive compounds, become involved in a complex of reactions which cycle nitrogen, hydrogen, oxygen, and certain hydrocarbon structures through a series of compounds and radicals. End products are primarily gaseous nitric acid and nitrate aerosols. Indirect aerosol effects include nitric acid adsorption onto many particle substrates, creating acidic aerosols. NO_x species also promote sulfate aerosol formation by the oxidation of gaseous SO_2 by ozone produced from NO_x -hydrocarbon interactions. The significance of these reactions to the analysis of NO_2 visibility impacts is that NO_2 in an urban atmosphere is likely to be accompanied by nitrate and other aerosols, and as a precursor to the formation of aerosols, NO_2 may contribute to violations of the state standards for "Visibility Reducing Particles" and PM_{10} .

10.2.6 Nitrate Visibility Reducing Particles

Fine particles in California are composed of a larger fraction of nitrate in contrast to the rest of the nation, where fine PM is composed mainly of sulfate. In fresh NO_x emissions, which primarily consist of nitric oxide (NO) and smaller amounts of nitrogen dioxide (NO_2), the NO undergoes reactions with ozone and peroxy radicals to form additional NO_2 . The NO_2 can be directly converted to nitric acid via a homogenous gas-phase reaction with the hydroxyl radical. This is the principal formation mechanism for nitric acid in the daytime. The major chemical loss process for gas-phase nitric acid (HNO_3) is its reaction with gaseous ammonia to form ammonium nitrate (NH_4NO_3). This reaction, which is reversible, is believed to be the major source of $\text{PM}_{2.5}$ nitrate aerosol in California's urban air (Motallebi et al. 2003).

The ocean (Pacific Ocean for California) influences the chemical composition of aerosols in the coastal zone. Sodium chloride (NaCl) is always present in aerosols in the form of large particles originating from seawater. Several studies have indicated the importance of the reaction of HNO_3 on sea salt particles, leading to thermally stable sodium nitrate (NaNO_3) production in the particle phase accompanied by liberation of gaseous hydrochloric acid (HCl) from the particles. This reaction may be the principal source of coarse (2.5 to 10 μm) nitrate, and plays an important role in atmospheric chemistry because it is a permanent sink for gas-phase nitrogen oxide species.

Ammonium and nitrate have a strong spatial variation with low concentrations at coastal locations and high concentrations at inland locations. This is partly due to transported precursor emissions having more time to react with nitric acid. Generally, NO and NO_2 concentrations are much higher than either gas-phase nitric acid concentrations or aerosol nitrate concentrations. Thus, the precursor gases needed for gas-phase nitric acid production are available in abundance. Excess ammonia is usually present; thus any gas-phase nitric acid formed usually will be driven quickly into the aerosol phase.

Of the gaseous air pollutants, only NO_2 absorbs visible light to any significant extent and thus contributes to visibility reduction. It is an orange-brown gas that absorbs radiation strongly at wavelength less than 430 nm; hence it acts as a filter for blue light (Franzblau et al. 1993). The brownish color of many polluted urban areas and the accompanying sunsets are at least partly due to the presence of NO_2 . The effect of increasing NO_2 on visibility and its degradation is reflected by the trends of increased ammonium nitrate concentrations measured during the haziest days. Visibility degradation is measured as the light extinction coefficient, which is the natural logarithm of the fractional reduction of light transmission per unit distance, usually expressed as Mm^{-1} ("inverse megameters").

Light extinction is a measure of how particles scatter and absorb incoming solar radiation. Since ammonium nitrate and ammonium sulfate have similar light extinction efficiencies (i.e., both scatter and absorb light to a similar extent), then increasing ammonium nitrate may begin to offset the visibility benefits that have been gained by sulfate aerosol reductions in many parts of the country. Particle diameter and density have more influence on the extinction efficiency than does particle composition. The calculated extinction efficiencies result from a consensus of theoretical calculations and empirical relationships that are reasonably consistent with more recent measurements (Lowenthal et al. 1995, Malm and Pitchford 1997). These efficiencies correspond to distributions peaking at ~ 0.3 and 1 μm for NH_4NO_3 .

Particles that contain sulfate and nitrate along with other soluble salts [e.g., sodium chloride (NaCl)], have long been known to absorb liquid water, thereby growing into size ranges that scatter more incident light (Tang et al. 1994). With increasing relative humidity, nitrate extinction efficiencies increase based on their tendency to absorb liquid water. Therefore, in the densely developed and industrialized coastal locations, the high humidity within the marine layer tends to cause hygroscopic aerosols to grow, thus increasing their relative visibility impact.

Further analysis of aerosol composition and visibility indicate that nitrate may be present in both the fine and coarse particle modes. Usually ammonium nitrate is in the fine mode. In coastal locations, the fine fraction may actually be the tail of the coarse fraction and sodium nitrate may be the main coarse mode constituent, suggesting that chemicals other than ammonia are important in stabilizing nitrate in aerosol form. Particle size is a very important characteristic determining whether particles will interfere with visibility. Coarse particles greater than 2.5 μm diameter are less efficient at scattering light and thus have little impact on visibility. Sodium nitrate (NaNO_3) formed by reaction of nitric acid with coarse sea salt particles or minerals probably will have a lower scattering efficiency than secondary NH_4NO_3 particles.

Fine particulate matter is the major cause of reduced visibility and can be a major source of contaminants imported into otherwise pristine environments. In terms of chemical composition, visibility degradation due to particulate matter is generally related most closely to scattering by sulfate and nitrate and absorption and scattering by elemental carbon. Relative humidity is also an important factor, with significant reduction in visibility occurring as the relative humidity increase from 50 to 90%. The effect of humidity on light scattering properties is also very dependent on chemical and microphysical variables, as components of fine particles (hygroscopic fraction of aerosol) will vary their ability to absorb water.

10.3 References

- ARB. 1992. Review of the One Hour Ambient Air Quality Standard for Nitrogen Dioxide. Technical Support Document and Staff Report.
- Franzblau, E., Popp, C.J., Prestbo, E.W., Marley, N.A., and Gaffney, J.S. (1993)., "Remote Measurements of NO₂ in the Brown Cloud over Albuquerque, New Mexico, Environmental Monitoring and Assessment, 24, pp.231-242
- Gard, E. E, Kleeman, M. J., Gross, D. S., Hughes, L. S., Allen, J. O., Morrical, B. D., Fergenson, D. P., Dienes, T., Galli, M. E., Johnson, R. J., Cass, G. R., and Prather, K. A. 1998: Direct observation of heterogeneous chemistry in the atmosphere, *Science*, , 279, 1184–1187
- Latimer DA, Ireson RG. 1980. Workbook for Estimating Visibility Impairment, U.S. EPA, EPA-450/4-80-031, Research Triangle Park, North Carolina.
- Lowenthal, D.H.; Rogers, C.F.; Saxena, P.; Watson, J.G.; Chow, J.C. 1995 Sensitivity of Estimated Light Extinction Coefficients to Model Assumptions and Measurement Errors; *Atmos. Environ.*, 29 (7), 751-766.
- Malm, W.C.; Pitchford, M.L. 1997 Comparison of Calculated Sulfate Scattering Efficiencies as Estimated from Size-Resolved Particle Measurements at Three National Locations; *Atmos. Environ.*, 31 (9), 1315-1325.
- Motallebi N., B.E. Croes, C.A. Taylor, and K. Turkiewicz 2003. "Spatial, temporal, and compositional patterns of PM_{2.5}, PM(10-2.5), and PM₁₀ in California". *J. Air Waste Manage. Assoc.*, 53:1517-1530.
- Tang, I.N.; Munkelwitz, H.R. Water Activities, Densities, and Refractive Indices of Aqueous Sulfates and Sodium Nitrate Droplets of Atmospheric Importance; *J. Geophys. Res.* 1994, 99 (D9), 18,801- 18,808.
- Tse PU, Martinez-Conde S, Schlegel AA, Macknik SL. 2005 Visibility, visual awareness, and visual masking of simple unattended targets are confined to areas in the occipital cortex beyond human V1/V2 *PNAS* 102: 17178-17183.
- Todd HN and Zakia RD 1969 Photographic Sensitometry, Morgan & Morgan, Inc., Publishers, New York
- U.S. EPA 1979 Protecting Visibility, An EPA Report to Congress, EPA-450/5-79-008.

Appendices A through D

Appendix A

OEHHA Recommendation

RECOMMENDATION FOR AN AMBIENT AIR QUALITY STANDARD FOR NITROGEN DIOXIDE

Submitted to the California Air Resources Board

Office of Environmental Health Hazard Assessment
Oakland and Sacramento, California

Office of Environmental Health Hazard Assessment



Linda S. Adams
Secretary for Environmental Protection

Joan E. Denton, Ph.D., Director
Headquarters • 1001 I Street • Sacramento, California 95814
Mailing Address: P.O. Box 4010 • Sacramento, California 95812-4010
Oakland Office • Mailing Address: 1515 Clay Street, 16th Floor • Oakland, California 94612



Arnold Schwarzenegger
Governor

MEMORANDUM

TO: Catherine Witherspoon
Executive Officer
Air Resources Board

FROM: Joan E. Denton, Ph.D.
Director

Joan E. Denton, Ph.D.

DATE: December 8, 2006

SUBJECT: RECOMMENDATION FOR AMBIENT AIR QUALITY STANDARDS FOR
NITROGEN DIOXIDE

I am transmitting to you a document describing the Office of Environmental Health Hazard Assessment (OEHHA) recommendations for the ambient air quality standards for nitrogen dioxide (NO₂). Our initial recommendations and their underlying scientific rationale have undergone public comment and a full review by the Air Quality Advisory Committee, our independent scientific review board. Based on their review, as well as public comments that we received, we have revised our recommendations accordingly. The document has been sent electronically to your staff for incorporation into the review of the NO₂ standard under SB 25.

California ambient air quality standards have four elements (California Health and Safety Code Section 39014, and Title 17, California Code of Regulations, Article 2, Section 70101): (1) definition of the air pollutant, (2) an averaging time, (3) a pollutant concentration, and (4) a monitoring method to determine attainment of the standard. OEHHA recommends the following revisions be made to the California ambient air quality standard for nitrogen dioxide:

1. Retain nitrogen dioxide as the air pollutant indicator.
2. Lower the current 1-hour-average standard for nitrogen dioxide to 0.18 ppm, not to be exceeded.
3. Establish a new annual average standard for nitrogen dioxide at 0.030 ppm, not to be exceeded.
4. These recommendations are based on the following reasons:
 - a. Evidence of enhanced airway inflammatory response after allergen challenge in asthmatics exposed to nitrogen dioxide at 0.26 ppm for 15 min to 30 min (in single or repeated doses).

California Environmental Protection Agency

The energy challenge facing California is real. Every Californian needs to take immediate action to reduce energy consumption.

♻️ Printed on Recycled Paper

Catherine Witherspoon
December 6, 2006
Page 2

- b. Evidence of increased airway reactivity among asthmatics after exposures to nitrogen dioxide in the range of 0.2-0.3 ppm for 30 min to 2 hour.
- c. The need for a margin of safety since only relatively healthy asthmatics are exposed in the chamber studies. Additionally, the chamber studies show effects on allergen responsiveness after 15-30 minutes of exposure at 0.26 ppm. Lowering the one-hour standard to 0.18 ppm would provide additional protection against brief periods of NO₂ at or near the current standard of 0.25 ppm.
- d. Robust evidence from epidemiological studies of both short-term and long-term effects of nitrogen dioxide on respiratory disease outcomes, many of which found effects which were independent of other pollutants such as particulate matter. Short-term studies included hospital admissions for respiratory disease in children and adults, emergency room visits for asthma in children, and increased symptoms and medication use in asthmatic children. Long-term effects (i.e. exposure over several months to several years) of nitrogen dioxide included reduced lung function growth in children. The annual average nitrogen dioxide levels in many of these studies are between 0.023 and 0.037 ppm.
- e. Weaker but suggestive evidence from epidemiologic studies of the effect of nitrogen dioxide on other health outcomes such as premature mortality, cardiovascular disease, low birth weight and preterm birth.
- f. Infants and children have disproportionately higher exposure to nitrogen dioxide than adults due to their greater ventilation rate and greater exposure duration.
- g. Children may be more susceptible to the effects of NO₂ than the general population due to effects on the developing lung.

We would like to thank staff in both the Research Division and Planning and Technical Support Division for working with us to provide information and technical support during the development of our recommendations.

Should you have any questions or concerns, please call me at (916) 322-6325.

Attachment

cc: George V. Alexeeff, Ph.D.
Melanie A. Marty, Ph.D., Chief
Bart D. Ostro, Ph.D., Chief

Bart Croes, ARB, Chief, Research Division
Richard Bode, ARB, Research Division

Recommendation for an Ambient Air Quality Standard for Nitrogen Dioxide

Revised November 30, 2006

OEHHA Recommendation for Standard

Introduction

This chapter presents the OEHHA recommendations for the nitrogen dioxide ambient air quality standard (AAQS) for California for the Board's consideration. The chapter begins with a brief history of the California and federal AAQS for nitrogen dioxide and a discussion of the Children's Environmental Protection Act and other general considerations in determining air quality standards. It then reviews the scientific evidence regarding the health effects of NO₂, discusses the findings on the overall adequacy of the current standards for nitrogen dioxide with respect to protecting the health of the public, including infants and children, and concludes with recommendations for the pollution indicators, averaging times, forms, and concentrations adequate to protect public health.

History of the Ambient Air Quality Standards (State and Federal)

In January of 1966, separate health and welfare standards for NO₂ were set by the Department of Public Health. A standard based on atmospheric discoloration alone was set at 0.25 ppm averaged over one hour. A separate health-based standard was set at 3.0 ppm averaged over one hour based on limited information on health effects available at that time.

The standard was reviewed and revised by the newly formed Air Resources Board in September 1969. Human Health data were still limited and the Board chose to adopt a single standard of 0.25 ppm averaged over one hour. This standard was chosen to protect both health and welfare based on the effects of NO₂ on laboratory animals and on atmospheric discoloration.

The CA standard was subsequently reviewed in October and December of 1985. Although the averaging time was retained at 0.25 ppm averaged over one hour, the language in Title 17 describing the most relevant effects was revised to reflect current health information. Evidence available at that time indicated the need for a standard to protect sensitive people from bronchial irritation and to prevent biochemical and cellular alterations that are indicative of adverse health effects in both normal and sensitive groups. Contribution to atmospheric discoloration also remained as a basis for the standard.

The California Air Resources Board and the Office of Environmental Health Hazard Assessment last reviewed the CA standard in December of 1992 (CARB 1992). It was recommended that the level of the California Air Quality Standard for NO₂ be retained at 0.25 parts per million, averaged over one hour. This level was deemed necessary because of the "potential to aggravate chronic respiratory disease and respiratory symptoms in sensitive groups." Additionally, "Risk to public health (is) implied by pulmonary and extra-pulmonary biochemical and cellular changes and pulmonary structural changes, observed in short-term animal tests at or above the concentration of the standard". Contribution to atmospheric discoloration also remained as a basis for the standard.

The Environmental Protection Agency first promulgated a National Ambient Air Quality Standard (NAAQS) for Nitrogen Dioxide of 0.053 ppm (annual average) in 1971. The NAAQS for NO₂ was again reviewed in 1985 and 1996 and a standard of 0.053 ppm, annual average was retained. In the 1995 EPA staff paper, an annual primary standard of 0.53 ppm was deemed adequate to protect also against the occurrence of 1-hour NO₂ values greater than 0.2 ppm in most areas of the country.

Considerations in Setting an Air Quality Standard for NO₂

The Children's Environmental Health Protection Act [Senate Bill 25, Escutia; Stats. 1999, Ch. 731, specifically California Health & Safety Code Section 39606(d)(2)] requires a standard that "adequately protects the health of the public, including infants and children, with an adequate margin of safety." In the development of standards, SB25 called for, to the extent that information is available, that the following information be assessed:

1. Exposure patterns among infants and children that are likely to result in disproportionately high exposures relative to the general population
2. Special susceptibility of infants and children to ambient air pollution relative to the general population. The effects on infants and children of exposure to ambient air pollution and other substances that have common mechanisms of toxicity
4. The interaction of multiple air pollutants on infants and children, including between criteria air pollutants and toxic air contaminants

The governing statutory language indicates that California's ambient air quality standards should also protect other vulnerable populations, in addition to infants and children, and the general public [(H&SC sections 39606(d)(2) and 39606(d)(3)]. This legislative directive is consistent with historical practice in California, where ambient air quality standards have been formulated to protect identifiable susceptible subgroups, as well as the general population. For instance, the one-hour sulfur dioxide standard was developed in order to protect the most sensitive recognized subgroup, exercising asthmatics. Nonetheless, even with standards tailored to shield vulnerable populations, there may be exquisitely sensitive individuals that will not be fully protected by the standards.

Although both the California Health & Safety Code (section 39606) and the federal Clean Air Act (section 109) refer to an adequate margin of safety, no specific legislative definition of "adequate" is provided. This judgment is left to the responsible regulatory agencies. As described in the preceding chapters, data from controlled human exposure studies demonstrate that asthmatics experienced an enhanced immune response to an inhaled allergen after NO₂ exposures at 0.26 ppm but has not been adequately investigated at lower concentrations. Also, some studies of asthmatics have found increased airway reactivity at 0.2-0.3 ppm, whereas others have not, suggesting that asthmatics may vary in their response to NO₂.

The incorporation of a safety margin has been recognized by the California Supreme Court as integral to the process of promulgating ambient air quality standards [Western Oil and Gas Association v. Air Resources Board, 22 ERC 1178, 1184 (1984)]. To the extent that health effects associated with ambient nitrogen dioxide occur at low levels of exposure, and that there is substantial inter-individual variability in response to environmental insults, it is unlikely that any nitrogen dioxide standard will provide universal protection for every individual against all possible nitrogen dioxide-related effects. Thus, in this instance, applying the notion of an “adequate margin of safety” for nitrogen dioxide standards becomes somewhat challenging. Nevertheless, taking into account the limitations of the scientific data, we have operationalized the concept of an adequate margin of safety by recommending standards that, when attained, should protect nearly all of the California population, including infants, children, asthmatics, the elderly, and individuals with chronic diseases, such as cardiovascular disease, against nitrogen dioxide-associated effects throughout the year.

The Children’s Environmental Health Protection Act required the ARB and OEHHA to review all health-based ambient air quality standards to determine whether the standards were protective of the health of the public, including infants and children, with an adequate margin of safety. The Act also required that, depending on the outcome of these reviews, the various ambient air quality standards be prioritized for full review and possible revision. Five factors were considered in assessing the health protectiveness of each ambient air quality standard during the prioritization process:

- 1) The extent of the evidence of effects reported to occur at or near the existing ambient air quality standard.
- 2) The nature and severity of those effects.
- 3) The magnitude of risk of effects anticipated when ambient (outdoor) levels are at or near the level of the existing standard.
- 4) Any evidence indicating that children may be more susceptible to effects than adults.
- 5) The degree of outdoor exposure in California relative to the level of the standard.

Following these reviews, the various ambient air quality standards were prioritized for full review (California Air Resources Board and Office of Environmental Health Hazard Assessment 2000). The standard for nitrogen dioxide was prioritized to undergo full review after the standards for particulate matter and sulfates and ozone. The SB25 review found that several clinical studies suggested effects of nitrogen dioxide exposure on enhancement of the immune response to aeroallergen in asthmatics at concentrations at or below that of the current State standard of 0.25 ppm, averaged over one hour. The epidemiological studies found relationships between both outdoor and indoor NO₂ levels and respiratory illness, decrements in lung function, and exacerbation of asthma, especially in children. Such evidence could indicate the need for a more stringent standard, an averaging time different from the current one-hour average, or both.

Defining an Adverse Effect

A key issue in evaluating the public health consequences of nitrogen dioxide exposure is consideration of the definition of an “adverse health effect”. The term “adverse health effect” is incorporated in the legislative background of the Federal Clean Air Act, as well as the California Health and Safety Code, although neither provides a definition for the term. Because it is helpful to the standard review process to consider the available scientific literature in the context of guidelines as to what is meant by the term, we have used guidelines published by the Scientific Assembly for Environmental and Occupational Health of the American Thoracic Society, which developed the most commonly used guidelines in the US (American Thoracic Society 1985; American Thoracic Society 2000). Both USEPA and ARB have referred to these guidelines over the intervening years in assessing the significance of pollutant-associated physiological, biological or pathological changes.

It is important to keep in mind the differences between statistical significance and medical or biological significance when considering what constitutes an adverse health effect. The 1985 ATS statement defined “adverse respiratory health effects” as medically significant physiologic or pathologic changes generally evidenced by one or more of the following: (1) interference with the normal activity of the affected person or persons, (2) episodic respiratory illness, (3) incapacitating illness, (4) permanent respiratory injury, and/or, (5) progressive respiratory dysfunction. The 2000 ATS statement expanded on the 1985 statement to include consideration of biomarkers, quality of life, physiological impact, symptoms, clinical outcomes, mortality, and population health versus individual risk when evaluating whether or not a change should be designated as an adverse health effect. The 2000 ATS review committee’s recommendations are summarized here:

1. *Biomarkers*: These should be considered, however it must be kept in mind that few biomarkers have been validated sufficiently to establish their use for defining a point at which a response becomes adverse, consequently, not all changes in biomarkers should necessarily be considered adverse.
2. *Quality of life*: In recent years, decreased health-related quality of life has become widely accepted as an adverse health effect. The review committee concluded that reduction in quality of life, whether in healthy persons or persons with chronic respiratory disease, should be considered as an adverse effect.
3. *Physiological impact*: The committee recommended that small, transient reductions in pulmonary function should not necessarily be regarded as adverse, although permanent loss of lung function should be considered adverse. The committee also recommended that reversible loss of lung function in conjunction with symptoms should be considered adverse.
4. *Symptoms*: Air pollution-related symptoms associated with reduced quality of life or with a change in clinical status (i.e., requiring medical care or a change in medications) should be considered adverse at the individual level. At the population level, the committee suggested that any detectable increase in symptom frequency should be considered adverse.

5. *Clinical outcomes:* Detectable effects of air pollution on clinical measures should be considered adverse. More specifically, the ATS committee cited as examples increases in emergency department visits for asthma or hospitalizations for pneumonia, at the population level, or an increased need to use bronchodilator medication, at the individual level. The committee recommended that: “no level of effect of air pollution on population-level clinical indicators can be considered acceptable.”
6. *Mortality:* Increased mortality should clearly be judged as adverse.
7. *Population health versus individual risk:* The committee concluded that a shift in risk factor distribution, and hence the risk profile of an exposed population, should be considered adverse when the relationship between the risk factor and the disease is causal, even if there is no immediate occurrence of obvious illness.

Based on these recommendations, many health outcomes found to be associated with nitrogen dioxide could be considered adverse including clinical outcomes such as emergency department visits for asthma, hospitalization for respiratory and cardiovascular disease, including life-threatening cardiac arrhythmias, and mortality.

In addition, controlled human exposure studies in asthmatics have found increased airway reactivity, inflammation, and enhancement of the allergic response to allergen at levels near the current CA standard. These endpoints may be considered adverse as they signify increases in the potential risk profile of the population of asthmatics.

In California, 8.8% of the population (nearly 3 million) had asthma symptoms at least once in the previous year, including 9.6% (nearly 900,000) of California's children (CHIS, 2001). Asthma is a chronic inflammatory disease of the airways characterized by an influx of inflammatory cells including eosinophils, and bronchial hyper-reactivity. Given our current scientific understanding of the pathophysiology of asthma, the observed NO₂ effects in controlled studies of asthmatics would be considered adverse. Specifically, the clinical significance of increased airway reactivity after NO₂ exposures in individuals with pre-existing respiratory diseases is the potential for a flare up or exacerbation of their underlying respiratory disease. Enhancement of the inflammatory response to allergen would contribute to the cycle of chronic inflammation, airway injury, and remodeling characteristic of asthma, especially in the more severe asthmatic.

Summary of the Scientific Evidence

Nitrogen Dioxide is an oxidant and strong respiratory irritant. Because of its low solubility in water, NO₂ penetrates deeper into the respiratory tract. The bronchoalveolar regions are the sites with the highest local concentrations. This area of the lung is especially vulnerable to NO₂ because the protective fluid that lines the mucosal surface of the deep airways (epithelial lining fluid) is relatively sparse in this region.

There is evidence from clinical, toxicological, and epidemiological studies that NO₂ can affect human health. Each investigative approach possesses advantages but also carries limitations. Controlled human exposure studies (i.e. clinical or chamber studies)

provide valuable information about the acute effects of NO₂ exposure in humans under controlled conditions. However, the studies have, in general, been limited to healthy subjects and mild asthmatics. Furthermore, acute responses seen in clinical studies cannot necessarily be used to predict health effects of chronic or repeated exposure.

Inhalation studies in animals allow precision in quantifying exposure duration and concentration, measurement of a wide variety of physiologic, biochemical, and histological endpoints, and examination of extremes of the exposure-response relationship. Interpretation of these studies may be constrained by difficulty in extrapolating findings from animals to humans, especially when exposure concentrations are unrealistically high. Studies done on human cells (or tissues) *in vitro* can help investigate mechanisms of toxicity but lack all (or some) of the naturally occurring defense mechanisms. Epidemiological investigations examine exposures in free-living populations and can study a wide range of subgroups. However, precise exposure characterization is difficult, and important confounders, e.g. other co-pollutant, socioeconomic status, and occupational factors, may not be fully characterized.

Summary of Findings from Controlled Human Exposure Studies of NO₂:

Design considerations in Controlled Exposure Studies:

Experimental exposure of human volunteers to air pollutants under controlled conditions provides useful data on pathophysiological changes that can be of direct relevance to standard setting. The carefully controlled environment allows investigators to identify responses to individual pollutants, to characterize exposure-response relationships, and to examine interactions among pollutants *per se* or with other variables such as exercise. Endpoint assessment traditionally has included symptoms, pulmonary function (e.g., FEV₁, the amount of air one can exhale in one second after a deep inspiration), and airway responsiveness. More recently, studies have been extended using a variety of markers of pulmonary, systemic, and cardiovascular effects. Responses after exposure to NO₂ are compared with responses after exposures using filtered air as a control. The exposure protocols for some chamber studies involve single exposures to NO₂ of varying duration (30 min. to up to six hours) or short (15-30 min.), repeated exposures to NO₂. This intermittent exposure protocol might better reflect the short-episodic high exposures to NO₂ seen in real-life exposure.

Human clinical studies also have limitations often due to small sample size, including limited statistical power and limited ability to adequately study the range of responses in the general population or specific subpopulations. In addition to specific subpopulations with underlying disease, such as asthmatics, there is increasing scientific evidence that genetics and other individual host factors (e.g. smoking status, prior exposure to ambient pollutant, dietary factors) may be important determinants of an individual's susceptibility to a given pollutant, and small clinical studies are unable to adequately evaluate the wide variation in susceptibility due to these host factors. Additionally, for safety and ethical reasons, among those with chronic medical conditions such as asthma or cardiovascular disease, only those with mild or moderate disease are usually

studied. The more vulnerable populations such as the fetus, young children, the elderly, asthmatics during or after a recent respiratory tract infection, and more severe asthmatics have not been studied in this setting. This selection bias in recruiting volunteers reduces the ability to generalize the findings of such studies. Finally, controlling the experimental conditions may result in failure to capture effects found in complex real-world exposures.

Additionally, studies must be limited to short durations of exposure (i.e., minutes to hours) and to pollutant concentrations that are expected to produce only mild and transient responses. The acute, transient responses seen in clinical studies are not necessarily predictive of health effects of chronic or repeated episodic peak exposures, which might be the more relevant exposure scenario for populations.

It should be emphasized, however, that these limitations all tend to underestimate pollutant effects. Therefore, finding a response that can be related to specific exposure conditions constitutes a valuable component to the standard setting process. In contrast, given the potential limitations of human clinical studies, negative findings may in some cases reflect the constraints of study design more than biological reality.

Below, we first summarize the studies of healthy subjects exposed to NO₂ alone, and then consider studies of subjects with asthma, infants and children, and other potentially susceptible subgroups. Finally, we summarize the studies of NO₂ in combination with other pollutants.

Healthy individuals

The clinical data suggests that young healthy subjects exposed to NO₂ at concentrations below 4 ppm for several hours do not experience symptoms, changes in pulmonary function or increased airway resistance. However, exposures to NO₂ in the range of 1.5-2.0 ppm can cause small, statistically significant effects on airway responsiveness in healthy individuals (Mohsenin et al., 1987b, Frampton et al., 1991). These levels are of potential concern primarily in occupational settings (see Chapter 5 of the Technical Support Document). Additionally, exposures to NO₂ in the range of 1.5 to 2.0 ppm for four to six hours induced mild airways inflammation, based on several different markers. These inflammatory response were unaccompanied by symptoms or changes in lung function. Short exposures (20 min.) at similar concentrations did not show evidence of airway inflammation (Strand et al., 1990, 1991). A limited number of clinical studies in healthy individuals have reported effects of NO₂ on host defenses at concentrations above 1.5 ppm. Taken together, these studies suggest that in healthy adults there may be a threshold for airway inflammatory effects of single, multi-hour NO₂ exposures at an approximate concentration of 1 ppm. Few studies have examined responses in healthy elderly; one study suggests there may be significant decrease in lung function (FEV₁) in older smokers exposed to 0.3 ppm NO₂ for several hours (Morrow et al., 1992).

Asthmatics

Clinical studies indicate that individuals with asthma are more susceptible to the effects of NO₂ compared with healthy individuals (Table 1). As discussed in Chapter 6 of the Technical Support Document, most studies of asthmatics have found no effects of NO₂ on symptoms or lung function at 0.1-0.5 ppm. Some studies of asthmatics found evidence of increased airway reactivity at NO₂ levels in the range of 0.2-0.3 ppm (Kleinman et al. 1983 (0.2 ppm/ 2hr); Jorres et al. 1990 (0.25 ppm/30 min, Bylin et al. 1988 (0.27 ppm, 30 min.), Bauer et al. 1986, (0.3 ppm/30 min), Strand et al., 1996 (0.26 ppm/30 min), whereas others using similar (but not identical) exposure protocols have not found evidence of increased airway reactivity as low as 0.1-0.5 ppm. One exception was a study by Orhek et al. (1976) that found increased airway responsiveness in 13/20 subjects at 0.1 ppm/1 hr. This study was challenged because of questionable statistical analysis, and other studies have been unable to confirm effects on airway responsiveness or lung function at 0.1-0.12 ppm (see Chapter 5 of the Technical Support Document). An examination of the data on responses for individuals suggest that there is substantial inter-individual variability in airway reactivity in response to NO₂ at levels near the current CA 1 hr-standard of 0.25 ppm. A pooled analysis of asthmatics found evidence of increased airway responsiveness at 0.2-0.3 ppm, primarily in studies with exposures at rest (Folinsbee 1992). Thus, the lack of findings in some studies may reflect, in part, lack of statistical power due to small sample size, differences in subjects (inter-individual variability) and exposure protocols.

Few subjects have evaluated the effects of NO₂ on airway inflammation in asthmatics. One study found evidence of airway inflammatory mediators in BAL of asthmatics exposed at 1 ppm for 3 hr; these changes were concomitant with small decrements in lung function (Jorres et al. 1995). Healthy subjects in the same study showed a lower response as measured by these markers of inflammation and no evidence of effect on lung function.

Recent studies from the UK and Sweden suggest that, overall, subjects with asthma exposed to NO₂, at rest, have an enhanced response to allergen challenge at concentrations as low as 0.26 ppm for 15 min (Barck et al., 2005) to 30 min. (Strand et al., 1997, 1998; Barck et al., 2002) and 0.4 ppm for 1 hr (Tunncliffe et al. 1994). There was no evidence of attenuation of the enhanced response to allergen after repeated exposures to 0.26 ppm NO₂ (30 min. exposure each day on four consecutive days) (Strand et al. 1998)

Compared with filtered air, single and repeated NO₂ exposures at rest for short durations (30 min.) enhanced responses of asthmatics to allergen challenge at concentrations as low as 0.26 ppm. Enhanced responses included: a more pronounced early and/or late-phase decrement in lung function (peak expiratory flow or FEV₁) and evidence of increased cellular inflammation (neutrophils) and eosinophil activity in lung lavage and/or sputum samples (Strand et al., 1997, 1998; Barck et al., 2002, 2005). Barck et al., (2005) demonstrated that brief repeated exposures on two consecutive days (15 min Day 1, 15 min x 2 Day 2) followed by allergen challenge on Day 2)

increased eosinophil activity in sputum and blood. Serum levels of eosinophilic cationic protein (ECP), a product of eosinophils that contributes to airway injury in asthmatics, are increased in individuals with asthma and atopy and correlate with disease activity (Venge et al. 1999). Although all of the endpoints (e.g. lung function decrements) were not consistently seen in different studies with very similar protocols, using more sensitive endpoints, investigators have found a biologically plausible coherent body of evidence that brief exposures to NO₂ (0.26 ppm for 30 min) enhances the allergic response in mild asthmatics.

Although the NO₂ exposures in these studies did not lead to a clinical asthma exacerbation in the laboratory setting, the response could be more pronounced and deleterious in those with more severe asthma. There is increasing evidence that air pollutants with strong oxidant properties (e.g. NO₂, ozone, and diesel exhaust particles) can potentiate the allergic response by similar mechanisms (Krishna et al., 1999). Animal models of allergic asthma support the observation (Gilmour et al., 1995).

The studies above clearly find that asthmatics are more susceptible to the effects of NO₂ compared with healthy individuals. Of note, the concentration dose-response has not been adequately studied; the one study that evaluated the allergen responses for filtered, 0.1 ppm, and 0.4 ppm for 1 hr found a significant drop in % FEV₁ between filtered air and 0.4 ppm but not between filtered air and 0.1 ppm (Tunnicliffe et al. 1994).

There are no studies on effects of NO₂ on host defenses in asthmatics; however, there is no reason to believe that the NO₂ effects on host defenses seen in healthy individuals (alterations in ciliary motility, oxidative stress, and enhanced susceptibility to epithelial cell injury *in vitro*) would not apply in asthmatics. Furthermore, these processes (ciliary dysmotility, epithelial cell injury, and oxidative stress) are part of the pathophysiology of asthma, and it is possible that the effects of NO₂ on host defenses in asthmatics might be seen at lower levels compared with normal healthy individuals. Effects of NO₂ on host defenses could lead to clinical consequences (e.g. exacerbations or increased severity or duration of asthma after respiratory infection), in more severe asthmatics that already have airway compromise due to their underlying disease.

Table 1. Summary of Human Chamber Studies on Nitrogen Dioxide: Healthy Individuals vs. Asthmatics *

	Healthy Individuals	Asthmatics
Symptoms	No effect as high as 4 ppm for up to 5 hr	Most studies showed no effect at 0.1 ppm-0.5 ppm for 30 min-1 hr
Lung function	No effects as high as 4ppm for up to 5 hr	Most studies showed no effect at 0.1-0.5 ppm for 30 min-1 hr
Airway responsiveness	Increased at 1.5-2 ppm for 1-3 hr	Increased at 0.2-0.3 ppm for 30 min-2 hr in some studies. Substantial between subject variability in response.
Airway Inflammation	Exposures at 1.5-2 ppm (3-6 hours), increased neutrophils and epithelial cytokines in bronchoalveolar lavage fluid (BAL). At 1 ppm for 3 hr: BAL showed increase in one inflammatory mediator (eicosanoid) but no increase in cell counts.	Only one study to date has evaluated asthmatics using BAL: At 1 ppm for 3 hr: BAL showed increase in several inflammatory mediators along with decrease FEV ₁ but no increase in cell counts. Wider inflammatory response suggests that asthmatics more responsive at 1ppm compared with healthy individuals in same study.
Response to NO ₂ + allergen compared with filtered air + allergen	Not applicable	Effects of NO ₂ plus allergen at 0.26 for 30 min (compared with filtered air) ¹ <ul style="list-style-type: none"> • larger decrement in lung function (FEV₁ or peak flow)increased neutrophils (BAL) • evidence of eosinophil activation (BAL, blood, sputum).

*Lowest level at which effects observed. Unless indicated, data not available on threshold level (i.e. level were no effect seen).

¹However, these responses were not consistently observed in each study. Eosinophil activation seen with repeated exposures as low as 15 min duration (Barck et al., 2005)
No change in FEV₁ after NO₂ + allergen exposure at 0.1 ppm (Tunnicliffe et al. 1994).

Studies in Children

Only two controlled studies of lung function after NO₂ exposures have examined children. Ten children with mild asthma (age 11-18 yr) exposed to NO₂ 0.12 ppm / 40 min did not experience changes in lung function. There was a non-significant trend of increased symptoms after NO₂ exposures. Asthma medications were not withheld and may have decreased the ability to detect an effect of NO₂ (Koenig et al., 1985). A second study of 34 asthmatics (age 8-16 yr) exposed to NO₂ at 0.30 ppm/ 3 hr found no effect on airway reactivity. A transient decrease in FEV₁ was noted for the first hour of exposure but returned to baseline during the latter part of the exposure (Avol et al. 1988). Thus, the only two studies in children with asthma did not find a clear effect of NO₂ on lung function or airway reactivity. Younger children with asthma, those with recent respiratory infections, and children with more severe asthma or other chronic lung diseases have not been studied in this setting.

Other Susceptible Populations:

Two studies of individuals with chronic obstructive pulmonary disease found small (3-5%), statistically significant, decrements in lung function (FEV₁) at 0.3 ppm NO₂ with intermittent exercise (Morrow et al. 1992 (0.3 ppm/4 hr), Vagaggini et al. 1996 (0.3 ppm/1 hr), whereas several others did not. The lack of findings is likely due to small samples, patient selection (subject variability), and/or differences in protocol. Older smokers and non-smokers (mean age 61 years) were also studied by Morrow et al. (1992). Smokers experienced a slight, statistically significant decrease in FEV₁ after NO₂ exposures at 0.3 ppm

Several clinical studies suggest there may be systemic and cardiovascular effects of NO₂ exposure. These data are insufficient to be conclusive, and do not provide adequate concentration-response data. Additional studies are needed to determine whether there are cardiovascular effects of NO₂ exposure, and the mechanisms involved.

Based on the current evidence from chamber studies, some of these findings might be extended to other potentially susceptible populations. For example, immunocompromised individuals, those with chronic lung diseases, and infants with immature immune systems might be at increased risk for respiratory infection after NO₂ exposures, although the chamber studies are not helpful at determining at what level.

Increased airway responsiveness or airway hyper-reactivity (AHR) is more severe in children with asthma compared with adults with asthma, even after adjusting for airway size (Peat 1994) Therefore, it is plausible that children with asthma, especially younger children, may experience greater AHR to a given concentration of NO₂ or experience increased AHR after a lower dose compared to adolescent and adult asthmatics. Also AHR is more prevalent in children without asthma (10% of 7-9 year olds) (Peat 1996, Forastiere et al. 1996). It is possible that others with AHR (e.g., infants post lower respiratory tract infections or those with certain chronic lung diseases) may experience increased AHR after NO₂ exposures.

Pollutant Concentration/Dose-response functions:

There is somewhat conflicting results among the few studies that have investigated which of the exposure parameters, the peak concentration of NO₂, duration of exposure, or the total dose (concentration x duration), is more important. Studies have shown evidence of airway inflammation in healthy individuals following prolonged exposure (four to six hours) to NO₂ at a concentration of 2.0 ppm (Azadniv et al. 1998, Blomberg et al. 1997, Devlin et al. 1999), whereas short (20 min.) exposures to NO₂ at 1.5-2 ppm did not (Sandstrom et al. 1990, 1992b). These results suggest that duration, not peak concentrations are more important. In contrast, a recent study by Jenkins et al. found that in a group of mild asthmatics exposed for 3 hr to NO₂ (400 ppb) FEV₁ was decreased, whereas the same total dose but delivered at a lower concentration and over longer duration (6 hr at 200 ppb) had no effect on FEV₁. The latter set of results suggests that the threshold concentration, rather than the total amount of pollutant inhaled over time, was more important. No studies have been conducted to investigate whether peak concentrations or total dose are more important at lower concentrations that might be directly applicable to standard setting.

Pollutant Mixtures

The database on NO₂ as part of air pollution mixtures remains limited, in part because of the complexity of the experimental design and the difficulty in studying the most susceptible subjects. Several studies found no effects of NO₂ – ozone mixtures on pulmonary function in young healthy individuals. Jorres et al. (1990) found increased airway responsiveness to SO₂ after exposure to 0.3 ppm NO₂ (Jorres et al 1990). Rusznak et al. (1996) and Devalia et al. (1994), showed increased allergen responsiveness after exposure to NO₂ (0.4 ppm) and SO₂ (0.2 ppm), but not to either gaseous pollutant alone. Drechsler-Parks (1995) found a decrease in cardiac output in elderly individuals for mixtures of NO₂ (0.6 ppm) and ozone (0.45 ppm) but not with individual pollutants. Overall, the data suggest that, in asthmatics, NO₂ at levels only slightly above the California standard of 0.25 ppm may enhance airways responsiveness to other pollutant challenges, and may act synergistically with SO₂ in enhancing responses to allergen challenge.

Concentrations where adverse effects have been observed

Studies indicate that asthmatics may have enhanced response to an inhaled allergen and increased airway responsiveness with NO₂ exposures at 0.2-0.3 ppm . These are two important endpoints for asthmatics. Asthma is a chronic inflammatory disease of the airways characterized by an influx of inflammatory cells including eosinophils, and bronchial hyper-reactivity. Those with allergic asthma would have a greater inflammatory response to an inhaled allergen, e.g. pollen when breathing ambient air with NO₂, and may experience allergic symptoms at lower pollen concentrations in the presence of NO₂. In more severe asthmatics this may contribute to a worsening of

asthma symptoms. The clinical significance of increased airway reactivity after NO₂ exposures in individuals with pre-existing respiratory diseases is the potential for a flare up or exacerbation of their underlying respiratory disease. There is little data on the effects of NO₂ at levels below 0.2 ppm. Studies on airway reactivity in asthmatics at 0.1-0.12 ppm have been largely negative. The one study that evaluated the allergen responses for filtered, 0.1 ppm, and 0.4 ppm for 1 hr found a significant drop in % FEV₁ between filtered air and 0.4 ppm but not between filtered air and 0.1 ppm (Tunnicliffe et al. 1994).

Thus, the studies to date call into question whether a standard of 0.25 ppm is adequately protective of people with asthma. Elderly smokers, and those with COPD may also have decrements in lung function at the current ambient standard of 0.25 ppm.

Conclusion:

Overall, the clinical studies suggest NO₂ exposures near the current ambient air quality standard for NO₂ (0.25 ppm, 1 hour average) may enhance the response to inhaled allergen in people with allergic asthma. Responses included: decrements in lung function, an increased inflammatory (neutrophil) response in airways, and evidence of activation of eosinophils. However, these responses were not consistently observed in each study. For a subset of asthmatics, exposures to NO₂ at levels near the current ambient air quality standard may have increased airway reactivity. Limited data suggest that elderly smokers, and those with COPD may also have decrements in lung function at the current ambient standard of 0.25 ppm.

Summary of Findings from Toxicological Studies

The health effects data of NO₂ exposures in animals and human *in vitro* test systems that is pertinent to the re-evaluation of the one-hour ambient air quality standard and the establishment of an annual average standard has been thoroughly reviewed. The focus of the animal health effects review was on those studies conducted at levels of NO₂ considered relevant for decision-making processes (i.e., exposures \leq 1.0 ppm NO₂). The review emphasized studies published after the CARB, 1992 document (CARB, 1992).

Dosimetry modeling studies support the animal and human exposure studies in that the primary site of lung damage due to inhalation of NO₂ is the bronchiolar-alveolar duct region (Overton, 1984). Dosimetry modeling has also estimated tissue dose of inhaled NO₂ in various species at different airway levels and in alveoli. Although pharmacodynamic differences (e.g., cellular defense and repair mechanisms) in oxidant protection exist between species, dosimetry modeling indicates that humans may receive 2-4 times greater maximal tissue dose of NO₂ at the centriacinar region relative to experimental animals (Miller *et al.*, 1982).

No measurable inflammatory effects were apparent with acute or short-term repeated NO₂ exposures at 1.0 ppm or less. However, epithelial cell labeling techniques have noted increased cell proliferation in bronchiolar tissue with one-day exposure to 0.8 ppm (Barth et al. 1994). This would indicate that epithelial cell labeling is a more sensitive indicator of cellular damage than conventional methods of measuring NO₂-induced pulmonary inflammation. In multi-day exposure studies, pulmonary inflammation was detected as an increase in protein content in bronchoalveolar lavage fluid with continuous exposure of mice and guinea pigs to NO₂ levels as low as 0.40 ppm for 7-10 days (Sherwin and Carlson, 1973; Sherwin and Layfield, 1976). In a six-week study, exposure of mice to 0.35 ppm NO₂ caused damage to the pulmonary microvasculature, thus apparently permitting an injected murine melanoma cell line to take hold, resulting in an increased metastatic lung burden relative to the control group (Richters and Richters, 1989).

Transient reductions in levels of particular arachidonate metabolites in BAL fluid following acute and short-term exposure to 0.5 ppm NO₂ and similar results in *ex vivo* studies suggest the potential for impeding the host's defense against microbial infection by damaging alveolar macrophages (AMs) (Robison et al., 1993). Exposure of rabbits to 0.3 ppm 2 hr/day for 2 days resulted in decreased AM phagocytosis and mobility (Schlesinger, 1987). In other studies of the host lung defense system, a transient increase in alveolar clearance rate was observed 4 to 5 days after exposure to 0.3 ppm NO₂ for 2 hrs that suggests a mild irritant action on lung tissue (Vollmuth et al., 1986).

These individual effects on various components of lung host defense by NO₂ have not translated into an enhancement of pulmonary infection by microorganisms with acute exposures of 1 ppm or less (Davis et al. 1991; Nisizawa et al 1988; Rose et al. 1989). However, chronic/subchronic exposures of mice to 0.5 ppm NO₂ have resulted in enhanced susceptibility to challenge by microorganisms (Ehrlich et al., 1979; Ehrlich and Henry, 1968). Greater mortality from *streptococcus* infection was demonstrated in mice exposed for one year to 0.2 ppm NO₂ with 0.8 ppm one-hr spikes twice per day (Miller et al., 1987).

Studies with prolonged exposure protocols have observed some morphological and biochemical changes in developing mice with NO₂ concentrations at 0.25 ppm. Six-week intermittent exposure to 0.25 ppm beginning at 3 weeks of age, during which lung development is still occurring, resulted in increased number and size of alveolar Type II cells (Sherwin and Richters, 1995a). While this effect was noted immediately following exposure, it was not statistically significant until 32 weeks post-exposure. Type II cell alterations long after NO₂ exposure has ended suggest permanent structural changes have occurred to alveolar tissue.

In addition to these effects, there were alterations in measures of elastic fiber abundance in alveolar tissue up to 32 weeks post-exposure (Sherwin and Richters, 1995b). The increased amount and density of elastin in alveolar tissue would suggest an interstitial fibrotic consequence resulting from exposure during lung development.

In other prolonged exposure studies, morphometric changes in alveolar tissue components (i.e. thickened alveolar walls, increased cellularity, altered epithelial cell volumes) also occurred during lung development in young mice intermittently exposed

to 0.3 ppm for 6 weeks, and in young ferrets intermittently exposed to 0.5 ppm for 15 weeks (Sherwin et al. 1985; Rasmussen and McClure; 1992).

Chronic NO₂ exposure studies averaging 0.4 ppm or higher in adult animals have not observed morphological changes in centriacinar region tissue (Mercer et al., 1995; Tepper et al., 1993; Ichinose et al., 1991), suggesting that the developing lung is a susceptible target of NO₂ toxicity. However, six-week exposure at similar exposure concentrations have resulted in morphometric changes in alveolar tissue suggestive of an irritant-type response (Chang et al., 1986). Moreover, chronic, continuous exposure to 0.4-0.5 ppm NO₂ in other rat studies have observed thickening of alveolar walls and other possible inflammatory changes (Hayashi et al., 1987; Kubota et al., 1987).

In addition to increased developmental susceptibility, use of animal models with predisposition to specific diseases provides evidence of increased susceptibility to low NO₂ concentrations. Obese rat strains prone to cardiovascular-type diseases exhibited increased blood levels of triglycerides and decreased HDL and HDL/total cholesterol ratio when exposed to 0.16 ppm NO₂ for 24 weeks (Takano et al., 2004). A related normal rat strain exposed similarly only showed decreased HDL levels.

The only acute exposure studies on pulmonary function explored the effect of NO₂ in combination with cold, dry air or warm, humid air. NO₂ (1 ppm) reduced the lungs ability to attenuate the airway constrictive effects of repeated, 10 min exposures to cold dry air (Halinen *et al.*, 2000a). This may have relevance for asthmatics in cold environments. However, NO₂ did not enhance this airway constrictive effect with longer exposures (1-hour) to cold, dry air and warm, humid air (Halinen et al., 2000b). With longer exposures, decreased vital capacity was observed in mice exposed to a background concentration of 0.2 ppm NO₂ with 0.8 ppm 1-hr spikes twice per day (Miller *et al.*, 1987). Background exposure to 0.2 ppm alone did not result in any changes in pulmonary function.

A transient reduction in $\Delta FEF_{25\%}$ was observed in adult rats with intermittent NO₂ exposure (0.5 ppm base with daily 2 hr peaks of 1.5 ppm) for 78 weeks (Tepper et al. 1993). In adult and 1-day-old rats exposed to 0.5 ppm NO₂ with daily 1-hr spikes to 1.5 ppm for 6 weeks, the adult rats experienced a decrease in lung compliance after 6 weeks, but recovered by 3 weeks post-exposure (Stevens *et al.*, 1988). Neonatal rats showed an increase in lung volume and compliance after 3 weeks of exposure but not after six weeks of exposure.

Because of the high sensitivity of human asthmatics exposed to NO₂, a number of studies have investigated the effect of NO₂ on allergic asthma in animal models. At low NO₂ concentrations (≤ 1.0 ppm), indicators of allergic asthma in antigen-sensitized animal models were either negative or produced effects contrary to the original hypothesis (Fujimaki *et al.*, 1998; Hubbard *et al.*, 2002). However, exposure to higher concentrations of NO₂ (about 5 ppm and greater) have more consistently produced one or more indicators of allergic asthma including, enhancement of delayed-type dyspneic symptoms, increased serum IgE levels, increased pulmonary eosinophilia and epithelial injury, and increased bronchial hyperresponsiveness (Kitabatake *et al.*, 1995; Gilmour *et al.*, 1996; Ohashi *et al.*, 1998; Papi *et al.*, 1999; Mi *et al.*, 2002). In a prolonged NO₂ exposure study, increased airway hyperresponsiveness with histamine challenge was

observed after exposure of guinea pigs to 1.0 ppm NO₂ for 6 weeks (Kobayashi and Miura, 1995).

Splenic lymphocytes have important roles in immune surveillance, antigen recognition, and overall control of immune responses. Systemic immune system cells and organs showed no differences with long-term exposure of rats to 0.5 ppm NO₂ with daily peaks to 1.5 ppm, with the exception of a transient reduction in splenic natural killer activity early in exposure (Selgrade et al. 1991). Reduction in natural killer activity may result in increased susceptibility to viral infections or neoplastic disease. In mice, however, subchronic exposures to 0.25-0.35 ppm NO₂ suppressed splenic T-lymphocyte subpopulations and altered spleen weight (Richters and Damji, 1988; Kuraitis and Richters, 1989).

In human *in vitro* test systems, Schierhorn *et al.*, (1999) observed elevated histamine in culture medium, a marker of mast cell degranulation, following 1 day exposure of nasal mucosa to 0.17 ppm NO₂. Notably, mast cell degranulation and increased mast cell number have been observed in lung tissue of rats acutely exposed to 0.5 and 0.2 ppm NO₂, respectively (Thomas *et al.*, 1967; Hayashi and Kohno, 1985). Acute exposure of human bronchial epithelial cells (HBEC) to NO₂ concentrations as low as 0.2 ppm from asthmatic and non-asthmatic individuals showed increased release of certain inflammatory mediators from HBECs of asthmatic individuals only (Bayram *et al.*, 2001). Also, NO₂-induced increases in cell permeability were greater in HBECs from asthmatic individuals relative to non-asthmatic individuals (Bayram *et al.*, 2002).

In vitro exposure of human AMs to NO₂ concentrations as low as 0.1 ppm resulted in an elevation of spontaneous reactive oxygen intermediates and altered release of cytokines (Kienast *et al.*, 1994; Kienast *et al.*, 1996). Cell activation by oxidant gases leading to secretion or altered release of various bioactive products and mediators may induce interstitial lung injury.

In vitro data provide valuable insight into potentially sensitive indicators of acute NO₂-induced cellular injury. However, *in vitro* data generate another level of uncertainty for interpretation of NO₂ exposures resulting in toxic effects in intact animals and may produce inconsistent results when compared to *in vivo* studies. Thus, *in vitro* studies provide mechanistic information but are not useful for dose-response assessment.

Experimental studies that reproduce multi-pollutant interactions can represent more realistic environmental conditions than studies with NO₂ alone.. Rats exposed to NO₂ (0.4 ppm) and ozone (0.4 ppm) for 2 weeks resulted in synergistic or additive increases in anti-oxidant substances and enzymes in lung and no increase in lipid peroxides (Ichinose and Sagai, 1989). Guinea pigs under the same exposure regimen produced the opposite effect; no change in pulmonary anti-oxidant levels but increased lipid peroxide formation. However, longer exposures in rats to NO₂ (0.4 or 0.04 ppm) and ozone (0.05 average with daily 0.1 ppm peak) did demonstrate a synergistic increase in lipid peroxides, but in general, no change in anti-oxidant substances and enzymes. Thus, pollutant interactions may be dependent on the timing and intensity of exposure as well as the species.

A repeated 4-week exposure to a complex mixture of urban-type pollutants, including 0.2-0.4 ppm NO₂, observed inflammatory, morphological, and pulmonary function

effects (Mautz *et al.*, 2001). Combined diesel exhaust and NO₂ exposure during fetal and neonatal development have observed reproductive and immunological effects (Watanabe and Ohsawa, 2002; Watanabe, 2005). However, in some cases, the co-pollutant studies did not include groups exposed to NO₂ alone or without NO₂ to determine the contribution of NO₂ to the effect.

Conclusions

Key non-cancer health concerns that have been associated with ambient exposure of experimental animals to NO₂ include: (1) morphological changes in bronchiolar-alveoli junction epithelium; (2) Immunological and biochemical changes associated with respiratory tract defense, and; (3) developmental changes. In particular, the NO₂-induced effects observed by Sherwin and Richters (1995a, 1995b) suggest that potentially permanent structural changes to the lung during development may occur from prolonged exposure to relatively low levels of NO₂ (0.25 ppm), but may not become fully apparent until later in life.

This evidence is supported by other reports of lung alterations following exposure to low concentrations of NO₂ in mice (0.3 ppm) and ferrets (0.5 ppm) during lung development (Sherwin *et al.* 1985; Rasmussen and McClure; 1992). Additionally, alteration of AM function appears to be a more sensitive indicator of pulmonary oxidant damage by NO₂ than indicators of pulmonary oxidant inflammation. Potentially deleterious alterations in AM function have been observed with acute and short-term NO₂ exposures in the range of 0.3-0.5 ppm. Because AMs are a critical component of the host lung defense against infectious microorganisms and *in vitro* NO₂ exposure studies on human AMs have resulted in effects, this is also a key area for NO₂ toxicology research.

Summary of Findings from Epidemiologic Studies

The experimental studies such as the chamber studies reported in this document provide valuable information about the acute effects of NO₂ exposure in humans under controlled conditions. Epidemiologic studies add to this evidence by evaluating both short and long-term (i.e., a year or more) effects of outdoor and indoor NO₂ in free-living populations. Epidemiologic studies of NO₂ have reported associations with such outcomes as lung function, respiratory symptoms, emergency department visits, hospitalizations and premature mortality. As such, these studies provide some additional evidence of an adverse effect of NO₂, subject to certain important limitations and uncertainties.

As with all epidemiological studies on air pollution, there are both advantages and disadvantages to observational studies of NO₂. Epidemiologic studies are able to examine a wide range of individuals, behaviors, subgroups, and exposure conditions. However, as indicated in Chapter 7 of the Technical Support Document, there are several disadvantages including some that are specific to the study of NO₂.

First, epidemiologic studies of NO₂ may be subject to measurement errors. It is not possible to characterize exposure in a precise manner similar to that of a chamber study. This is particularly true for NO₂, a pollutant that is more local and less regional in scope (unlike PM_{2.5} and ozone). In addition, as a reactive gas, it does not fully penetrate into the indoor environment. In addition, there are known indoor sources of NO₂ such as gas stoves and fireplaces. Therefore, there will be errors in assigning exposures to individuals which will likely result in biased effect estimates.

Second, epidemiologic studies may be subject to bias from uncontrolled or poorly controlled confounders such as seasonality, weather and co-pollutants. The latter is of particular concern since NO₂ is often highly correlated with OC, EC, PM_{2.5} and UF (Zhu et al 2002, Seaton 2003, Gauderman et al. 2002). Therefore, determining the independent effect of NO₂ can be challenging. Given the problems outlined above, the available outdoor studies were more informative when direct or indirect adjustment for measured particles concentration (PM₁₀, PM_{2.5}, Black Smoke) were possible, or when the studies were conducted in areas where the variability of NO₂ was larger than that of fine particles, or when modification of the effect of particulate matter by NO₂ has been evaluated indicating consequences of exposure to traffic derived particles. However, the role of pollutants that are typically correlated and unmeasured remains unknown.

Third, the epidemiologic studies in this review used different averaging times of NO₂ for their exposure measurements. Many used a 24-hour average while others reported results for 1-hour maximum or 8-hour average levels. Since these metrics tend to be highly correlated, if there is a positive association between NO₂ and a given health effect, it is difficult to attribute the effect to a precise averaging time.

Despite these limitations, a large number of epidemiological studies published in the last several years have demonstrated associations between NO₂ concentrations and several health effects including mortality, cardiopulmonary mortality, decreased lung function, respiratory symptoms, and emergency room visits for asthma. The overall findings from these studies are supported by the coherence of effects, the biological plausibility (at least at higher concentrations) obtained from animal studies, and the finding of a concentration-response relationship in many of the studies. While any given epidemiologic study may have some limitations, taken together these studies suggest the possibility of significant adverse effects on the free-living population. However, given the problems outlined above, it is difficult to use these studies to determine a likely effects level. Nevertheless, prudent public health policy suggests that these studies should contribute to important margin of safety considerations. In addition, the epidemiological studies suggest the need for an additional standard that includes an averaging time greater than one-hour.

A summary of the most important findings is presented below.

Studies of respiratory disease

The strongest epidemiological evidence of an effect of NO₂ on human health is derived from the studies of respiratory disease, including studies measuring both short (24

hour)- and long (one or more years)-term exposure. The studies on asthmatics are particularly important because they support the findings of NO₂ effects on asthmatics in controlled clinical studies (see Technical Support Document, Chapter 6). The time series studies evaluating the relationship between NO₂ and both hospital admissions and emergency department visits for asthma in children and adults are fairly consistent and robust (Peel et al. 2005, Simpson et al. 2005, Galan et al. 2003, Atkinson et al. 1999, Hajat et al. 1999, Anderson et al. 1998, Sunyer et al. 1997, Lee et al. 2006). The associations between NO₂ and these health outcomes often remained significant in models that included both NO₂ and particulate matter, even when the latter pollutant was also statistically significant.

Overall, an effect of NO₂ has been noted in many panel studies evaluating aggravation of asthma. Panel studies involve the charting of health outcomes among a pre-selected cohort of individuals who are prospectively followed over a period of time, often lasting several months. Several asthma panel studies, including some from California, showed an effect of NO₂ on symptoms, medication use, and lung function (Schildcrout et al. 2006; Delfino et al. 2004; Delfino et al. 2003; Mortimer et al. 2002; Ostro et al. 2001; Linaker et al. 2000; Boezen et al. 1999).

The respiratory health effects of long-term exposure to NO₂ (which may represent NO₂ per se or be a marker of traffic-related pollutants) have been clearly demonstrated in European studies (Kramer et al. 2000, Janssen et al. 2003), in a cross-sectional study of children in Alameda, California (Kim et al. 2004) and in the Children's Health Study (CHS) in Southern California (Gauderman et al. 2004, Gauderman et al. 2005). The finding from the CHS of reduced lung function growth in children exposed to higher levels of NO₂ over an eight-year period is especially important, since it is a risk factor for chronic diseases and premature mortality later in life. These respiratory health effects have been observed in areas with average NO₂ level of 18 to 57 ppb, with many in the range of 23 to 37 ppb.

Although the findings from studies of respiratory disease are the most robust, several other health outcomes have been associated with both outdoor and indoor NO₂ exposure. These studies are briefly summarized below.

Mortality

NO₂ has been associated with mortality in both short-term time series studies and in long-term exposure studies. Time series studies report associations between daily changes in NO₂ and resultant changes in daily counts of mortality. Several of these studies have reported statistically significant associations between NO₂ and all-cause, cardiovascular and respiratory mortality in the United States (Dominici et al. 2003), in Australia (Simpson et al. 2005a) and in Europe (Samoli et al. 2006). Some, but not all, of these studies found NO₂ effects even after controlling for other pollutants such as PM10 and ozone. (See Technical Support Document, Chapter 7).

The Harvard Six City study (Dockery et al. 1993; Krewski et al. 2000) provides some evidence from the United States of an association between long-term NO₂ concentrations and both all-cause and cardiopulmonary mortality. The investigators did not fit multi-pollutant models to these data, and NO₂ was highly correlated with PM_{2.5}, TSP, PM_{1.5} and SO₂ (r=0.78, 0.82, 0.77 and 0.84, respectively). The American Cancer Society (ACS) study (Pope, III et al, 2002) failed to find any effect of long-term exposure to NO₂ on cardiopulmonary mortality, while data from Europe (Nafstad et al. 2004), suggested an increased risk of all-cause mortality. Likewise, European studies provided some evidence of an effect of long-term exposure on lung cancer (Nyberg et al. 2000; Nafstad et al. 2004).

Other health outcomes

NO₂ exposure has also been associated with cardiovascular disease. Studies by Wellenius (2005), Metzger et al. (2004), and Simpson et al. (2005b) all reported an effect of NO₂ on either hospital admissions or emergency room visits for cardiovascular disease after PM was taken into account. Peters et al. (2000) found a strong independent effect of NO₂ on increased risk of defibrillator discharges in patients with implanted defibrillators, while Rich et al. (2005) found that the effect of NO₂ on ventricular arrhythmia was null when PM_{2.5} was included in the model. Pekkanen et al. (2002) found significant associations between risk of ST segment depression and ambient lag 2 day NO₂ (OR 2.02, 95% CI: 1.34, 3.04) in 45 adults with coronary artery disease NO₂ was moderately correlated with the co-located particle measurements. Two pollutant models for PM and gases were not tested.

Several studies are suggestive of an effect of NO₂ or traffic on birth outcomes including the likelihood of pre-term birth (Wilhelm and Ritz 2003, Liu et al. 2003) intrauterine growth retardation (Liu et al. 2003), and low birth weight (Wilhelm and Ritz 2003, Ha et al. 2001). In some of these studies elevated risks for IUGR associated with NO₂ persisted after adjustment for other co-pollutants. For many of these studies, it is difficult to disentangle the relevance of NO₂ per se compared to NO₂ as a marker for traffic related air pollution.

Indoor studies

Several epidemiological investigations have been conducted in indoor settings. These studies have the advantage of lower measurement error and less confounding by co-pollutants, relative to the outdoor studies. Burning natural gas in gas stoves or cooking produces fine and ultrafine particles in addition to NO₂. Also, the effects of NO₂ on symptoms and lung function may be partly explained by nitrous acid, which is produced by gas appliances and is highly correlated with NO₂ levels. Thus, there may be toxicity from unmeasured indoor co-pollutants. Nevertheless, these studies are also suggestive

of potential adverse health outcomes in response to exposure to NO₂. For example, an increased incidence of lower respiratory symptoms among children in relation to indoor NO₂ has been suggested from a meta-analysis of the indoor studies (Hasselblad et al, 1992). A strong association between indoor NO₂ measured with passive samplers and respiratory symptoms among infants with an asthmatic sibling has been recently reported in the USA (New England) (van Strien et al, 2004). Investigators in the New England study also found associations between respiratory symptoms and NO₂ exposures dichotomized at or above 20 ppb in the older siblings with asthma (Belanger et al. 2006). Several indoor studies in Australia have found similar results. In Victoria, Australia Garrett et al. (1998) found that respiratory symptoms were more common in children exposed to a gas stove with a dose-dependent response between bedroom NO₂ levels and respiratory symptoms. The indoor air studies provide evidence that increased levels of NO₂ from gas stoves or other appliances are associated with respiratory symptoms. However, as discussed in Chapter 5 of the Technical Support Document (exposure chapter), operations of indoor combustion sources tend to have very high peak exposures (up to 400-1000 ppb). Therefore, it is difficult to ascertain whether the effect is due to high peak exposures or the averaged concentrations. Thus, it is difficult to extrapolate the findings in indoor air studies to ambient outdoor situations, limiting the use of these data in determining a long-term average for standard setting.

Conclusions

Studies of respiratory disease, including hospital admissions, asthma symptoms and lung function in panel studies and studies of long-term exposure are particularly robust with respect to NO₂ effects. Many of these studies show independent effects of NO₂. These respiratory health effects have been observed in areas with average NO₂ level of 18 to 57 ppb, with many in the range of 23 to 37 ppb.

In addition, recent studies of hospital admissions for cardiovascular disease, adverse pregnancy outcomes, acute mortality, and studies evaluating the indoor effects of NO₂, especially among infants at risk for asthma, suggest the possibility of an independent effect of NO₂. With respect to long-term effects of NO₂, analyses in the CHS reported significant effects of NO₂ on both bronchitis symptoms in asthmatics (McConnell et al. 2003) and reduced lung growth (Gauderman et al. 2004). The latter finding is particularly important since it followed lung development between the ages of 10 and 18 years of age. In both the short-term and long-term studies effects of NO₂ were observed at levels below the current state and federal standards.

Consideration of Infants and Children

As noted earlier, SB25 specifically asks that OEHHA assess the proposed standard in light of four factors related to infants and children, to the extent that information is available.

1. Exposure patterns among infants and children that are likely to result in disproportionately high exposures relative to the general population

As indicated above, children who are outdoors for extended periods of time, particularly while engaged in physical activity that increases their breathing rate, should be considered as a potentially susceptible subpopulation. Under these circumstances, their effective dose of NO₂ would be disproportionately high relative to the general population. Infants and children inhale more air per unit body weight than adults, even at rest. Thus, young children and infants experience a greater exposure per lung surface area than adults.

2. Special susceptibility of infants and children to ambient air pollution relative to the general population
3. Children with asthma have a higher degree of airway responsiveness compared with adult asthmatics.

A number of animal studies have indicated that the developing lung is altered by NO₂ exposure. In some exposure studies, morphometric changes in alveolar tissue components (i.e. thickened alveolar walls, increased cellularity, altered epithelial cell volumes) occurred during lung development in young mice intermittently exposed to 0.3 ppm for 6 weeks, and in young ferrets intermittently exposed to 0.5 ppm for 15 weeks (Sherwin et al. 1985; Rasmussen and McClure; 1992). Alternatively, long-term NO₂ exposure studies averaging 0.4 ppm or higher in adult animals have not observed morphological changes in centriacinar region tissue (Mercer et al., 1995; Tepper et al., 1993; Ichinose et al., 1991), suggesting that the developing lung may be a major target of NO₂ toxicity.

Thus, children may be more susceptible to the effects of NO₂ than the general population due to effects on the developing lung. Epidemiological studies have found reduced lung growth in association with NO₂ and its co-pollutants. In addition, there is some evidence of associations between long-term exposure to ambient NO₂ or other co-pollutants and adverse birth outcomes

4. The effects on infants and children of exposure to ambient air pollution and other substances that have common mechanisms of toxicity.

In considering the epidemiological studies (including field studies), it should be noted that exposures to highly correlated traffic related pollutants in the ambient air are inherently included in the evaluation.

5. The interaction of multiple air pollutants on infants and children, including between criteria air pollutants and toxic air contaminants.

There are limited studies of the interaction of multiple pollutants, and most have not addressed effects in infants and children. NO₂ may modify the effect of other pollutants, including PM in some epidemiologic studies (Katsouyanni et al. 2001). Interaction between NO₂ and PM₁₀ was significant in a panel study of asthmatic children (Delfino et al. 2002). The human controlled exposure studies are limited but suggest that, in asthmatics, NO₂ at levels only slightly above the California standard may enhance airways responsiveness to other pollutant challenges, and may act synergistically with SO₂ in enhancing responses to allergen challenge.

Other Susceptible Populations

The clinical and epidemiological studies indicate that individuals with asthma and other chronic lung diseases are more susceptible to NO₂. The epidemiological studies also indicate that elderly subjects and those with cardiovascular disease are more vulnerable to ambient NO₂ as highlighted by time-series studies on daily mortality and hospitalization. Other factors such as genetics, diet and other lifestyle factors may be important determinants of susceptibility to air pollutants. Gilliland et al. (2004) found that asthmatics with certain genetic polymorphisms were more responsive to diesel exhaust particles. In addition, life-style factors such as smoking, alcohol, diet and physical activity and socio-economic status may modify the effect of air pollutants. Lower education seems to be a risk factor for higher effect estimates in outdoor NO₂ studies in adults [Pope, III et al. 2002; Hoek et al. 2002; Schindler et al. 1998], independent of smoking, diet and alcohol consumption. Nitrogen dioxide is a potent oxidant, and pre-treatment with vitamin C can decrease airway responsiveness to NO₂ (Mohansen 1987b). Thus, life-style factors such as diet may modify the response to oxidative stress induced by NO₂. In addition, diet might influence allergic airway and cardiovascular diseases.

Recommended Pollutant Indicator

OEHHA recommends that NO₂ continue to be the appropriate indicator. However, NO₂ is highly correlated with all oxides of nitrogen as well as several other traffic-related pollutants. Although it is possible that other oxides of nitrogen can induce adverse health effects, most of the available controlled human studies, epidemiologic studies and toxicologic studies use NO₂ as the relevant exposure metric. Control of NO₂ and related pollutants is likely to provide significant public health protection to exposed populations. Therefore, this metric serves as a reasonable marker for standard setting.

Recommended Averaging Times and Forms

The current California ambient air quality standard for NO₂ uses a 1-hr averaging time. Selection of this averaging period was based on the desire to protect the public against health effects associated with peak short-term exposures to NO₂, based on typical NO₂ diurnal patterns experienced in California, particularly in the South Coast Air Basin. In addition, the State has had an NO₂ 1-hour average standard since 1969, and its retention has provided an historical record of trend for this pollutant. It was also recognized that a stringent 1-hr NO₂ standard would serve to reduce multi-hour and 24-hour average NO₂ concentrations, and thereby also provide protection against health effects associated with exposures longer than one hour. The studies on which the 1-hr standard was previously based (CARB 1992) indicated that exposures to NO₂ as low as 0.25 ppm for 30 minutes (Jorres et al. 1990) or 0.20 ppm for two hours (Kleinman et al. 1983) induced an increase in airway reactivity in asthmatics. Also, newer data suggests that short (15-30 min.) single or repeated exposures to NO₂ at 0.26 ppm enhance the allergic response in mild asthmatics. As we judge these effects to be adverse, the retention of a standard with a one-hour average is warranted. OEHHA recommends that a short-term 1-hour standard be retained to protect against these possible effects.

In a real life setting, of course, individuals are exposed to not only 1-hour concentrations but also multi-hour, 24-hour and multi-day averages. As indicated above, dozens of epidemiological studies demonstrate an association between 24-hour average concentrations of NO₂ and a wide range of adverse health effects including premature mortality, hospitalizations, emergency rooms visits, asthma exacerbation, and respiratory symptoms. Also, studies have shown that even longer-term averages of NO₂, including exposures of several months or years, may be of concern. As suggested above, exposure to NO₂ and its correlates is associated with permanent lung function decrements, symptoms and asthma. In addition, there is some evidence linking long-term exposure to NO₂ or traffic on birth outcomes including the likelihood of pre-term birth, intrauterine growth retardation, and low birth weight. Some of these studies have the potential to be confounded by season, weather and co-pollutants. Although other co-pollutants with oxidant properties, such as PM_{2.5} and ultrafine particles, are likely to contribute to some of the observed effects in the longitudinal epidemiological studies, human exposure studies indicate that NO₂ is a strong oxidant that causes airway inflammation and enhanced allergic response in asthmatics. Thus, we cannot rule out that NO₂ plays a role in the observed adverse health outcomes. In addition, some of the effects may be likely due to multi-hour exposures to NO₂, which are highly correlated with one-hour averages. Nevertheless, we cannot say with certainty that the effects observed in epidemiological studies are due to short-term effects. Thus, there is a non-zero probability that these effects are, in fact, associated with 24-hour and multi-year exposures to NO₂. While the one-hour standard will protect against peaks on a given day, it may not provide enough protection from effects that may be related to longer-term averages of NO₂. Therefore, prudent public health policy suggests that a standard with a longer-term average of NO₂ also be considered. An annual average standard would provide protection against potential effects of long-term (i.e., several months or years) exposures. In addition, by lowering the annual mean, the entire distribution of NO₂ would decrease as well. Thus the annual average standard would afford protection against 24-hour averages as well. Therefore, OEHHA recommends retaining a one-hour standard and adding an annual average standard for NO₂.

Recommended Concentrations

Considerations for the Margin of Safety

Both the California Health & Safety Code (section 39606) and the federal Clean Air Act (section 109) refer to an adequate margin of safety, although neither includes a specific legislative definition of this term. The Children's Environmental Health Protection Act [Senate Bill 25, Escutia; Stats. 1999, Ch731, sec. 3; Health & Safety Code section 39404(d)(2)] requires a standard that "*adequately* protects the health of the public, including infants and children, *with an adequate margin of safety.*" Given the current state of the science, which is limited by uncertainties in the existing data sets and methods available to analyze the impacts of low-level exposures, it is not possible to set standards for NO₂ that absolutely protect all individuals.

The governing statutory language indicates that California's ambient air quality standards should also protect other vulnerable populations, in addition to infants and

children, and the general public [(Health & Safety Code sections 39606 (d)(2) and 39606 (d)(3)]. This legislative directive is consistent with historical practice in California, where ambient air quality standards have been formulated to protect identifiable susceptible subgroups, as well as the general population. Nonetheless, even with standards tailored to protect vulnerable populations, there may be exquisitely sensitive individuals who still have adverse responses.

In addition, NO₂ concentrations reported at central site monitors may be substantially lower than those found in close proximity to mobile sources such as roads and highways. Ambient concentrations of NO₂ vary spatially within a community due to localized emissions of NO₂ especially from traffic (Kim et al., 2004; Gauderman et al. 2005). In these same studies increased levels of NO₂ were associated with asthma symptoms in the past 12 months (Kim et al., 2004) and prevalence of asthma (Gauderman et al. 2005). A recent study (Green et al. 2004) found that 9.5% of K-12 public schools in California are located within 150 meters of a busy road (25,000 or more vehicles per day). Thus a substantial number of school children, whose lungs are still developing, experience exposures that are much higher than those indicated by central site monitors alone.

Several other factors were incorporated into the margin of safety considerations. The margin was based on the available scientific data describing population effects and variability, and on epidemiologic studies examining endpoints and subgroups that can't be studied in exposure chambers. Specifically, the following evidence was utilized:

(1) chamber studies indicating variability in human response with the existence of particularly large individual responses; (2) chamber studies indicating, at levels close to the current standard, both bronchial reactivity and enhanced airway inflammatory response to allergen challenge; (3) knowledge that individuals that may be particularly susceptible such as severe asthmatics or asthmatics with an ongoing respiratory infection, elderly people with pre-existing respiratory or cardiovascular conditions, and infants and children cannot be tested in the exposure chambers; (4) animal toxicology studies supporting many of these findings and also suggesting the possibility of decreases in lung defense mechanism; and (5) epidemiologic studies reporting associations between ambient NO₂ and a suite of adverse outcomes including premature mortality, hospitalization, emergency room visits, respiratory symptoms and changes in lung function. The results are particularly robust for respiratory outcomes, which include premature mortality due to respiratory causes, emergency room visits for asthma, increased symptom reporting in asthma panel studies, and decreased lung function growth in long-term studies. While it is difficult to use all of the epidemiological studies quantitatively in developing a standard, the significant potential of adverse effects clearly should factor into the margin of safety considerations. Below, we provide the scientific rationale for the one-hour standards.

One-hour average

We recommend that the current state standard of 0.25 ppm, not to be exceeded, be reduced to 0.18 ppm, not to be exceeded. Most of the new controlled chamber studies studying enhanced allergen response indicate group-level effects at concentrations at 0.26 ppm for short (30 minutes to one hour) durations of exposure. Additionally, some,

but not all, of the controlled chamber studies have found increased airway reactivity in asthmatics after NO₂ exposures at 0.2-0.3 ppm for 30 min to 2 hr.

Given these findings, the OEHHA recommendation is based on several factors:

1. Asthmatics exposed to NO₂ at 0.26 ppm for 15-30 min (in single or repeated doses) developed an enhanced airway inflammatory response after allergen challenge in several carefully controlled human exposure studies. This enhanced response included: small decreases in lung function (Strand et al (1997, 1998), increased neutrophils and markers of eosinophil activation in airways (Barck 2002) and markers of eosinophil activation in blood and sputum (Barck et al., 2005). The eosinophil markers measured are a product of eosinophils that contributes to asthmatics; and serum levels of this marker are correlated with disease activity (Venge et al. 1999). Thus, the increased allergic response could lead to more prolonged asthma symptoms or clinical asthma attack, especially in the more severe asthmatic. The ultimate impact of the inflammatory response is unclear but repeated exposures to high NO₂ levels may result in restructuring of the airways, fibrosis, and possibly permanent respiratory injury. These latter outcomes are supported by animal toxicology studies, which also suggest the possibility of decreases in lung defense mechanism.
2. Several studies of NO₂ exposures in the range of 0.2-0.3 ppm have found that asthmatics exposed to NO₂ have increased airway reactivity (Kleinman et al.1983 (0.2 ppm/ 2hr); Jorres et al. 1990 (0.25 ppm/30 min, Bylin et al. 1988 (0.27 ppm, 30 min), Bauer et al. 1986, (0.3 ppm/30 min), Strand et al., 1996 (0.26 ppm/30 min),, whereas other studies using similar protocols have not. The lack of findings in some studies reflects, in part, differences in NO₂ response among subjects (inter-individual variability).

Because the data suggests that some asthmatics experience increased airway reactivity to NO₂ at levels near the current standard, we recommend that the air quality standard should be lowered to protect these more vulnerable subpopulations. Increased airway reactivity, a hallmark of asthma, is also seen in individuals with other chronic lung diseases, such as cystic fibrosis and COPD. Increased airway reactivity is also seen in children without clinical history suggestive of asthma or reactive airway disease.

The clinical significance of increased airway reactivity after NO₂ exposures in individuals with pre-existing respiratory diseases is the potential for a flare up or exacerbation of their underlying respiratory disease. In support of this, COPD patients demonstrated small decrements in lung function after NO₂ exposure at 0.3 ppm for 1-4 hr (Vagaggini et al., 1996, Morrow et al. 1992). Additionally, some infants and young children develop bronchial hyper-reactivity and wheezing after viral respiratory tract infections and may or may not go on to develop asthma (Martinez et al. 1995). Thus, the observed increase in airway reactivity after NO₂ exposure may also affect infants and young children who wheeze with an active or recent viral respiratory tract infection but are not confirmed with asthma. In addition, the chamber studies, by design, do not

include especially vulnerable populations (e.g., people with moderate to severe asthma, COPD, or heart disease, and asthmatics with concurrent respiratory infections), which may be incorporated in the epidemiologic studies. These factors were important considerations in allowing a margin of safety in OEHHA's determination of a health-protective standard.

3. Few studies have been undertaken to establish a threshold level for which no effects are observed. Tunnicliffe et al. (1994) evaluated the allergen responses for filtered, 0.1 ppm, and 0.4 ppm for 1 hr and found a significant drop in % FEV₁ between filtered air and 0.4 ppm but not between filtered air and 0.1 ppm for 1 hr. Jenkins et al. (1999) found that NO₂ at 0.4 ppm for 1 hr followed by allergen challenge resulted in a decrease in FEV₁ in asthmatics, whereas 0.2 ppm exposures for 3 hr did not. No studies have looked at the effects of NO₂-enhanced allergen response as measured by lung inflammation or markers of eosinophil activation at levels below 0.26 ppm. Thus, the limited studies did not find an NO₂ effect on allergen response at 0.1-0.2 ppm. Airway reactivity in asthmatics has been documented in several studies at 0.2-0.3 ppm, but the data below 0.2 ppm is less certain.
4. The studies on enhanced allergen response were seen with brief exposures (15-30 min duration) at 0.26 ppm. It is likely that the current standard of 0.25 ppm for 1 hr will not adequately protect against 15-30 min. peaks of 0.26 ppm. Lowering the one-hour standard to 0.18 ppm would provide additional protection against brief periods of NO₂ at or near 0.25 ppm. It is important to note that as discussed in Chapter 5 of the Technical Support Document, ambient levels of NO₂ vary during the day, with peaks concurrent with morning and afternoon commute times when people are more likely to be outside.
5. There have been a number of short-term and long term epidemiological studies completed over the last 10 years indicating the potential for severe adverse health outcomes including premature mortality, hospitalizations, emergency room visits, preterm births, and reduced lung growth. These studies include concentrations to which the public is currently being exposed (range of study means = 0.025-0.045 ppm). One-hour peak concentrations are highly correlated with 24-hr averages and longer-term averages. Based on California air quality data, the empiric ratio of 1-hr maximum to annual average is approximately 4 to 6 for the more populated air basins (Table 1). Thus, a 1-hr standard of 0.18 ppm would provide some protection against longer-term averages of 0.03 – 0.044 ppm.

Although the time-series and panel studies support the need for a short-term effect of NO₂, it is difficult to attribute these adverse outcomes to a specific NO₂ averaging time or concentration in observational epidemiological studies. Most of the studies used linear non-threshold models and did not explicitly test for thresholds. As indicated in the above reviews, these studies need to be viewed with some caution since it is difficult to separate out the effects of NO₂ from other co-varying pollutants. In addition, significant measurement error exists for NO₂ in relating ambient NO₂ to personal NO₂ exposure (Sarnat et al., 2001, 2005).

Finally, a larger margin of safety (relative to the 1-hour 0.26 ppm from the chamber studies) may be necessary to account for the possibility of adverse impacts associated with multiple peak exposures of NO₂ occurring over a long period of time (i.e., one year or more). While we are proposing an annual average standard to more directly take these effects into account, a lowering of the one-hour peaks will result in a lowering of the entire NO₂ distribution

6. The toxicology studies provide a body of evidence supporting the potential health effects seen in humans although it is difficult to use this data for determining a concentration or averaging time for the short-term (1-hr) standard. Toxicology studies have looked primarily at subacute and longer-term exposures. There is some *in vitro* data on the biochemical effects of NO₂ on airway epithelium and alveolar macrophages at 0.2 ppm for 2hr.

Thus, the short-term average is based on controlled human exposure studies in asthmatics with support from time-series and panel epidemiological studies on asthmatics or those with respiratory illness and toxicology studies.

Airway hyper-responsiveness and an enhanced allergic response with eosinophil activation are hallmarks of asthma. Although there have been no consistent decrements in lung function or increased symptoms at these levels there is a biologically coherent body of data that exposures of ~0.26 ppm may be detrimental to asthmatics.

For the determination of the standard for the one-hour average we used the studies that found exposure to NO₂ at 0.26 ppm for 30 min enhances the allergic immune response in asthmatics (Strand et al 1997, 1998, Barck et al 2002, 2005). We also used the four studies finding increased airway reactivity in asthmatics between 0.25-0.30 ppm for (30 min – 1 hr). With the exception of one peer-reviewed study, other studies have not found evidence of airway reactivity at 0.1 ppm. Evidence of these health effects were reported for relatively healthy asthmatics exposed in the range of the current standard for 30 minutes. In order to protect against a 30 min exposure at 0.26 ppm we would need to lower the 1-hr std to below 0.25 ppm (i.e., a level of 0.26 ppm for 30 minutes followed by a 30 minute exposure at a lower concentration, would yield a 1-hr average below 0.26 ppm).

To provide an adequate margin of safety, we chose a level of 0.18 ppm (1 hr average). This value is half-way between 0.26 ppm where effects have been consistently demonstrated and 0.1 ppm which is lowest level studied which appears to have no clear effect,

Annual average

We recommend an annual average standard of 0.030 ppm, not to be exceeded. Our recommendation for the annual average standard is based primarily on the

epidemiologic studies of respiratory disease, including asthma, involving longer-term (a week to several years) exposure as well as those using daily 24-hour averages. The respiratory health effects have been observed in areas with average NO₂ level of 18 to 57 ppb, with many in the range of 23 to 37 ppb.

The time series studies evaluating the relationship between NO₂ and both hospital admissions and emergency department visits for asthma in children and adults are consistent and robust (Peel et al. 2005, Simpson et al. 2005, Galan et al. 2003, Atkinson et al. 1999, Hajat et al. 1999, Anderson et al. 1998, Sunyer et al. 1997, Lee et al. 2006). Often, along with significant particle associations, these outcomes remained associated with NO₂ in regression models that included both pollutants.

Overall, an effect of NO₂ has been noted in many panel studies evaluating aggravation of asthma. Several asthma panel studies, including some from California, showed an effect of NO₂ on symptoms, medication use, and lung function (Delfino et al. 2002, Delfino et al. 2003, Delfino et al. 2004, Mortimer et al. 2002, Ostro et al. 2001, Boezen et al. 1999, Linaker et al. 2000, Schildcrout et al. 2006).

The respiratory health effects of long-term exposure to NO₂ as an individual pollutant or as a marker of traffic related pollutants have been clearly demonstrated in European studies (Kramer et al. 2000, Janssen et al. 2003), in a cross-sectional study of children in Alameda, California (Kim et al. 2004) and in the Children's Health Study (CHS) in Southern California (Gauderman et al. 2004, Gauderman et al. 2005). The finding from the CHS of reduced lung function growth in children exposed to higher levels of NO₂ over an eight-year period is especially important, since it is a risk factor for chronic diseases and premature mortality later in life.

Although the findings from studies of respiratory disease are the most robust, several other health outcomes have been associated with outdoor NO₂ exposure. These studies provide further support for the annual average recommendation. Longer-term exposures (one to several months average) to NO₂ have associated with several adverse birth outcomes including the likelihood of pre-term birth, intrauterine growth retardation, and low birth weight (Wilhelm and Ritz 2003; Liu et al. 2003; Ha et al. 2001). While two of these studies were conducted in Asia and might be less applicable to conditions here, Wilhelm and Ritz (2003) was conducted in the Los Angeles basin. The mean NO₂ level in this study was 44 ppb.

Regarding the short-term studies, analysis of the largest 90 U.S. cities reported an association between NO₂ and all-cause mortality (Dominici et al. 2003), which remained the same but became statistically insignificant in multi-pollutant models. The longer-term average of 24-hr NO₂ levels in those cities ranged from 11.0 to 39.4 ppb. A limited number of European studies have found independent effects of NO₂ (Saez et al. 2002; Hoek et al. 2000; Samoli et al. 2006). The APHEA-2 study on daily mortality including

29 cities (Katsouyanni et al., 2001) found that NO₂ modified the effect of PM₁₀. The effects of PM₁₀ on daily mortality were stronger in areas with higher levels of NO₂ (greater than or equal to 37 ppb).

In addition, several studies outside of the United States and Western Europe found associations between NO₂ and mortality that appear to be independent of other pollutants (Simpson et al. 2005a; Burnett et al. 1998; Kwon et al. 2001; Hong et al. 2002). Mean NO₂ levels in these studies ranged from about 16.3 to 32.5 ppb.

NO₂ exposure has also been associated with cardiovascular disease. In the U.S. a panel study of cardiac arrhythmias and studies of hospitalizations and emergency room visits for cardiovascular disease found potentially independent effects of NO₂ in areas with average NO₂ levels between 23 and 37 ppb (Peters et al. 2000; Metzger et al. 2004; Wellenius et al. 2005). These findings are supported by similar findings in a study conducted outside of the U.S. (Simpson et al. 2005 b.)

In summary, epidemiologic studies of both short-term and long term effects of NO₂ on respiratory disease in the U.S. and Europe have been particularly robust, and many have found independent effects. (Figure 1) The range of means in these studies was 18 to 57 ppb with many in the range of 23 to 37 ppb. While some short-term studies have suggested an independent effect of NO₂, there is a real possibility that the NO₂ effects in both short and long-term studies may be due to measured or unmeasured indoor or outdoor co-pollutants that are products of traffic and/or fuel combustion such as ultrafines, elemental carbon, acid vapor, fine particles or NO. For example, Seaton and Dennekamp (2003) proposed that the association of NO₂ levels with cardiovascular mortality and morbidity occurs because NO₂ is closely associated with release of ultrafine particles in ambient air. In addition, animal toxicology studies demonstrate adverse effects on the lung from prolonged NO₂ exposure. Thus, prudent public health policy warrants that some level of protection from exposure to NO₂ be specified.

The empirical relation between the one-hour and annual average provides additional support for this proposed standard. Clearly the one-hour and annual average standards are linked. For example, attainment of the 0.18 ppm one-hour standard would succeed in lowering the entire distribution of daily exposures at all durations. Therefore, this standard will afford some increased degree of protection from longer-term exposures, and vice versa.

Our analysis of data in California indicates that that 99th percentile and the single highest value of the one-hour average NO₂ is roughly 4 to 6 times that of the annual average (see Table 1). Therefore, a 1-hour standard of 0.18 ppm is associated with an annual average of between 0.044 and 0.029 ppm. However, the ratio varies by district so the controlling standard will also vary by air district, as well. Taken together, the recommended annual average of 0.030 ppm is consistent with the proposed short-term standard of 0.18 ppm and should provide the additional protection needed from long-term exposures.

As such, given the seriousness of the potential effects, we recommend an annual average standard for NO₂ be adopted. Based on these studies we recommend that an

annual average standard of 0.030 ppm be adopted. While some studies with a mean concentration below this level suggest the possibility of effects related to exposure to NO₂, substantial uncertainties in the exposure metric remain. Therefore, OEHHA staff has not recommended an annual average standard that is below the means observed in all of these studies. The recommended standard is likely to afford a sufficient level of protection for both individuals.

The empirical relation between the one-hour and annual average provides additional support for this proposed standard. Clearly the one-hour and annual average standards are linked. For example, attainment of the 0.18 ppm one-hour standard would succeed in lowering the entire distribution of daily exposures at all durations. Therefore, this standard will afford some increased degree of protection from longer-term exposures, and vice versa.

Consideration of Infants and Children in Recommending the NO₂ Standard

The recommended 1 hr standard of 0.18 ppm is based on results of the human exposure studies. These studies indicate that asthmatics are more susceptible to NO₂. Also, in general, the time series studies have found that the effects of NO₂ on asthma outcomes appear to be more robust than either the time-series mortality or hospitalization studies, supporting that asthmatics are a susceptible population. There is no direct data to suggest that children with asthma are more susceptible to NO₂ than adults, so this was not explicitly taken into consideration when determining the recommended standards. However, asthma is the most common chronic childhood disease; nearly 9.6% of California's children have had asthma symptoms in the last year. Further, children with asthma have a higher degree of airway responsiveness compared with adult asthmatics, and thus children with asthma may be more responsive to NO₂.

Several epidemiological studies in children were considered in weighing the evidence to support a longer-term standard (annual average). These studies, including the longitudinal epidemiological studies on the association between measured NO₂ (indoors) and increased risk of wheezing and persistent cough in infants with a strong family history of asthma (van Strien et al. 2004, Belanger et al. 2003, 2006), are evidence that NO₂ has respiratory effects on young children. Additionally, using personal monitors, Chauhan et al. (2003) found that higher exposures to NO₂ were associated with more severe viral-related asthma exacerbations in children with asthma or at risk for asthma. Finally, the Children's Health Study conducted in Southern California between 1993 and 2001, followed children through adolescence and found that those children who resided in communities with high levels of NO₂ and other co-pollutants had reduced lung growth compared to those living in less polluted communities (Gauderman et al. 2004). This reduction in lung growth is especially important, as reduced attained lung function as a young adult is a strong predictor for cardiovascular disease and mortality in adulthood. Although we cannot ascribe all the effects of lung growth to NO₂, there is evidence that NO₂, a strong respiratory oxidant, causes airway inflammation.

Additionally, animal studies suggest that potentially permanent structural changes to the lung during development may occur from prolonged exposure to relatively low levels of

NO₂ (0.25 ppm), but may not become fully apparent until later in life. This underscores the potential that NO₂ at the ambient levels observed in the Children's Health Study impacts lung function growth in children. Further, additional human exposure studies, toxicology studies, and epidemiological studies are needed to determine the extent of NO₂ toxicity both separately and in combination with other ambient co-pollutants.

Finally, there is considerable spatial variability in exposures to NO₂ and current monitoring locations may not capture the areas with higher concentrations within the region. Children living in census tracts with high traffic density and attending CA public schools located near busy roads are likely to be exposed to concentrations that are higher than those reported by most NO₂ monitors, which by design, measure general population exposure. There is some evidence that these highly exposed groups may be disproportionately minority and economically disadvantaged (Green et al., 2004; Gunier et al., 2003).

Summary of OEHHA Recommendation:

- 1) Nitrogen dioxide continues to be the indicator for nitrogen oxide air pollutants.
- 2) Retention of a 1-hour standard and the addition of an annual average standard.
- 3) Decreases the 1-hour average standard of 0.18 ppm, not to be exceeded and add an annual average of 0.030 ppm, not to be exceeded. Such a standard would protect against both 1-hour concentrations and repeated or long-term exposures to nitrogen dioxide.

References

- American Thoracic Society. 1985. Guidelines as to what constitutes an adverse respiratory health effect, with special reference to epidemiologic studies of air pollution. *Am Rev Respir Dis* 131:666-8.
- American Thoracic Society. 2000. What constitutes an adverse health effect of air pollution? *Am J Respir Crit Care Med* 161:665-73.
- Anderson HR, Ponce de Leon A, Bland JM, Bower JS, Emberlin J, Strachan DP. Air pollution, pollens, and daily admissions for asthma in London 1987-92. *Thorax* 1998; 53: 842-848.
- Atkinson RW, Anderson HR, Strachan DP, Bland JM, Bremner SA, Ponce de Leon A. Short-term associations between outdoor air pollution and visits to accident and emergency departments in London for respiratory complaints. *Eur Respir J*. 1999a;13:257-265.
- Avol, E. L., Linn, W. S., Peng, R. C., Whynot, J. D., Shamoo, D. A., Little, D. E., Smith, M. N., and Hackney, J. D. Experimental exposures of young asthmatic volunteers to 0.3 ppm nitrogen dioxide and to ambient air pollution. *Toxicol Ind Health*. 1989 Dec; 5(6):1025-34.
- Azadniv M, Utell M.J., Morrow P.E., Gibb F.R., Nichols J., Roberts N.J. Jr., Speers D.M., Torres A., Tsai Y., Abraham M.K., Voter K.Z., Frampton M.W. 1998. Effects of nitrogen dioxide exposure on human host defense. *Inhal Toxicol* 10(6):585-601.
- Barck C, Lundahl J, Hallden G, Bylin G. 2005. Brief exposures to NO₂ augment the allergic inflammation in asthmatics. *Environ Res* 95:58-66.
- Barck C, Sandstrom T, Lundahl J, Hallden G, Svartengren M, Strand V, Rak S, Bylin G. 2002. Ambient level of NO₂ augments the inflammatory response to inhaled allergen in asthmatics. *Respir Med* 96(11):907-917.
- Barnett AG, Williams GM, Schwartz J, Neller AH, Best TL, Petroeschevsky AL, Simpson RW. Air pollution and child respiratory health: a case-crossover study in Australia and New Zealand. *Am J Respir Crit Care Med*. 2005 Jun 1;171(11):1272-8. Epub 2005 Mar 11.
- Barth PJ, Muller B, Wagner U, Bittinger A. Assessment of proliferative activity in type II pneumocytes after inhalation of NO₂ by AgNOR-analysis. *Exp Toxicol Pathol* 1994; 46:335-42.
- Bauer, M. A., Utell M. J., Morrow P. E., Speers D. M., and Gibb F. R. 1986. Inhalation of 0.30 ppm nitrogen dioxide potentiates exercise-induced bronchospasm in asthmatics. *Am Rev Respir Dis* 134:1203-8.
- Bayram, H., Rusznak, C., Khair, O. A., Sapsford, R. J., and Abdelaziz, M. M. (2002). Effect of ozone and nitrogen dioxide on the permeability of bronchial epithelial cell cultures of non-asthmatic and asthmatic subjects. *Clin Exp Allergy* 32, 1285-1292.
- Bayram, H., Sapsford, R. J., Abdelaziz, M. M., and Khair, O. A. (2001). Effect of ozone and nitrogen dioxide on the release of proinflammatory mediators from bronchial

epithelial cells of nonatopic nonasthmatic subjects and atopic asthmatic patients in vitro. *J Allergy Clin Immunol* **107**, 287-294.

Belanger K, Beckett W, Triche E, Bracken MB, Holford T, Ren P et al. Symptoms of wheeze and persistent cough in the first year of life: associations with indoor allergens, air contaminants, and maternal history of asthma. *Am J Epidemiol* 2003;158:195-202.

Belanger K, Gent JF, Triche EW, Bracken MB, Leaderer BP. Association of Indoor Nitrogen Dioxide Exposure with Respiratory Symptoms in Asthmatic Children. *Am J Respir Crit Care Med* 2006; 173:297-303.

Blomberg, A., Krishna M. T., Bocchino V., Biscione G. L., Shute J. K., Kelly F. J., Frew A. J., Holgate S. T., and Sandstrom T. 1997. The inflammatory effects of 2 ppm NO₂ on the airways of healthy subjects. *Am J Respir Crit Care Med* 156:418-24.

Burnett RT, Cakmak S, Brook JR. The effect of the urban ambient air pollution mix on daily mortality rates in 11 Canadian cities. *Can J Publ Health* 1998;89:152-156.

Bylin G, Hedenstierna G, Lindvall T, Sundin B. Ambient nitrogen dioxide concentrations increase bronchial responsiveness in subjects with mild asthma. *Eur Respir J.* 1988 Jul; 1(7):606-12.

California Air Resources Board (CARB). Review of the One-Hour Ambient Air Quality Standard for Nitrogen Dioxide: Technical Report and Staff Report, December 1992.

California Health Interview Survey (CHIS), 2001. Meng, Y-Y, Babey, SH, Malcom, E, Brown, ER, Neetu, C. Asthma in California: Findings from the 2001 California Health Interview Survey. Available at URL:
<http://www.healthpolicy.ucla.edu/pubs/publication.asp?pubID=83>

Chang, L. Y., Graham, J. A., Miller, F. J., Ospital, J. J., and Crapo, J. D. (1986). Effects of subchronic inhalation of low concentrations of nitrogen dioxide. I. The proximal alveolar region of juvenile and adult rats. *Toxicol Appl Pharmacol* **83**, 45-61

Chauhan AJ, Inskip HM, Linaker CH, Smith S, Schreiber J, Johnston SL et al. Personal exposure to nitrogen dioxide (NO₂) and the severity of virus-induced asthma in children. *Lancet* 2003;61:1939-1944.

Davis JK, Davidson M, Schoeb TR. Murine respiratory mycoplasmosis: a model to study effects of oxidants. *Res Rep Health Eff Inst* 1991:1-29; discussion 31-43.

Delfino RJ, Gong H Jr, Linn WS, Pellizzari ED, Hu Y. Asthma symptoms in Hispanic children and daily ambient exposures to toxic and criteria air pollutants. *Environ Health Perspect* 2003;111:647-656.

Delfino RJ, Zeiger RS, Seltzer JM, Street DH, McLaren CE. Association of asthma symptoms with peak particulate air pollution and effect modification by anti-inflammatory medication use. *Environ Health Perspect* 2002;110:A607-17.

Delfino RJ, Quintana PJE, Floro J, Gastañaga VM, Samimi BS, Kleinman MT, Liu L-JS, Bufalino C, Wu C-F, McLaren CE. Association of FEV₁ in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. *Environ Health Perspect.* 2004; 112: 932-41.

- Devalia, J. L., Rusznak C., Herdman M. J., Trigg C. J., Tarraf H., and Davies R. J. 1994. Effect of nitrogen dioxide and sulphur dioxide on airway response of mild asthmatic patients to allergen inhalation. *Lancet* 344:1668-71.
- Devlin, R. B., Horstman D. P., Gerrity T. R., Becker S., Madden M. C., Biscardi F., Hatch G. E., and Koren H. S. 1999. Inflammatory response in humans exposed to 2.0 ppm nitrogen dioxide. *Inhal Toxicol* 11:89-109.
- Dockery DW, Luttmann-Gibson H, Rich DQ, Link MS, Mittleman MA, Gold DR, Koutrakis P, Schwartz JD, Verrier RL. Association of air pollution with increased incidence of ventricular tachyarrhythmias recorded by implanted cardioverter defibrillators. *Environ. Health. Perspect.* 2005; 113: 670-674.
- Dockery DW, Pope AC, Xu X et al. An association between air pollution and mortality in six U.S. cities. *N Engl J Med* 1993;329:1753-1759.
- Dominici F, McDermott A, Daniels M, Zeger SL, Samet JM. 2003. Mortality among residents of 90 cities. Health Effects Institute. Revised analyses of time-series studies of air pollution and health. Special Report. Capital City Press, Montpelier VT.
- Drechsler-Parks, D. M. 1995. Cardiac output effects of O₃ and NO₂ exposure in healthy older adults. *Toxicol Ind Health* 11:99-109.
- Ehrlich, R., Findlay, J. C., and Gardner, D. E. (1979). Effects of repeated exposures to peak concentrations of nitrogen dioxide and ozone on resistance to streptococcal pneumonia. *J Toxicol Environ Health* 5, 631-642.
- Ehrlich, R., and Henry, M. C. (1968). Chronic toxicity of nitrogen dioxide. I. Effect on resistance to bacterial pneumonia. *Arch Environ Health* 17, 860-865
- Folinsbee LJ. 1992. Does nitrogen dioxide exposure increase airways responsiveness? *Toxicol Ind Health* 8(5):273-83.
- Forastiere, F.; Corbo, G. M.; Dell'Orco, V.; Pistelli, R.; Agabiti, N., and Kriebel, D. A longitudinal evaluation of bronchial responsiveness to methacholine in children: role of baseline lung function, gender, and change in atopic status. *Am J Respir Crit Care Med.* 1996 Mar; 153(3):1098-104.
- Frampton, M. W., Morrow P. E., Gibb F. R., Speers D. M., and Utell M. J. 1991. Effects of nitrogen dioxide exposure on pulmonary function and airway reactivity in normal humans. *Am Rev Respir Dis* 143:522-27.
- Fujimaki, H., Ohmori, T., Ushio, H., and Saneyoshi, K. (1998). Timing of low-level NO₂ exposure alters antigen-specific IgE, IgG1, and IgG2a antibody production in mice. *Inhal Toxicol* 10, 1079-1093
- Galan I, Tobias A, Banegas JR, Aránguez E. Short-term effects of air pollution on daily asthma emergency room admissions. *Eur Respir J.* 2003; 22: 802-08.
- Garrett MH, Hooper MA, Hooper BM, Abramson MJ. Respiratory symptoms in children and indoor exposure to nitrogen dioxide and gas stoves. *Am J Respir Crit Care Med* 1998; 158:891-5.

Gauderman WJ, Avol E, Gilliland F, Vora H, Thomas D, Berhane K, McConnell R, Kuenzli N, Lurmann F, Rappaport E, Margolis H, Bates D, Peters JM. The effect of air pollution on lung development from 10 to 18 years of age. *N.Engl.J Med.* 2004; 351 (11):1057-1067, 2004.

Gauderman WJ, Avol E, Lurmann F, Kuenzli N, Gilliland F, Peters J, McConnell R. Childhood asthma and exposure to traffic and nitrogen dioxide. *Epidemiology* 2005; 16:737-43.

Gauderman WJ, Gilliland F, Vora H, Avol E, Stram D, McConnell R, Thomas D, Lurmann F, Margolis HG, Rappaport EB, Berhane K, Peters J. Association between air pollution and lung function growth in southern California children. Results from a second cohort. *Am J Respir Crit Care Med* 2002; 166: 76-84.

Gehring U, Cyrus J, Sedlmeir G et al. Traffic-related air pollution and respiratory health during the first 2 yrs of life. *Eur.Respir.J.* 2002; 19: 690-698.

Gilmour, M. I. 1995. Interaction of air pollutants and pulmonary allergic responses in experimental animals. *Toxicology* 105:335-42.

Gilmour, M. I., Park, P., and Selgrade, M. J. (1996). Increased immune and inflammatory responses to dust mite antigen in rats exposed to 5 ppm NO₂. *Fundam Appl Toxicol* **31**, 65-70

Green RS, Smorodinsky S, Kim JJ, McLaughlin R, Ostro B. 2004. Proximity of California public schools to busy roads. *Environ Health Perspect* 112:61-6.

Gunier RB, Hertz A, Von Behren J, Reynolds P. Traffic density in California: Socioeconomic and ethnic differences among potentially exposed children. *J Expo Anal Environ Epidemiol* 2003; 13:240-6.

Ha EH, Hong YC, Lee BE, Woo BH, Schwartz J, Christiani DC. Is air pollution a risk factor for low birth weight in Seoul? *Epidemiology* 2001; 12: 643-648.

Hajat S, Haines A, Goubet SA, Atkinson RW, Anderson HR. Association of air pollution with daily GP consultations for asthma and other lower respiratory conditions in London. *Thorax.* 1999;54 (7):597-605.

Halinen AI, Salonen RO, Pennanen AS, Kosma VM. Combined respiratory effects of cold air with SO₂ or NO₂ in single 1-hour exposures of hyperventilating guinea pigs. *Inhal Toxicol* 2000a; 12:693-713.

Halinen AI, Salonen RO, Pennanen AS, Kosma VM. Combined respiratory effects of cold air with SO₂ or NO₂ in repeated 10-minute exposures of hyperventilating guinea pigs. *Inhal Toxicol* 2000b; 12:671-91.

Hasselblad V, Eddy DM, Kotchmar DJ. Synthesis of environmental evidence: nitrogen dioxide epidemiology studies. *J Air Waste Manage Assoc.* 1992; 42: 662-71. 9;54:597-605.

Hayashi, Y., and Kohno, T. (1985). A pathological study on effects of nitrogen dioxide on the respiratory system in rats. In: *Experimental studies on health effects of nitrogen dioxides*. Special Research Project of Environmental Science, Ministry of Education

Science and Culture, Japan Researches on Human Health Effects. Kagawa Medical School, Kagawa, Japan. (Environ. Science Res. Report, B233-R20-1). 31-44.

Hayashi, Y., Kohno, T., and Ohwada, H. (1987). Morphological effects of nitrogen dioxide on the rat lung. *Environ Health Perspect* **73**, 135-145

Hoek G, Brunekreef B, Verhoeff A, van Wijnen J, Fischer P. Daily mortality and air pollution in the Netherlands. *J Air Waste Manage Assoc* 2000; 50:1380-1389

Hong YC, Lee JT, Kim H, Ha EH, Schwartz J, Christiani DC. Effects of air pollutants on acute stroke mortality. *Environ Health Perspect* 2002;110:187-191.

Hubbard AK, Symanowicz PT, Thibodeau M, Thrall RS, Schramm CM, Cloutier MM, Morris JB. Effect of nitrogen dioxide on ovalbumin-induced allergic airway disease in a murine model. *J Toxicol Environ Health A* 2002; 65:1999-2005.

Ichinose T, Fujii K, Sagai M. Experimental studies on tumor promotion by nitrogen dioxide. *Toxicology* 1991; 67:211-25.

Ichinose T, Sagai M. Biochemical effects of combined gases of nitrogen dioxide and ozone. III. Synergistic effects on lipid peroxidation and antioxidative protective systems in the lungs of rats and guinea pigs. *Toxicology* 1989; 59:259-70.

Janssen NA, Brunekreef B, van Vliet P, et al. The relationship between air pollution from heavy traffic and allergic sensitization, bronchial hyperresponsiveness, and respiratory symptoms in Dutch schoolchildren. *Environ Health Perspect* 2003; 111: 1512-1518.

Jenkins, H. S., Devalia J. L., Mister R. L., Bevan A. M., Rusznak C., and Davies R. J. 1999. The effect of exposure to ozone and nitrogen dioxide on the airway response of atopic asthmatics to inhaled allergen. *Am J Respir Crit Care Med* 160:33-39.

Johnson DA, Winters RS, Lee KR, Smith CE. Oxidant effects on rat and human lung proteinase inhibitors. *Res Rep Health Eff Inst* 1990:1-39.

Jörres, R., and Magnussen H. 1990. Airways response of asthmatics after a 30 min exposure, at resting ventilation, to 0.25 ppm NO₂ or 0.5 ppm SO₂. *Eur Respir J* 3:132-37.

Jörres, R., Nowak D., Grimminger F., Seeger W., Oldigs M., and Magnussen H. 1995. The effect of 1 ppm nitrogen dioxide on bronchoalveolar lavage cells and inflammatory mediators in normal and asthmatic subjects. *Eur Respir J* 8:416-24.

Katsouyanni K, Touloumi G, Samoli E, Gryparis A, Le Tertre A, Monopolis Y, Rossi G, Zmirou D, Ballester F, Boumghar A, Anderson HR, Wojtyniak B, Paldy A, Braunstein R, Pekkanen J, Schindler C, Schwartz J. Confounding and effect modification in the short-term effects of ambient particles on total mortality: results from 29 European cities within the APHEA2 project. *Epidemiology*. 2001; 12: 521-31.

Kienast, K., Knorst, M., Lubjuhn, S., Muller-Quernheim, J., and Ferlinz, R. (1994). Nitrogen dioxide-induced reactive oxygen intermediates production by human alveolar macrophages and peripheral blood mononuclear cells. *Arch Environ Health* **49**, 246-250.

- Kienast, K., Knorst, M., Muller-Quernheim, J., and Ferlinz, R. (1996). Modulation of IL-1 beta, IL-6, IL-8, TNF-alpha, and TGF-beta secretions by alveolar macrophages under NO₂ exposure. *Lung* **174**, 57-67.
- Kim JJ, Smorodinsky S, Lipsett M, Singer BC, Hodgson AT, Ostro B. Traffic-related air pollution near busy roads: the East Bay Children's Respiratory Health Study. *Am J Respir Crit Care Med* 2004; 170:520-6.
- Kitabatake, M., Yamamoto, H., Yuan, P. F., Manjurul, H., Murase, S., and Yamauchi, T. (1995). Effects of exposure to NO₂ or SO₂ on bronchopulmonary reaction induced by *Candida albicans* in guinea pigs. *J Toxicol Environ Health* **45**, 75-82
- Kleinman, M. T., R. M. Bailey, W. S. Linn, K. R. Anderson, J. D. Whynot, D. A. Shamoo, and J. D. Hackney. 1983. Effects of 0.2 ppm nitrogen dioxide on pulmonary function and response to bronchoprovocation in asthmatics. *J Toxicol Environ Health* 12: 815-26.
- Kobayashi T, Miura T. Concentration- and time-dependent increase in specific airway resistance after induction of airway hyperresponsiveness by subchronic exposure of guinea pigs to nitrogen dioxide. *Fundam Appl Toxicol* 1995; 25:154-8.
- Koenig, J. Q., D. S. Covert, M. S. Morgan, M. Horike, N. Horike, S. G. Marshall, and W. E. Pierson. 1985. Acute effects of 0.12 ppm ozone or 0.12 ppm nitrogen dioxide on pulmonary function in healthy and asthmatic adolescents. *Am Rev Respir Dis* 132: 648-51.
- Kramer U, Koch T, Ranft U, Ring J, Behrendt H. Traffic-related air pollution is associated with atopy in children living in urban areas. *Epidemiology* 2000; 11: 64-70.
- Krewski D, Burnett RT, Goldberg MS, Hoover K, Siemiatycki J, Jerrett M, Abrahamowicz M, White WH, and others; Reanalysis of the Harvard Six Cities Study and the American Cancer Society study of particulate air pollution and mortality. Health Effects Institute. July 2000.
- Krishna MT, Holgate ST. 1999. Inflammatory mechanisms underlying potentiation of effects of inhaled aeroallergens in response to nitrogen dioxide in allergic airways disease. *Clin Exp Allergy* 29(2):150-4.
- Kubota, K., Murakami, M., Takenaka, S., Kawai, K., and Kyono, H. (1987). Effects of long-term nitrogen dioxide exposure on rat lung: morphological observations. *Environ Health Perspect* **73**, 157-169
- Kuraitis, K. V., and Richters, A. (1989). Spleen cellularity shifts from the inhalation of 0.25-0.35 PPM nitrogen dioxide. *J Environ Pathol Toxicol Oncol* **9**, 1-11
- Kwon HJ, Cho SH, Nyberg F, Pershagen G. Effects of ambient air pollution on daily mortality in a cohort of patients with congestive heart failure. *Epidemiology* 2001;12:413-419
- Lee JT, Kim H, Song H, Hong YC, Cho YS, Shin SY, Hyun YJ, Kim YS. Air pollution and asthma among children in Seoul, Korea. *Epidemiology* 2002; 13: 481-4.
- Lee SL, Wong WH, Lau YL. Association between air pollution and asthma admission among children in Hong Kong. *Clin Exp Allergy* 2006;36:1138-46.

Lin M, Chen Y, Burnett RT, Villeneuve PJ, Krewski D. The influence of ambient coarse particulate matter on asthma hospitalization in children: case-crossover and time-series analyses. *Environ Health Perspect* 2002; 110: 575-81.

Lin M, Chen Y, Burnett RT, Villeneuve PJ, Krewski D. Effect of short-term exposure to gaseous pollution on asthma hospitalisation in children: a bi-directional case-crossover analysis. *J Epidemiol Community Health* 2003; 57(1): 50-5.

Liu S, Krewski D, Shi Y, Chen Y, Burnett RT. Association between gaseous ambient air pollutants and adverse pregnancy outcomes in Vancouver, Canada. *Environ Health Perspect* 2003; 111: 1773-1778.

Mann JK, Tager IB, Lurmann F, Segal M, Quesenberry CP Jr, Lugg MM, Shan J, Van Den Eeden SK. Air pollution and hospital admissions for ischemic heart disease in persons with congestive heart failure or arrhythmia. *Environ Health Perspect* 2002; 110: 1247-52.

Mar TF, Norris GA, Koenig JQ, Larson TV. Associations between air pollution and mortality in Phoenix, 1995-1997. *Environ Health Perspect* 2000;108:347-353

Martinez FD, Wright AL, Taussig LM, Holberg CJ, Halonen M, Morgan WJ. 1995. Asthma and wheezing in the first six years of life. The Group Health Medical Associates. *N Engl J Med* 332(3):133-8. Mohsenin, V. 1987a. Airway responses to nitrogen dioxide in asthmatic subjects. *J Toxicol Environ Health* 22: 371-80.

Mautz, W. J., Kleinman, M. T., Bhalla, D. K., and Phalen, R. F. (2001). Respiratory tract responses to repeated inhalation of an oxidant and acid gas-particle air pollutant mixture. *Toxicol Sci* **61**, 331-341

McConnell R, Berhane K, Gilliland F et al. Prospective Study of Air Pollution and Bronchitic Symptoms in Children with Asthma. *Am.J.Respir.Crit Care Med.* 2003; 168: 790-797.

Mercer RR, Costa DL, Crapo JD. Effects of prolonged exposure to low doses of nitric oxide or nitrogen dioxide on the alveolar septa of the adult rat lung. *Lab Invest* 1995; 73:20-8.

Metzger KB, Tolbert PE, Klein M, Peel JL, Flanders WD, Todd K, Mulholland JA, Ryan PB, Frumkin H. Ambient air pollution and cardiovascular emergency department visits. *Epidemiology* 2004; 15: 46-56.

Mi, H., Hiramoto, K., Kujirai, K., Ando, K., Ikarashi, Y., and Kikugawa, K. (2002). Effects of vitamin E-deficiency and/or nitrogen dioxide inhalation on allergen-sensitized type IV and type I allergy responses of mice. . *Journal of Health Science* **48**, 22-29

Miller, F. J., Graham, J. A., Raub, J. A., Illing, J. W., Menache, M. G., House, D. E., and Gardner, D. E. (1987). Evaluating the toxicity of urban patterns of oxidant gases. II. Effects in mice from chronic exposure to nitrogen dioxide. *J Toxicol Environ Health* **21**, 99-112

Miller, F. J., Overton, J. H., Jr., Myers, E. T., and Graham, J. A. (1982). Pulmonary dosimetry of nitrogen dioxide in animals and man. In: *Air Pollution by Nitrogen Oxides*.

Proceedings of the US-Dutch International Symposium, Maastricht, The Netherlands, May 24-28, 1982. T. Schneider and L. Grant Eds. Elsevier Scientific Publishing Co. New York, NY :pp. 377-85

Mohsenin, V. 1987b. Effect of vitamin C on NO₂-induced airway hyperresponsiveness in normal subjects. *Am Rev Respir Dis* 136: 1408-11.

Morrow, P. E., Utell M. J., Bauer M. A., Smeglin A. M., Frampton M. W., Cox C., Speers D. M., and Gibb F. R. 1992. Pulmonary performance of elderly normal subjects and subjects with chronic obstructive pulmonary disease exposed to 0.3 ppm nitrogen dioxide. *Am Rev Respir Dis* 145:291-300.

Moshhammer H, Hutter HP, Hauck H, Neuberger M. Low levels of air pollution induce changes of lung function in a panel of school children. *European Respiratory Journal Express*. 2006; Published online February 2, 2006.

Nafstad P, Haheim LL, Wisloff T et al. Urban air pollution and mortality in a cohort of norwegian men. *Environ. Health Perspect*. 2004; 112: 610-615.

Nisizawa T, Saito M, Nakayama K, Nishihara T, Imai S, Nakagawa M. Effects of nitrogen dioxide on Mycoplasma Pulmonis infection and humeral immune responses in mice. *Japan J Med Sci Biol* 1988; 41:175-87.

Norris G, Young Pong SN, Koenig JQ, Larson TV, Sheppard L, Stout JW. An association between fine particles and asthma emergency department visits for children in Seattle. *Environ Health Perspect*. 1999; 107(6): 489-93.

Nyberg F, Gustavsson P, Jarup L et al. Urban air pollution and lung cancer in Stockholm. *Epidemiology* 2000; 11: 487-495.

Ohashi, Y., Nakai, Y., Okamoto, H., Sugiura, Y., Ohno, Y., Hashimoto, M., and Uozumi, M. (1998). Nitrogen dioxide modifies allergic inflammation in tracheal mucosa. *Acta Otolaryngol Suppl* **538**, 227-232

Orehek, J., J. P. Massari, P. Gayrard, C. Grimaud, and J. Charpin. 1976. Effect of short-term, low-level nitrogen dioxide exposure on bronchial sensitivity of asthmatic patients. *J Clin Invest* 57: 301-7.

Overton, J. H., Jr. (1984). Physicochemical processes and the formulation of dosimetry models. *J Toxicol Environ Health* **13**, 273-294

Papi, A., Amadesi, S., Chitano, P., Boschetto, P., Ciaccia, A., Geppetti, P., Fabbri, L. M., and Mapp, C. E. (1999). Bronchopulmonary inflammation and airway smooth muscle hyperresponsiveness induced by nitrogen dioxide in guinea pigs. *Eur J Pharmacol* **374**, 241-247

Peat JK, Gray EJ, Mellis CM, Leeder SR, Woolcock AJ. 1994. Differences in airway responsiveness between children and adults living in the same environment: an epidemiological study in two regions of New South Wales. *Eur Respir J* 7(10):1805-13.

Peat JK, Salome CM, Xuan W. 1996. On adjusting measurements of airway responsiveness for lung size and airway caliber. *Am J Respir Crit Care Med* 154(4 Pt 1):870-5.

Peel JL, Tolbert PE, Klein M, Metzger KB, Flanders WD, Todd K, Mulholland JA, Ryan PB, Frumkin H. 2005. Ambient air pollution and respiratory emergency department visits. *Epidemiology* 16:164-74.

Pekkanen J, Peters A, Hoek G, Tiittanen P, Brunekreef B, de Hartog J, et al. 2002. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the Exposure and Risk Assessment for Fine and Ultrafine Particles in Ambient Air (ULTRA) study. *Circulation* 106:933-938

Peters A, Liu E, Verrier RL et al. Air pollution and incidence of cardiac arrhythmia. *Epidemiology* 2000; 11: 11-17.

Peters JM, Avol E, Navidi W et al. A study of twelve Southern California communities with differing levels and types of air pollution. I. Prevalence of respiratory morbidity. *Am J Respir Crit Care Med* 1999b; 159: 760-767.

Pilotto LS, Nitschke M, Smith BJ, Pisaniello D, Ruffin RE, McElroy HJ, Martin J, Hiller JE. Randomized controlled trial of unflued gas heater replacement on respiratory health of asthmatic schoolchildren. *Int J Epidemiol* 2004; 33:208-14.

Pope CA, III, Burnett RT, Thun MJ et al. Lung cancer, cardiopulmonary mortality, and long-term exposure to fine particulate air pollution. *JAMA* 2002; 287: 1132-1141.

Rasmussen RE, McClure TR. Effect of chronic exposure to NO₂ in the developing ferret lung. *Toxicol Lett* 1992; 63:253-60.

Rich DQ, Schwartz J, Mittleman MA, Link M, Luttmann-Gibson H, Catalano PJ, Speizer FE, Dockery DW. Association of short-term ambient air pollution concentrations and ventricular arrhythmias. *Am J Epidemiol*. 2005 Jun 15;161(12):1123-32.

Richters, A., and Damji, K. S. (1988). Changes in T-lymphocyte subpopulations and natural killer cells following exposure to ambient levels of nitrogen dioxide. *J Toxicol Environ Health* **25**, 247-256

Richters A, Richters V. Nitrogen dioxide (NO₂) inhalation, formation of microthrombi in lungs and cancer metastasis. *J Environ Pathol Toxicol Oncol* 1989; 9:45-51.

Robison, T. W., Murphy, J. K., Beyer, L. L., Richters, A., and Forman, H. J. (1993). Depression of stimulated arachidonate metabolism and superoxide production in rat alveolar macrophages following in vivo exposure to 0.5 ppm NO₂. *J Toxicol Environ Health* **38**, 273-292.

Rose RM, Pinkston P, Skornik WA. Altered susceptibility to viral respiratory infection during short-term exposure to nitrogen dioxide. *Res Rep Health Eff Inst* 1989:1-24.

Rusznak, C., Devalia J. L., and Davies R. J. 1996. Airway response of asthmatic subjects to inhaled allergen after exposure to pollutants. *Thorax* 51:1105-8.

Ryan PB, Frumkin H. Ambient air pollution and respiratory emergency department visits. *Epidemiology*. 2005; 16:164-74.

Saez M, Ballester F, Barcelo MA, Perez-Hoyos S, Bellido J, Tenias JM, Ocana R, Fidueiras A, Arribas F, Aragonés N, Tobias A, Cirera L, Canada A on behalf of the

EMECAM group. A combined analysis of the short-term effects of photochemical air pollutants on mortality within the EMECAM project. *Environ Health Perspectives* 2002; 110:221-228

Samet JM, Lambert E, Skipper BJ et al. Nitrogen dioxide and respiratory illnesses in infants. *Am Rev Respir Dis* 1993; 148:1258–65.

Samoli E, Aga E, Touloumi G, Nisiotis K, Forsberg B, Lefranc A, Pekkanen J, Wojtyniak B, Schindler C, Niciu E, Brunstein R, Dodic Fikfak M, Schwartz J, Katsouyanni K. Short-term effects of nitrogen dioxide on mortality: an analysis within the APHEA project. *European Respiratory Journal Express*. 2006; Published online March 15, 2006.

Sandstrom, T., Andersson M. C., Kolmodin-Hedman B., Stjernberg N., and Angstrom T. 1990. Bronchoalveolar mastocytosis and lymphocytosis after nitrogen dioxide exposure in man: a time-kinetic study. *Eur Respir J* 3:138-43.

Sandstrom, T., Ledin M. C., Thomasson L., Helleday R., and Stjernberg N. 1992b. Reductions in lymphocyte subpopulations after repeated exposure to 1.5 ppm nitrogen dioxide. *Br J Ind Med* 49:850-854.

Sarnat JA, Brown KW, Schwartz J, Coull BA, Koutrakis P. 2005. Ambient gas concentrations and personal particulate matter exposures: implications for studying the health effects of particles. *Epidemiology* 2005; 16:385-95.

Sarnat JA, Schwartz J, Catalano PJ, Suh HH. Gaseous pollutants in particulate matter epidemiology: confounders or surrogates? *Environ Health Perspect* 2001; 109:1053-61.

Schierhorn, K., Zhang, M., Matthias, C., and Kunkel, G. (1999). Influence of ozone and nitrogen dioxide on histamine and interleukin formation in a human nasal mucosa culture system. *Am J Respir Cell Mol Biol* **20**, 1013-1019

Schildcrout JS, Sheppard L, Lumley T, Slaughter JC, Koenig JQ, Shapiro GG. Ambient air pollution and asthma exacerbations in children: an eight-city analysis. *Am J Epidemiol* 2006;164:505-17.

Schlesinger, R. B. (1987). Intermittent inhalation of nitrogen dioxide: effects on rabbit alveolar macrophages. *J Toxicol Environ Health* **21**, 127-139

Seaton A, Dennekamp M. Hypothesis: ill health associated with low concentrations of nitrogen dioxide--an effect of ultrafine particles? *Thorax*. 2003; 58: 1012-5.

Selgrade MK, Daniels MJ, Grose EC. Evaluation of Immunotoxicity of an Urban Profile of Nitrogen Dioxide: Acute, Subchronic, and Chronic Studies. *Inhal Toxicol* 1991; 3:389-403.

Sherwin, R. P., and Carlson, D. A. (1973). Protein content of lung lavage fluid of guinea pigs exposed to 0.4 ppm nitrogen dioxide. *Arch Environ Health* **27**, 90-93

Sherwin, R. P., and Layfield, L. J. (1976). Protein leakage in the lungs of mice exposed to 0.5 ppm nitrogen dioxide. *Arch Environ Health* **31**, 116-118

Sherwin RP, Richters V, Richters A. Image analysis quantitation of type 2 cells and alveolar walls part II: Influence of 0.3 ppm nitrogen dioxide exposure on the developing mouse lung. *Journal of the American College of Toxicology* 1985; 4:27-43.

Sherwin RP, Richters V. Effects of 0.25 PPM nitrogen dioxide on the developing mouse lung. Part 1: Quantitation of type 2 cells and measurements of alveolar walls. *Inhal Toxicol* 1995a; 7:1173-82.

Sherwin RP, Richters V. Effects of 0.25 ppm nitrogen dioxide on the developing mouse lung. Part 2: Quantitation of elastic tissue and alveolar walls. *Inhal Toxicol* 1995b; 7:1183-94.

Simpson R, Williams G, Petroeshevsky A, Best T, Morgan G, Denison L, Hinwood A, Neville G, Neller A. The short-term effects of air pollution on daily mortality in four Australian cities. *Aust N Z J Public Health* 2005a; 29:205-12.

Simpson R, Williams G, Petroeshevsky A, Best T, Morgan G, Denison L, Hinwood A, Neville G. The short-term effects of air pollution on hospital admissions in four Australian cities. *Aust N Z J Public Health* 2005b; 29:213-21.

Stevens, M. A., Menache, M. G., Crapo, J. D., FJ, M. I., and Graham, J. A. (1988). Pulmonary function in juvenile and young adult rats exposed to low-level NO₂ with diurnal spikes. *J Toxicol Environ Health* **23**, 229-240

Stieb DM, Judek S, Burnett RT. Meta-analysis of time-series studies of air pollution and mortality: effects of gases and particles and the influence of cause of death, age, and season. *J Air Waste Manag Assoc.* 2002; 52: 470-84.

Stieb DM, Judek S, Burnett RT. Meta-analysis of time-series studies of air pollution and mortality: update in relation to the use of generalized additive models. *J Air Waste Manag Assoc.* 2003; 53: 258-61.

Strand, V., Rak S., Svartengren M., and Bylin G. 1997. Nitrogen dioxide exposure enhances asthmatic reaction to inhaled allergen in subjects with asthma. *Am J Respir Crit Care Med* 155:881-87.

Strand, V., Salomonsson P., Lundahl J., and Bylin G. 1996. Immediate and delayed effects of nitrogen dioxide exposure at an ambient level on bronchial responsiveness to histamine in subjects with asthma. *Eur Respir J* 9:733-40.

Strand, V., Svartengren M., Rak S., Barck C., and Bylin G. 1998. Repeated exposure to an ambient level of NO₂ enhances asthmatic response to a nonsymptomatic allergen dose. *Eur Respir J* 12:6-12.

Sunyer J, Spix C, Quénel P, et al. Urban air pollution and emergency admissions for asthma in four European cities: the APHEA project. *Thorax* 1997; 52:760-765

Sunyer J, Puig C, Torrent M, Garcia-Algar O, Calico I, Munoz-Ortiz L, Barnes M, Cullinan P; Asthma Multicentre Infants Cohort Study. Nitrogen dioxide is not associated with respiratory infection during the first year of life. *Int J Epidemiol.* 2004 Feb;33(1):116-20.

Takano H, Yanagisawa R, Inoue K, Shimada A, Ichinose T, Sadakane K, Yoshino S, Yamaki K, Morita M, Yoshikawa T. Nitrogen dioxide air pollution near ambient levels is an atherogenic risk primarily in obese subjects: a brief communication. *Exp Biol Med* (Maywood) 2004; 229:361-4.

- Tepper JS, Costa DL, Winsett DW, Stevens MA, Doerfler DL, Watkinson WP. Near-lifetime exposure of the rat to a simulated urban profile of nitrogen dioxide: pulmonary function evaluation. *Fundam Appl Toxicol* 1993; 20:88-96.
- Thomas, H. V., Mueller, P. K., and Wright, R. (1967). Response of rat lung mast cells to nitrogen dioxide inhalation. *J Air Pollut Control Assoc* **17**, 33-35
- Triche EW, Belanger K, Bracken MB, Beckett WS, Holford TR, Gent JF, McSharry JE, Leaderer BP. Indoor heating sources and respiratory symptoms in nonsmoking women. *Epidemiology* 2005; 16:377-84.
- Tunncliffe, W. S., Burge P. S., and Ayres J. G. 1994. Effect of domestic concentrations of nitrogen dioxide on airway responses to inhaled allergen in asthmatic patients. *Lancet* 344:1733-36.
- Vagaggini, B., Paggiaro P. L., Giannini D., Franco A. D., Cianchetti S., Carnevali S., Taccola M., Bacci E., Bancalari L., Dente F. L., and Giuntini C. 1996. Effect of short-term NO₂ exposure on induced sputum in normal, asthmatic and COPD subjects. *Eur Respir J* 9:1852-57.
- van Strien RT, Gent JF, Belanger K, Triche E, Bracken MB, Leaderer BP. Exposure to NO₂ and nitrous acid and respiratory symptoms in the first year of life. *Epidemiology*. 2004; 15: 471-8.
- Venge P, Bystrom J, Carlson M, Hakansson L, Karawaczyk M, Peterson C, Seveus L, Trulsson A. 1999. Eosinophil cationic protein (ECP): molecular and biological properties and the use of ECP as a marker of eosinophil activation in disease. *Clin Exp Allergy* 29(9):1172-86.
- Vollmuth, T. A., Driscoll, K. E., and Schlesinger, R. B. (1986). Changes in early alveolar particle clearance due to single and repeated nitrogen dioxide exposures in the rabbit. *J Toxicol Environ Health* **19**, 255-266
- Watanabe, N. (2005). Decreased number of sperms and Sertoli cells in mature rats exposed to diesel exhaust as fetuses. *Toxicol Lett* **155**, 51-58
- Watanabe, N., and Ohsawa, M. (2002). Elevated serum immunoglobulin E to *Cryptomeria japonica* pollen in rats exposed to diesel exhaust during fetal and neonatal periods. *BMC Pregnancy Childbirth* **2**, 2
- Wellenius GA, Bateson TF, Mittleman MA, Schwartz J. Particulate air pollution and the rate of hospitalization for congestive heart failure among medicare beneficiaries in Pittsburgh, Pennsylvania. *Am J Epidemiol*. 2005 Jun 1;161(11):1030-6.
- Wilhelm M, Ritz B. Residential proximity to traffic and adverse birth outcomes in Los Angeles county, California, 1994-1996. *Environ. Health Perspect*. 2003; 111: 207-216.
- Zhu Y, Hinds WC, Kim S, Sioutas C. Concentration and size distribution of ultrafine particles near a major highway. *J Air Waste Manag Assoc* 2002b; 52:1032-42.

Recommendation Appendix

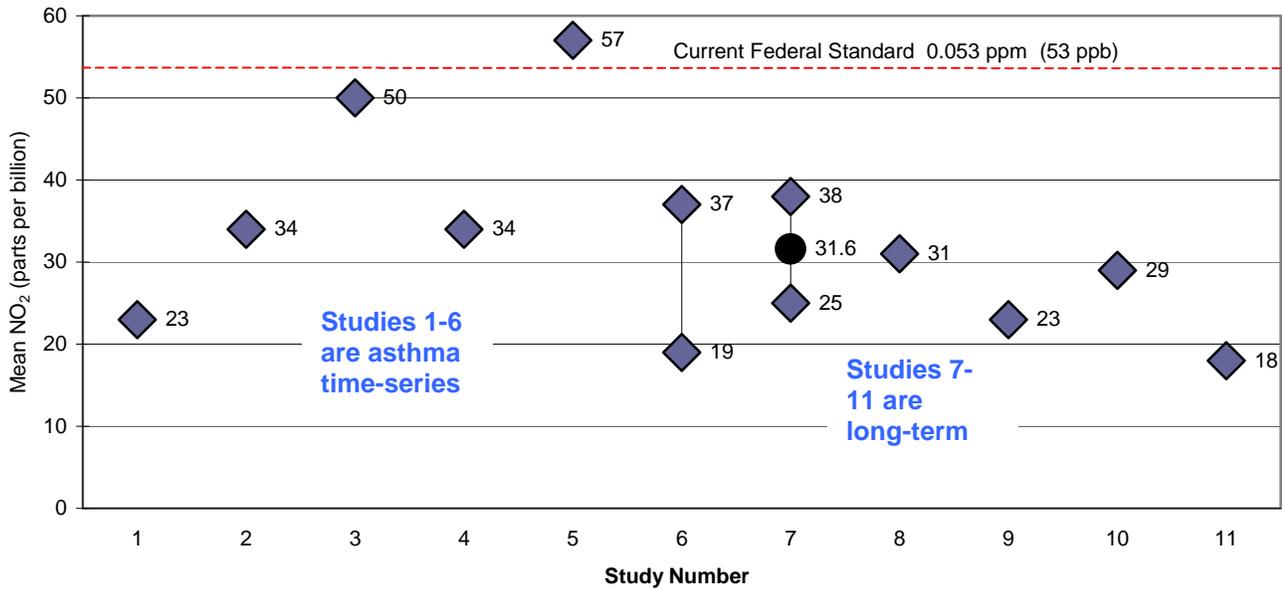
Table 1. Ratios of one-hour maximum to annual mean, by air basin, 2004

Basin	Annual Mean (ppm)	Ratios of quantiles of 1-hour maximum to annual mean		
		95th percentile	99th percentile	Maximum
Lake Tahoe	0.00462	8.22	11.47	14.71
Mexico	0.01900	3.95	5.26	10.11
Mojave Desert	0.01431	4.40	5.17	7.20
North Central Coast	0.00562	5.52	6.58	24.73
North Coast	0.00826	3.39	3.87	4.48
Sacramento Valley	0.01173	4.01	5.03	12.44
Salton Sea	0.01327	4.15	5.13	8.14
San Diego	0.01710	3.45	4.39	7.31
San Francisco Bay Area	0.01300	3.23	4.00	5.62
San Joaquin Valley	0.01393	3.59	4.52	5.96
South Central Coast	0.00685	5.11	6.42	10.36
South Coast	0.02422	2.97	3.80	6.48

Table 2. California air monitoring sites with average annual NO₂ levels greater than 0.0290 ppm, 2004

Air Basin_Name	Monitoring Site_Name	Annual average NO ₂ (ppm)
South Coast	Los Angeles-North Main Street	0.0337
South Coast	Burbank-W Palm Avenue	0.0332
South Coast	Pomona	0.0312
South Coast	Pico Rivera	0.0305
South Coast	Upland	0.0305
South Coast	Hawthorne	0.0304
South Coast	Lynwood	0.0302

Figure 1. Key epidemiological studies showing associations between NO₂ and respiratory disease in the U.S. and Europe



 = average NO₂ in single city study
  = Range of averages in multi-city study
  = Overall average NO₂ in multi-city study

Study	Author	Outcome	Location
1	Peel (05)	Asthma ERV, child	Atlanta
2	Galan (03)	Asthma HA	Madrid
3	Atkinson (99)	Asthma ER, child	London
4	Hajat (99)	MD asthma, child	London
5	Anderson (98)	Asthma HA	London
6	Sunyer (97)	Asthma HA, child	3 Euro cities
7	Gauderman (04)	Lung function	So. Calif.
8	Gauderman (05)	Asthma, wheeze	So. Calif.
9	Kim (04)	Asthma, bronchitis	S.F. Bay Area
10	Kramer (00)	Allergic sx	Dusseldorf
11	Janssen (03)	Allergic sensitization	Netherlands

Appendix B

Summary Comments of the Air Quality Advisory Committee on the Scientific Basis of the California Ambient Air Quality Standard for Nitrogen Dioxide



Community and Environmental Medicine
College of Medicine

Irvine, CA 92697-1825

December 5, 2006

Dr. Richard Bode
Research Division
California Air Resources Board
1001 I Street
Sacramento, CA 95812

Sacramento, CA

Dear Dr. Bode:

The Air Quality Advisory Committee met on June 12 and 13, 2006 to evaluate the draft document "Scientific Basis of the California Ambient Air Quality Standard for Nitrogen Dioxide." The examination of the current air quality standards and the recommendations for modification of those standards derived from the Children's Environmental Health Protection Act (Senate Bill 25) and a resulting document "Adequacy of California Ambient Air Quality Standards: Children's Environmental Health Protection Act" which was published as a staff report in 2000. SB 25 prompted an analysis of the scientific basis of the California air quality standards for particulate matter, sulfates, ozone, carbon monoxide, nitrogen dioxide, lead, and sulfur dioxide.

In response to SB 25, an up to date examination of the scientific information relevant to each of these standards that was published in peer reviewed documents was commissioned to determine if the current California standards were adequately protective of children's health. The staff of the Office of Environmental Health Hazard Assessment (OEHHA) made an analysis of the findings and recommended a list of standards that required re-review. The OEHHA analysis was deliberated by AQAC in a public meeting and the list of standards to be reviewed was prioritized. The standard for nitrogen dioxide was among those that were identified for review.

The committee went on record to complement the staffs of the ARB and OEHHA for performing a very comprehensive and careful compilation and analysis of the peer reviewed literature on sources, monitoring and health effects of ambient nitrogen dioxide. In most respects, the committee was pleased with the Technical Support Document "Scientific Basis of the California Ambient Air Quality Standard for Nitrogen Dioxide" and the Staff Report in which recommendations were made for modification of the existing standard.

Based on its review of the Staff Report and the Technical Support Document the Air Quality Advisory Committee endorses the Staff recommendations for a long term Standard

- Annual Average NO₂ at 0.030 ppm
- Not to be exceeded

The Committee also endorses the reduction of the 1-hr standard to a level below the current 0.25 ppm NO₂ and agrees with the Staff Report's recommendation of a 0.18 ppm 1-hr average standard (not to be exceeded). However, the committee requests expanded documentation to support the contention that this level of standard provides an adequate margin of safety for sensitive populations.

While the Committee endorses a 1-hr standard as the appropriate averaging time to capture acute events, the Committee suggests that the NO₂ monitoring network be realigned to provide better spatial resolution and include monitoring of "hotspots" and that ARB consider conversion of the form of the standard from ppm(v) to ppb(v) to avoid ambiguities due to rounding

The specific comments of the AQAC on the draft document are appended to this letter.

The AQAC is extremely appreciative of the responsiveness and expertise of the the staffs of OEHHA and the ARB. We commend them on the excellent job they did in reviewing and summarizing the scientific literature in the complex area of nitrogen dioxide and its effects on human health, and in establishing a set of ambient air quality standards that will better protect the health of California's citizens and especially their children.

Finally, the AQAC strongly recommends that additional research is needed on the possible effects of nitrogen dioxide on fetal and neonatal development, and that the nitrogen dioxide standard should be reviewed in 5 years or less if significant new research results become available.

Sincerely,



Michael T. Kleinman, Chair
Air Quality Advisory Committee

Cc: Bart Croes, Research Division

Summary Comments of the Air Quality Advisory Committee on the Scientific Basis of the California Ambient Air Quality Standard for Nitrogen Dioxide

The staffs of OEHHA and the ARB provided an excellent review of the current literature relevant to the sources, transport and health effects of ambient nitrogen dioxide (NO₂). The review provided a firm basis for establishing the needs for modification of the current NO₂ air quality standards and the committee was unanimous in its appreciation of the effort and diligence involved in producing the report.

The Air Quality Advisory Committee (AQAC) has provided comments on a chapter by chapter basis and also addressed specific overarching questions that were submitted to them during their review of the report.

In conducting its review the Committee specifically considered whether the documentation adequately addressed:

- The extent of evidence of effects at or below the existing ambient air quality standard.
- The nature and severity of those effects.
- The magnitude of risk when ambient levels are at or near the level of the existing standard.
- The available evidence that children may be more susceptible than adults.
- The degree of outdoor exposure relative to the level of the standard.

Children's protection, with an adequate margin of safety, is of paramount importance to public health. As the committee report indicates, this is an area in which more work is needed. Children with chronic lung diseases such as bronchopulmonary dysplasia, asthma and cystic fibrosis could be at special risk but, with the possible exception of asthma, there has been little research effort on health effects in these potentially susceptible groups. Since asthma affects nearly 10% of the child population, the effects of NO₂ on this group is of special importance. Having said this, the committee was particularly impressed with the efforts taken in the preparation of the reviewed documentation to thoroughly evaluate what is presently known about the effects of NO₂ on the health of children.

A previous evaluation of the health protection afforded by the current ambient air quality standards in California was mandated by SB25. The SB25 review which has been previously published identified clinical and epidemiological studies that suggested effects of NO₂ on pulmonary function, asthma exacerbation and acute morbidity in children and adults at or below the 1-hr CA standard of 0.25 ppm. Accordingly OEHHA and ARB staff have compiled and critically reviewed the scientific literature to determine whether:

- The current NO₂ standard provided an adequate margin of safety,
- A different averaging time was warranted.

In the Technical Support Document that was prepared, the published literature information was integrated and interpreted and the potential for exposures was assessed, the individuals at risk were identified, the potential health outcomes were determined and recommendations were made to establish new air quality standards that will better protect health for California citizens.

Based on its review of the Staff Report and the Technical Support Document the Air Quality Advisory Committee endorses the Staff recommendations for a long term Standard

- Annual Average NO₂ at 0.030 ppm
- Not to be exceeded

The Committee also endorses the reduction of the 1-hr standard to a level below the current 0.25 ppm NO₂ and agrees with the SR recommendation of a 0.18 ppm 1-hr average standard (not to be exceeded). However, the committee requests improved documentation of the support that this level of standard provides an adequate margin of safety for sensitive populations. While the Committee endorses a 1-hr standard as the appropriate averaging time to capture acute events, the Committee suggests that the NO₂ monitoring network be realigned to provide better spatial resolution and include monitoring of “hotspots” and that ARB consider conversion of the form of the standard from ppm(v) to ppb(v) to avoid ambiguities due to rounding

The Committee has identified some issues that should be addressed in a revised Technical Support Document. These issues are presented below.

Critique

Chapter 1.

Chapter 1 provides summary information of historical interest. Current Standards were summarized. The NAAQS provides an annual NO₂ standard but does not include a short term standard. CA currently has a short term but not a long term standard.

Standard	1 hr (ppb)	Basis	Annual (ppb)	Basis	Comment
NAAQS			53	Arithmetic mean of 1-hr measurements	
WHO	106		21		Guidelines
CA (Current)	250	1hr Arithmetic Mean			Not to be exceeded
CA (Proposed)	180	1hr Arithmetic Mean	30		Not to be exceeded

It would be appropriate to include in the summary the rationale for not having a Secondary standard. This might be an important consideration since in Chapter 2 the large contribution (50% during winter in SC basin) of NO₂ to fine secondary PM formation is discussed. In the Staff Summary of Welfare effects, visibility degradation which might be a basis for a secondary standard it was determined (1992 review) that meeting the 250 ppb NO₂ standard would adequately protect against visibility degradation because “the majority of the effect was due to fine particulate matter.”

The reduction to 180 ppb will reduce visibility impacts further and this could be mentioned as an added potential benefit of the proposed standard.

Chapter 2.

Chapter 2 discusses issues of atmospheric chemistry. The complex interplay between NO₂ and other components of the atmosphere such as NO (the other portion of NO_x), ozone, particulate matter and VOCs is described in good detail. Future research will undoubtedly refine details, but NO₂ physics, chemistry, measurement, sources and sinks are all adequately well understood to regulate, and this review thoroughly covers the topics needed for updating and establishing new regulations. The section on visibility impairment (2-9) separates the direct light absorption of the gas from that of the secondary aerosol. It would be very useful to indicate NO₂-related PM contribution and what the effect would be of lowering the CA short term standard to 180 ppb.

Specific Comments

Definitions of NO_x and NO_y

- should be defined carefully and consistently (they are not--see pp. vii, 2-11, 3-1)
- should be defined when the term is first used in each chapter (e.g., p. 2-2 needs NO_x definition)

p. 2-2. last sentence in the 1st paragraph after equation 2; this sentence is awkward (although technically correct, "remainder" usually refers to the smaller portion, not 90%)

Make sure all equations are balanced (e.g., see p. 2-2, equations 2 and 3)

p. 2-4, section 2.3.2, 1st paragraph, last sentence--drop "Thus"

p. 2-4, next to last line:improve "in this chemistry" (perhaps with "similar reactions")

p. 2-15, 4th line: do the authors really mean NO_x?

p. 2-15, section 2.9, 8th line--get correct Section number.

Chapter 3.

Chapter 3 deals with measurement methods and endorses the chemiluminescence method as the approved method in CA. Measurement of NO₂ is well-defined, sensitive, quantitative and selective. To avoid the need for correction due to elevation or weather changes in barometric pressure, it is appropriate to continue measuring, reporting and regulating in units of volume fraction – rather than mass concentration such as ug/m³. For clarity, it might be helpful to move toward uniformly using ppb(v) units (for example: 180 for 1 hour, 30 for annual average) -- rather than ppm(v) which requires a trailing zero that can lead to confusion about rounding/truncating data and hence determining resulting exceedances. The literature uses both ppm(v) and ppb(v), as with ozone, so either is acceptable. The measurement precision is not discussed. What is the degree of uncertainty around a 1-hr average concentration? Given that the standard is listed as “not to be exceeded”, an analysis of precision vs. the expected number of exceedances at the level of the standard might provide useful guidance. Also in Chapter 5 the calculation of a “peak indicator value” which is used to exclude “extreme concentration events” is discussed. How does measurement error and instrument precision factor into the peak indicator value?

Chapter 4.

Chapter 4 discusses sources and emissions. The report adequately describes the combustion sources of NO₂. It would be appropriate to also discuss non-combustion sources of NO, which inter-converts with NO₂. There are entirely natural (sometimes called biogenic) emissions from soil, grasses and trees, as well as anthropogenic non-combustion sources, generally in the area of managed annual and perennial plants, as well as animal agriculture. These processes include fertilizing, composting, feed and waste management, etc...and including non-commercial activities such as gardening. As management of combustion sources steadily improves, non-combustion sources will rise in relative importance. Natural/biogenic sources must be included since they contribute to the background, even if they are relatively uncontrollable; managed/anthropogenic sources must be included since they are becoming a larger factor on a relative basis – and possibly even on an absolute basis in some regions and/or seasons. Improving the summer-time ozone problem in the San Joaquin Valley

will probably only be achieved with reductions in NO_x. One could therefore mention that NO₂ regulation will have a secondary benefit i.e. reducing ozone and PM, and may actually be essential. It is clear from the data that the fractional contribution of mobile sources to ambient nitrogen emissions is decreasing. Stationary source emissions are expected to increase slowly over the next few decades due to population pressures. How the projections were made is not presented. Were changes in fuels considered given the increased costs and decreased availability of the fuels currently in use? The extent to which these changes are driven by NO₂ regulations per se or by reductions in combustion emissions related to reduction of PM could be made clearer.

Specific Comments

p. 4-1 and 4-2--same sentence repeated (1st sentence of 4.1.1 4th sentence of 4.2)
the graph on p. 4-2 and figure on p. 4-3 are difficult to read

Chapter 5.

Chapter 5 discusses ambient air quality with respect to NO₂ for CA. Data for each air basin in the state are presented. The discussion however centers on overall trends and ignores the increasing trends in the North Central Coast and Sacramento Valley basins.

General Comments

An explanation of the peak indicator needs to be moved from 5-43 to 5-3. It is not clear why the Statewide average of maximum 1-hr NO₂ is greater than in any of the individual air basins. Tables 5.3 and Figure 5.4 need some explanation of this.

Table 5.1 shows all air basins in CA average below the proposed annual average standard of .030 ppm, but presumably the standard has to be met at every monitor? If so, then data for individual monitors should also be shown. Table 2 in the staff report shows several monitors in the South Coast district exceeded 0.030 ppm in 2004.

Chapter 5 reports that no districts are out of compliance with the current 1-hour standard after adjustments for the Expected Peak Daily Concentration (EPDC), but it does look like Salton Sea and South Coast districts are at risk of exceeding the proposed new 1-hour standard. However, Table 5.7 shows that the EPDC based on 3 years of data is below the proposed new hourly standard in all districts.

Data reported in Chapter 3 show declining concentrations of NO₂ in most districts, and especially in those that have been reducing emissions to meet the federal standards for PM and ozone. Reducing NO_x emissions is one of the strategies being used to meet the PM and ozone standards, because NO_x is a precursor to both PM and ozone.

All of this means that the new standards are either currently met or not far out of reach and may be met soon as a result of efforts to meet the PM and ozone standards. The standards are supposed to be health and welfare based so this is not a limiting consideration, but as a practical matter the effect of these changes to the standards will

be mostly to encourage districts to continue to reduce NO_x emissions as part of their strategies to meet PM and ozone standards.

Section 5.5 presents an Analysis of Peak Nitrogen Dioxide Exposure in California. This section used inverse-distance weighting (IDW) from monitor location to estimate population averaged exposures. However, actual population exposures are likely to be higher on average because of in-vehicle and other personal exposures, and more importantly because a subpopulation will have high exposures simply based on proximity to sources such as traffic that are not included in the IDW model. This results in over-smoothing of the true spatial pattern of exposure (see Jerrett 2005, JEAEE 15:185-204). Some estimate based on this should be included given the indication from the epidemiologic studies that NO₂ effects are found at concentrations much lower than standards. NO₂ is serving at least in part as an indicator for traffic and other sources of unmeasured air pollutants. The spatial distribution of NO₂ secondary to traffic should receive some additional attention (see below).

Section 5.7.1.5 starting on page 5-74, presents important information on the spatial variability of ambient NO₂ concentrations. The information presented suggests that because NO₂ reacts quickly in the atmosphere, central monitors may not fully reflect concentrations relevant for the population living, working, or attending school near major traffic sources. An important topic for future research is whether the exposures measured at stationary monitors are sufficiently protective of public health. The report notes that 10% of public school children spend their school days within 150 meters of a busy road. Given the apparent effects of NO₂ exposure on lung function development, it will be important to determine whether this population is adequately protected by these standards. There probably are not sufficient data available at this time to answer this question, but it is important for ongoing research.

Specific Comments

Pg 5-3 Para 3 L 4- Peak indicator was not previously described. The information from 5-43 should be placed here.

Pg 5-12-Section 5.4.3, first sentence: it's NO₂ not ozone. It's 0.25 not 0.025 ppm.

Pg 5-14 Para 2 L1– Table 5.3 (not 5.4).

Pg 5-15 The note on Table 5.3 is not clear. Are these ppm concentrations or counts?

Pg 5-55 Section 5.6.2.1.1 Concentrations in Homes: What is meant by “Indoor/outdoor NO₂ ratios were positively associated with the community”?

Pg 5-74 Section 5.7.1.5 Spatial Variability of NO₂ Concentrations-This section was limited compared with the long section on indoor sources. Given that the ambient standard is the topic of concern, it would be appropriate to place a considerably larger emphasis on how spatial variability affects the inaccuracy of NO₂ measurement at stations in relation to population exposure. Additional information from the Singer 2004 study for instance would be helpful. They found a school located directly adjacent to a major freeway and a shopping center showed normalized NO₂ and NO_x were around 60% and 100% higher than regional background levels. At three schools within 130–

230m downwind of a freeway, normalized NO₂ and NO_x were around 20–30% and 50–80% higher than regional levels. The levels at the regional site in the East Bay study would underestimate their exposure. Given that children are a susceptible subpopulation, this is an important issue. Wu et al found overall within-community variability of personal exposures was highest for NO₂ (+/- 20-40%), and that traffic was a major determinant:

Wu J, Lurmann F, Winer A, et al. Development of an individual exposure model for application to the Southern California children's health study. *ATMOSPHERIC ENVIRONMENT* 39 (2): 259-273 JAN 2005.

Ross et al reference below was not discussed. This that might shed more light on spatial variability:

Ross Z, English PB, Scalf R, et al. Nitrogen dioxide prediction in Southern California using land use regression modeling: potential for environmental health analyses. *JOURNAL OF EXPOSURE SCIENCE AND ENVIRONMENTAL EPIDEMIOLOGY* 16 (2): 106-114 MAR 2006

Chapter 6.

Chapter 6 describes data from controlled human exposures. These data are used as the primary basis for reducing the short term standard from 250 ppb to 180 ppb. The chapter adequately discusses the recent toxicology information available.

General comments

It would be good to be a bit more consistent about the meaning of the variable findings in some subjects with asthma. The wording in section 6.1, paragraph 3 (e.g. "...suggest that some individuals experience increased airway responsiveness to NO₂ in the range of 0.2-0.3 ppm" seems more on target than the wording on page 6-18, para 3 "These recent studies involving allergen challenge appear consistent in demonstrating effects...." Otherwise, the chapter did an excellent job of capturing a challenging body of literature.

Specific Comments

P6-7, para 3 The description of effects of IL-5 and IL-13 is slightly inaccurate. These are cytokines produced by Th2 lymphocytes, but neither "can induce a Th2 response in T helper cells." Actually, T cells don't express receptors for these cytokines. IL-4 is the major cytokine that induces Th2 cell differentiation.

P6-16, para 4. Do you mean "decreased peak flow" rather than "increased"?

P6-24, para 2 – The statement that "The divergence of findings from various studies suggests that some individuals with asthma are particularly susceptible..." might be

overstated. It might be preferable to simply say “....suggests that some individuals with asthma might be particularly susceptible....”

Chapter 7.

Chapter 7 presents an evaluation of the epidemiological data reviewed.

General comments:

Overall, this is a comprehensive review of the epidemiologic literature on NO₂. It points to well-known methodological weaknesses that are inherent to the study of ambient air pollution in free-living human populations, or weaknesses that have not been addressed yet by researchers. None of these weaknesses takes away from the coherence of the epidemiologic evidence with the clinical and toxicological data. The choice of an NO₂ standard based on susceptible populations is well supported by the evidence presented. Susceptible subpopulations were clearly identified in several reviewed studies, including children with asthma, infants, patients with pre-existing cardiovascular or respiratory disease, and the elderly. The time series studies evaluating the relationship between hospital admission or ED visits and asthma in children were remarkably consistent and robust for NO₂. Often in the face of significant particle associations, the associations with NO₂ remained after inclusion of the particle measurements. The chapter's organization could be improved by adding some summary figures or tables that provide an overview of the available science.

An important issue discussed was that in many of the epidemiologic studies, NO₂ is likely acting as a good indicator of the complex gas-particle mixture originating from vehicular traffic. Depending on the region, other important sources significantly contribute to this mixture (e.g., ports). What is important in this concept is that the regulatory standards currently used focus on a very limited set of pollutants, most of which are in part surrogates of other potentially more harmful pollutants. The ultimate focus of air pollutant regulation is rightly on sources, and the ability of the pollutant to function as an indicator of sources is important in this regard, apart from its independent effect on health.

The summary conclusion 7.3.1.1 after the text on cohort studies is inaccurate and misleading. It reads as follows:

“The studies in this review show little evidence for effects of long-term concentrations of NO₂ on prevalence and/or incidence of asthma, allergic rhinitis, and atopic eczema. For asthma diagnoses and symptoms, two cross-sectional studies show positive and three show negative associations.”

The summary conclusion does not reflect what is in Tables 7-10. The word negative is not correct. It might be better to refer to the findings as “null”, and the count does not reflect the tables. The Table shows no negative associations and in general, the ORs or RRs are positive but not always statistically significant. The words “little evidence” is misleading. For instance, in the case of allergic sensitization, the words should be “there are few studies.” Describing the literature as “little evidence” suggests that many

studies find no association. The one cross-sectional study (Janssen 2003) with high power showed associations between NO₂ and total IgE and positive skin prick tests to allergens. This finding was consistent with the robust findings of the smaller study by Kramer et al. 2000 for atopic sensitization and allergic rhinitis in relation to outdoor home NO₂. The conclusion about the surrogate nature of NO₂ holds, but does not diminish its usefulness in the regulation of unmeasured and largely unregulated air pollutants that NO₂ probably represents. The CHS findings for OC and EC (solely measured for the CHS) along with NO₂ further support that view.

The authors have been very careful to acknowledge the limitations of the epi literature in terms of being able to specifically identify NO₂ as the causative pollutant. The co-occurrence of the set of traffic-related pollutants that includes NO₂ is the primary difficulty. However, it is clear that this mix of pollutants is associated with adverse health effects. When the epi results are considered along with the clinical and toxicological evidence, there is reasonable support for the conclusion that NO₂ is at least one of the harmful constituents of this mix. This is a prudent interpretation of the evidence in terms of protecting public health.

The epidemiology results are strongest for an association between NO₂ and respiratory illness, especially asthma exacerbations. This is consistent with the evidence from the clinical studies. These associations are observed in the epi studies at ambient concentrations that exist in CA.

Gauderman et al. (2004) and related studies seem especially important because they suggest lung function development decrements in children over an 8-year study. This is a very serious effect that is a risk factor for chronic disease and premature mortality later in life. This elevated risk is observed at long-term concentrations of 25-30 ppb, which exist in some CA locations. Questions regarding co-pollutants are still important, but this association is consistent with toxicological study results showing adverse effects of NO₂ on lung function development in some animal studies. It is also important to note that this effect could lead to premature mortality, but it would not show up in time-series mortality studies because it is a function of childhood exposure, not short-term exposure fluctuations.

It should be pointed out that little is known about the impact of NO₂ inhalation on vulnerable pediatric populations which include the fetus, infants born prematurely, newborn infants, early infancy, infants and children with chronic lung conditions, such as chronic lung disease of infancy (BPD), cystic fibrosis, interstitial lung disease. The target population usually studied in assessing the response to inhaled environmental pollutants has been healthy children, usually older than 7 years old, who are often compared to children with asthma, a surrogate for children with airway or lung disease. These studies are difficult to interpret due to the grouping of the children and adolescents who cough and/or wheeze in the same study without controlling for sex, race, socio-economic status or age groups [0-1 year, 1-2 years, 2-5 years, and 5-13 years]. There are developmental and physiological reasons for the necessity to study children in these age groups. First, establishing the diagnosis of asthma in young

children prior to the age of 4-5 years old is difficult, often impossible, even those with atopy or a family history of asthma. Wheezy bronchitis is common in infants and young children from birth to 4 years. In fact, of the infants and young children [less than 4 years old] with chronic or recurrent cough or wheeze, less than 25% will have persisting cough or wheeze by 5 years of age. Some reasons for this diagnostic dilemma are:

1. boys being born with smaller airways than girls (Taussig), making cough and wheeze more common in infant males than females during and following routine respiratory tract infections. In the first two years boys airways grow more rapidly than girls so that after 2 years of age airway caliber of males exceed that of females of the same age, so that after 2 years old females experience more cough and wheeze than females;
2. the lack of a specific serologic or lung function test to make the diagnosis asthma which makes the diagnosis of asthma problematic in the child less than 4-5 years of age in the absence of a strong family history.
3. Difficulty in performing reliable pulmonary function tests in very young children.

Specific comments:

P 7-1: Clarify the comment about epidemiologic studies that:

“it is not possible to quantify exposure for individuals, as is commonly done in chamber studies.”

I assume you are excluding personal exposure monitors because hourly sampling is not yet available.

For 95% CI, I would suggest using commas to separate upper and lower limits instead of dashes. Some journals do this to avoid the misinterpretation of interval sign and to make reading easier.

P 7-6, bottom: The following sentences are unclear

“For asthma, a stronger effect was detected considering distributed lag models (lags 0 to 13 days), with PM10, NO₂ (4.7% for 20 pbb, 95%CI=1.1-8.5%), and CO, showing a statistically significant effect.”

Suggest separating out the numbers for the NO₂ association.

“In multipollutant models, the NO₂ effects were attenuated when PM10, NO₂, and CO were considered simultaneously. However, the effect of NO₂ on emergency visits for asthma was not attenuated in multi-pollutant models while the estimates for the other pollutants suggested weaker or no associations.”

Attenuated or not?

Throughout, for the time series results, there was a shift in the use RR and % change. For instance, in the text on page 7-7, results for Simpson et al. 2005 are in RR, but the table on p 7-52 is in % and not consistent if $100 \times \text{RR} = \%$ change in admissions.

Last line and word p 7-9: typo.

P 7-11 last paragraph: should be "...after adjusting for outdoor pollens and fungal spores."

P 7-13: Just et al, 2002: Larger associations were seen between respiratory infections and NO₂ and BS. This is missing in text and table.

P 7-13: Moshammer et al 2006: This is a general population study of children as noted in Table 6. There is no information about clinical status, so this paper does not belong in a section on children with asthma. It is nevertheless important that they did find lung function deficits in relation to increased NO₂. There are several other studies that have studied otherwise healthy children or mixed populations, although the clinical relevance is lessened by this approach to sample selection.

Table 4 and related section: The two outcome and age groups are unrelated and is confusing to see asthma in children combined with arrhythmias in adults. Panel studies of medication use in asthmatic children is separated but would be more appropriately combined with the other panel studies of asthmatic children looking at a variety of other outcomes. The section could be "Panel Studies" and then subsections with the outcome groups as presented, including General Population and Other Pediatric Panels.

Table 6: Lung function in Asthmatic Children: Again, the title is inaccurate since many of the studies were not of asthmatics. In addition, nearly all studies have only looked at PEF, an inaccurate measurement of large airway function compared with FEV₁. Therefore, it is important to report in Table 6 and the text on panel studies using FEV₁, which are few in number. The review missed two recent papers in this regard:

1) Delfino RJ, Quintana PJE, Floro J, Gastañaga VM, Samimi BS, Kleinman MT, Liu L-JS, Bufalino C, Wu C-F, McLaren CE. Association of FEV₁ in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. *Environ Health Perspect.* 2004; 112: 932-41.

Delfino et al (2004) followed a panel of 19 children with asthma for two weeks with personal PM nephelometers. They found central-site 5-day average 8-hr maximum NO₂ was inversely associated with percent predicted FEV₁ (per IQR increase in NO₂ of 10.5 ppb, -1.16%; 95% CI, -2.4 to 0.1), and associations were similar for the 3- and 4-day average and for 1-hr maximum NO₂. However, NO₂ was confounded by personal PM with parameter estimates falling near zero. Associations of FEV₁ with personal PM were largely independent of NO₂.

P 7-13, Cardiovascular Effects: An important paper was left out that is currently the only repeated measures study of ECG-measured ST segment depression. This is important because transient myocardial ischemia is clinically and/or biologically relevant to more severe outcomes such as MI:

Pekkanen J, Peters A, Hoek G, Tiittanen P, Brunekreef B, de Hartog J, et al. 2002. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the Exposure and Risk Assessment for Fine and Ultrafine Particles in Ambient Air (ULTRA) study. *Circulation* 106:933-938.

This was a study of 45 adults with stable coronary artery disease that analyzed data from repeated biweekly in-clinic ECG measurements during submaximal exercise testing and outdoor ultrafine and fine particles measured at a central regional site of Helsinki, Finland. They found significant associations between risk of ST segment depression and ambient lag 2 day PM_{2.5} mass (OR 2.8, 95% CI: 1.42, 5.66). Similar magnitudes of association were found for ultrafine and accumulation mode particle number concentrations, but smaller but significant associations were also found for lag 2 day NO₂ (OR 2.02, 95% CI: 1.34, 3.04) and CO (OR 1.73, 95% CI: 1.26, 2.39), which were moderately correlated with the co-located particle measurements. Two pollutant models for PM and gases were not tested.

Table 7 and 8 titles would be clearer to contrast with 9 and 10 if it was “between-community”

Pp 7-66 to 7-67: ORs are for what increase in NO₂ in ppb?

P 7-17 statement: “In a West German study (Kramer et al. 2000), outdoor levels of NO₂, ...” To be clear, it’s outdoor home, a point that strengthens the following statement in the text on the importance of traffic given the null results for personal NO₂.

Table 9, Kramer: The report is somewhat inaccurate since I believe it includes the rural subjects, which biased estimates downwards. Here is what I found:

Associations were dominated by the urban subgroup as follows:

Outdoor home NO₂, but not personal NO₂, was significantly associated with reports of at least 1 week with symptoms of wheezing: OR for 10 µg/m³ increase, 14.9 (95% CI, 2.59, 86.4); and with symptoms of allergic rhinitis: OR 1.81 (95% CI 1.02, 3.21), which in pollen season increased to OR 3.09 (95% CI 1.38, 6.92).

An ever diagnosis of hay fever was associated with outdoor NO₂, OR 4.24 (95% CI: 1.01, 17.8), asthma was not, OR 1.82 (95% CI : 0.36, 9.36).

Atopic sensitization to pollen, house dust mite or cat, and milk or egg were each significantly associated with outdoor NO₂ (ORs ranged from 3.5 to 5.0), but not personal NO₂. (see text and Figure 2 in Kramer).

P 7-23, statement: “In addition, more localized panel studies could be used to attempt to separate the effects of NO₂ from other pollutants.” I hope to provide the committee with results from my panel study currently under review that makes notable advances in this area using eNO from asthmatic children in relation to personal and ambient NO₂, PM_{2.5}, EC and OC.

Chapter 8.

Chapter 8 deals with toxicology of NO₂. The chapter is well written but most of the real information is contained in the Appendix. The information from the Appendix should be incorporated into the body of the TSD. The brief presentation made to the Committee provided a very good overview of the key factors and salient features of that presentation should be added to the TSD also. The chapter mentions dosimetry, but the use of dosimetry for bridging between data in animal models to application to humans needs to be discussed. For example the TSD mentions that estimation from Miller et al. suggests that, for the same exposure, the dose to the rat's epithelium would be ¼ of that delivered to a human's. The Miller modeling should be checked but, if correct, one could use such information to put the data from rat studies at concentrations from .5 to 5 ppm NO₂ into context of “equivalent” human exposures at ~0.1 to 1 ppm. This suggestion is obviously an oversimplification of a very complex issue – the Committee provides it as an example of one method to strengthen the link between the mechanistic studies available from toxicological studies to possible mechanisms in humans. It would be useful to mention that while there are some areas in which specific mechanisms in rodents might differ from those in human and non-human primates, there are several biological pathways that are sufficiently similar that useful comparisons can be drawn.

Since the mandate for this review was specific for the health/welfare of infants and children, it would be helpful if this chapter emphasized the issues that are specific to

infants and children such as: growth, proliferation, differentiation, respiratory rates/pulmonary functions, time/activity outdoors. This then leads into a discussion of choosing the proper model and the advantages and limitations of available models. Also, since allergic/asthmatic individuals are discussed, some discussion regarding proper choice of the immunologic models would be helpful.

Also, some mention of in utero exposures and issues would be helpful (if for no other reason than to highlight the lack of information available).

Specific Comments regarding the Appendix:

Page A-3-5: The dosimetry section is well-written, but under utilized. This information could be used to help extrapolate the doses used for the animal studies (especially since the animal tissue dose is 2-4 times less than humans). It could be useful to point out that after taking dosimetry into account a rat study at 0.25 ppm is approximately equivalent to a human study at 0.0625 to 0.125 ppm.

Page A-4, last ¶: Is there a reference for measuring reduction in lung lining fluid thickness in distal airways? Was this inferred or actually measured?

Page A-5-6: Clarify whether this refers to tissue or BAL effects. Also, it would be helpful to contrast the kind of information that can be obtained from BAL vs. tissue (i.e. site-specific data vs whole lung data).

Page A-6: 1st full ¶: line 6: define "continuous" exposure (also p9, 2nd ¶, line5). If "continuous" actually means 24 h/day, then these studies should be moved to a separate section and given little weight. A continuous exposure will result in an adaptive or tolerant pulmonary response completely different from the response to a more realistic intermittent or episodic exposure.

Page A-9, lines 6-7: It would be helpful to specify which studies in the 1992 review were used.

Page A-10: ferret work: Please discuss the appropriateness of the ferret as a model. For example, the lung development of the ferret may be similar to the human, but it would be appropriate to mention that their long trachea can scrub out pollutants before they reach the lungs, therefore underestimating the effective dose in extrapolation.

Page A-11: In vitro studies: need to clarify that the morphological lesions for NO₂ are focal, therefore caution should be used in interpreting negative data from BAL or whole lung homogenates (the small percentage of tissue affected may be overwhelmed by the large percentage of tissue not affected in these non-specific methods).

Page A-11: In vitro studies, 1st ¶, last sentence: What studies specifically in the 1992 review are being referenced?

Morphological data should come first in the Tox studies. Knowing where the injury is will affect how the biochemical effects are interpreted.

Page 26: 4th ¶: same issues for morphological affects in ferret as described above.

Page 26-27: The study of newborn mice with the structural changes should be placed to have more emphasis.

Chapter 9.

Chapter 9 discusses effects on vegetation. Welfare effects are not being used as the basis for the proposed changes in the standards, but it is important to note that some welfare benefits are likely to occur as a result of reducing NO₂ emissions (or preventing increases), especially in the South Coast and Central Valley areas. The summary statements in the Staff Report (p. 13-14) are too weak on this and unnecessarily suggest minimal benefit.

Chapter 9 focuses a lot on foliar injury and it may be that most areas do not have ambient concentrations of NO₂ sufficiently high to cause visible foliar damage. However, a more significant ecosystem concern is total nitrogen deposition. This is discussed in Chapter 9, but not carried over to the summary in the Staff Report. The discussion on page 9-23 suggests that critical loads (deposition rates that can be tolerated without harmful effects on an ongoing basis) for California mountain ecosystems may be higher than in other locations, but the specific critical loads for these areas have not been established. Nitrogen deposition rates reported in Figure 9.5 are some of the highest in the country. NAPAP (2005) reports that the highest annual total nitrogen deposition rates in the Midwest and Northeast range 8-11 kg/ha/yr. Figure 9.5 shows rates at 9 kg/ha/yr or higher (up to 97.5!) at multiple sites in Sequoia, Angeles, and San Bernardino National Forests. The superintendent of Rocky Mountain National Park recently proposed a critical load standard of 1.5 kg/ha/yr for the park because it is now showing signs of nitrogen saturation (with annual N deposition rates in the range of 3-4). NAPAP (2005) notes evidence of elevated concentrations of nitrate in

surface and ground water in the San Gabriel and San Bernardino Mountains, which suggests possible N saturation in those forests. Reducing NO₂ emissions, especially in the South Coast basin, will result in reduced nitrogen deposition and this can be expected to benefit the forest ecosystems and reduce nitrogen concentrations in surface and ground water.

National Acid Precipitation Assessment Program. NAPAP Report to Congress: An Integrated Assessment. Washington DC, August 2005
<http://www.al.noaa.gov/AQRS/reports/napapreport05.pdf>

Staff Report and Recommendations.

The SR is generally well written but some areas need to be improved. There is no discussion of whether or not there is a threshold for NO₂ effects. There are some articles that were not cited in the TSD that could be added. Samoli and Vedal, respectively, discuss epidemiological data from European and Canadian studies (Samoli et al., 2003; Vedal et al., 2003) that provide some discussion on the identification of thresholds and why measurement errors could obscure detection of a threshold. Another factor that could be mentioned is that if a contaminant was a surrogate for another contaminant a threshold might not be detectable. Vedal et al. report that “increases in air pollutant concentrations, even when concentrations are low, are associated with adverse effects on daily mortality. Although this observation may support the argument that there are no threshold concentrations of air pollution below which adverse effects cannot be detected, it also raises concern that the associations are not reflecting the effects of the measured pollutants, but rather some factor or combination of factors, such as, for example, unmeasured air pollutants or uncontrolled features of meteorology that are correlated with the measured pollutants.” The APHEA-2 data (Samoli et al., 2003) was unable to detect a threshold (i.e. a linear non-threshold model could adequately describe the data), however they provide the caution “The NO₂–mortality association in the cities included in the present analysis could be adequately estimated using the linear model. However, it became evident that the linear model should not be applied without investigating the city specific dose-response curves first.”

The committee endorses the addition of the long term 30 ppb annual standard and also endorses the “not to be exceeded” form of the proposed standard. The short term standard is based primarily on human clinical studies rather than on epidemiological studies. The TSD and SR both make the point that effects are relatively robust at or above the current 250 ppb standard but that some studies also demonstrate significant changes at levels of about 200 ppb. Data used in Germany to set a short term standard

(Kraft et al., 2005) showed effects down to about 200 ppb but effects on patients with mild asthma were not observed after short-term exposure to concentrations below about 100 ppb. This is consistent with the data summarized in the TSD. The logic applied to arrive at the proposed lowered short term standard (180 ppb) needs to be better described. The criteria for assuring an adequate margin of safety should be transparent. There is a dilemma in that the epidemiological data could be interpreted as indicating that a lower short term standard is warranted. However the committee also recognizes that causality in the epidemiological studies is difficult to ascribe solely to NO₂, hence the use of the chamber studies to develop the standard is acceptable. The committee is also concerned that the location of the NO₂ ambient monitors is not adequate to provide protection to individuals living in “hot spots.” The relocation of monitors to provide better spatial representation of NO₂ exposures in each of the air basins, similar to the approach used for CO, would benefit protection of public health.

The welfare benefits of controlling NO₂ could be expanded. On page 14 the Staff Report suggests that there may be little improvement in visibility as a result of the reduction in the NO₂ standard. It was mentioned that the 0.25 hourly standard was expected to be protective of the discoloring effect that NO₂ causes (the brown color to the air). Has it really been established that there is no brown color at concentrations below 0.25 ppm? Also, the statement that most of the haze is caused by particulate fails to acknowledge that NO₂ emissions contribute to the formation of secondary particulate. Thus, some visibility improvements can be expected as a result of further reduction in NO₂ emissions even if the discoloration is no longer an issue.

APPENDIX

Some members of the committee provided extensive comments which were integrated into the above summary. This necessitated extraction of material for insertion into comments on specific chapters. To ensure that the sense of these comments was not lost, they are included below in their entirety.

Individual Member Comments

Russell P. Sherwin, M.D.

A first consideration for standard setting is a definition of adverse health effect. I believe the definition should encompass the following major areas of concern: Mortality, Morbidity, and Morbidity, the latter including clinically covert disease (subclinical disease), pathobiological alterations, and the depletion of health reserves (hypeinopenia). With respect to the body of data presently available that address a large part of those concerns, I wish to commend the Staff for their excellent work in reviewing the vast amount of literature regarding the adverse health effects of ambient levels of nitrogen dioxide. In my opinion, the data presented in the Staff Report fully support the Staff's recommendations for a 0.18 NO₂ one-hour and a 0.03ppm yearly average standards. A reservation in the latter respect is an understatement of Morbidity concerns. Some degree of Morbidity in the form of serious subclinical disease is

ubiquitous in the adult population and is reflected in the large proportion of especially susceptible individuals found in the general population, from infants to the elderly. Relatively little data are available on ambient NO₂ exposure and effects on Morbidity and a critical question has received little attention, namely whether or not NO₂ exposure in community air is playing a significant role in the causation, promotion, facilitation, and/or exacerbation of subclinical disease. An important case in point is pulmonary emphysema, now the fourth leading cause of death nationally but expected to rise to become the third leading cause of death. While cigarette smoking is clearly a major etiological factor, emphysema is ubiquitous in all adults. Of interest, emphysema in Antelope Valley is said to be the second leading cause of death, presumably related in part to the severe dust storms but the principle of multicausative factors is undoubtedly operative. Of special pertinence to Antelope Valley in particular is the lack of adequate technology to measure lung reserve depletion (the pathological hallmark of emphysema) with respect to rate and magnitude. The inadequacy of presently available technologies in general is a major concern for setting reasonable air pollution quality standards. For appropriate insight in the absence of hard data, I would recommend greater emphasis on pathobiological findings that suggest an adverse health effect with the potential of serious harm to the body. Mention should be made that a few personal research studies and related reports by others may warrant consideration for inclusion in the Staff Report, in particular protein leakage in the respiratory tract. Leaky lungs predispose the individual to infection, impair gaseous exchange, alter metabolic functions, facilitate thrombotic events and metastases, and place an added burden on the cardiovascular system. In the latter respect, Wellenius GA, et al have recently reported a salient finding with respect to leaky lungs, bearing in mind that pulmonary edema is the major complication of congestive failure (cf., below). They pointed out that triggering by particulate exposure of acute decompensation in patients with congestive heart failure has not been evaluated in a systematic manner, but when carried out the "results support the hypothesis that elevated levels of particulate air pollution, below the current limits set by the United States Environmental Protection Agency, are associated with an increase in the rate of hospital admission for exacerbation of CHF" -- Wellenius GA, Schwartz J, Mittleman MA. Particulate air pollution and hospital admissions for congestive heart failure in seven United States cities. *Am J Cardiol.* 2006;97:404-8; cf. also, #10 and following citations, below). With the foregoing in mind as examples of the Morbidity problem, it is apparent that adoption of the recommended standard will provide some margin of safety but will nevertheless leave in question the proportion of the general population that will be adequately protected.

I. A review of pertinent literature cannot establish a no-harm level for NO₂ and advances in technologies can be expected to uncover presently unrecognized injuries from exposure to ambient NO₂. To reach a level of Best Judgmental Value (BJV), a very broad spectrum of health effects reports should be evaluated. Note judgmental differences in reviews by German and French sources, below). From a brief review of key issues involved in NO₂ standard setting, I believe that some studies, not cited in the

draft Staff Report (in part recent publications), may warrant consideration for inclusion in the final Staff Report:

1: McConnell R, Berhane K, Yao L, Jerrett M, Lurmann F, Gilliland F, Kunzli N, Gauderman J, Avol E, Thomas D, Peters J. Traffic, susceptibility, and childhood asthma. *Environ Health Perspect.* 2006;114:766-72.

2: Millstein J, Gilliland F, Berhane K, Gauderman WJ, McConnell R, Avol E, Rappaport EB, Peters JM. Effects of ambient air pollutants on asthma medication use and wheezing among fourth-grade school children from 12 Southern California communities enrolled in The Children's Health Study. *Arch Environ Health.* 2004;59:505-14.

3. Hwang BF, Lee YL, Lin YC, Jaakkola JJ, Guo YL. Traffic related air pollution as a determinant of asthma among Taiwanese school children. *Thorax.* 2005;60:467-73.

"The results are consistent with the hypothesis that long term exposure to traffic related outdoor air pollutants such as NO_x, CO, and O₃ increases the risk of asthma in children".

4. Hwang JS, Chen YJ, Wang JD, Lai YM, Yang CY, Chan CC. Subject-domain approach to the study of air pollution effects on schoolchildren's illness absence. *Am J Epidemiol.* 2000 1;152:67-74.

"School children's risk of illness absence were significantly related to acute exposures to nitrogen dioxide and nitrogen oxides with a 1-day lag ($p < 0.01$) at levels below the World Health Organization's guidelines. By contrast, the authors could not detect significant associations between air pollution and schoolchildren's absenteeism using time-domain approaches. Such findings imply that the models built on subject domain may be a general solution to the problem of the ecologic fallacy, which is commonly encountered in environmental and social epidemiologic studies".

5. Richters A, Damji KS. Changes in T-lymphocyte subpopulations and natural killer cells following exposure to ambient levels of nitrogen dioxide. *J Toxicol Environ Health.* 1988;25:247-56. [Intermittent exposure to NO₂ at 0.25 ppm for 27 days or 0.35 ppm for 60 days]

"This is the first report providing evidence linking alterations in T-lymphocyte subpopulations and natural killer cells to NO₂ exposure at ambient levels. Changes in

T-lymphocyte subpopulations detected by FACS and correlated to impaired immune function may provide an extremely sensitive means of demonstrating NO₂-induced changes in the immune system.

6: Richters A, Richters V. Nitrogen dioxide (NO₂) inhalation, formation of microthrombi in lungs and cancer metastasis. *J Environ Pathol Toxicol Oncol.* 1989;9:45-51.

“The main lesions observed were microthrombi and injury to capillary endothelial cells, following 6 weeks of 0.35 +/- 0.05 ppm NO₂ exposure. --- A correlation was observed between increased incidence of microthrombi, endothelial cell injury and lung metastasis in exposed animals --- more metastases developed in the exposed group (p<.04)”.

7: Kuraitis KV, Richters A. Spleen cellularity shifts from the inhalation of 0.25-0.35 PPM nitrogen dioxide. *J Environ Pathol Toxicol Oncol.* 1989;9:1-11.

“The effects of ambient level (0.25-0.35 ppm)NO₂ on percent spleen cell counts, relative percentages of spleen lymphocyte subpopulations, spleen lymphoid nodule size, and differential peripheral blood cell counts were investigated in 170 young adult male mice following various NO₂ exposure periods. The total spleen cell counts, surface IgM-positive lymphocytes and spleen mean lymphoid nodule area were all significantly decreased in the groups exposed to NO₂ following extended time periods”.

(cf. 6-8: “NO₂ levels as low as 4 ppm”; compare with above citations)

8. Protein leakage in the lungs of mice exposed to 0.5 ppm nitrogen dioxide. Sherwin RP, Layfield LJ. *Arch Environ Health.* 1976;31:116-8.

(Forty-four mice continuously exposed to 0.47 ppm nitrogen dioxide for ten, 12, and 14 days. --- homogenized lung tissue assayed fluorometrically intravenous fluorescamine -- exposed animals had increased levels (p<.025).

See also:

Sherwin RP, Carlson DA. Protein content of lung lavage fluid of guinea pigs exposed to 0.4 ppm nitrogen dioxide. *Arch Environ Health.* 1973 Aug;27(2):90-3.

Tohyama Y, Kanazawa H, Fujiwara H, Hirata K, Fujimoto S, Yoshikawa J. Role of nitric oxide on airway microvascular permeability in patients with asthma. *Osaka City Med J*. 2005;5:1-9.

(significant correlation between exhaled NO level and airway vascular permeability index -- Interaction between airway microcirculation and NO may be a key element in disordered airway function in asthma).

9. Gehring U, Heinrich J, Kr Amer U, Grote V, Hochadel M, Sugiri D, Kraft M, Rauchfuss K, Eberwein HG, Wichmann HE. Long-Term Exposure to Ambient Air Pollution and Cardiopulmonary Mortality in Women. *Epidemiology*. 2006 May 30; [Epub ahead of print]

("Living close to major roads and chronic exposure to NO₂ and PM₁₀ may be associated with an increased mortality due to cardiopulmonary causes).

10. Samoli E, Aga E, Touloumi G, Nisiotis K, Forsberg B, Lefranc A, Pekkanen J, Wojtyniak B, Schindler C, Niciu E, Brunstein R, Dodic Fikfak M, Schwartz J, Katsouyanni K. Short-term effects of nitrogen dioxide on mortality: an analysis within the APHEA project. *Eur Respir J*. 2006 Mar 15; [Epub ahead of print]

("We found a significant association of NO₂ with total, cardiovascular and respiratory mortality, with stronger effects on cause-specific mortality. -- The results of this large study are consistent with an independent effect of NO₂ on mortality, but the role of NO₂ as a surrogate of other unmeasured pollutants cannot be completely ruled out".

11. Liu S, Krewski D, Shi Y, Chen Y, Burnett RT. Association between maternal exposure to ambient air pollutants during pregnancy and fetal growth restriction. *J Expo Sci Environ Epidemiol*. 2006 May 31; [Epub ahead of print]

("Previous research demonstrated consistent associations between ambient air pollution and emergency room visits, hospitalizations, and mortality. -- A 20 ppb increase in NO₂ -- in the first, second, and third trimesters) and a 10 µg/m³ increase in PM_{2.5} -- were also associated with an increased risk of IUGR (intrauterine growth restriction). Consistent results were found when ORs were calculated by month rather than trimester of pregnancy. Our findings add to the emerging body of evidence that exposure to relatively low levels of ambient air pollutants in urban areas during pregnancy is associated with adverse effects on fetal growth"

12. Kraft M, Eikmann T, Kappos A, Kunzli N, Rapp R, Schneider K, Seitz H, Voss JU, Wichmann HE. The German view: effects of nitrogen dioxide on human health--derivation of health-related short-term and long-term values. *Int J Hyg Environ Health*. 2005;208(4):305-18.

("Ministry of the Environment and Conservation, Agriculture and Consumer Protection of the state of North Rhine-Westphalia, Dusseldorf, Germany. – The presented overview concerning health relevant effects caused by nitrogen dioxide (NO₂) resumes the current state of results from animal experiments and human studies (epidemiology and short-term chambers studies). NO₂ concentrations applied in animal experiments were mostly considerably higher than in ambient air. Therefore, short- and long-term limit values were derived from human data. Experimental studies conducted with humans demonstrate effects after short-term exposure to concentrations at or above 400 microg NO₂/m³. Effects on patients with light asthma could not be observed after short-term exposure to concentrations below 200 microg/m³. On basis of epidemiological long-term studies a threshold below which no effect on human health is expected could not be specified. Two short-term limit values have been proposed to protect public health: a 1-h value of 100 microg/m³ and a 24-h mean value of 50 microg/m³. Due to the limitations of epidemiological studies to disentangle effects of single pollutants, a long-term limit value cannot be easily derived. However, applying the precautionary principle, it is desirable to adopt an annual mean of 20 microg NO₂/m³ as a long-term mean standard to protect public health").

13. Eilstein D, Declercq C, Prouvost H, Pascal L, Nunes C, Filleul L, Cassadou S, Le Tertre A, Zeghnoun A, Medina S, Lefranc A, Saviuc P, Quenel P, Campagna D. The impact of air pollution on health. The "Programme de Surveillance Air et Sante 9 villes" (Air and Health surveillance program in 9 cities *Presse Med*. 2004 Nov 6;33(19 Pt 1):1323-7.

("If the levels of air pollution were reduced to 10 microg/m³ in the nine cities, 2800 premature deaths and 750 hospitalisations for respiratory disorders in children would be avoided, every Year").

II. On susceptible populations:

1. An update on estimated proportions of susceptible populations would be desirable (? Available from the American Lung Association --- early one by Gladys Meade)

2. Examples of key issues may have merit for judgment purposes, particularly with respect to arguments that only clinically manifested responses constitute an adverse

health effect. Emphysema may especially warrant singling out for evaluation, particularly since it has not been clearly defined and pathological as well as clinical diagnosis is often inaccurate or entirely unreliable. From a clinical standpoint, a lung function evaluation for a person being tested for the first time may not indicate an abnormality until 25% of lung tissue has been irreversibly lost. Data are presently insufficient data to establish whether the 25% estimate regarding a Pulmonary Function Test (PFT) should be lower or higher. In view of the relative insensitivity of PFTs, the lack of an altered PFT following an NO₂ challenge is by no means assurance that injury has not occurred. Moreover, tests carried out on healthy young volunteers will necessarily have variable results in view of individual variation that, from our studies of youths who died suddenly from violence had shown, will most likely if not invariably include some individuals with serious lung disease at clinical and/or subclinical levels. From a pathological standpoint, a scientifically valid diagnosis of emphysema is obviated by a virtually total failure nationally if not universally to process the lung properly at autopsy. Yet, there is no question from the results of appropriate studies that some degree of emphysema is ubiquitous in the general population and is contributing to the rise of emphysema to become the fourth leading cause of death.

Lastly, as is the case with emphysema, subclinical disease involving the body in general and the lung in particular, is ubiquitous in the general population. Standard setting for NO₂ should be directed at reducing the frequency and severity of subclinical disease (more properly, Morbidity) by asking what role does an ambient level of NO₂ play in the causation, promotion, facilitation, and/or exacerbation of disease in general. Compensation by the body in response to injury may lead to false reassurance that a noxious effect is no longer harmful. However, the remodeling of tissues and reactive proliferative processes generally have a cost in structural and functional integrity, and also in long term potential for chronic and/or neoplastic disease. The public should be made aware of critical questions that investigators face in their assessment of adverse health effects in addition to well known cardiovascular and lung effects. Examples are the role of ambient NO₂ levels in: bronchiolitis in infants and children, endothelin and platelet alterations related to thrombotic phenomena (stroke, pulmonary embolism, deep vein thrombosis), cancer metastasis (seeding of cancer cells), and diverse immunodeficiencies. Our ongoing work with asthmatic bronchitis has shown an unexpectedly high frequency of severe Eosinophil Airway Disease of uncertain cause.

Additional References

Kraft, M., Eikmann, T., Kappos, A., Kunzli, N., Rapp, R., Schneider, K., Seitz, H., Voss, J. U., and Wichmann, H. E. (2005). The German view: effects of nitrogen dioxide on human health--derivation of health-related short-term and long-term values. *Int J Hyg Environ Health* 208, 305-318.

Samoli, E., Touloumi, G., Zanobetti, A., Le Tertre, A., Schindler, C., Atkinson, R., Vonk, J., Rossi, G., Saez, M., Rabczenko, D., Schwartz, J., and Katsouyanni, K. (2003). Investigating the dose-response relation between air pollution and total mortality in the APHEA-2 multicity project. *Occup Environ Med* 60, 977-982.

Vedal, S., Brauer, M., White, R., and Petkau, J. (2003). Air pollution and daily mortality in a city with low levels of pollution. *Environ Health Perspect* 111, 45-52.

Arnold C.G. Platzker, MD

GOALS

1. Protection of the health of infants, children and adolescents
2. Protection of the most vulnerable pediatric populations
3. Allow normal outdoor activities for all children
- 4.

BACKGROUND

Little is known about the impact of NO₂ inhalation on the most vulnerable pediatric populations which include the fetus, infants born prematurely, newborn infants, early infancy, infants and children with chronic lung conditions, such as chronic lung disease of infancy (BPD), cystic fibrosis, interstitial lung disease. The target population studied in assessing the response to inhaled environmental pollutants has been healthy children, usually older than 7 years old, who are often compared to children with asthma, a surrogate for children with airway or lung disease. These studies are difficult to interpret due to the grouping of the children and adolescents who cough and/or wheeze in the same study without controlling for sex, race, socio-economic status or age groups [0-1 year, 1-2 years, 2-5 years, and 5-13 years]. There are developmental and physiological reasons for the necessity to study children in these age groups. First, establishing the diagnosis of asthma in young children prior to the age of 4-5 years old is difficult, often impossible, even those with atopy or a family history of asthma. Wheezy bronchitis is common in infants and young children from birth to 4 years. In fact, of the infants and young children [less than 4 years old] with chronic or recurrent cough or wheeze, less than 25% will have persisting cough or wheeze by 5 years of age. The reasons for this diagnostic dilemma stems from:

1. boys being born with smaller airways than girls (Taussig), making cough and wheeze more common in infant males than females during and following routine respiratory tract infections. In the first two years boys airways grow more rapidly than girls so that after 2 years of age airway caliber of males exceed that of females of the same age, so that after 2 years old females experience more cough and wheeze than females;

2. the lack of a specific serologic or lung function test to make the diagnosis asthma which makes the diagnosis of asthma problematic in the child less than 4-5 years of age in the absence of a strong family history.

Another intriguing unresolved issue in early childhood the impact of prenatal exposure to inhalant pollution on the fetus, that is, mother to fetus transmission of an inhaled environmental pollutants on lung development and lung function at birth and in infancy. For evidence of this potential impact on fetal development, one need only review the fetal impact of maternal cigarette smoking during pregnancy and lung function at birth and during infancy (Hanrahan, et al). Hanrahan studied pregnant women from an East Boston Health Clinic. He compared the neonatal and infancy lung function of infants whose mother's did and did not smoke during pregnancy through questionnaire and measurement of cotinine, a metabolite of nicotine, in the urine of mother and infant. Hanrahan found that the impact of in utero tobacco smoke exposure on the lung development and function in infancy was greater than that of post-natal environmental tobacco smoke exposure [ETS] during infancy and early childhood. Other studies published subsequently have confirmed the findings of Hanrahan, et al. Other major findings of in utero ETS which have been reported include reduced DNA, leading to lower birth weights smaller lungs (reduced TLC), higher total respiratory resistance [Rrs] indicative of smaller caliber of the airways, and lower maximal expiratory flow rates at functional residual capacity [V'maxFRC] and disordered breathing during sleep leading to increased risk of infant apnea or sudden death.

While studies of ETS on the fetus has revealed a major impact of ETS on birth weight and on fetal lung growth and function at birth, there are no comparable studies of NO₂ and related (fellow traveler) pollutant exposure on the fetus and newborn infant. NO₂ has been postulate to have a small effect on the odds ratio for low birth weight and for an increase in sudden infant death, but there have been no corresponding studies of lung function at birth or in early infancy focusing on the impact of NO₂ exposure of the fetus and in infancy. These studies been primarily on the impact in school-age children and longitudinal studies have been conducted to record the impact of NO₂ or NO₂ + PM₁₀ over time on school children. In summary, there have been no studies in which the impact of NO₂ have focused on the fetus, newly born, infant or in the early childhood pre-school years when the airway caliber is small and very reactive with airway obstruction is common with respiratory illnesses such as metapneumovirus or RSV infection.

CONCLUSIONS:

The studies of nitrogen oxide air pollutants are compromised by a lack of ability to discriminate between the effects of nitrogen dioxide and its companion air pollutants. There are inadequate or no pediatric studies which:

1. Define the relationship between maternal exposure and the impact on the fetus;

2. Post-natal exposures and respiratory function in
 - a. Prematurely born
 - b. Full term infants
 - c. Infants born with neonatal and early respiratory illnesses, RDS, chronic lung disease of infancy (BPD), cystic fibrosis, wheezy bronchitis;
 - d. Following sentinel lung infection (metapneumovirus, RSV infection, mycoplasma pneumonia, etc)
3. Studies of at risk populations
 - a. Proximity to freeways (traffic)
 - b. Socio-economically disadvantaged
 - i. Indoor pollutants + outdoor
4. Include impact of exposure on inflammatory markers, allergic inflammation

Appendix C

Staff Responses to Comments of the Air Quality Advisory Committee (AQAC) for NO₂

Appendix C
Staff Responses to Comments of the
Air Quality Advisory Committee (AQAC) for NO₂

Based on:

Summary Comments of the Air Quality Advisory Committee on the Scientific Basis of the California Ambient Air Quality Standard for Nitrogen Dioxide

Note: Staff responses are given in bold italics following each point raised by AQAC.

The staffs of OEHHA and the ARB provided an excellent review of the current literature relevant to the sources, transport and health effects of ambient nitrogen dioxide (NO₂). The review provided a firm basis for establishing the needs for modification of the current NO₂ air quality standards and the committee was unanimous in its appreciation of the effort and diligence involved in producing the report.

The Air Quality Advisory Committee (AQAC) has provided comments on a chapter by chapter basis and also addressed specific overarching questions that were submitted to them during their review of the report.

In conducting its review, the Committee specifically considered whether the documentation adequately addressed:

- The extent of evidence of effects at or below the existing ambient air quality standard.
- The nature and severity of those effects.
- The magnitude of risk when ambient levels are at or near the level of the existing standard.
- The available evidence that children may be more susceptible than adults.
- The degree of outdoor exposure relative to the level of the standard.

Children's protection, with an adequate margin of safety, is of paramount importance to public health. As the committee report indicates, this is an area in which more work is needed. Children with chronic lung diseases such as bronchopulmonary dysplasia, asthma and cystic fibrosis could be at special risk but, with the possible exception of

asthma, there has been little research effort on health effects in these potentially susceptible groups. Since asthma affects nearly 10% of the child population, the effects of NO₂ on this group is of special importance. Having said this, the committee was particularly impressed with the efforts taken in the preparation of the reviewed documentation to thoroughly evaluate what is presently known about the effects of NO₂ on the health of children.

A previous evaluation of the health protection afforded by the current ambient air quality standards in California was mandated by SB25. The SB25 review which has been previously published identified clinical and epidemiological studies that suggested effects of NO₂ on pulmonary function, asthma exacerbation and acute morbidity in children and adults at or below the 1-hr CA standard of 0.25 ppm. Accordingly OEHHA and ARB staff have compiled and critically reviewed the scientific literature to determine whether:

- The current NO₂ standard provided an adequate margin of safety,
- A different averaging time was warranted.

In the Technical Support Document that was prepared, the published literature information was integrated and interpreted and the potential for exposures was assessed, the individuals at risk were identified, the potential health outcomes were determined and recommendations were made to establish new air quality standards that will better protect health for California citizens.

Based on its review of the Staff Report and the Technical Support Document the Air Quality Advisory Committee endorses the Staff recommendations for a long-term standard:

- Annual Average NO₂ at 0.030 ppm
- Not to be exceeded

The Committee also endorses the reduction of the 1-hr standard to a level below the current 0.25 ppm NO₂ and agrees with the SR recommendation of a 0.18 ppm 1-hr average standard (not to be exceeded). However, the committee requests improved documentation of the support that this level of standard provides an adequate margin of safety for sensitive populations. While the Committee endorses a 1-hr standard as the appropriate averaging time to capture acute events, the Committee suggests that the NO₂ monitoring network be realigned to provide better spatial resolution and include monitoring of “hotspots” and that ARB consider conversion of the form of the standard from ppm(v) to ppb(v) to avoid ambiguities due to rounding.

The Committee has identified some issues that should be addressed in a revised Technical Support Document. These issues are presented below.

Critique

Chapter 1.

Chapter 1 provides summary information of historical interest. Current Standards were summarized. The NAAQS provides an annual NO₂ standard but does not include a short term standard. CA currently has a short-term but not a long-term standard.

Standard	1 hr (ppb)	Basis	Annual (ppb)	Basis	Comment
NAAQS			53	Arithmetic mean of 1-hr measurements	
WHO	106		21		Guidelines
CA (Current)	250	1hr Arithmetic Mean			Not to be exceeded
CA (Proposed)	180	1hr Arithmetic Mean	30		Not to be exceeded

It would be appropriate to include in the summary the rationale for not having a secondary standard. This might be an important consideration since in Chapter 2 the large contribution (50% during winter in SC basin) of NO₂ to fine secondary PM formation is discussed. In the Staff Summary of Welfare effects, visibility degradation which might be a basis for a secondary standard it was determined (1992 review) that meeting the 250 ppb NO₂ standard would adequately protect against visibility degradation because “the majority of the effect was due to fine particulate matter.”

The reduction to 180 ppb will reduce visibility impacts further and this could be mentioned as an added potential benefit of the proposed standard.

Additional language in both the Staff Report and the Technical Support Document now support the statement in the proposed regulatory text that the 180 ppb standard would help limit adverse effects on welfare, including atmospheric discoloration by NO₂. Further, the added benefit of visibility improvements based on the decrease in fine airborne nitrate has also been added to both reports.

Chapter 2.

Chapter 2 discusses issues of atmospheric chemistry. The complex interplay between NO₂ and other components of the atmosphere such as NO (the other portion of NO_x), ozone, particulate matter and VOCs is described in good detail. Future research will undoubtedly refine details, but NO₂ physics, chemistry, measurement, sources and sinks are all adequately well understood to regulate, and this review thoroughly covers the topics needed for updating and establishing new regulations. The section on visibility impairment (2-9) separates the direct light absorption of the gas from that of the secondary aerosol. It would be very useful to indicate NO₂-related PM contribution and what the effect would be of lowering the CA short term standard to 180 ppb.

Specific Comments

1. Definitions of NO_x and NO_y
 - should be defined carefully and consistently (they are not--see pp. vii, 2-11, 3-1)
 - should be defined when the term is first used in each chapter (e.g., p. 2-2 needs NO_x definition)
2. p. 2-2, last sentence in the 1st paragraph after equation 2: this sentence is awkward (although technically correct, "remainder" usually refers to the smaller portion, not 90%)
3. Make sure all equations are balanced (e.g., see p. 2-2, equations 2 and 3)
4. p. 2-4, section 2.3.2, 1st paragraph, last sentence--drop "Thus"
5. p. 2-4, next to last line: improve "in this chemistry" (perhaps with "similar reactions")
6. p. 2-15, 4th line: do the authors really mean NO_x?
7. p. 2-15, section 2.9, 8th line--get correct Section number

All suggested changes in the above "Specific Comments" have been made, except for #6, where NO_x is the intended chemical formula.

Chapter 3.

Chapter 3 deals with measurement methods and endorses the chemiluminescence method as the approved method in CA. Measurement of NO₂ is well-defined, sensitive, quantitative and selective. To avoid the need for correction due to elevation or weather changes in barometric pressure, it is appropriate to continue measuring, reporting and regulating in units of volume fraction – rather than mass concentration such as ug/m³. For clarity, it might be helpful to move toward uniformly using ppb(v) units (for example: 180 for 1 hour, 30 for annual average) -- rather than ppm(v) which requires a trailing zero that can lead to confusion about rounding/truncating data and hence determining

resulting exceedances. The literature uses both ppm(v) and ppb(v), as with ozone, so either is acceptable. The measurement precision is not discussed. What is the degree of uncertainty around a 1-hr average concentration? Given that the standard is listed as “not to be exceeded”, an analysis of precision vs. the expected number of exceedances at the level of the standard might provide useful guidance. Also in Chapter 5 the calculation of a “peak indicator value” which is used to exclude “extreme concentration events” is discussed. How does measurement error and instrument precision factor into the peak indicator value?

Agency policy is to use ppm, and we have done so throughout the report. Chapter 5 now includes an expanded discussion of peak indicator values and extreme concentration events. Staff has determined that degree of uncertainty around the determination of NO₂ concentrations is sufficiently low to justify standards that can be expressed as either ppb units or thousandths of a ppm. ARB’s equipment specifications (Total Oxides of Nitrogen [NO_x] Analyzer, July 17, 1999) indicate that the precision of the NO_x analyzer is plus or minus 0.5 ppb. Chapter 3, Section 3.2 now includes this information. Further, method precision based on performance audits on 78 NO_x monitors in 2004 resulted in a standard deviation of 5%, which translates to plus or minus 1 ppb on 30 ppb values. A discussion of accuracy and precision of the measurements has been added to the text in Chapter 3 of the Technical Document, and to Section 2.1.5 in the Staff Report.

Chapter 4.

Chapter 4 discusses sources and emissions. The report adequately describes the combustion sources of NO₂. It would be appropriate to also discuss non-combustion sources of NO, which inter-converts with NO₂. There are entirely natural (sometimes called biogenic) emissions from soil, grasses and trees, as well as anthropogenic non-combustion sources, generally in the area of managed annual and perennial plants, as well as animal agriculture. These processes include fertilizing, composting, and feed and waste management, and include non-commercial activities such as gardening. As management of combustion sources steadily improves, non-combustion sources will rise in relative importance. Natural/biogenic sources must be included since they contribute to the background, even if they are relatively uncontrollable; managed/anthropogenic sources must be included since they are becoming a larger factor on a relative basis – and possibly even on an absolute basis in some regions and/or seasons. Improving the summer-time ozone problem in the San Joaquin Valley will probably only be achieved with reductions in NO_x. One could therefore mention that NO₂ regulation will have a secondary benefit, i.e., reducing ozone and PM, and may actually be essential.

These issues are discussed in the technical support document, in section 2.7.2 (Natural Processes that Remove Nitrogen Dioxide), and in section 2.10.2 (Biological Processes and NO_x Emissions).

It is clear from the data that the fractional contribution of mobile sources to ambient nitrogen emissions is decreasing. Stationary source emissions are expected to increase slowly over the next few decades due to population pressures. How the projections were made is not presented.

Emissions projections are based on historical emission inventory data, expected economic and population growth, and expected emissions controls. This is described in ARB's annual "California Almanac of Emissions and Air Quality". We have added this statement to Section 4.2 in the TSD which addressing emissions.

Were changes in fuels considered given the increased costs and decreased availability of the fuels currently in use? The extent to which these changes are driven by NO₂ regulations *per se* or by reductions in combustion emissions related to reduction of PM could be made clearer.

The ARB receives emissions projections from several local planning agencies that use differing models to generate their estimates. Fuel cost is one of the factors used to determine what mode of travel is used, how many miles are traveled, and how many individual trips are made. Therefore, fuel cost impacts emissions projections, but the extent is difficult to quantify. ARB regulations that affect fuel use have been driven more by the need to control PM and ozone, rather than NO₂ per se. Chapter 4 of the Technical Report includes a discussion of the decrease in NO_x emissions from 1980 to 1995 due to a change in fuel from oil to natural gas.

Specific Comments

p. 4-1 and 4-2--same sentence repeated (1st sentence of 4.1.1 4th sentence of 4.2)

the graph on p. 4-2 and figure on p. 4-3 are difficult to read

Corrections to the repeated sentence have been made. Figure 4-2 has now been enlarged to be more readable.

Chapter 5.

Chapter 5 discusses ambient air quality with respect to NO₂ for CA. Data for each air basin in the state are presented. The discussion, however, centers around overall trends and ignores the increasing trends in the North Central Coast and Sacramento Valley basins.

Chapter 5 now includes a discussion on air basins with increasing trends. We note that the Sacramento Valley basin shows more variability in the maximum 1-hour concentrations, compared to other basins. The variability may be due to changes in emission sources and may also reflect year to year changes in

meteorology. A decline in NO₂ concentrations is expected in the coming years (California Almanac of Emissions and Air Quality, 2006).

General Comments

An explanation of the peak indicator needs to be moved from 5-43 to 5-3. It is not clear why the Statewide average of maximum 1-hr NO₂ is greater than in any of the individual air basins. Tables 5.3 and Figure 5.4 need some explanation of this.

The explanation of the peak indicator has been moved from section 5-43 to 5-3. The Statewide monthly average of maximum 1-hr NO₂ concentrations is slightly higher than the average provided for the South Coast air basin (Table 5-3), which is the air basin that usually has the highest levels. This is because on some days within a given month, higher NO₂ levels have been observed in an air basin other than South Coast. This information has been added to the Table and Figure legends.

Table 5.1 shows all air basins in CA average below the proposed annual average standard of .030 ppm, but presumably the standard has to be met at every monitor? If so, then data for individual monitors should also be shown. Table 2 in the staff report shows several monitors in the South Coast district exceeded 0.030 ppm in 2004.

Table 5.1 is a summary of the annual average for each year. The annual average is calculated by taking the maximum hourly concentration for a site within the air basin. The maximum from all sites in the air basin for the entire year was used to calculate the annual average. To clarify this point, the legend for Table 5.1 now states: "Annual average for all sites within the air basin was calculated from the maximum hourly values taken daily from the highest recorded site." Detailed daily concentrations for each basin is available from the ARB.

Chapter 5 reports that no districts are out of compliance with the current 1-hour standard after adjustments for the Expected Peak Daily Concentration (EPDC), but it does look like Salton Sea and South Coast districts are at risk of exceeding the proposed new 1-hour standard. However, Table 5.7 shows that the EPDC based on 3 years of data is below the proposed new hourly standard in all districts.

Data reported in Chapter 3 show declining concentrations of NO₂ in most districts, and especially in those that have been reducing emissions to meet the federal standards for PM and ozone. Reducing NO_x emissions is one of the strategies being used to meet the PM and ozone standards, because NO_x is a precursor to both PM and ozone.

All of this means that the new standards are either currently met or not far out of reach and may be met soon as a result of efforts to meet the PM and ozone standards. The standards are supposed to be health and welfare based so this is not a limiting consideration, but as a practical matter the effect of these changes to the standards will

be mostly to encourage districts to continue to reduce NO_x emissions as part of their strategies to meet PM and ozone standards.

Staff agrees that, pursuant to the Health and Safety Code, the establishment of a new standard does not depend upon the risk of exceeding that new standard.

Section 5.5 presents an Analysis of Peak Nitrogen Dioxide Exposure in California. This section used inverse-distance weighting (IDW) from monitor location to estimate population averaged exposures. However, actual population exposures are likely to be higher on average because of in-vehicle and other personal exposures, and more importantly because a subpopulation will have high exposures simply based on proximity to sources such as traffic that are not included in the IDW model. This results in over-smoothing of the true spatial pattern of exposure (see Jerrett 2005, JEAEE 15:185-204). Some estimate based on this should be included given the indication from the epidemiologic studies that NO₂ effects are found at concentrations much lower than standards. NO₂ is serving at least in part as an indicator for traffic and other sources of unmeasured air pollutants. The spatial distribution of NO₂ secondary to traffic should receive some additional attention (see below).

Section 5.7.1.5 starting on page 5-74, presents important information on the spatial variability of ambient NO₂ concentrations. The information presented suggests that because NO₂ reacts quickly in the atmosphere, central monitors may not fully reflect concentrations relevant for the population living, working, or attending school near major traffic sources. An important topic for future research is whether the exposures measured at stationary monitors are sufficiently protective of public health. The report notes that 10% of public school children spend their school days within 150 meters of a busy road. Given the apparent effects of NO₂ exposure on lung function development, it will be important to determine whether this population is adequately protected by these standards. There probably are not sufficient data available at this time to answer this question, but it is important for ongoing research.

New sections on the Spatial Variability of NO₂ have been placed into the Staff Report (Section 2.4.4) and the Technical Support Document (Section 5.8). These sections discuss the Jerrett et al. (2005), Wu et al. (2005), and Ross et al. (2006) studies. Section 1.4 of the Staff Report now includes a staff recommendation for further research in this area: "The spatial distribution of the air monitoring sites for NO₂ should be reviewed to determine if these adequately characterize exposures to NO₂, especially for infants, children, asthmatics, and individuals living near high volume roadways. Based on the results of this review, staff should evaluate and recommend the locations of monitoring sites to adequately determine Californian's exposures to NO₂."

Specific Comments

Pg 5-3 Para 3 L 4: Peak indicator was not previously described. The information from 5-43 should be placed here.

The discussion on peak indicator was moved up to Chapter 5.2.

Pg 5-12-Section 5.4.3, first sentence: it's NO₂ not ozone. It's 0.25 not 0.025 ppm.

Staff corrected the text.

Pg 5-14 Para 2 L1: Table 5.3 (not 5.4).

Staff corrected the text.

Pg 5-15: The note on Table 5.3 is not clear. Are these ppm concentrations or counts?

The table includes ppm concentrations. Therefore, staff changed the note to read: "The seasonality is represented by the monthly average of daily maximum values that were measured at one or more monitoring sites in an air basin or planning area for the years 1990 to 2004."

Pg 5-55 Section 5.6.2.1.1 Concentrations in Homes: What is meant by "Indoor/outdoor NO₂ ratios were positively associated with the community"?

Three factors were found to be positively associated with indoor NO₂ levels: community (outdoor) levels, the presence of a gas range, and the presence of an air conditioner. The Technical Support document has been revised accordingly.

Pg 5-74 Section 5.7.1.5 Spatial Variability of NO₂ Concentrations

This section was limited compared with the long section on indoor sources. Given that the ambient standard is the topic of concern, it would be appropriate to place a considerably larger emphasis on how spatial variability affects the inaccuracy of NO₂ measurement at stations in relation to population exposure. Additional information from the Singer 2004 study for instance would be helpful. They found a school located directly adjacent to a major freeway and a shopping center showed normalized NO₂ and NO_x were around 60% and 100% higher than regional background levels. At three schools within 130–230m downwind of a freeway, normalized NO₂ and NO_x were around 20–30% and 50–80% higher than regional levels. The levels at the regional site in the East Bay study would underestimate their exposure. Given that children are a susceptible subpopulation, this is an important issue.

Wu et al. found overall within-community variability of personal exposures was highest for NO₂ (+/- 20-40%), and that traffic was a major determinant:

Wu J, Lurmann F, Winer A, et al. Development of an individual exposure model for application to the Southern California children's health study. *ATMOSPHERIC ENVIRONMENT* 39 (2): 259-273 JAN 2005.

Ross et al. reference below was not discussed. This that might shed more light on spatial variability:

Ross Z, English PB, Scalf R, et al. Nitrogen dioxide prediction in Southern California using land use regression modeling: potential for environmental health analyses. *JOURNAL OF EXPOSURE SCIENCE AND ENVIRONMENTAL EPIDEMIOLOGY* 16 (2): 106-114 MAR 2006

The staff report and technical support document now include sections on spatial variability of NO₂, including studies by Singer (2004), Wu et al. (2005), and Ross et al. (2006).

Chapter 6.

Chapter 6 describes data from controlled human exposures. These data are used as the primary basis for reducing the short term standard from 250 ppb to 180 ppb. The chapter adequately discusses the recent toxicology information available.

General comments

It would be good to be a bit more consistent about the meaning of the variable findings in some subjects with asthma. The wording in section 6.1, paragraph 3 (e.g. "...suggest that some individuals experience increased airway responsiveness to NO₂ in the range of 0.2-0.3 ppm" seems more on target than the wording on page 6-18, para 3 "These recent studies involving allergen challenge appear consistent in demonstrating effects...." Otherwise, the chapter did an excellent job of capturing a challenging body of literature.

Thank you. We appreciate your review and comments. As suggested, the wording on page 6-18 has been changed to reflect the variable findings in some subjects.

Specific Comments

P6-7, para 3: The description of effects of IL-5 and IL-13 is slightly inaccurate. These are cytokines produced by Th2 lymphocytes, but neither "can induce a Th2 response in T helper cells." Actually, T cells don't express receptors for these cytokines. IL-4 is the major cytokine that induces Th2 cell differentiation.

This has been corrected in the chapter revision.

P6-16, para 4: Do you mean “decreased peak flow” rather than “increased”?

The correct word is “increased” and has been corrected in the chapter revision.

P6-24, para 2: The statement that “The divergence of findings from various studies suggests that some individuals with asthma are particularly susceptible...” might be overstated. It might be preferable to simple say “...suggests that some individuals with asthma might be particularly susceptible...”

The sentence has been rephrased.

Chapter 7.

Chapter 7 presents an evaluation of the epidemiological data reviewed.

General comments:

Overall, this is a comprehensive review of the epidemiologic literature on NO₂. It points to well-known methodological weaknesses that are inherent to the study of ambient air pollution in free-living human populations, or weaknesses that have not been addressed yet by researches. None of these weaknesses takes away from the coherence of the epidemiologic evidence with the clinical and toxicological data. The choice of an NO₂ standard based on susceptible populations is well supported by the evidence presented. Susceptible subpopulations were clearly identified in several reviewed studies, including children with asthma, infants, patients with pre-existing cardiovascular or respiratory disease, and the elderly. The time series studies evaluating the relationship between hospital admission or ED visits and asthma in children were remarkably consistent and robust for NO₂. Often in the face of significant particle associations, the associations with NO₂ remained after inclusion of the particle measurements. The chapter’s organization could be improved by adding some summary figures or tables that provide an overview of the available science.

Besides the summary table of all of the studies provided at the end of the technical chapter on epidemiology, we have summarized the most important studies for the development of the standard in a figure and table in the OEHHA recommendation chapter.

An important issue discussed was that in many of the epidemiologic studies, NO₂ is likely acting as good indicator of the complex gas-particle mixture originating from vehicular traffic. Depending on the region, other important sources significantly contribute to this mixture (e.g., ports). What is important in this concept is that the regulatory standards currently used focus on a very limited set of pollutants, most of which are in part surrogates of other potentially more harmful pollutants. The ultimate focus of air pollutant regulation is rightly on sources, and the ability of the pollutant to function as an indicator of sources is important in this regard, apart from its independent effect on health.

Comment noted.

The summary conclusion 7.3.1.1 after the text on cohort studies is inaccurate and misleading. It reads as follows:

“The studies in this review show little evidence for effects of long-term concentrations of NO₂ on prevalence and/or incidence of asthma, allergic rhinitis, and atopic eczema. For asthma diagnoses and symptoms, two cross-sectional studies show positive and three show negative associations.”

The summary conclusion does not reflect what is in Tables 7-10. The word negative is not correct. It might be better to refer to the findings as “null”, and the count does not reflect the tables. The Table shows no negative associations and in general, the ORs or RRs are positive but not always statistically significant. The words “little evidence” is misleading. For instance, in the case of allergic sensitization, the words should be “there are few studies.” Describing the literature as “little evidence” suggests that many studies find no association. The one cross-sectional study (Janssen 2003) with high power showed associations between NO₂ and total IgE and positive skin prick tests to allergens. This finding was consistent with the robust findings of the smaller study by Kramer et al. 2000 for atopic sensitization and allergic rhinitis in relation to outdoor home NO₂. The conclusion about the surrogate nature of NO₂ holds, but does not diminish its usefulness in the regulation of unmeasured and largely unregulated air pollutants that NO₂ probably represents. The CHS findings for OC and EC (solely measured for the CHS) along with NO₂ further support that view.

Descriptions of the Janssen et al. (2003) study and the Kramer et al. (2000) study have been added to this section on cohort studies. We have revised the conclusions to better reflect the full scope of evidence.

The authors have been very careful to acknowledge the limitations of the epi literature in terms of being able to specifically identify NO₂ as the causative pollutant. The co-occurrence of the set of traffic-related pollutants that includes NO₂ is the primary difficulty. However, it is clear that this mix of pollutants is associated with adverse health effects. When the epi results are considered along with the clinical and toxicological evidence, there is reasonable support for the conclusion that NO₂ is at least one of the harmful constituents of this mix. This is a prudent interpretation of the evidence in terms of protecting public health.

The epidemiology results are strongest for an association between NO₂ and respiratory illness, especially asthma exacerbations. This is consistent with the evidence from the clinical studies. These associations are observed in the epi studies at ambient concentrations that exist in CA.

We have revised our summaries of the evidence to indicate that, indeed, the epidemiologic, toxicologic and clinical evidence all consistently suggest effects of NO₂ on asthma exacerbations and other respiratory outcomes.

Gauderman et al. (2004) and related studies seem especially important because they suggest lung function development decrements in children over an 8-year study. This is a very serious effect that is a risk factor for chronic disease and premature mortality later in life. This elevated risk is observed at long-term concentrations of 25-30 ppb, which exist in some CA locations. Questions regarding co-pollutants are still important, but this association is consistent with toxicological study results showing adverse effects of NO₂ on lung function development in some animal studies. It is also important to note that this effect could lead to premature mortality, but it would not show up in time-series mortality studies because it is a function of childhood exposure, not short-term exposure fluctuations.

It should be pointed out that little is known about the impact of NO₂ inhalation on vulnerable pediatric populations which include the fetus, infants born prematurely, newborn infants, early infancy, infants and children with chronic lung conditions, such as chronic lung disease of infancy (BPD), cystic fibrosis, interstitial lung disease. The target population usually studied in assessing the response to inhaled environmental pollutants has been healthy children, usually older than 7 years old, who are often compared to children with asthma, a surrogate for children with airway or lung disease. These studies are difficult to interpret due to the grouping of the children and adolescents who cough and/or wheeze in the same study without controlling for sex, race, socio-economic status or age groups [0-1 year, 1-2 years, 2-5 years, and 5-13 years]. There are developmental and physiological reasons for the necessity to study children in these age groups. First, establishing the diagnosis of asthma in young children prior to the age of 4-5 years old is difficult, often impossible, even those with atopy or a family history of asthma. Wheezy bronchitis is common in infants and young children from birth to 4 years. In fact, of the infants and young children [less than 4 years old] with chronic or recurrent cough or wheeze, less than 25% will have persisting cough or wheeze by 5 years of age. Some reasons for this diagnostic dilemma are:

1. boys being born with smaller airways than girls (Taussig), making cough and wheeze more common in infant males than females during and following routine respiratory tract infections. In the first two years boys airways grow more rapidly than girls so that after 2 years of age airway caliber of males exceed that of females of the same age, so that after 2 years old females experience more cough and wheeze than females;
2. the lack of a specific serologic or lung function test to make the diagnosis asthma which makes the diagnosis of asthma problematic in the child less than 4-5 years of age in the absence of a strong family history.
3. Difficulty in performing reliable pulmonary function tests in very young children.

We have indicated the lack of the studies on potentially susceptible populations, including infants and children and, in our summary of research needs, have added that this population needs to be studied.

Specific Comments:

P 7-1: Clarify the comment about epidemiologic studies that:

“it is not possible to quantify exposure for individuals, as is commonly done in chamber studies.”

I assume you are excluding personal exposure monitors because hourly sampling is not yet available.

The text has been revised to clarify the comment on measurement error.

For 95% CI, I would suggest using commas to separate upper and lower limits instead of dashes. Some journals do this to avoid the misinterpretation of interval sign and to make reading easier.

The dashes were used because they reflected the presentation in the written scientific articles. This style was determined jointly with other chapter authors and cannot be altered at this point.

P 7-6, bottom: The following sentences are unclear.

“For asthma, a stronger effect was detected considering distributed lag models (lags 0 to 13 days), with PM10, NO₂ (4.7% for 20 pbb, 95%CI=1.1-8.5%), and CO, showing a statistically significant effect.”

Suggest separating out the numbers for the NO₂ association.

The text has been revised to clarify the sentences and incorporate these suggestions.

“In multipollutant models, the NO₂ effects were attenuated when PM10, NO₂, and CO were considered simultaneously. However, the effect of NO₂ on emergency visits for asthma was not attenuated in multi-pollutant models while the estimates for the other pollutants suggested weaker or no associations.”

Attenuated or not?

The text has been revised for clarity.

Throughout, for the time series results, there was a shift in the use RR and % change. For instance, in the text on page 7-7, results for Simpson et al. 2005 are in RR, but the table on p 7-52 is in % and not consistent if $100 \times RR = \% \text{ change in admissions}$.

In the text, the results are given in the form presented in the individual papers. In the tables describing the time-series studies the results are all converted to % excess risk, calculated as $(Relative Risk - 1) \times 100$, in order to make it easier to compare results of studies. This formula is now listed in the text on page 2.

Last line and word p 7-9: typo.

Correction made.

P 7-11 last paragraph: should be "...after adjusting for outdoor pollens and fungal spores."

The text has been revised to incorporate this change.

P 7-13: Just et al. 2002: Larger associations were seen between respiratory infections and NO₂ and BS. This is missing in text and table.

This text and the table have been revised to incorporate this information. The actual results for respiratory infections are now shown in the table.

P 7-13: Moshhammer et al. 2006: This is a general population study of children as noted in Table 6. There is no information about clinical status, so this paper does not belong in a section on children with asthma. It is nevertheless important that they did find lung function deficits in relation to increased NO₂. There are several other studies that have studied otherwise healthy children or mixed populations, although the clinical relevance is lessened by this approach to sample selection.

We placed this paper in the section on children with asthma because there were asthmatics in the population and there would be no other table in which to place it otherwise. We realize that this was a mixed population.

Table 4 and related section: The two outcome and age groups are unrelated and is confusing to see asthma in children combined with arrhythmias in adults. Panel studies of medication use in asthmatic children is separated but would be more appropriately combined with the other panel studies of asthmatic children looking at a variety of other outcomes. The section could be "Panel Studies" and then subsections with the outcome groups as presented, including General Population and Other Pediatric Panels.

We have a section of panel studies in this chapter.

Table 6: Lung function in Asthmatic Children: Again, the title is inaccurate since many of the studies were not of asthmatics. In addition, nearly all studies have only looked at PEF, an inaccurate measurement of large airway function compared with FEV₁. Therefore, it is important to report in Table 6 and the text on panel studies using FEV₁, which are few in number. The review missed two recent papers in this regard:

Delfino RJ, Quintana PJE, Floro J, Gastañaga VM, Samimi BS, Kleinman MT, Liu L-JS, Bufalino C, Wu C-F, McLaren CE. Association of FEV₁ in asthmatic children with personal and microenvironmental exposure to airborne particulate matter. *Environ Health Perspect.* 2004; 112: 932-41.

Delfino et al. (2004) followed a panel of 19 children with asthma for two weeks with personal PM nephelometers. They found central-site 5-day average 8-hr maximum NO₂ was inversely associated with percent predicted FEV₁ (per IQR increase in NO₂ of 10.5 ppb, -1.16%; 95% CI, -2.4 to 0.1), and associations were similar for the 3- and 4-day average and for 1-hr maximum NO₂. However, NO₂ was confounded by personal PM with parameter estimates falling near zero. Associations of FEV₁ with personal PM were largely independent of NO₂.

Discussion of this paper has been added to the chapter and to the tables. We have also added another new asthma panel study of symptoms and medication use by Schildcrout et al. (2006).

P 7-13, Cardiovascular Effects: An important paper was left out that is currently the only repeated measures study of ECG-measured ST segment depression. This is important because transient myocardial ischemia is clinically and/or biologically relevant to more severe outcomes such as MI:

Pekkanen J, Peters A, Hoek G, Tiittanen P, Brunekreef B, de Hartog J, et al. 2002. Particulate air pollution and risk of ST-segment depression during repeated submaximal exercise tests among subjects with coronary heart disease: the Exposure and Risk Assessment for Fine and Ultrafine Particles in Ambient Air (ULTRA) study. *Circulation* 106:933-938.

This was a study of 45 adults with stable coronary artery disease that analyzed data from repeated biweekly in-clinic ECG measurements during submaximal exercise testing and outdoor ultrafine and fine particles measured at a central regional site of Helsinki, Finland. They found significant associations between risk of ST segment depression and ambient lag 2 day PM_{2.5} mass (OR 2.8, 95% CI: 1.42, 5.66). Similar magnitudes of association were found for ultrafine and accumulation mode particle number concentrations, but smaller but significant associations were also found for lag 2 day NO₂ (OR 2.02, 95% CI: 1.34, 3.04) and CO (OR 1.73, 95% CI: 1.26, 2.39), which were moderately correlated with the co-located particle measurements. Two pollutant models for PM and gases were not tested.

A discussion of this paper has been added to the text.

Table 7 and 8 titles would be clearer to contrast with 9 and 10 if it was “between-community”

The titles of Tables 7 and 8 have been edited to reflect this comment.

Pp 7-66 to 7-67: ORs are for what increase in NO₂ in ppb?

The odds ratios are for a 24 ppb increase. The heading rows of the tables will repeat to show this information.

P 7-17 statement: “In a West German study (Kramer et al. 2000), outdoor levels of NO₂, ...” To be clear, it’s outdoor home, a point that strengthens the following statement in the text on the importance of traffic given the null results for personal NO₂.

Table 9, Kramer: The report is somewhat inaccurate since I believe it includes the rural subjects, which biased estimates downwards. Here is what I found:

Associations were dominated by the urban subgroup as follows:

Outdoor home NO₂, but not personal NO₂, was significantly associated with reports of at least 1 week with symptoms of wheezing: OR for 10 µg/m³ increase, 14.9 (95% CI, 2.59, 86.4); and with symptoms of allergic rhinitis: OR 1.81 (95% CI 1.02, 3.21), which in pollen season increased to OR 3.09 (95% CI 1.38, 6.92).

An ever diagnosis of hay fever was associated with outdoor NO₂, OR 4.24 (95% CI: 1.01, 17.8), asthma was not, OR 1.82 (95% CI: 0.36, 9.36).

Atopic sensitization to pollen, house dust mite or cat, and milk or egg were each significantly associated with outdoor NO₂ (ORs ranged from 3.5 to 5.0), but not personal NO₂. (See text and Figure 2 in Kramer).

The text has been revised to incorporate this information. The Kramer paper was consulted to ensure accuracy of the report.

P 7-23, statement: “In addition, more localized panel studies could be used to attempt to separate the effects of NO₂ from other pollutants.” I hope to provide the committee with results from my panel study currently under review that makes notable advances in this area using eNO from asthmatic children in relation to personal and ambient NO₂, PM2.5, EC and OC.

Comment noted.

Chapter 8.

Chapter 8 deals with toxicology of NO₂. The chapter is well written but most of the real information is contained in the summary. The information from the summary should be incorporated into the body of the TSD.

The toxicology information in the summary, identified as Appendix A. Toxicological Effects, has now replaced the considerably briefer toxicology information that was previously located in Chapter 8. This information will

provide a stronger presentation of the toxicology literature towards the front of the TSD.

The brief presentation made to the Committee provided a very good overview of the key factors and salient features of that presentation should be added to the TSD also.

The information in the brief presentation given to the Committee at the meeting is now essentially provided in brief summaries at the end of each major section in Chapter 8. In addition, a summary of key effects observed at concentrations below 0.25 ppm (the standard 1 hr level prior to this NO₂ Review) and above 0.25 ppm are presented in separate sections at the end of the chapter. These sections have been expanded to emphasize the key effects with acute and prolonged exposures to low NO₂ concentrations.

The chapter mentions dosimetry, but the use of dosimetry for bridging between data in animal models to application to humans needs to be discussed. For example the TSD mentions that estimation from Miller et al. suggests that, for the same exposure, the dose to the rat's epithelium would be ¼ of that delivered to a human's. The Miller modeling should be checked but, if correct, one could use such information to put the data from rat studies at concentrations from .5 to 5 ppm NO₂ into context of "equivalent" human exposures at ~0.1 to 1 ppm. This suggestion is obviously an oversimplification of a very complex issue – the Committee provides it as an example of one method to strengthen the link between the mechanistic studies available from toxicological studies to possible mechanisms in humans.

To use the dosimetry modeling data as a bridge between animal and human NO₂ exposure, the following sentences were added to the Introduction: "...emphasis on animal studies using exposures up to 1.0 ppm is indicated by pharmacokinetic modeling data that estimate humans receive a 2-4 times greater tissue dose of NO₂ at sensitive pulmonary sites relative to rodents. Assuming equivalent pharmacodynamic responses between species, this would suggest that exposures as low as 0.25 ppm NO₂ in humans could result in the same degree of injury as exposure to 1 ppm NO₂ in rodents".

No further dosimetry modeling research could be located in the literature since the work by Miller et al. (1982) that investigated rodent-to-human differences in tissue doses of inhaled NO₂ in sensitive areas of the lung (e.g., centriacinar region). However, a recent report by Tsujino et al. (2005) has been added to the dosimetry section that modeled NO₂ concentrations in airways of several species. It was observed that the mean NO₂ concentrations in the 5th and 10th generation bronchi of humans was 12-fold and 8-fold higher, respectively, than that of rats. However, modeled NO₂ concentrations in the most oxidant-sensitive airways, considered to be the 16th or 17th airway generation in rats and humans, was not estimated.

It would be useful to mention that while there are some areas in which specific mechanisms in rodents might differ from those in human and non-human primates, there are several biological pathways that are sufficiently similar that useful comparisons can be drawn.

Comparisons of NO₂ effects between human and animal experimental studies have been highlighted in several sections of the report. For example, morphological findings and experimental dosimetry data in both human and experimental animal studies identify the bronchiolar-alveolar duct junction, or centriacinar region, as the primary target site of lung damage due to NO₂ inhalation.

Studies examining the interaction of NO₂ with allergens in experimental animals have now been thoroughly reported and compared with the human data. Unlike human exposures, experimental animal exposures to higher concentrations of NO₂ (about 5 ppm and greater) have more consistently produced indicators of allergic asthma. However, indicators of allergic asthma were similar to that found in humans, including enhancement of delayed-type dyspneic symptoms, increased serum IgE levels, increased pulmonary eosinophilia and epithelial injury, and increased bronchial hyperresponsiveness.

Also emphasized in the report is that human studies have suggested a link between NO₂ exposure and cardiovascular effects in patients with diabetes mellitus and those with cardiovascular diseases who have high risks of atherogenesis. Similar findings have been observed by Takano et al. (2004) in an obese rat strain prone to cardiovascular-type diseases. Increased blood levels of triglycerides and decreased HDL and HDL/total cholesterol ratio occurred when the rats were exposed to 0.16 ppm NO₂ for 24 weeks. A related normal rat strain similarly exposed also showed decreased HDL levels.

In addition, a section reviewing the toxicological effects of NO₂ in human in vitro test systems has now been included in Chapter 8. Alteration of alveolar macrophage function is a key sensitive indicator of pulmonary oxidant damage by NO₂. Pro-inflammatory changes were noted in exposed alveolar macrophages in the Staff Report in both human and experimental animal in vitro studies.

Since the mandate for this review was specific for the health/welfare of infants and children, it would be helpful if this chapter emphasized the issues that are specific to infants and children such as: growth, proliferation, differentiation, respiratory rates/pulmonary functions, time/activity outdoors. This then leads into a discussion of choosing the proper model and the advantages and limitations of available models.

A new section has now been added to Chapter 8 (Section 8.5, Effects on Development) that reviews in detail all pre- and post-natal NO₂ exposure studies investigating primarily effects during lung development. In addition, a discussion of animal model choices and comparison with recent episodic ozone monkey

exposure studies are now included in the Effects of Development Summary in Section 8.5.1.

Also, since allergic/asthmatic individuals are discussed, some discussion regarding proper choice of the immunologic models would be helpful.

Text has now been added to Chapter 8, Section 8.3.6: Effects on the Pulmonary Immune Response and Interaction with Allergens, which includes advantages and disadvantages of various animal models used in NO₂/allergen exposure studies, primarily guinea pigs, mice, rats, and rabbits.

Also, some mention of *in utero* exposures and issues would be helpful (if for no other reason than to highlight the lack of information available).

A review specific for in utero exposure studies is now included in Section 8.5 (Effects on Development). The scarcity of studies in this area, considering the potential sensitivity of developing animals to NO₂, is discussed in Section 8.10 (Conclusion).

Chapter 9

Chapter 9 discusses effects on vegetation. Welfare effects are not being used as the basis for the proposed changes in the standards, but it is important to note that some welfare benefits are likely to occur as a result of reducing NO₂ emissions (or preventing increases), especially in the South Coast and Central Valley areas. The summary statements in the Staff Report (p. 13-14) are too weak on this and unnecessarily suggest minimal benefit.

The Staff Report now includes new section 1.1.1 (Summary of Non-Health Issues) and section 2.6 that discuss critical loads of nitrogen deposition in California and visibility effects. The Technical Support Document now includes a new Chapter 10, "Effects on Visibility" that summarizes formation of NO₂-derived PM nitrates and their effects on visibility.

Chapter 9 focuses a lot on foliar injury and it may be that most areas do not have ambient concentrations of NO₂ sufficiently high to cause visible foliar damage. However, a more significant ecosystem concern is total nitrogen deposition. This is discussed in Chapter 9, but not carried over to the summary in the Staff Report. The discussion on page 9-23 suggests that critical loads (deposition rates that can be tolerated without harmful effects on an ongoing basis) for California mountain ecosystems may be higher than in other locations, but the specific critical loads for these areas have not been established. Nitrogen deposition rates reported in Figure 9.5 are some of the highest in the country. NAPAP (2005) reports that the highest annual total nitrogen deposition rates in the Midwest and Northeast range 8-11 kg/ha/yr. Figure 9.5 shows rates at 9

kg/ha/yr or higher (up to 97.5!) at multiple sites in Sequoia, Angeles, and San Bernardino National Forests. The superintendent of Rocky Mountain National Park recently proposed a critical load standard of 1.5 kg/ha/yr for the park because it is now showing signs of nitrogen saturation (with annual N deposition rates in the range of 3-4). NAPAP (2005) notes evidence of elevated concentrations of nitrate in surface and ground water in the San Gabriel and San Bernardino Mountains, which suggests possible N saturation in those forests. Reducing NO₂ emissions, especially in the South Coast basin, will result in reduced nitrogen deposition and this can be expected to benefit the forest ecosystems and reduce nitrogen concentrations in surface and ground water.

National Acid Precipitation Assessment Program. NAPAP Report to Congress: An Integrated Assessment. Washington DC, August 2005

<http://www.al.noaa.gov/AQRS/reports/napapreport05.pdf>

Chapter 9 was revised to refer to the NAPAP findings and report.

Appendix

Some members of the committee provided extensive comments which were integrated into the above summary. This necessitated extraction of material for insertion into comments on specific chapters. To ensure that the sense of these comments was not lost, they are included below in their entirety.

We appreciate the additional comments and suggestions of the AQAC members. They have been considered in revising staff report and technical report.

Specific Comments regarding the Appendix:

Page A-3-5: The dosimetry section is well-written, but under utilized. This information could be used to help extrapolate the doses used for the animal studies (especially since the animal tissue dose is 2-4 times less than humans). It could be useful to point out that after taking dosimetry into account a rat study at 0.25 ppm is approximately equivalent to a human study at 0.0625 to 0.125 ppm.

To highlight this important point, as noted above, text has been added to the introduction to emphasize the potential pharmacokinetic sensitivity of humans relative to rodent species. This pharmacokinetic comparison between species provides the basis for reviewing animal studies using NO₂ exposures up to 1 ppm.

Page A-4, last ¶: Is there a reference for measuring reduction in lung lining fluid thickness in distal airways? Was this inferred or actually measured?

The lung lining fluid thickness parameters used in the references cited (Miller et al. 1992, Overton et al. 1995) are inferred from previous modeling studies by the

researchers with ozone. Lining fluid thickness in distal airways is based on actual measurements by other authors but appear to vary greatly in thickness without specific reference by airway generation (i.e., 0.1-8 μm fluid thickness for airway generations 3-19). Certain assumptions appear to have been made in the model, using a range of about 0.25 to 0.062 μm for lining fluid thickness at airway generation 20. The text will clarify that lung lining fluid thickness is based, in part, on actual measurements in conducting airways.

Page A-5-6: Clarify whether this refers to tissue or BAL effects. Also, it would be helpful to contrast the kind of information that can be obtained from BAL vs. tissue (i.e. site-specific data vs. whole lung data).

The text has been clarified to indicate whether tissue/histopathological or BAL techniques were used to assess NO_2 -induced pulmonary inflammation. Text has been added to describe the focal nature of NO_2 -induced pulmonary inflammation, and that BAL and whole lung homogenate analysis are likely to be too broad-spectrum in approach to detect injury.

Page A-6, 1st full ¶: line 6: define “continuous” exposure (also p. 9, 2nd ¶, line 5). If “continuous” actually means 24 h/day, then these studies should be moved to a separate section and given little weight. A continuous exposure will result in an adaptive or tolerant pulmonary response completely different from the response to a more realistic intermittent or episodic exposure.

The text will clarify continuous exposure as 24 hr/day in the section in question. A number of studies employing continuous exposure are located throughout the chapter in nearly all sections. However, the studies highlighted in Section 8.9, Summary of Relevant Effects, are primarily intermittent exposure studies. Those studies discussed in Sections 8.9 and 8.10 (Conclusion) that use continuous exposure are now identified as such and the text includes a recommendation that less emphasis should be given to those studies employing continuous exposure conditions.

Page A-9, lines 6-7: It would be helpful to specify which studies in the 1992 review were used.

The studies covered in the 1992 Review are now mainly presented in the first or second paragraph of each section and are identified as such. To provide a more complete history of NO_2 exposure studies in animals, more of the most relevant studies (e.g., emphasis on studies using 0.5 ppm or less) presented in the 1992 Review have been included in the current review. This inclusion will allow easy comparison with more recent studies.

Page A-10; ferret work: Please discuss the appropriateness of the ferret as a model. For example, the lung development of the ferret may be similar to the human, but it would be appropriate to mention that their long trachea can scrub out pollutants before they reach the lungs, therefore underestimating the effective dose in extrapolation.

The ferret has been used as a model for air pollution work because ferret lung physiology/anatomy more closely resembles human physiology/anatomy than the rat or mouse. The validity of the ferret model relative to the rat model has been experimentally investigated. A study by Sterner-Kock et al. (2000) looked at ozone-induced lung damage and found that the lesions in ferrets more closely resembled the damage in primates than the rats. Primates are considered the best human model. Additional text will be added to support the validity of exposure studies in ferrets relative to rats.

It is possible that the relatively long trachea in ferrets compared to humans may act to scrub out pollutants and thus reduce the relative dose, with the most significant relative reductions likely to be for the more water soluble pollutants. We were unable to locate scientific studies quantitatively modeling or measuring the relative possible differences in dosimetry. However, the ferret is an improvement over the rodent model in terms of anatomical/physiological similarity to humans.

Page A-11: In vitro studies: need to clarify that the morphological lesions for NO₂ are focal, therefore caution should be used in interpreting negative data from BAL or whole lung homogenates (the small percentage of tissue affected may be overwhelmed by the large percentage of tissue not affected in these non-specific methods).

The text has been revised to incorporate this information.

Page A-11: In vitro studies, 1st ¶, last sentence: What studies specifically in the 1992 review are being referenced?

The reference referred to has now been cited in the text:

Sagai M, Ichinose T, Kubota K. 1984. Studies on the biochemical effects of nitrogen dioxide. IV. Relation between the change of lipid peroxidation and the antioxidative protective system in rat lungs upon life span exposure to low levels of NO₂. Toxicol Appl Pharmacol 73: 444-456.

Morphological data should come first in the Tox studies. Knowing *where* the injury is will affect how the biochemical effects are interpreted.

The morphological data has now been moved up to the beginning of Section 8.3, Respiratory Tract Effects

Page 26, 4th ¶: same issues for morphological affects in ferret as described above.

Text was also added here to emphasize the validity of the ferret model relative to the rat model for oxidant pulmonary damage research.

Page 26-27: The study of newborn mice with the structural changes should be placed to have more emphasis.

To emphasize the importance of this study along with other developmental studies, developmental animal data is now included in a new section of Chapter 8 (Section 8.5, Effects on Development) that reviews in detail all pre- and post-natal NO₂ exposure studies investigating primarily effects during lung development. In addition, this study and other developmental studies are highlighted at the end of the chapter in Section 8.9, Summary of Relevant Effects, and in Section 8.10, Conclusion.

Staff Report and Recommendations.

The SR is generally well written but some areas need to be improved. There is no discussion of whether or not there is a threshold for NO₂ effects. There are some articles that were not cited in the TSD that could be added. Samoli and Vedal, respectively, discuss epidemiological data from European and Canadian studies (Samoli et al. 2003, Vedal et al. 2003) that provide some discussion on the identification of thresholds and why measurement errors could obscure detection of a threshold. Another factor that could be mentioned is that if a contaminant was a surrogate for another contaminant a threshold might not be detectable. Vedal et al. report that “increases in air pollutant concentrations, even when concentrations are low, are associated with adverse effects on daily mortality. Although this observation may support the argument that there are no threshold concentrations of air pollution below which adverse effects cannot be detected, it also raises concern that the associations are not reflecting the effects of the measured pollutants, but rather some factor or combination of factors, such as, for example, unmeasured air pollutants or uncontrolled features of meteorology that are correlated with the measured pollutants.” The APHEA-2 data (Samoli et al. 2003) was unable to detect a threshold (i.e., a linear non-threshold model could adequately describe the data), however they provide the caution “The NO₂-mortality association in the cities included in the present analysis could be adequately estimated using the linear model. However, it became evident that the linear model should not be applied without investigating the city specific dose-response curves first.”

As we indicated in our recommendations chapter, most of the time-series studies used linear non-threshold models and did not explicitly test for thresholds. We also indicated that these studies need to be viewed with some caution since it is difficult to separate out the effects of NO₂ from other co-varying pollutants.

The committee endorses the addition of the long-term 30 ppb annual standard and also endorses the “not to be exceeded” form of the proposed standard. The short-term standard is based primarily on human clinical studies rather than on epidemiological studies. The TSD and SR both make the point that effects are relatively robust at or above the current 250 ppb standard but that some studies also demonstrate significant changes at levels of about 200 ppb. Data used in Germany to set a short term standard (Kraft et al. 2005) showed effects down to about 200 ppb but effects on patients with mild asthma were not observed after short-term exposure to concentrations below about 100 ppb. This is consistent with the data summarized in the TSD. The logic applied to arrive at the proposed lowered short term standard (180 ppb) needs to be better described. The criteria for assuring an adequate margin of safety should be transparent. There is a dilemma in that the epidemiological data could be interpreted as indicating that a lower short term standard is warranted. However the committee also recognizes that causality in the epidemiological studies is difficult to ascribe solely to NO₂, hence the use of the chamber studies to develop the standard is acceptable.

We have clarified the basis for the proposed short-term standard of 0.18 ppm in the revision of the OEHHA Recommendations and Staff Report.

For the determination of the standard for the one-hour average, we used the studies that found exposure to NO₂ at 0.26 ppm for 30 min enhances the allergic immune response in asthmatics (Strand et al. 1997, 1998; Barck et al. 2002, 2005). We also used the four studies finding increased airway reactivity in asthmatics between 0.25-0.30 ppm for (30 min – 1 hr). With the exception of one peer-reviewed study, other studies have not found evidence of airway reactivity at 0.1 ppm. Evidence of these health effects was reported for relatively healthy asthmatics exposed in the range of the current standard for 30 minutes. In order to protect against a 30 min exposure at 0.26 ppm, we would need to lower the 1-hr standard to below 0.25 ppm (i.e., a level of 0.26 ppm for 30 minutes followed by a 30 minute exposure at a lower concentration, would yield a 1-hr average below 0.26 ppm).

To provide an adequate margin of safety, we chose a level of 0.18 ppm (1 hr average). This value is half-way between 0.26 ppm where effects have been consistently demonstrated, and 0.1 ppm, which is the lowest level studied that appears to have no clear effect.

The committee is also concerned that the location of the NO₂ ambient monitors is not adequate to provide protection to individuals living in “hot spots.” The relocation of monitors to provide better spatial representation of NO₂ exposures in each of the air basins, similar to the approach used for CO, would benefit protection of public health.

Staff has started a review of the siting of California’s NO₂ ambient monitors to provide better spatial representation of NO₂ exposures in each of the air basins, in consideration of the concern regarding “hot spot” exposures.

The welfare benefits of controlling NO₂ could be expanded. On page 14, the Staff Report suggests that there may be little improvement in visibility as a result of the reduction in the NO₂ standard. It was mentioned that the 0.25 hour standard was expected to be protective of the discoloring effect that NO₂ causes (the brown color to the air). Has it really been established that there is no brown color at concentrations below 0.25 ppm? Also, the statement that most of the haze is caused by particulate fails to acknowledge that NO₂ emissions contribute to the formation of secondary particulate. Thus, some visibility improvements can be expected as a result of further reduction in NO₂ emissions even if the discoloration is no longer an issue.

The staff reports include the new Chapter 10 on visibility in the TSD, and additional discussion on visibility in the SR. These reports now state that some brown color can be perceived at concentrations below 0.25 ppm, although the perception of color at this low level is quite small. These reports also state that reducing NO₂ emissions should lead to visibility improvements through the reduced formation of secondary particulate matter.

Appendix D

Summary of Public Comments (by Commenter) and Staff Responses

Appendix D

Summary of Public Comments (by Commenter) and Staff Responses

Note: Comments are in regular type, and responses are in bold italics.

Western State Petroleum Association

1. “For the 1-hour standard, OEHHA relied on two key findings on making recommendations – one study on enhanced allergic response and several inconsistent studies on airway reactivity.”

Table 6.6 of the Technical Support Document shows a number of studies finding enhanced allergic response after NO₂ exposures. All four studies at 0.26 ppm exposures found evidence of enhanced allergic response.

Airway reactivity in asthmatics was increased after NO₂ exposures at 0.2-0.3 ppm in five studies. Although there were some negative studies with similar but not identical protocols, the fact that five studies were positive makes it extremely unlikely that this is due to chance. Differences in subjects selected for the study are likely reasons for negative findings in some studies. Even in studies with a negative group mean response, there are “responders” as supported by the pooled analysis by Folinsbee (1992). Thus, some asthmatics appear sensitive to NO₂. Health standards are set to protect vulnerable populations.

2. “OEHHA relied heavily on Barck et al. (2005) for the 1-hour standard. This study reported on subclinical effects (increases of two inflammation markers in the blood), but there were no clinical effects. Therefore, it is unclear that this study justifies revision of the standard.”

Table 6.6 Pg A-13 of the Staff Report shows that OEHHA relied on a body of evidence finding enhanced allergic response after NO₂ exposures. Several studies found increased decrements in lung function at 0.26 ppm, and others found evidence of increased markers of allergic inflammation (but not lung function changes) at 0.26 ppm. Allergic inflammation is part of the pathophysiology of asthma. Although “subclinical”, the marker, ECP, has been correlated with increased asthma symptoms.

3. “OEHHA noted three areas of concern involving the epidemiological studies cited in support of the annual standard: 1) determining actual exposure concentrations, 2) separating out confounding variables, and 3) determining averaging times. Because of these uncertainties, these studies do not provide sufficient support for the annual standard.”

4. “The Staff Report and Technical Support Documents should include sections addressing uncertainties.”

In response to items 3 and 4, we agree that uncertainties exist and will always remain, and we have cited the limitations of the scientific studies specific to examining NO₂ in the Staff Report and Technical Support Document and provided our rationale in support of the need for an annual standard.

Joint submission by the Alliance of Automobile Manufacturers, and the Engine Manufacturers Association

1. “Section 1.9 (Levels of NO₂) should present additional data on the distribution of ambient levels for the purpose of evaluating the plausibility of health effects implied in the epidemiological studies.”

The Staff Report contains concise summarized air quality and exposure data. However, the Technical Support Document contains more detailed air quality information by air quality basin. Included in this document are frequency distributions of maximum 1-hour daily NO₂ concentrations by air basin, for example.

2. “Because people spend most of their time indoors, and because ambient NO₂ levels can be lower indoors, some epidemiological studies can be confounded.”

The short-term epidemiological studies and some of the long-term studies cited in support of an annual average standard utilize ambient concentrations of NO₂ as an estimate of exposures. For the time series studies that examine day-to-day changes, it is unlikely that the indoor sources will confound the association since they would have to change on a daily basis and be correlated with outdoor NO₂. There is no evidence of an association between ambient concentrations of NO₂ and presence of indoor sources of NO₂. Any contribution of indoor sources of NO₂ to overall exposures to NO₂ would lead to exposure misclassification and could bias results of the association towards the null, making it more difficult to find an association, if one existed.

3. “This statement is not defensible: Although the staff reports notes the difficulty of separating NO₂ effects from all other air pollution effects, staff states that the results of the epidemiological studies are consistent with the health effects when only NO₂ alone is tested in chamber and toxicological studies.”

As discussed in the Technical Support Document, the epidemiological, clinical (chamber) and toxicological studies provide consistent evidence for respiratory health effects of NO₂ particularly for asthmatics or those with bronchial hyperresponsiveness. Although it is difficult to separate out the independent

effect of NO₂ versus other pollutants, it is the case that the epidemiological results are consistent with those found using other study modalities.

4. “Prudent public health policy may warrant some level of protection from exposure to NO₂, but the current and proposed 1-hour standard will provide protection, so that a long-term standard is not needed.”

As noted in the Staff Report and the Technical Support Document, the epidemiological studies suggest the need for an additional standard that includes an averaging time greater than one-hour. In several parts of California, the one-hour standard may be met but elevated longer-term averages will remain. Given the severity of the health effects observed in the epidemiologic studies (e.g., mortality, hospital admissions), effects related to this longer averaging time are of concern.

5. “Referring to “potential enhancement of asthma in some asthmatics”, “Even these effects occur very rarely, since 99.9% of peak daily 1-hr NO₂ concentrations are below 0.13 ppm at the 114 monitoring sites in California.”

Controlled human exposure studies show effects near the current 1-hr standard of 0.25 ppm. Our standards are based on the existing scientific evidence of health effects, not on current concentrations or attainment status. Although most areas of California are well below the recommended 1-hr standard of 0.18 ppm, several monitoring sites have had exceedances in recent years.

6. “The staff report and Technical Support Document should carefully evaluate animal studies instead of using a blanket statement that enhancement of allergic response by NO₂ is consistent with the human data. The staff report was not very informative and should be rewritten to include dose information needed to elicit effects.”

The toxicology section will include a more specific explanation regarding animal/human similarities for NO₂ enhancement of allergic response. In allergic animal models, NO₂ exposure elevated IgE, pulmonary eosinophilia, and airway hyper-responsiveness, all hallmarks of asthma seen in humans. Although exposure to elicit response is greater in animals (≤5 ppm vs. 0.25 ppm), dosimetry studies indicate that exposure of the distal airways is 2 to 4 times greater for humans than animals at the same concentration. The staff report’s toxicology summary will be modified and expanded and include dose levels and durations.

The Staff Report sections covering the toxicological studies are intended to be short summaries. The bulk of the information including exposure information is in the Technical Support document. However, these sections will be modified and expanded somewhat to present a more complete summary of the animal toxicology, and will include exposure concentrations and durations used.

7. "Several factors limit interpretation of epidemiologic association of NO₂ and health effects: publication bias, model selection uncertainty, and biologic impossibility."

Publication bias is unlikely in multi-city studies or when considering NO₂ in PM-centric studies. OEHHA did not rely on "single-estimate, single site analysis" but rather attempted to survey all of the available literature. Model selection was shown in the HEI re-analysis to have small effect on estimates. The Koop and Tole review was conducted for one city, assumed no prior information, and was not recommended by HEI. We agree that uncertainties exist and will always remain, and we have cited the problems specific to examining NO₂. Regarding biological plausibility, the most robust findings in the short-term and long-term epidemiological studies are related to respiratory health effects of NO₂. The results of the human controlled chamber studies, and the animal and in vitro toxicology studies, support the respiratory effects seen in the epidemiological studies. The effects on asthmatics appear particularly plausible, given the findings in all of the study designs. These respiratory studies form the basis for the recommendations of the annual average standard.

8. "The staff findings overstate the confidence of the reported associations with real health effects. Given the many qualifications in the document, there is little confidence in the health effects evidence justifying the proposed standard reduction. The OEHHA report notes the contradictory and inconsistent results in many studies."

Staff provided the full range of study findings and the issues that are specific to both clinical and epidemiology studies of NO₂. While some factors (high correlation of pollutants) may tend to lead to false positive outcomes for an effect of NO₂ exposure, some factors (epidemiologic measurement error, limits of clinical studies) may lead to false negative outcomes. While the existing studies are not totally consistent, staff's review of the chamber, toxicology, and epidemiology studies suggests support for an NO₂ effect on asthmatics and possibly other sensitive sub-populations.

As indicated in the document, it would be imprudent to ignore findings from both the chamber and epidemiology studies. This includes findings in chamber studies at sub-clinical effects that could lead to asthma exacerbation, and epidemiology studies that indicate premature mortality, possible loss in lung function, and asthma exacerbation causing increased hospitalization and ER visits. In addition, effects such as birth outcomes are broadly related to traffic. Therefore, staff recommendations represent a reasonable and responsible public health policy.

Comments were received from:

1. Western States Petroleum Association
2. Alliance of Automobile Manufacturers/Engine Manufacturers Association